Hair cortisol as a neuroendocrine biomarker to evaluate the impact of chronic stress on the interaction between neuropsychiatric disorders and metabolic syndrome

by

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Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

This dissertation includes one original paper published in peer-reviewed journals or books and six unpublished publications. The development and writing of the papers (published and unpublished) were the principal responsibility of myself and, for each of the cases where this is not the case, a declaration is included in the dissertation indicating the nature and extent of the contributions of co-authors.

Date: March 2020

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Summary

Individuals with neuropsychiatric disorders (NPDs) demonstrate increased rates of cardiovascular disease (CVD) and metabolic syndrome (MetS). There is evidence of dysregulated hypothalamic pituitary adrenal (HPA) axis functioning in both NPDs and CVD and the HPA-axis may be a shared mechanistic pathway contributing to NPD-CVD comorbidity. Very few studies have, however, directly examined the association between NPDs, CVD risk and HPA-axis function. Hair cortisol concentrations (HCC), reflecting longer-term systemic cortisol levels, can provide insight into the role of HPA-axis dysregulation in the occurrence of CVD risk, as defined by MetS, in NPDs.

This study was a neuroendocrine ancillary study to 'Understanding the SHARED ROOTS (SR) of Neuropsychiatric Disorders and Modifiable Risk Factors for Cardiovascular Disease'. SR was a cross-sectional matched case-control study investigating the pathways contributing to the comorbidity of MetS in NPDs and included three NPD cohorts (posttraumatic stress disorder (PTSD), schizophrenia and Parkinson's disease). This study investigated the role of HPA-axis dysfunction, as measured by HCC, in the three NPDs as compared to controls and in relation to NPD-MetS co-occurrence.

We demonstrated that HPA-axis function was altered in the three NPDs, with higher HCC in PTSD patients than trauma exposed controls, lower HCC in patients with schizophrenia than controls and higher hair cortisone levels, but not HCC, in Parkinson's disease patients than controls. MetS was not associated with HCC in any of the individual cohorts. The lack of significant findings related to MetS may have been due to limited statistical power to detect significant associations in the individual cohorts.

Additionally, as this is one of the first studies investigating HCC in South Africa and the majority of studies have been conducted in developed regions, we sought to identify basic determinants of HCC in a South African mixed ancestry control sample. The main determinants associated with HCC were age, level of education, duration of sun exposure,

hair product use, duration of sample storage and breastfeeding in women. We also demonstrated that resilience, but not self-perceived stress, was significantly inversely associated with HCC, underscoring the importance of identifying stress-resilience indicators of HCC in non-pathological samples. Finally we also found that poorer working memory performance was associated with higher HCC, suggesting an association between a neuroendocrine marker of chronic stress and working memory deficits.

This is the first study to utilise a measure of longer-term HPA axis function to investigate the links between HPA-axis, NPDs and CVD risk. Considering the high burden of CVD in NPDs, this study provides a step towards better understanding the role played by chronic stress, as reflected by long-term HPA axis dysfunction, in the co-occurrence of CVD in NPDs. Furthermore, this study provides insights into the role of HPA-axis dysfunction in relation to clinical conditions and subjects of relevance to South Africa and contributes to broader geographic, cultural and ethnic representation in hair cortisol research.

Opsomming

Individue met neuropsigiatriese versteurings (NPVs) demonstreer ‘n verhoogde voorkoms van kardiovaskulêre siekte (KVS) en metaboliese sindroom (MetS). Daar is bewyse dat die hipotalamus pituïtêre adrenale (HPA) as in beide NPVs en KVS wanfunksioneer en die HPA-as is moontlik 'n gemeenskaplike meganistiese baan wat tot NPD-KVS komorbiditeit bydra. Baie min studies het egter al die verwantskap tussen NPVs, KVS risiko en HPA-as funksie direk ondersoek. Haar kortisol konsentrasies (HKK), wat langtermyn sistemiese kortisolvlakke weerspieël, kan insig aangaande die rol van HPA-as disregulasie in die voorkoms van KVS risiko, soos gedefinieer deur Mets, in NPVs bied.

Hierdie studie was 'n neuro-endokriene aanvullende studie tot ‘Understanding the SHARED ROOTS (SR) of Neuropsychiatric Disorders and Modifiable Risk Factors for Cardiovascular Disease’. SR was 'n deursnit gepaarde gevallekontrolestudie wat die roetes wat tot die komorbiditeit van MetS in NPVs bydra in drie NPV kohorte (post-traumatiese stresversteuring (PTSV), skisofrenie en Parkinson se siekte) ondersoek het. Hierdie studie het die rol van HPA-as disfunksie, soos gemeet deur HKK, in die drie NPVs met kontroles vergelyk, asook in verhouding tot NPV-MetS mede-voorkoms ondersoek.

Ons het getoon dat HPA-as funksie in die drie NPVs gewysig is, met hoër HKK in PTSV pasiënte as trauma blootgestelde kontroles, laer HKK in pasiënte met skisofrenie as kontroles en hoër hare kortisoon vlakke, maar nie HKK nie, in pasiënte met Parkinson se siekte as kontroles. MetS was nie geassosieer met HKK in enige van die individuele kohorte nie. Die gebrek aan betekenisvolle bevindinge met betrekking tot MetS is moontlik te wyte aan beperkte statistiese onderskeidingsvermoë om betekenisvolle assosiasies in die individuele kohorte te demonstreer.

Aangesien hierdie een van die eerste studies is wat HKK in Suid-Afrika ondersoek en die meerderheid van die studies in ontwikkelde streke gedoen is, het ons beoog om die basiese bepalingsfaktore van HKK in 'n Suid-Afrikaanse gemengde afkoms kontrole steekproef te

identifiseer. Die belangrikste bepalingsfaktore wat met HKK verband gehou het was ouderdom, opvoedingsvlak, duur van blootstelling aan die son, gebruik van haarprodukte, duur van monster berging en borsvoeding in vroue. Ons het ook getoon dat veerkragtigheid, maar nie self-waargenome stres nie, aansienlik omgekeerd met HKK geassosieer was, wat beklmetoon hoe belangrik dit is om stres-veerkragtigheid aanwysers van HKK in nie-patologiese steekproewe te identifiseer. Laastens het ons ook bevind dat swakker werkende geheue prestasie, met hoër HKK geassosieer was, wat dui op ‘n assosiasie tussen 'n neuro-endokriene merker van chroniese stres en werkende geheue terkorte.

Hierdie is die eerste studie wat ‘n maatstaf van langtermyn-HPA-as funksie gebruik om die verband tussen HPA-as, NPVs en KVS risiko te ondersoek. In ag genome die hoë las van KVS in NPVs, bied hierdie studie 'n tree tot ‘n beter begrip van die rol wat kroniese stres, soos weerspieël deur lang termyn HPA-as disfunksie, in die mede-voorkoms van KVS in NPVs speel. Verder bied hierdie studie insigte aangaande die rol van HPA-as disfunksie in kliniese toestande en onderwerpe van belang vir Suid-Afrika en lewer ‘n bydrae tot breër geografiese, kulturele en etniese verteenwoordiging in haar kortisol navorsing.

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Table of contents

CHAPTER 1

Introduction

Introduction

This chapter provides a brief overview of themes and concepts and conveys the study context. As the chapters are written in manuscript format, additional background information is presented in each chapter. The study objectives and hypotheses are then described. This is followed by a brief overview of the parent study within which the dissertation is nested. Finally, each chapter is outlined and a rationale for the approach used throughout is provided.

1 Background

1.1 The hypothalamic pituitary adrenal axis

The hypothalamic pituitary adrenal (HPA) axis is one of the primary pathways involved in the stress response. Following exposure to a stressor the HPA-axis response is initiated in the hypothalamus by connected neural pathways, resulting in the release of corticotrophin releasing hormone (CRH) which induces the anterior pituitary to secrete adrenocorticotropic hormone (ACTH) into the circulation. ACTH stimulates the adrenal cortex to produce and secrete cortisol which then mediates various aspects of the stress response primarily through binding to intracellular glucocorticoid receptors (GR) and influencing gene transcription (Chrousos, 2009; McEwen & Gianaros, 2010). The physiological effects of cortisol are related to mobilising energy stores by the breakdown of fats and proteins and increased gluconeogenesis, by suppressing certain inflammatory and immune responses, and by influencing behaviour (Chrousos, 2009). The stress response is regulated by a negative feedback loop, whereby cortisol binding to GR receptors in the hypothalamus and anterior pituitary halts further release of CRH and ACTH. Prolonged or severe stress can result in dysregulation of the HPA-axis with either blunted or increased cortisol release (McEwen, 2008). When the HPA-axis and related systems, such as immune and autonomic systems, become maladapted through prolonged or severe stress this is known as allostatic load (Juster, McEwen, & Lupien, 2010; McEwen, 1998). HPA-axis dysfunction and associated allostatic load can have significant detrimental effects on various systems, including the cardiovascular and central nervous systems (CNS), and contribute to illness development (McEwen & Gianaros, 2010).

1.2 Methods used to analyse hypothalamic pituitary adrenal axis function

Different methods have been employed to evaluate HPA-axis function, with measurement of cortisol levels the most frequently employed. Cortisol has traditionally been measured in blood (plasma and serum), saliva and urine and these include basal measurements of cortisol, repeat measures used to obtain diurnal secretion patterns and the cortisol-awakening response (CAR) which estimates the increase in cortisol following awakening from sleep (J.Yeo, Babic, Hannoush, & Weiss, 2000; Kudielka & Wüst, 2010). Other tests of HPA-axis function include measuring levels of other HPA-axis related hormones and proteins, such as ACTH, CRH, cortisol binding globulin (CBG), and 11-deoxycortisol (compound S) (J.Yeo et al., 2000). Further tests involve psychological and pharmacological challenge tests, where cortisol (and ACTH) levels are measured following exposure to either a psychological or pharmacological challenge. There are various pharmacological stress tests including the insulin tolerance test, the ACTH stimulation test, the CRH stimulation test, the metyrapone test, the naloxone challenge test, and the most frequently employed pharmacological stress test is the dexamethasone suppression test (DST) where the feedback inhibition of dexamethasone on the HPA-axis is measured (de Kloet et al., 2006; J.Yeo et al., 2000). Psychological stress tests involve exposure to various stressful paradigms, such as startling stimuli, novel situations, exposure to physically or psychologically painful stimuli, trauma related cues, and social performance and evaluation tasks, of which the Trier Social Stress Test (TSST) is the most frequently utilised (Allen et al., 2017; de Kloet et al., 2006; Gunnar, 2010; Zorn et al., 2017). Further measures that can be employed include utilising structural and functional imaging modalities of the hypothalamus, pituitary and adrenal glands (Borges, Gayer-Anderson, & Mondelli, 2013; J.Yeo et al., 2000). Each method of assessment provides different insights into HPA-axis function, however none of these methods are ideally suited to ascertain longer-term HPA-axis function and within this context measuring cortisol levels in hair samples has increasingly been investigated for this purpose.

1.3 Hair cortisol concentrations

Endogenous cortisol levels were first quantified in hair by Raul et al. in 2004 (Raul, Cirimele, Ludes, & Kintz, 2004). Since then hair cortisol concentrations (HCC) have increasingly been used as a measure

of long-term cortisol release in both medical and psychiatric conditions (Gray et al., 2018; Stalder et al., 2017; Wosu, Valdimarsdóttir, Shields, Williams, & Williams, 2013). As hair grows on average 1cm per month, compounds that are deposited in hair during its formation and growth come to reflect longer-term retrospective levels (Wennig, 2000). Traditional measurements of cortisol (e.g. in blood, saliva and urine samples) are better suited to determine dynamic or acute circulating levels with repeated sampling required to obtain estimates of longer-term cortisol exposure (Stalder et al., 2017; Wosu et al., 2013). Furthermore, these samples can be influenced by sampling and environmental factors impacting on acute cortisol secretion, such as diurnal fluctuations, physical activity, substance use, food intake and the stress associated with sampling (Gow, Thomson, Rieder, Van Uum, & Koren, 2010; Manenschijn, Koper, Lamberts, & Van Rossum, 2011; Stalder & Kirschbaum, 2012; Wosu et al., 2013). Hair sampling is non-invasive and simple and samples can be stored and transported at room temperature thereby increasing its utility for neuroendocrine measurement (Abell et al., 2016; Stalder & Kirschbaum, 2012). HCC have demonstrated consistency, stable intra-individual variability and show good correlation with traditional measures of cortisol (Manenschijn et al., 2011; Papafotiou et al., 2017; Russell et al., 2015; Stalder & Kirschbaum, 2012; Stalder et al., 2017, 2012; Wosu et al., 2013; Q. Zhang, Chen, Chen, Xu, & Deng, 2017). HCC thus provide a useful measure of chronic stress as reflected by long-term HPA-axis dysfunction.

1.4 The burden of neuropsychiatric disorders and cardiovascular disease

Non-communicable diseases (NCDs) including neurological conditions, mental disorders and cardiovascular disease (CVD), are the largest contributors to disability adjusted life years (DALY's) globally (Kyu et al., 2018). Of the non-communicable diseases, CVD, namely ischaemic heart disease and stroke, cause the greatest percentage of healthy years of life lost (Kyu et al., 2018; Whiteford et al., 2013). Psychiatric disorders are the largest contributor to years lived with disability (YLDs) and the burden of psychiatric disorders, as well as CVD and neurological disease has increased over the last decade (Kyu et al., 2018; Patel et al., 2018; Whiteford et al., 2013). In South-Africa, NCDs as well as the risk factors for NCDs, are on the rise and are also the leading cause of mortality (43.4%), with cerebrovascular disease, ischaemic heart disease, diabetes and hypertensive heart disease among

the top ten causes of death (Mayosi et al., 2012; Pillay-van Wyk et al., 2016). Mental disorders are also very prevalent in South Africa and in the South African Stress and Health (SASH) study mental disorders were rated as more disabling than physical conditions (Herman et al., 2009; Suliman, Stein, Myer, Williams, & Seedat, 2010). Despite mental disorders being perceived as more disabling, South Africans are much less likely to receive treatment for their mental health problems (Seedat et al., 2008; Suliman et al., 2010).

Individuals with neuropsychiatric disorders (NPDs) have increased rates of CVD, and risk factors for CVD, including metabolic syndrome (MetS), compared to the general population (Bradley & Dinan, 2010; Penninx & Lange, 2018; Vancampfort et al., 2015). MetS denotes a cluster of risk factors for CVD and type 2 diabetes mellitus. Although there is some variation in diagnostic criteria proposed by different scientific organisations and societies (Alberti et al., 2009; Huang, 2009; Kassi, Pervanidou, Kaltsas, & Chrousos, 2011), risk factors for MetS include central obesity, raised blood pressure, raised fasting glucose, and dyslipidaemia, namely raised triglycerides and low levels of high-density lipoprotein (HDL) cholesterol (Alberti et al., 2009). Individuals with MetS have double the risk of developing CVD in the next 5-10 years and MetS contributes to disease progression in individuals with established CVD and diabetes (Alberti et al., 2009). MetS is considered a public health problem, due to a rise in its prevalence, secondary to factors, such as obesity and sedentary lifestyles. Very high rates of type 2 diabetes (28.8%) and MetS (62.0%) have also been demonstrated in a South African mixed-ancestry (coloured) population from the Western Cape (Erasmus, R. T. Soita, D. J. Hassan, M. S. Blanco-Blanco, E. Vergotine, Z. Kengne, A. P. Matsha, 2012). In South Africa, patients with mental illness also demonstrate higher rates of risk factors for CVD and comorbid CVD is associated with greater disability in individuals with mental disorders (Saloojee, Burns, & Motala, 2016; Suliman et al., 2010). Individuals with NPDs have increased mortality risk and lower life expectancies and this is mainly secondary to CVD (Correll et al., 2017; De Hert, Detraux, & Vancampfort, 2018; Penninx & Lange, 2018; Walker, McGee, & Drus, 2015). Despite higher rates of CVD and associated mortality, individuals with mental disorders have decreased access to and receipt of adequate medical care (Laursen, Nordentoft, & Mortensen, 2014; Penninx & Lange, 2018; Walker et al., 2015). Furthermore,

the pathways linking NPDs and CVD remain poorly delineated (Correll et al., 2017; De Hert et al., 2018; Vancampfort et al., 2015) and need to expedite research investigating the factors that contribute to comorbidity that can inform prevention and treatment initiatives.

1.5 Hypothalamic pituitary adrenal axis dysfunction in neuropsychiatric disorders and cardiovascular disease

The HPA-axis is one of the pathways postulated to play a role in the co-occurrence of NPDs and CVD (Bradley & Dinan, 2010; Penninx & Lange, 2018). Altered HPA-axis function, as manifested largely by elevated basal cortisol levels, has been demonstrated in NPDs and in relation to CVD and MetS, suggesting a shared pathway contributing to NPD-CVD comorbidity (Bradley & Dinan, 2010; De Hert et al., 2018; Penninx & Lange, 2018). However, very few studies have directly examined the association between NPDs, CVD risk and HPA-axis function. HCC, a reflection of longer-term systemic cortisol levels, can provide insights into the role of HPA-axis dysregulation in the occurrence of MetS in NPDs.

1.6 The neuropsychiatric disorders included in this study

1.6.1 Posttraumatic stress disorder (PTSD)

Posttraumatic stress disorder (PTSD) denotes a characteristic set of symptoms that develop secondary to exposure to severely stressful, or traumatic events, involving potential or actual harm to self or others (American Psychiatric Association [APA], 2013). In PTSD, persisting symptoms involving intrusion and avoidance of the traumatic event occur alongside negative changes in cognitions and mood and increased reactivity, causing notable distress and impairment of functioning (American Psychiatric Association [APA], 2013). In the World Mental Health Surveys the global prevalence of trauma exposure was 69.7% and of those exposed 5.6% and 2.8% had a lifetime and current PTSD diagnosis, respectively (Koenen et al., 2017). Although the course varies, PTSD symptoms persist for an average of 6 years and the burden of PTSD is estimated at 77.7 lifetime person-years/100 respondents (Kessler et al., 2017). In the SASH study 73.8% of respondents had a history of trauma exposure and the lifetime prevalence of PTSD was 2.3%, with 36% of those with PTSD presenting with a severe form (Atwoli et al., 2013; Herman et al., 2009). PTSD is associated with increased rates of

MetS, CVD and associated mortality (Ahmadi et al., 2011; Bartoli, Carra, Crocamo, Carretta, & Clerici, 2013; Kubzansky, Koenen, & Spiro, 2007; Rosenbaum et al., 2015).

There is evidence of a dysregulated HPA-axis in relation to trauma and PTSD, with PTSD cases demonstrating lower basal cortisol levels than controls, although findings have been mixed (Meewisse, Reitsma, de Vries, Gersons, & Olff, 2007; Morris, Compas, & Garber, 2012; Schumacher et al., 2019). The number of studies examining HCC in PTSD have steadily been rising and these have contributed to a better understanding of the effects of trauma on HPA-axis function and how that relates to PTSD symptomatology (Dajani, Hadfield, van Uum, Greff, & Panter-Brick, 2018; Groer, Kane, Williams, & Duffy, 2015; Luo et al., 2012; Steudte-Schmiedgen, Kirschbaum, Alexander, & Stalder, 2016; Steudte et al., 2013, 2011; Straub, Klaubert, Schmiedgen, Kirschbaum, & Goldbeck, 2017; van Zuiden et al., 2019). There have, however, been very few studies (Blessing et al., 2017) examining the association between cortisol levels, PTSD and CVD risk, and none utilising HCC.

1.6.2 Schizophrenia

Schizophrenia is a serious psychiatric disorder where symptoms such as delusions, hallucinations, disorganisation of behaviour and speech, and reduced emotional expression persist and cause significant impairment (American Psychiatric Association [APA], 2013). The lifetime prevalence of schizophrenia is around 0.7% and individuals with schizophrenia have a 3 fold higher risk of dying on the basis of the standardized mortality ratio (McGrath, Saha, Chant, & Welham, 2008). Although schizophrenia is not a prevalent disorder it contributes 1.7% of years lived with disability (YLD) and the burden of disease is increasing, particularly in low- and middle-income countries (Charlson et al., 2018). Individuals with schizophrenia demonstrate increased rates of MetS and CVD and amongst the highest mortality risk observed among psychiatric disorders (Correll et al., 2017; Mitchell et al., 2013; Vancampfort et al., 2013; Walker et al., 2015). There is evidence of HPA-axis dysregulation in schizophrenia, with patients generally demonstrating higher basal cortisol levels than controls (Borges et al., 2013; Bradley & Dinan, 2010; Gajsak, Gelemanovic, Kuzman, & Puljak, 2017; Girshkin, Matheson, Shepherd, & Green, 2014; Hubbard & Miller, 2019). There have now also been a number of studies examining HCC in patients with schizophrenia and first-episode psychosis (FEP) providing

insights into long-term HPA-axis function in schizophrenia (Aas et al., 2019; Andrade et al., 2016; Streit et al., 2016; Touskova et al., 2018). Very few studies (Manzanares et al., 2014; Vuksan-Cúsa, Săgud, Mihaljevic-Peleš, Jaksĭć, & Jakovljević, 2014) have examined cortisol levels in relation to CVD risk in schizophrenia and none have utilised HCC.

1.6.3 Parkinson's disease

Parkinson's disease is a neurodegenerative disorder with the core symptoms of bradykinesia, rigidity and resting tremor associated with dopaminergic neuronal death in the substantia nigra (Wirdefeldt, Adami, Cole, Trichopoulos, & Mandel, 2011). Parkinson's disease is the second most common neurodegenerative disorder and affects 1% of people older than 60 years (Tysnes & Storstein, 2017). The prevalence of, and mortality and DALYs secondary to, Parkinson's disease has increased globally, as well as in South Africa (Ray Dorsey et al., 2018). There is mounting evidence that CVD risk factors and MetS can increase the risk for Parkinson's disease and that MetS comorbidity in patients with Parkinson's disease contributes to worse outcomes (Bainbridge et al., 2017; Nam et al., 2018; P. Zhang & Tian, 2014). Patients with Parkinson's disease demonstrate HPA-axis dysregulation and in general have higher basal cortisol levels than controls (Du & Pang, 2015; Herrero, Estrada, Maatouk, & Vyas, 2015; Soares, Pereira, Altmann, de Almeida, & Rieder, 2019). There have been no studies examining HCC in Parkinson's disease patients and no studies examining cortisol levels in relation to CVD in patients with Parkinson's disease.

HCC, reflecting long-term systemic cortisol exposure, can provide further insights into the role of HPA-axis dysregulation in PTSD, schizophrenia, and Parkinson's disease and the relationship with co-occurring CVD risk.

2 Study objectives

Primary

1. To evaluate the role of chronic stress, as measured by hair cortisol, in the interaction between three NPDs (PTSD, schizophrenia and Parkinson's disease) and MetS;

2. To determine and compare the neuroendocrine signatures in these three NPDs (PTSD, SCZ and PD) and undertake a case-control comparison of HCC in these NPDs.

Secondary

1. To characterise the neuroendocrine status of individuals with schizophrenia prior to diagnosis and following a course of flupenthixol decanoate treatment;
2. To determine which clinical, behavioural and biological factors influence HCC in a sample of mixed ancestry;
3. To evaluate whether a neuroendocrine marker of chronic stress is associated with measures of self-perceived stress and resilience;
4. To evaluate whether a neuroendocrine marker of chronic stress is associated with performance on cognitive testing.

3 Hypotheses

1. HCC will be raised in those with NPDs with MetS versus those with NPDs without MetS.
2. HCC will be altered in patients with PTSD versus controls, with a complex interaction between trauma related variables (time since trauma exposure, trauma severity and load) and clinical features of PTSD. Considering the mixed findings demonstrated in relation to PTSD we did not postulate in which direction the association would be.
3. HCC will be raised in those with Parkinson's disease versus controls, but the effect may not be as significant as for PTSD and schizophrenia.
4. HCC will be raised in those recently diagnosed with schizophrenia versus controls and HCC will decline following flupenthixol decanoate treatment.
5. In a sample of South African mixed ancestry individuals HCC will demonstrate analogous relationships with some of the basic determinants (clinical, behavioural and biological) previously examined in other settings, with some dissimilarities anticipated.
6. HCC will be associated with self-perceived stress and resilience in opposite directions, being higher in relation to stress and lower in relation to resilience.

7. HCC will correlate positively with cognitive impairments, particularly in domains known to be sensitive to the effects of cortisol, such as declarative memory, working memory, and executive functioning.

4 Overview of the parent study

This study is a neuroendocrine ancillary study to 'Understanding the SHARED ROOTS of Neuropsychiatric Disorders and Modifiable Risk Factors for Cardiovascular Disease' or SHARED ROOTS (SR). SR is a South African Medical Research Council (SAMRC) funded flagship project [Grant no. MRC-RFA-IFSP-01-2013/SHARED ROOTS]. The overarching aim of SR is the interrogation of genomic, neural, cellular and environmental signatures that are common to NPDs and CVD risk, as defined by the MetS, and that contribute to co-morbidity, symptom severity, and treatment outcomes. SR was designed to use a multi-omics approach combining genomic, transcriptomic, epigenetic, and complementary phenotypic and multimodal neuroimaging data, to disentangle mechanistic pathways that lead to the development of comorbidity of these disorders. This research question was investigated in three cohorts of NPDs of relevance to the South African context, namely posttraumatic stress disorder (PTSD), schizophrenia and Parkinson's disease. SR was a cross-sectional matched case-control study and enrolled participants over 3 years from May 2014 until June 2017. The study aimed to sample 200 patients for each NPD cohort (100 with MetS and 100 without MetS). An equivalent number of non-clinical controls were also recruited for each disease cohort and were matched to the NPD cohort based on age, gender and MetS. There was a longitudinal component to the schizophrenia cohort; patients with first episode psychosis (FEP) were treated with open-label flupenthixol decanoate (a depot antipsychotic) and the cohort (patients and controls) returned for follow-up assessments 12 months later.

The study sample was limited to individuals who self-identified as belonging to the mixed ancestry (coloured) ethnic group. The decision to limit SR to one ethnic group was chiefly to avoid the effects of population stratification on genomic analyses. The mixed ancestry group was chosen for the following reasons: (i) the mixed ancestry ethnic group is the predominant population group in the Western Cape, (ii) in previous studies conducted in the NPD cohorts in our setting the majority of participants had been

mixed ancestry, (ii) high rates of metabolic syndrome and related conditions (e.g. diabetes) observed in this ethnic group (Erasmus, R. T. Soita, D. J. Hassan, M. S. Blanco-Blanco, E. Vergotine, Z. Kengne, A. P. Matsha, 2012).

In total 922 individuals were enrolled in SR (461 patients and 461 controls), of these 441 patients and 444 controls completed participation. The PTSD cohort comprised 307 patients and 321 controls. The Parkinson's disease cohort comprised 89 patients and 87 controls. The schizophrenia cohort comprised 62 patients and 56 controls and 31 FEP schizophrenia patients and 30 controls returned for month-12 longitudinal assessments. Participants were characterised for the presence or absence of MetS, using international harmonised JIS criteria (Alberti et al., 2009).

Factors that influenced the final sample size and distribution obtained included the following: (i) difficulties in recruiting adequate numbers of Parkinson's disease and schizophrenia patients overall; (ii) PTSD patient versus trauma-exposed control (TEC) status could only be determined once diagnostic measures had been completed; (iii) MetS status could only be confirmed once participants had already been enrolled and the study investigations (such as biochemical laboratory tests) had been completed; (iv) the baseline prevalence of MetS is lower than 50% and thus efforts to reach the target of a 100 PTSD patients with MetS required over-sampling; (v) we initially planned to include only FEP patients of mixed ancestry ethnicity in the schizophrenia cohort, but due to the difficulties recruiting FEP patients fulfilling the study criteria, a decision was made to remove the ethnicity criterion for that cohort, as well as to recruit chronic schizophrenia patients from a cohort who previously participated in an FEP study, called the EONKCS study (Chiliza et al., 2016).

Participants in SR attended two to three study visits, each lasting around 2-3 hours. At the first visit, diagnostic and clinical measures were administered by a clinician and participant-administered measures were completed. The second visit occurred within 24-96 hours of the first visit and involved the physical procedures (e.g. fasting blood sampling, physical measurements, hair sampling), additional lifestyle measures, and neurocognitive testing. The subset of participants included in the nested neuroimaging study attended a third study visit within 3 weeks of the first visit.

SR was approved by the Health Research Ethics Committee at Stellenbosch University (HREC N13/08/115) and conducted according to the ethical guidelines and principles of the seventh revision of the Declaration of Helsinki (World Medical Association, 2013).

4.1 The neuroendocrine ancillary study

This neuroendocrine ancillary study was conceived prior to initiating recruitment on SR and thus all participants were eligible for inclusion. Hair samples were obtained from participants with scalp hair 3cm or longer who provided informed consent for hair sampling. Samples were analysed with liquid chromatography tandem mass spectrometry (LC-MS/MS) at the laboratory of Prof Clemens Kirschbaum at Dresden University of Technology. The LC-MS/MS method allows for simultaneous identification of five steroid hormones [cortisol, cortisone, testosterone, progesterone, and dehydroepiandrosterone (DHEA)] in human hair. Prof Clemens Kirschbaum and Dr Tobias Stalder are collaborators on this study and have extensive expertise in cortisol and stress research.

4.2 Role in the study

I was the project manager and lead clinician on SR and was thus involved with all aspects of SR since study initiation. Under the guidance of my supervisor, Prof Soraya Seedat, the Principal Investigator on SR and recipient of the SR grant, I conceived the neuroendocrine ancillary study, formulated the protocol, conducted statistical analyses in consultation with Prof Carl Lombard, and first-authored all the manuscripts and chapters contained within this thesis.

5 Outline of Chapters and rationale

The central focus of this thesis is on HCC, as a measure of longer-term HPA-axis dysfunction, in relation to various outcomes in a case-control design. As prior HCC studies have not been conducted in South Africa and this was one of the first HCC studies the goal was to utilise HCC to address a variety of research questions applicable to the SR study as well as broader questions pertaining to the role of longer-term neuroendocrine dysfunction in various conditions/contexts. The primary aims of the thesis align with the primary aims of SR, namely to investigate HCC in relation to NPDs, and their comorbidity with MetS. The primary aims are addressed in Chapters 2 to 5 of the thesis. In Chapters 2

to 4 these aims are addressed in each of the three NPD cohorts separately and in Chapter 5 across the three cohorts. The secondary aim investigating the longitudinal trajectory of HCC in patients with FEP schizophrenia is also addressed in Chapter 4; this chapter addresses the main aims in the schizophrenia cohort. The remaining aims are addressed in the control sample - these secondary aims investigate the role of longer-term neuroendocrine dysfunction in relation to other factors of interest, but that are not the primary focus of the study. Two of the secondary aims are addressed in Chapter 6, namely identifying the basic determinants of HCC in individuals of mixed ancestry, as well as investigating whether HCC are associated with measures of self-perceived stress and resilience. The remaining secondary aim, addressed in Chapter 7, investigates whether HCC are associated with performance on cognitive testing. In the manuscripts, a number additional aims that are not outlined in the original protocol, but that were of interest, are also addressed as outlined below in the summary of each of the chapters. We examined for associations between HCC and clinical severity of each of the NPDs as these associations can provide a quantitative measure of how HCC are related to the NPDs. We also included exploratory analyses of the associations between individual CVD risk factors and HCC as these analyses are aligned with other studies examining both total CVD risk and individual CVD risk factors in relation to HCC (Kuehl et al., 2015; Langerak et al., 2015; Stalder et al., 2013). Caution needs to be exercised in interpreting these findings as the final sample sizes were underpowered to detect significant associations between MetS and HCC, based on a-priori sample size calculations. Other aims were formulated in the process of reviewing the literature and identifying areas of importance as well as gaps in the knowledge. Some of these were also based on preliminary analyses of the data, such as including an analysis of hair cortisone levels alongside HCC in the Parkinson's disease cohort. In the concluding chapter the main outcomes are summarised and the original contributions to the field outlined along with recommendations for future research. In Appendix A a published systematic review protocol directly relating to this thesis is included 'Cortisol levels in different tissue samples in posttraumatic stress disorder patients versus controls: a systematic review and meta-analysis protocol'. In Appendix B other published manuscripts pertaining to SR where I have been involved as co-author are referenced.

As HCC, a biomarker of longer-term HPA-axis function, form the central focus of the thesis as well as the core unifying theme addressed in each of the objectives, the analysis approach that was utilised centers on an investigation of various factors related to HCC. Thus HCC is the dependent variable in linear regression models constructed to address the different objectives. Although some of the components in each chapter may differ depending on the features of the sample and the outcomes addressed, an overarching and uniform approach was applied across the different chapters. Thus, for the most part, common data variables are included in the analysis framework for each chapter and the analysis strategy is also applied consistently throughout, with some modifications made as indicated. One of the major limitations of this study was that the majority of males did not have hair of sufficient length to participate in the neuroendocrine study, thus in all the chapters, excluding Chapter 4, analyses are limited to females. The sample sizes, as well as flow-diagrams illustrating inclusion and exclusion of participants in each analysis run are included in the individual chapters.

Each of the Chapters has been written in manuscript format and the goal is to publish each of the manuscripts. Manuscripts were drafted in accordance with the STROBE guidelines (von Elm et al., 2014), with the aim of increasing transparency and ensuring that the manuscripts adhere to international standards of reporting for observational studies. Two of the manuscripts are currently under review and the remaining manuscripts have been circulated for co-author contributions and review and will be submitted shortly to peer-reviewed journals for publication. As the chapters are in manuscript format there will be some repetition, particularly relating to study methodology. In Chapter 5, the chapter comparing HCC across the disorders and in Chapter 7, the chapter investigating cognitive function in relation to HCC, the methods and results have been abbreviated as these have been described in the preceding chapters pertaining to the same samples.

5.1 Chapter 2 - Hair cortisol levels in posttraumatic stress disorder and metabolic syndrome: A case-control study in South African mixed ancestry females

In this manuscript the main aims of this study are addressed in the PTSD cohort. Here we investigate whether HCC are associated with PTSD caseness, by comparing HCC in patients with PTSD and trauma exposed controls (TEC). We also investigate whether MetS is associated with HCC and

assess for interaction effects between PTSD and MetS on HCC. We also address the following additional aims: we investigate whether clinical factors related to PTSD, namely PTSD severity and trauma severity (as defined by a count of traumatic event exposures), as well as self-perceived stress, are associated with HCC and, in exploratory analyses, whether individual CVD risk factors are associated with HCC.

5.2 Chapter 3 - Hair glucocorticoid levels in females with Parkinson's disease

In this manuscript the main aims of this study are addressed in the Parkinson's disease cohort. Here we investigate whether HCC are associated with Parkinson's disease, by comparing HCC in patients with Parkinson's disease and controls. We also investigate whether MetS is associated with HCC and assess for the presence of a statistically significant interaction between Parkinson's disease and MetS on HCC. We also address the following additional aims: we investigate whether clinical factors related to Parkinson's disease (e.g., severity of motor and non-motor symptoms of Parkinson's disease), as well as self-perceived stress are associated with HCC. In exploratory analyses we further investigate whether individual CVD risk factors are associated with HCC. In the Parkinson's disease cohort we noted differential patterns between HCC and hair cortisone levels, another marker of long-term HPA-axis function, and clinical factors. We have thus conducted analyses examining the associations between hair cortisone levels and relevant clinical variables, in addition to HCC.

5.3 Chapter 4 – Hair cortisol levels in schizophrenia and metabolic syndrome

In this manuscript the main aims of this study are addressed in the schizophrenia cohort. Here we investigate whether HCC are associated with schizophrenia, by comparing HCC in patients with schizophrenia and controls. We also investigate whether MetS is associated with HCC and examine interaction effects between schizophrenia diagnosis and MetS on HCC. The schizophrenia sample consists of two groups, a FEP psychosis group and a chronic schizophrenia group. We conduct analyses for the cohorts combined as well as analyses focused on the FEP cohort. The secondary aim investigating the longitudinal trajectory of HCC in FEP is also addressed in this chapter. We also address the following additional aims: we investigate whether clinical factors related to schizophrenia (e.g., severity of core schizophrenia symptoms and insight), as well as self-perceived stress, are

associated with HCC and in exploratory analyses whether the individual CVD risk factors are associated with HCC.

5.4 Chapter 5 - Hair cortisol as a neuroendocrine biomarker to evaluate the impact of chronic stress on the interaction between neuropsychiatric disorders and metabolic syndrome in females

In this chapter we conduct analyses across the cohorts to address components of the primary aims that could not be addressed in the individual cohorts. We compare HCC among the three NPDs and assess for shared effects of MetS across NPDs on HCC. Although there are significant limitations to undertaking these analyses, such as significant differences between the NPDs cohorts (e.g. average age) and unequal sample sizes across the cohorts, the combined analyses may provide preliminary insights that are not apparent in addressing the individual cohorts in each of the chapters. This chapter also synthesizes and integrates the key findings in accordance with the main study aims.

5.5 Chapter 6 - Hair cortisol as a biomarker of stress and resilience in South African mixed ancestry females

In this chapter we address two of the secondary aims of the thesis. We seek to determine socio-demographic, hair related, clinical and behavioural factors that are associated with HCC in a South African mixed ancestry sample and assess whether self-perceived stress and resilience scores in the sample are associated with HCC. As this is one of the first studies investigating HCC in South Africa, with the majority of studies having been conducted in developed regions and in samples of primarily Caucasian origin, there is a need to identify the basic determinants of HCC in samples that have broader ethnic representation. We thus examine a wide array of factors that have the potential to influence HCC in non-clinical populations as exemplified by our control sample who represent fairly healthy individuals (common somatic conditions were permitted e.g. atopy, hypertension and diabetes) without any current significant psychopathology. Originally these factors were planned to be used as covariates across all analyses in the thesis, however this approach needed to be adjusted due to the varying composition and sample size of the different cohorts. In each of the NPD cohorts, we therefore investigated which factors were associated with HCC once NPD diagnosis and age were controlled for

and used these as the covariates of HCC. As cortisol is released in response to stress, various studies have investigated whether HCC are associated with measures of self-perceived stress and the results have been mixed, with largely negative findings (Stalder et al., 2017; Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, 2013; Wosu et al., 2013). Very few studies have investigated whether cortisol levels are associated with resilience or stress-coping ability (Dockray & Steptoe, 2010; García-León, Pérez-Mármol, Gonzalez-Pérez, García-Ríos, & Peralta-Ramírez, 2019; Milam, Slaughter, Verma, & McConnell, 2014; Steptoe, Dockray, & Wardle, 2009; Ullmann et al., 2016) and the precise biological processes underpinning resilience as compared to stress have yet to be elucidated. As such, to provide insights into the relationship between self-perceived stress, resilience and HPA-axis function, we examined whether measures of self-perceived stress and resilience were associated with HCC.

5.6 Chapter 7 - The association between cognitive functioning and hair cortisol levels in South African mixed ancestry females

In Chapter 7 we assess whether performance on neurocognitive testing is associated with HCC, as a biomarker of chronic stress and associated HPA-axis dysfunction. Cortisol can directly influence brain structure and function, particularly in regions with increased density of GR and mineralocorticoid (MR) receptors, such as the hippocampus and prefrontal cortices (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007; McEwen, Nasca, & Gray, 2016). There is also evidence of impairments in cognitive performance in domains associated with these brain regions, namely declarative memory, executive functions and working memory (Het, Ramlow, & Wolf, 2005; Sauro, Jorgensen, & Pedlow, 2003; Shields, Bonner, & Moons, 2015). We sought to examine whether performance on a battery of neurocognitive tests were associated with HCC in the same control sample included in Chapter 6, but with additional exclusions applied pertaining to factors that can influence cognitive performance (e.g. prior head injuries with loss of consciousness). Our aim was to investigate whether HCC were associated with cognitive domains known to be sensitive to glucocorticoids as compared to other domains and overall cognitive functioning.

5.7 Appendix A - Cortisol levels in different tissue samples in posttraumatic stress disorder patients versus controls: A systematic review and meta-analysis protocol

The protocol is for a systematic review evaluating basal cortisol levels in different tissue samples (e.g. plasma, serum, urine, saliva, hair and nails) in PTSD patients versus controls. The systematic review has been placed on hold as a related systematic review evaluating basal cortisol levels in PTSD (Schumacher et al., 2019) was published shortly following the publication of this protocol. Work on this systematic review will proceed within a year or two to allow new research, including the results pertaining to Chapter 2, to be included in the systematic review. As the focus of the systematic review is on cortisol levels in different tissue samples, more studies utilising newer sample types, such as hair and nails, are required to be able to conduct meta-analyses and to make meaningful deductions about how cortisol measurements using these newer methods of tissue sampling compare with measurements in traditional tissue samples.

Table 1 Overview of aims and in which chapters they are addressed

Aims	Chapters in which aims are addressed
Primary	
To evaluate the role of chronic stress, as measured by hair cortisol, in the interaction between three NPDs (PTSD, schizophrenia and Parkinson's disease) and MetS	Chapter 2 - Hair cortisol levels in posttraumatic stress disorder and metabolic syndrome Chapter 3 - Hair glucocorticoid levels in Parkinson's disease Chapter 4 – Hair cortisol levels in schizophrenia and metabolic syndrome Chapter 5 - Hair cortisol as a neuroendocrine biomarker to evaluate the impact of chronic stress on the interaction between neuropsychiatric disorders and metabolic syndrome
To determine and compare the neuroendocrine signatures in these three NPDs (PTSD, SCZ and PD) and undertake a case-control comparison of HCC in these NPDs	Chapter 2 - Hair cortisol levels in posttraumatic stress disorder and metabolic syndrome Chapter 3 - Hair glucocorticoid levels in Parkinson's disease Chapter 4 – Hair cortisol levels in schizophrenia and metabolic syndrome Chapter 5 - Hair cortisol as a neuroendocrine biomarker to evaluate the impact of chronic stress on the interaction between neuropsychiatric disorders and metabolic syndrome
Secondary	

To characterise the neuroendocrine status of individuals with schizophrenia prior to diagnosis and following flupenthixol decanoate treatment.	Chapter 4 - Hair cortisol levels in schizophrenia and metabolic syndrome
To determine which clinical, behavioural and biological factors influence hair cortisol levels in a sample of mixed ancestry.	Chapter 6: Hair cortisol as a biomarker of stress and resilience in females
To evaluate whether a neuroendocrine marker of chronic stress is associated with measures of self-perceived stress and resilience	Chapter 6: Hair cortisol as a biomarker of stress and resilience in females
To evaluate whether a neuroendocrine marker of chronic stress is associated with performance on cognitive testing	Chapter 7: The association between cognitive functioning and hair cortisol levels in females

6 References

Aas, M., Pizzagalli, D. A., Laskemoen, J. F., Reponen, E. J., Ueland, T., Melle, I., … Andreassen, O. A. (2019). Elevated hair cortisol is associated with childhood maltreatment and cognitive impairment in schizophrenia and in bipolar disorders. *Schizophrenia Research*, (xxxx), 1–7. https://doi.org/10.1016/j.schres.2019.01.011

Abell, J. G., Stalder, T., Ferrie, J. E., Shipley, M. J., Kirschbaum, C., Kivimäki, M., & Kumari, M. (2016). Assessing cortisol from hair samples in a large observational cohort : The Whitehall II study. *Psychoneuroendocrinology*, *73*, 148–156. https://doi.org/10.1016/j.psyneuen.2016.07.214

Ahmadi, N., Hajsadeghi, F., Mirshkarlo, H. B., Budoff, M., Yehuda, R., & Ebrahimi, R. (2011). Post-traumatic stress disorder, coronary atherosclerosis, and mortality. *American Journal of Cardiology*, *108*(1), 29–33. https://doi.org/10.1016/j.amjcard.2011.02.340

Alberti, K. G., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., … Smith Jr, S. C. (2009). Harmonizing the Metabolic Syndrome A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International A. *Circulation*, *120*(16), 1640–1645. https://doi.org/10.1161/CIRCULATIONAHA.109.192644 [doi]

Allen, A. P., Kennedy, P. J., Dockray, S., Cryan, J. F., Dinan, T. G., & Clarke, G. (2017). The Trier

Social Stress Test: Principles and practice. *Neurobiology of Stress*, *6*, 113–126. https://doi.org/10.1016/j.ynstr.2016.11.001

American Psychiatric Association [APA]. (2013). *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5®)*. Arlington, VA.

Andrade, E. H., Rizzo, L. B., Noto, C., Ota, V. K., Gadelha, A., Daruy-Filho, L., … Brietzke, E. (2016). Hair cortisol in drug-naïve first-episode individuals with psychosis. *Revista Brasileira de Psiquiatria*, *38*(1), 11–16. https://doi.org/10.1590/1516-4446-2014-1634

Atwoli, L., Stein, D. J., Williams, D. R., Mclaughlin, K. A., Petukhova, M., Kessler, R. C., & Koenen, K. C. (2013). Trauma and posttraumatic stress disorder in South Africa: Analysis from the South African Stress and Health Study. *BMC Psychiatry*, *13*. https://doi.org/10.1186/1471-244X-13-182

Bainbridge, J., Boyd, J., Cambi, F., Christine, C., Dhall, R., Leehey, M., … Zhang, Y. (2017). Association of metabolic syndrome and change in Unified Parkinson Disease Rating Scale scores. *Neurology*, *89*(17), 1789–1794. https://doi.org/https://doi.org/10.1212/WNL.0000000000004572

Bartoli, F., Carra, G., Crocamo, C., Carretta, D., & Clerici, M. (2013). Metabolic Syndrome in People Suffering from Posttraumatic Stress Disorder: A Systematic Review and Meta-Analysis. *Metabolic Syndrome and Related Disorders*, *11*(5), 301–308. https://doi.org/10.1089/met.2013.0010

Blessing, E. M., Reus, V., Mellon, S. H., Wolkowitz, O. M., Flory, J. D., Bierer, L., … Marmar, C. R. (2017). Biological predictors of insulin resistance associated with posttraumatic stress disorder in young military veterans. *Psychoneuroendocrinology*, *82*(April), 91–97. https://doi.org/10.1016/j.psyneuen.2017.04.016

Borges, S., Gayer-Anderson, C., & Mondelli, V. (2013). A systematic review of the activity of the hypothalamic-pituitary-adrenal axis in first episode psychosis. *Psychoneuroendocrinology*, *38*(5), 603–611. https://doi.org/10.1016/j.psyneuen.2012.12.025

Bradley, A. J., & Dinan, T. G. (2010). A systematic review of hypothalamic-pituitary-adrenal axis function in schizophrenia: implications for mortality. *Journal of Psychopharmacology (Oxford, England)*, *24*(4 Suppl), 91–118. https://doi.org/10.1177/1359786810385491; 10.1177/1359786810385491

Charlson, F. J., Ferrari, A. J., Santomauro, D. F., Diminic, S., Stockings, E., Scott, J. G., … Whiteford, H. A. (2018). Global epidemiology and burden of schizophrenia: Findings from the global burden of disease study 2016. *Schizophrenia Bulletin*, *44*(6), 1195–1203. https://doi.org/10.1093/schbul/sby058

Chiliza, B., Ojagbemi, A., Esan, O., Asmal, L., Oosthuizen, P., Kidd, M., … Emsley, R. (2016). Combining depot antipsychotic with an assertive monitoring programme for treating first-episode schizophrenia in a resource-constrained setting. *Early Intervention in Psychiatry*, *10*(1), 54–62. https://doi.org/10.1111/eip.12141

Chrousos, G. P. (2009). Stress and disorders of the stress system. *Nature Reviews Endocrinology*, *5*(7), 374–381. https://doi.org/10.1038/nrendo.2009.106.

Correll, C. U., Solmi, M., Veronese, N., Bortolato, B., Rosson, S., Santonastaso, P., … Stubbs, B. (2017). Prevalence, incidence and mortality from cardiovascular disease in patients with pooled and specific severe mental illness: a large-scale meta-analysis of 3,211,768 patients and 113,383,368 controls. *World Psychiatry*, *16*(2), 163–180. https://doi.org/10.1002/wps.20420

Dajani, R., Hadfield, K., van Uum, S., Greff, M., & Panter-Brick, C. (2018). Hair cortisol concentrations in war-affected adolescents: A prospective intervention trial. *Psychoneuroendocrinology*, *89*(December 2017), 138–146. https://doi.org/10.1016/j.psyneuen.2017.12.012

De Hert, M., Detraux, J., & Vancampfort, D. (2018). The intriguing relationship between coronary heart disease and mental disorders. *Dialogues in Clinical Neuroscience*, *20*(1), 31–40.

de Kloet, C. S., Vermetten, E., Geuze, E., Kavelaars, A., Heijnen, C. J., & Westenberg, H. G. M. (2006). Assessment of HPA-axis function in posttraumatic stress disorder: Pharmacological and

non-pharmacological challenge tests, a review. *Journal of Psychiatric Research*, *40*(6), 550–567. https://doi.org/10.1016/j.jpsychires.2005.08.002

Dockray, S., & Steptoe, A. (2010). Positive affect and psychobiological processes. *Neuroscience and Biobehavioral Reviews*, *35*(1), 69–75. https://doi.org/10.1016/j.neubiorev.2010.01.006

Du, X., & Pang, T. Y. (2015). Is dysregulation of the HPA-axis a core pathophysiology mediating co-morbid depression in neurodegenerative diseases? *Frontiers in Psychiatry*, *6*(MAR), 1–33. https://doi.org/10.3389/fpsyt.2015.00032

Erasmus, R. T. Soita, D. J. Hassan, M. S. Blanco-Blanco, E. Vergotine, Z. Kengne, A. P. Matsha, T. E. (2012). High prevalence of diabetes mellitus and metabolic syndrome in a South. *SAMJ - South African Medical Journal; 2012*, *102*(11), 841–844. https://doi.org/10.7196/SAMJ.5670

Gajsak, L. R., Gelemanovic, A., Kuzman, M. R., & Puljak, L. (2017). Impact of stress response in development of first-episode psychosis in schizophrenia: An overview of systematic reviews. *Psychiatria Danubina*, *29*(1), 14–23.

García-León, M. Á., Pérez-Mármol, J. M., Gonzalez-Pérez, R., García-Ríos, M. del C., & Peralta-Ramírez, M. I. (2019). Relationship between resilience and stress: Perceived stress, stressful life events, HPA axis response during a stressful task and hair cortisol. *Physiology and Behavior*, *202*(September 2018), 87–93. https://doi.org/10.1016/j.physbeh.2019.02.001

Girshkin, L., Matheson, S. L., Shepherd, A. M., & Green, M. J. (2014). Morning cortisol levels in schizophrenia and bipolar disorder: A meta-analysis. *Psychoneuroendocrinology*, *49*(1), 187–206. https://doi.org/10.1016/j.psyneuen.2014.07.013

Gow, R., Thomson, S., Rieder, M., Van Uum, S., & Koren, G. (2010). An assessment of cortisol analysis in hair and its clinical applications. *Forensic Science International*, *196*(1), 32–37. https://doi.org/https://doi.org/10.1016/j.forsciint.2009.12.040

Gray, N. A., Dhana, A., Van Der Vyver, L., Van Wyk, J., Khumalo, N. P., & Stein, D. J. (2018). Determinants of hair cortisol concentration in children: A systematic review.

Psychoneuroendocrinology, *87*(August 2017), 204–214. https://doi.org/10.1016/j.psyneuen.2017.10.022

Groer, M. W., Kane, B., Williams, S. N., & Duffy, A. (2015). Relationship of PTSD symptoms with combat exposure, stress, and inflammation in American soldiers. *Biological Research for Nursing*, *17*(3), 303–310. https://doi.org/10.1177/1099800414544949

Gunnar, M. R. (2010). Stressor paradigms in developmental studies. *Psychoneuroendocrinology*, *34*(7), 953–967. https://doi.org/10.1016/j.psyneuen.2009.02.010.Stressor

Herman, A. A., Stein, D. J., Seedat, S., Heeringa, S. G., Moomal, H., & Williams, D. R. (2009). The South African Stress and Health (SASH) study: 12-month and lifetime prevalence of common mental disorders. *South African Medical Journal*, *99*(5), 339–344.

Herrero, M.-T., Estrada, C., Maatouk, L., & Vyas, S. (2015). Inflammation in Parkinson's disease: role of glucocorticoids. *Frontiers in Neuroanatomy*, *9*(April), 1–12. https://doi.org/10.3389/fnana.2015.00032

Het, S., Ramlow, G., & Wolf, O. T. (2005). A meta-analytic review of the effects of acute cortisol administration on human memory. *Psychoneuroendocrinology*, *30*(8), 771–784. https://doi.org/10.1016/j.psyneuen.2005.03.005

Huang, P. L. (2009). A comprehensive definition for metabolic syndrome. *DMM Disease Models and Mechanisms*, *2*(5–6), 231–237. https://doi.org/10.1242/dmm.001180

Hubbard, D. B., & Miller, B. J. (2019). Meta-analysis of blood cortisol levels in individuals with first-episode psychosis. *Psychoneuroendocrinology*, *104*(September 2018), 269–275. https://doi.org/10.1016/j.psyneuen.2019.03.014

J.Yeo, K.-T., Babic, N., Hannoush, Z. C., & Weiss, R. E. (2000). *Endocrine Testing Protocols: Hypothalamic Pituitary Adrenal Axis*. (K. Feingold, B. Anawalt, A. Boyce, G. Chrousos, K. Dungan, A. Grossman, … D. Wilson, Eds.), *Endotext*. South Dartmouth (MA): MDText.com, Inc. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/25905177

Juster, R. P., McEwen, B. S., & Lupien, S. J. (2010). Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neuroscience and Biobehavioral Reviews*, *35*(1), 2–16. https://doi.org/10.1016/j.neubiorev.2009.10.002

Kassi, E., Pervanidou, P., Kaltsas, G., & Chrousos, G. (2011). Metabolic syndrome: Definitions and controversies. *BMC Medicine*, *9*(1), 48. https://doi.org/10.1186/1741-7015-9-48

Kessler, R. C., Aguilar-Gaxiola, S., Alonso, J., Benjet, C., Bromet, E. J., Cardoso, G., … Koenen, K. C. (2017). Trauma and PTSD in the WHO World Mental Health Surveys. *European Journal of Psychotraumatology*, *8*. https://doi.org/10.1080/20008198.2017.1353383

Koenen, K. C., Ratanatharathorn, A., Ng, L., McLaughlin, K. A., Bromet, E. J., Stein, D. J., … Scott, K. (2017). Posttraumatic stress disorder in the World Mental Health Surveys. *Psychological Medicine*, *47*(13), 2260–2274. https://doi.org/https://doi.org/10.1017/S0033291717000708

Kubzansky, L. D., Koenen, K., & Spiro, A. (2007). Prospective study of posttraumatic stress disorder symptoms and coronary heart disease in the normative aging study. *Archives of General Psychiatry*, *64*(1), 109–116. https://doi.org/10.1001/archpsyc.64.1.109

Kudielka, B. M., & Wüst, S. (2010). Human models in acute and chronic stress: Assessing determinants of individual hypothalamuspituitaryadrenal axis activity and reactivity. *Stress*, *13*(1), 1–14. https://doi.org/10.3109/10253890902874913

Kuehl, L. K., Hinkelmann, K., Muhtz, C., Dettenborn, L., Wingenfeld, K., Spitzer, C., … Otte, C. (2015). Hair cortisol and cortisol awakening response are associated with criteria of the metabolic syndrome in opposite directions. *Psychoneuroendocrinology*, *51*, 365–370. https://doi.org/10.1016/j.psyneuen.2014.09.012

Kyu, H. H., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., … Murray, C. J. L. (2018). Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2017: A systematic analysis for the Global Burden of Disease Study 2017. *The Lancet*, 1859–1922.

https://doi.org/10.1016/S0140-6736(18)32335-3

Langerak, T., van den Dries, L. W. J., Wester, V. L., Staufenbiel, S. M., Manenschijn, L., van Rossum, E. F. C., & van Gorp, E. C. M. (2015). The relation between long-term cortisol levels and the metabolic syndrome in HIV-infected patients. *Clinical Endocrinology*, *83*(2), 167–172. https://doi.org/10.1111/cen.12790

Laursen, T. M., Nordentoft, M., & Mortensen, P. B. (2014). Excess Early Mortality in Schizophrenia. *Annual Review of Clinical Psychology*, *10*(1), 425–448. https://doi.org/10.1146/annurev-clinpsy-032813-153657

Luo, H., Hu, X., Liu, X. X. X. X., Ma, X., Guo, W., Qiu, C., … Li, T. (2012). Hair cortisol level as a biomarker for altered hypothalamic-pituitary- adrenal activity in female adolescents with posttraumatic stress disorder after the 2008 Wenchuan earthquake. *Biological Psychiatry*, *72*(1), 65–69. https://doi.org/10.1016/j.biopsych.2011.12.020

Lupien, S. J., Maheu, F., Tu, M., Fiocco, A., & Schramek, T. E. (2007). The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. *Brain and Cognition*, *65*(3), 209–237. https://doi.org/10.1016/j.bandc.2007.02.007

Manenschijn, L., Koper, J. W., Lamberts, S. W. J., & Van Rossum, E. F. C. (2011). Evaluation of a method to measure long term cortisol levels. *Steroids*, *76*(10), 1032–1036. https://doi.org/10.1016/j.steroids.2011.04.005

Manzanares, N., Monseny, R., Ortega, L., Montalvo, I., Franch, J., Gutiérrez-Zotes, A., … Labad, J. (2014). Unhealthy lifestyle in early psychoses: The role of life stress and the hypothalamic-pituitary-adrenal axis. *Psychoneuroendocrinology*, *39*(1), 1–10. https://doi.org/10.1016/j.psyneuen.2013.09.023

Mayosi, B. M., Lawn, J. E., Van Niekerk, A., Bradshaw, D., Abdool Karim, S. S., & Coovadia, H. M. (2012). Health in South Africa: Changes and challenges since 2009. *The Lancet*, *380*(9858), 2029–2043. https://doi.org/10.1016/S0140-6736(12)61814-5

McEwen, B. S. (1998). Stress, Adaptation, and Disease: Allostasis and Allostatic Load. *Annals of the New York Academy of Sciences*, *840*(1), 33–44. https://doi.org/10.1111/j.1749-6632.1998.tb09546.x

McEwen, B. S. (2008). Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *European Journal of Pharmacology*, *583*(2), 174–185. https://doi.org/10.1016/j.ejphar.2007.11.071

McEwen, B. S., & Gianaros, P. J. (2010). Central role of the brain in stress and adaptation: links to socioeconomic status, health, and disease. *Annals of the New York Academy of Sciences*, *1186*(1), 190–222. https://doi.org/10.1111/j.1749-6632.2009.05331.x

McEwen, B. S., Nasca, C., & Gray, J. D. (2016). Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex. *Neuropsychopharmacology*, *41*(1), 3–23. https://doi.org/10.1038/npp.2015.171

McGrath, J., Saha, S., Chant, D., & Welham, J. (2008). Schizophrenia: A concise overview of incidence, prevalence, and mortality. *Epidemiologic Reviews*, *30*(1), 67–76. https://doi.org/10.1093/epirev/mxn001

Meewisse, M. L., Reitsma, J. B., de Vries, G. J., Gersons, B. P., & Olff, M. (2007). Cortisol and post-traumatic stress disorder in adults: systematic review and meta-analysis. *The British Journal of Psychiatry : The Journal of Mental Science*, *191*, 387–392. https://doi.org/191/5/387 [pii]

Milam, J., Slaughter, R., Verma, G., & McConnell, R. (2014). Hair cortisol, perceived stress and dispositional optimism: A pilot study among adolescents. *Journal of Traumatic Stress Disorders & Treatment*, *3*(3), 1000126. https://doi.org/10.4172/2324-8947.1000126

Mitchell, A. J., Vancampfort, D., Sweers, K., Van Winkel, R., Yu, W., & De Hert, M. (2013). Prevalence of metabolic syndrome and metabolic abnormalities in schizophrenia and related disorders-a systematic review and meta-analysis. *Schizophrenia Bulletin*, *39*(2), 306–318. https://doi.org/10.1093/schbul/sbr148

Morris, M. C., Compas, B. E. B. E., & Garber, J. (2012). Relations among posttraumatic stress disorder, comorbid major depression, and HPA function: a systematic review and meta-analysis. *Clinical Psychology Review*, *32*(4), 301–315. https://doi.org/10.1016/j.cpr.2012.02.002.Relations

Nam, G. E., Kim, S. M., Han, K., Kim, N. H., Chung, H. S., Kim, J. W., … Choi, K. M. (2018). Metabolic syndrome and risk of Parkinson disease: A nationwide cohort study. *PLoS Medicine*, *15*(8), 1–15. https://doi.org/10.1371/journal.pmed.1002640

Papafotiou, C., Christaki, E., Akker, E. L. T. Van Den, Wester, V. L., van den Akker, E. L. T., Wester, V. L., … Pervanidou, P. (2017). Hair cortisol concentrations exhibit a positive association with salivary cortisol profiles and are increased in obese prepubertal girls. *Stress*, *20*(2), 217–222. https://doi.org/10.1080/10253890.2017.1303830

Patel, V., Saxena, S., Lund, C., Thornicroft, G., Baingana, F., Bolton, P., … UnÜtzer, Jü. (2018). The Lancet Commission on global mental health and sustainable development. *The Lancet*, *392*(10157), 1553–1598. https://doi.org/10.1016/S0140-6736(18)31612-X

Penninx, B. W. J. H., & Lange, S. M. M. (2018). Metabolic syndrome in psychiatric patients: Overview, mechanisms, and implications. *Dialogues in Clinical Neuroscience*, *20*(1), 63–73.

Pillay-van Wyk, V., Msemburi, W., Laubscher, R., Dorrington, R. E., Groenewald, P., Glass, T., … Bradshaw, D. (2016). Mortality trends and differentials in South Africa from 1997 to 2012: second National Burden of Disease Study. *The Lancet Global Health*, *4*(9), e642–e653. https://doi.org/https://doi.org/10.1016/S2214-109X(16)30113-9

Raul, J.-S. S., Cirimele, V., Ludes, B., & Kintz, P. (2004). Detection of physiological concentrations of cortisol and cortisone in human hair. *Clinical Biochemistry*, *37*(12), 1105–1111. https://doi.org/10.1016/j.clinbiochem.2004.02.010

Ray Dorsey, E., Elbaz, A., Nichols, E., Abd-Allah, F., Abdelalim, A., Adsuar, J. C., … Murray, C. J. L. (2018). Global, regional, and national burden of Parkinson's disease, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Neurology*, *17*(11), 939–953.

https://doi.org/10.1016/S1474-4422(18)30295-3

Rosenbaum, S., Stubbs, B., Ward, P. B., Steel, Z., Lederman, O., & Vancampfort, D. (2015). The prevalence and risk of metabolic syndrome and its components among people with posttraumatic stress disorder: A systematic review and meta-analysis. *Metabolism: Clinical and Experimental*, *64*(8), 926–933. https://doi.org/10.1016/j.metabol.2015.04.009

Russell, E., Kirschbaum, C., Laudenslager, M. L., Stalder, T., Rijke, Y. De, van Rossum, E. F. C., … Koren, G. (2015). Toward Standardization of Hair Cortisol Measurement : Results of the First International Interlaboratory Round Robin. *Therapeutic Drug Monitoring*, *37*(1), 71–75. https://doi.org/10.1097/FTD.0000000000000148

Saloojee, S., Burns, J. K., & Motala, A. A. (2016). Metabolic syndrome in South African patients with severe mental illness: Prevalence and associated risk factors. *PLoS ONE*, *11*(2), 1–14. https://doi.org/10.1371/journal.pone.0149209

Sauro, M. D., Jorgensen, R. S., & Pedlow, C. T. (2003). Stress, Glucocorticoids, and Memory: A Meta-analytic Review. *Stress*, *6*(4), 235–245. https://doi.org/10.1080/10253890310001616482

Schumacher, S., Niemeyer, H., Engel, S., Cwik, J. C., Laufer, S., Klusmann, H., & Knaevelsrud, C. (2019). HPA axis regulation in posttraumatic stress disorder: A meta-analysis focusing on potential moderators. *Neuroscience and Biobehavioral Reviews*, *100*(June 2018), 35–57. https://doi.org/10.1016/j.neubiorev.2019.02.005

Seedat, S., Stein, D. J., Herman, A., Kessler, R., Sonnega, J., Heeringa, S., … Williams, D. (2008). Twelve-month treatment of psychiatric disorders in the South African Stress and Health Study (World Mental Health Survey Initiative). *Social Psychiatry and Psychiatric Epidemiology*, *43*(11), 889–897. https://doi.org/10.1007/s00127-008-0399-9

Shields, G. S., Bonner, J. C., & Moons, W. G. (2015). Does cortisol influence core executive functions? A meta-analysis of acute cortisol administration effects on working memory, inhibition, and set-shifting. *Psychoneuroendocrinology*, *58*, 91–103.

https://doi.org/https://doi.org/10.1016/j.psyneuen.2015.04.017

Soares, N. M., Pereira, G. M., Altmann, V., de Almeida, R. M. M., & Rieder, C. R. M. (2019). Cortisol levels, motor, cognitive and behavioral symptoms in Parkinson's disease: a systematic review. *Journal of Neural Transmission*, *126*(3), 219–232. https://doi.org/10.1007/s00702-018-1947-4

Stalder, T., & Kirschbaum, C. (2012). Analysis of cortisol in hair–State of the art and future directions. *Brain, Behavior, and Immunity*, *26*(7), 1019–1029. https://doi.org/10.1016/j.bbi.2012.02.002

Stalder, T., Kirschbaum, C., Alexander, N., Bornstein, S. R., Gao, W., Miller, R., … Fischer, J. E. (2013). Cortisol in hair and the metabolic syndrome. *The Journal of Clinical Endocrinology & Metabolism*, *98*(6), 2573–2580. https://doi.org/10.1210/jc.2013-1056

Stalder, T., Steudte-Schmiedgen, S., Alexander, N., Klucken, T., Vater, A., Wichmann, S., … Miller, R. (2017). Stress-related and basic determinants of hair cortisol in humans: A meta-analysis. *Psychoneuroendocrinology*, *77*, 261–274. https://doi.org/10.1016/j.psyneuen.2016.12.017

Stalder, T., Steudte, S., Miller, R., Skoluda, N., Dettenborn, L., & Kirschbaum, C. (2012). Intraindividual stability of hair cortisol concentrations. *Psychoneuroendocrinology*, *37*(5), 602–610. https://doi.org/https://doi.org/10.1016/j.psyneuen.2011.08.007

Staufenbiel, S. M., Penninx, B. W. J. H., Spijker, A. T., Elzinga, B. M., & van Rossum, E. F. C. (2013). Hair cortisol, stress exposure, and mental health in humans: A systematic review. *Psychoneuroendocrinology*, *38*(8), 1220–1235. https://doi.org/10.1016/j.psyneuen.2012.11.015

Steptoe, A., Dockray, S., & Wardle, J. (2009). Positive Affect and Psychobiological Processes Relevant to Health. *Journal of Personality*, *77*(6), 1747–1776. https://doi.org/10.1111/j.1467-6494.2009.00599.x

Steudte-Schmiedgen, S., Kirschbaum, C., Alexander, N., & Stalder, T. (2016). An integrative model linking traumatization, cortisol dysregulation and posttraumatic stress disorder: Insight from recent hair cortisol findings. *Neuroscience and Biobehavioral Reviews*, *69*, 124–135. https://doi.org/10.1016/j.neubiorev.2016.07.015

Steudte, S., Kirschbaum, C., Gao, W., Alexander, N., Schönfeld, S., Hoyer, J. J., & Stalder, T. (2013). Hair cortisol as a biomarker of traumatization in healthy individuals and posttraumatic stress disorder patients. *Biological Psychiatry*, *74*(9), 639–646. https://doi.org/10.1016/j.biopsych.2013.03.011

Steudte, S., Kolassa, I.-T., Stalder, T., Pfeiffer, A., Kirschbaum, C., & Elbert, T. (2011). Increased cortisol concentrations in hair of severely traumatized Ugandan individuals with PTSD. *Psychoneuroendocrinology*, *36*(8), 1193–1200. https://doi.org/10.1016/j.psyneuen.2011.02.012

Straub, J., Klaubert, L. M., Schmiedgen, S., Kirschbaum, C., & Goldbeck, L. (2017). Hair cortisol in relation to acute and post-traumatic stress symptoms in children and adolescents. *Anxiety, Stress and Coping*, *30*(6), 661–670. https://doi.org/10.1080/10615806.2017.1355458

Streit, F., Memic, A., Hasandedić, L., Rietschel, L., Frank, J., Lang, M., … Rietschel, M. (2016). Perceived stress and hair cortisol: Differences in bipolar disorder and schizophrenia. *Psychoneuroendocrinology*, *69*, 26–34. https://doi.org/10.1016/j.psyneuen.2016.03.010

Suliman, S., Stein, D. J., Myer, L., Williams, D. R., & Seedat, S. (2010). Disability and treatment of psychiatric and physical disorders in South Africa. *Journal of Nervous and Mental Disease*, *198*(1), 8–15. https://doi.org/10.1097/NMD.0b013e3181c81708

Touskova, T. P., Bob, P., Pec, O., Mishara, A., Vanickova, Z., Raboch, J., & Lysaker, P. (2018). Insight and cortisol responses in women with first episode psychosis. *Schizophrenia Research*, *201*, 428–429. https://doi.org/10.1016/j.schres.2018.06.002

Tysnes, O. B., & Storstein, A. (2017). Epidemiology of Parkinson's disease. *Journal of Neural Transmission*, *124*(8), 901–905. https://doi.org/10.1007/s00702-017-1686-y

Ullmann, E., Barthel, A., Petrowski, K., Stalder, T., Kirschbaum, C., & Bornstein, S. R. (2016). Pilot study of adrenal steroid hormones in hair as an indicator of chronic mental and physical stress. *Scientific Reports*, *6*(May), 1–7. https://doi.org/10.1038/srep25842

van Zuiden, M., Savas, M., Koch, S. B. J., Nawijn, L., Staufenbiel, S. M., Frijling, J. L., … Olff, M.

(2019). Associations Among Hair Cortisol Concentrations, Posttraumatic Stress Disorder Status, and Amygdala Reactivity to Negative Affective Stimuli in Female Police Officers. *Journal of Traumatic Stress*, *32*(2), 238–248. https://doi.org/10.1002/jts.22395

Vancampfort, D., Stubbs, B., Mitchell, A. J., De Hert, M., Wampers, M., Ward, P. B., … Correll, C. U. (2015). Risk of metabolic syndrome and its components in people with schizophrenia and related psychotic disorders, bipolar disorder and major depressive disorder: A systematic review and meta-analysis. *World Psychiatry*, *14*(3), 339–347. https://doi.org/10.1002/wps.20252

Vancampfort, D., Wampers, M., Mitchell, A. J., Correll, C. U., De Herdt, A., Probst, M., & De Hert, M. (2013). A meta-analysis of cardio-metabolic abnormalities in drug naïve, first-episode and multi-episode patients with schizophrenia versus general population controls. *World Psychiatry*, *12*(3), 240–250. https://doi.org/10.1002/wps.20069

von Elm, E., Altman, D. G., Egger, M., Pocock, S. J., Gøtzsche, P. C., & Vandenbroucke, J. P. (2014). The strengthening the reporting of observational studies in epidemiology (STROBE) statement: Guidelines for reporting observational studies. *International Journal of Surgery*, *12*(12), 1495–1499. https://doi.org/10.1016/j.ijsu.2014.07.013

Vuksan-Cúsa, B., Săgud, M., Mihaljevic-Peleš, A., Jaksĭć, N., & Jakovljević, M. (2014). Metabolic syndrome and cortisol/dheas ratio in patients with bipolar disorder and schizophrenia. *Psychiatria Danubina*, *26*(2), 187–189.

Walker, E. R., McGee, R. E., & Drus, B. G. (2015). Mortality in Mental Disorders and Global Disease Burden Implications: A Systematic Review and Meta-analysis. *JAMA Psychiatry*, *72*(4), 334–341. https://doi.org/10.1001/jamapsychiatry.2014.2502

Wennig, R. (2000). Potential problems with the interpretation of hair analysis results. *Forensic Science International*, *107*(1), 5–12. https://doi.org/10.1016/S0379-0738(99)00146-2

Whiteford, H. A., Degenhardt, L., Rehm, J., Baxter, A. J., Ferrari, A. J., Erskine, H. E., … Johns, N. (2013). Global burden of disease attributable to mental and substance use disorders: findings

from the Global Burden of Disease Study 2010. *The Lancet*, *382*(9904), 1575–1586. https://doi.org/https://doi.org/10.1016/S0140-6736(13)61611-6

Wirdefeldt, K., Adami, H., Cole, P., Trichopoulos, D., & Mandel, J. (2011). Epidemiology and etiology of Parkinson's disease: a review of the evidence. *European Journal of Epidemiology*, *26*(Suppl 1), 0–58. https://doi.org/10.1007/s10654-011-9581-6

World Medical Association. (2013). World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA - Journal of the American Medical Association*, *310*(20), 2191–2194. https://doi.org/10.1001/jama.2013.281053

Wosu, A. C., Valdimarsdóttir, U., Shields, A. E., Williams, D. R., & Williams, M. A. (2013). Correlates of cortisol in human hair: Implications for epidemiologic studies on health effects of chronic stress. *Annals of Epidemiology*, *23*(12), 797–811. https://doi.org/10.1016/j.annepidem.2013.09.006

Zhang, P., & Tian, B. (2014). Metabolic syndrome: An important risk factor for Parkinson's disease. *Oxidative Medicine and Cellular Longevity*, *2014*. https://doi.org/10.1155/2014/729194

Zhang, Q., Chen, Z., Chen, S., Xu, Y., & Deng, H. (2017). Intraindividual stability of cortisol and cortisone and the ratio of cortisol to cortisone in saliva, urine and hair. *Steroids*, *118*, 61–67. https://doi.org/10.1016/j.steroids.2016.12.008

Zorn, J. V., Schür, R. R., Boks, M. P., Kahn, R. S., Joëls, M., & Vinkers, C. H. (2017). Cortisol stress reactivity across psychiatric disorders: A systematic review and meta-analysis. *Psychoneuroendocrinology*, *77*, 25–36. https://doi.org/10.1016/j.psyneuen.2016.11.036

CHAPTER 2

Hair cortisol levels in posttraumatic stress disorder and metabolic syndrome: A case-control study in South African mixed ancestry females

(*manuscript under review)*

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Hair cortisol levels in posttraumatic stress disorder and metabolic syndrome: A case-control study in South African mixed ancestry females

Abstract

Background: Individuals with posttraumatic stress disorder (PTSD) evidence increased rates of metabolic syndrome (MetS) and both PTSD and MetS are associated with alterations in hypothalamic pituitary adrenal (HPA) axis function. Few investigations have examined the possible role of HPA-axis dysfunction in the co-occurrence of PTSD and MetS.

Objectives: In a case-control study we aimed to determine whether hair cortisol concentrations (HCC) were associated with (i) PTSD caseness and severity and (ii) PTSD and CVD risk (as defined by the MetS) co-occurrence.

Methods: We utilised the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5) to determine PTSD diagnostic status and severity scores in 216 females of mixed ancestry aged between 20 and 79 years (M = 43.8, SD = 13.3). Hair samples, representing a three-month retrospective window of cortisol levels were obtained and analysed utilizing liquid chromatography tandem mass spectrometry. We constructed multivariate linear regression models to evaluate whether PTSD diagnosis, PTSD severity and MetS comorbidity were associated with HCC (reciprocal square root transformed) controlling for potential confounders.

Results: The prevalence of MetS was 30.0% in PTSD patients (n = 110) and 40.6% in trauma-exposed controls (TEC, n = 106). HCC were significantly higher (Cohen's d = 0.44) in PTSD patients than TEC (adj β = 0.09, 95% CI = 0.01; 0.18, p = 0.033). HCC were also significantly associated with CAPS severity scores (adj β = 0.00, 95% CI = 0.00; 0.01, p = 0.005). MetS was not associated with HCC and there were no significant interactions between PTSD caseness and MetS on HCC.

Conclusions: We demonstrate increased HCC in PTSD patients, with a directly proportional relationship. This study provides evidence of a chronically dysregulated neuroendocrine mediated stress response in PTSD. HCC do not, however, appear to have specificity for the comorbidity of PTSD and MetS in this sample.

Keywords: Post-traumatic stress disorder; hair cortisol concentrations; metabolic syndrome; trauma

1 Introduction

Due to its direct relationship to severe stress or trauma, posttraumatic stress disorder (PTSD) may represent the disorder with the most apparent link to the biological stress response. The hypothalamic pituitary adrenal (HPA) axis is one of the primary pathways involved in the stress response and has therefore been a logical focus of investigation into pathophysiological pathways involved in PTSD. Although HPA axis dysfunction has been clearly linked to PTSD, findings have been mixed (Steudte-Schmiedgen, Kirschbaum, Alexander, & Stalder, 2016). Systematic reviews and meta-analyses evaluating basal cortisol levels between PTSD patients and controls tend to find lower basal cortisol levels in PTSD patients, but also report frequent negative findings depending on the outcomes investigated (Meewisse, Reitsma, de Vries, Gersons, & Olff, 2007; Morris, Compas, & Garber, 2012; Schumacher et al., 2019). The results also appear to vary according to assay method and tissue type (e.g. saliva, plasma, urine), with differences possibly reflecting distinct facets of HPA-axis function (Steudte-Schmiedgen et al., 2016). The measurement of cortisol levels in hair, indicating more chronic HPA-axis function, is a fairly recent development and thus most systematic reviews have not included hair cortisol studies.

A synthesis of the existing literature suggests a two-stage model of cortisol secretion following trauma exposure; initially trauma exposure leads to increased cortisol secretion, which results in enhanced HPA-axis negative feedback sensitivity and higher glucocorticoid receptor sensitivity, and over the longer-term to a blunted HPA-axis and lower baseline cortisol levels in PTSD (Miller, Chen, & Zhou, 2007; Steudte-Schmiedgen et al., 2016). Context also plays an important role, with higher cortisol output demonstrated in PTSD patients in studies conducted in high trauma or high stress environments (Miller et al., 2007; Steudte-Schmiedgen et al., 2016).

Another question pertaining to PTSD is whether the HPA-axis alterations observed are related to the pathophysiology of PTSD or predominantly to trauma exposure. A recent meta-analysis examining the association between adversity, including trauma and childhood maltreatment, and HCC demonstrated a small significant positive relationship (d = 0.21) between adversity and HCC (Khoury, Bosquet Enlow, Plamondon, & Lyons-Ruth, 2019). However, a moderating effect for PTSD diagnostic status was not

found. Studies utilising HCC therefore tend to suggest that effects are chiefly trauma related, rather than specific to PTSD symptomatology (Steudte-Schmiedgen et al., 2016).

PTSD is associated with increased risk for cardiovascular disease (CVD) and mortality (Ahmadi et al., 2011; Kubzansky, Koenen, & Spiro, 2007). Furthermore patients with PTSD demonstrate increased rates (OR = 1.4; RR = 1.8) of metabolic syndrome (MetS) as compared to controls, with a pooled prevalence of MetS in PTSD patients of 38.7% (Bartoli, Carra, Crocamo, Carretta, & Clerici, 2013; Rosenbaum et al., 2015). The MetS denotes a cluster of risk factors for CVD and type 2 diabetes mellitus. Although there is some variation in diagnostic criteria proposed, risk factors include central obesity, hypertension, elevated fasting glucose, and dyslipidaemia, namely hypertriglyceridemia and low levels of high-density lipoprotein cholesterol (HDL-C) (Alberti et al., 2009). Longitudinal investigations tend to suggest that PTSD increases the risk for MetS, rather than the converse (Farr, Sloan, Keane, & Mantzoros, 2014; Wolf et al., 2016).

Similar to PTSD, HPA-axis dysfunction and altered glucocorticoid levels have also been demonstrated in relation to MetS and CVD (Anagnostis, Athyros, Tziomalos, Karagiannis, & Mikhailidis, 2009; Stalder et al., 2013). Authors have thus postulated that HPA-axis dysfunction secondary to trauma and PTSD may be a pathway leading to MetS and CVD morbidity in PTSD (Bartoli et al., 2013; Wolf et al., 2016). Various pathways potentially linking PTSD, the HPA axis and CVD risk are suggested. Chronically elevated cortisol, secondary to trauma or PTSD, can contribute to visceral fat accumulation, insulin resistance, dyslipidaemia and increased food intake (Michopoulos, Vester, & Neigh, 2016). MetS can also increase cortisol levels, by contributing to HPA axis dysfunction, as well as by adipose tissue, containing 11-hydroxysteroid dehydrogenase-1 (11HSD1), converting cortisone to cortisol (Anagnostis et al., 2009). Inflammation, immune alterations and oxidative stress secondary to HPA-axis dysfunction may also contribute to increased PTSD and CVD risk (Michopoulos et al., 2016). Furthermore, both MetS and a dysregulated HPA-axis can potentially increase the risk for developing PTSD by directly influencing brain function (Farr et al., 2014; Michopoulos et al., 2016). Although HPA-axis dysfunction is clearly linked to both PTSD and MetS, this relationship has not been extensively examined (Bartoli et al., 2013). One study in male military veterans that investigated biological pathways linking PTSD to

increased insulin resistance, found that morning plasma cortisol levels were not significantly associated with PTSD patient status, nor insulin resistance (Blessing et al., 2017).

HCC, reflecting chronic cortisol release, can potentially provide additional insights into HPA-axis dysfunction in relation to PTSD and associated CVD risk. Our objectives, in a sample of South African mixed ancestry adults, were to (i) determine whether HCC were associated with PTSD and clinical factors related to stress (PTSD severity, trauma severity and self-perceived stress); (ii) investigate interactions between PTSD and MetS (as well as individual CVD risk factors) on HCC.

2 Methods

2.1 Study design

This study is a neuroendocrine ancillary study to 'Understanding the SHARED ROOTS of Neuropsychiatric Disorders and Modifiable Risk Factors for Cardiovascular Disease' (SHARED ROOTS). The main aim of SHARED ROOTS (SR) is to determine the factors that contribute to comorbidity of NPDs and MetS, as a marker of CVD risk. This research question was investigated in three cohorts of NPDs of relevance to the South African context, namely PTSD, schizophrenia and Parkinson's disease. SR was a cross-sectional matched case-control study and controls were matched to each NPD cohort based on age, gender, and MetS status. Here we address the original main aims of the neuroendocrine study in the PTSD cohort.

2.1.1 Ethical aspects

This study was approved by the Health Research Ethics Committee at Stellenbosch University (HREC N13/08/115) and conducted according to principles of the of the seventh revision of the Declaration of Helsinki (World Medical Association, 2013). A tiered consenting process was adhered to, allowing participants to opt-out of the hair sampling procedure. Participants were referred to their usual health care service providers for any medical or psychiatric problems warranting further investigation and treatment.

2.2 Setting

Participants were enrolled over 3 years from May 2014 until June 2017 in Cape Town, Western Cape, South Africa. We utilised a purposive sampling approach and participants were recruited using multi-pronged strategies. PTSD cohort participants were recruited via (i) referrals from colleagues working at healthcare centres and from the Mental Health Information Centre; (ii) active recruitment by a registered nurse visiting various community centres; (iii) existing patient databases; (iv) print, radio and web advertisements; and (v) word of mouth by participants already recruited.

2.3 Participants

The study sample was limited to individuals who self-identified as belonging to the mixed ancestry (coloured) ethnic group. The decision to limit the study to one ethnic group was chiefly to avoid the effects of population stratification on genomic analyses. All participants had to be 18 years and older, willing and able to provide informed consent and be able to read and write in English or Afrikaans (the predominant languages spoken in this population). Participants were excluded from hair sampling if they had hair length shorter than 3cm (see Figure 1 for a flow diagram of participants included in this study).

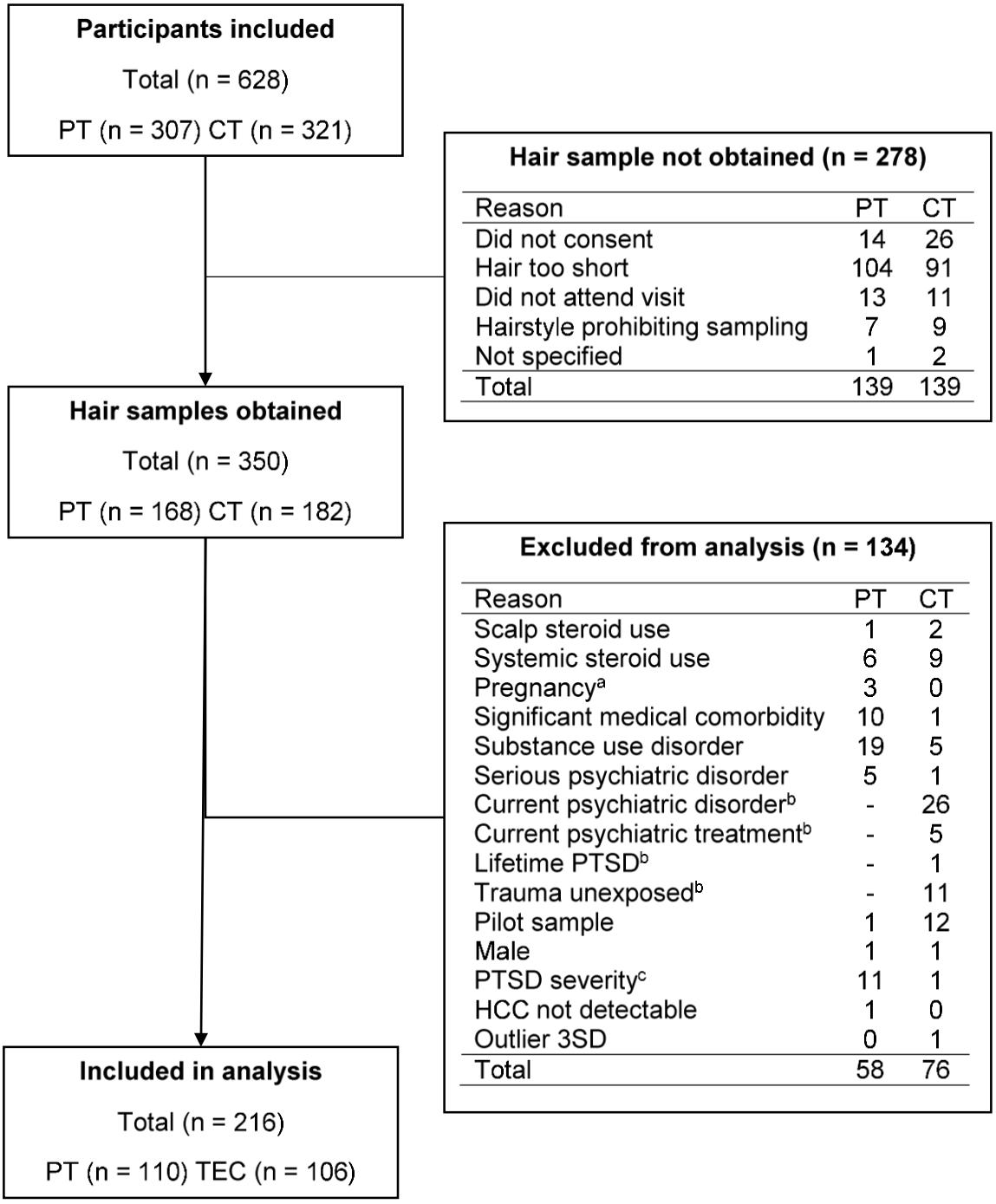
Further exclusion criteria applied in this study were, (i) systemic or scalp steroid use, (ii) current pregnancy, or pregnancy within the prior three months, (iii) significant medical comorbidity (e.g. cancer, HIV, auto-immune disorders), (iv) current substance use disorders, as determined with the Mini International Neuropsychiatric Interview (MINI), and (v) lifetime or current serious psychiatric disorder (a psychotic or bipolar disorder) as determined based on the psychiatric history or the MINI. The Diagnostic and Statistical Manual of Mental Disorders (DSM–5) definition of trauma-exposure was employed. PTSD case status was defined as meeting the DSM-5 criteria of PTSD according to the diagnostic evaluation with the Clinician Administered Posttraumatic Stress Disorder Scale for DSM–5 (CAPS-5). Trauma exposed participants in the PTSD cohort not meeting the DSM-5 criteria on the CAPS-5 were designated as trauma-exposed controls (TEC). We also applied a severity criterion excluding PTSD patients with a CAPS-5 severity score below 23 and TEC with a severity score above 22. Although we also enrolled trauma unexposed controls (TUC), these participants were excluded

from the current analysis due to the small number recruited (n = 11). Exclusion criteria only applied to controls were any current psychiatric disorder, as determined based on history, the MINI or current psychiatric medication use, as well as a lifetime diagnosis of PTSD based on history.

MetS status was determined based on the harmonized Joint Interim Statement (JIS) criteria (Alberti et al., 2009), with the presence of any three of the following five risk factors required for a diagnosis of MetS: (i) Raised blood pressure (BP), systolic ≥ 130 and/or diastolic ≥ 85 mmHg, or being on antihypertensive treatment; (ii) Elevated triglycerides (trig) > 1.70 mmol/l (150 mg/dl) or being on treatment for hypertriglyceridemia (fibrates or high dose omega-3 fatty acids); (iii) Reduced HDL-C, < 1.0 mmol/l (40mg/dl) in males, and < 1.3 mmol/l (50mg/dl) in females, or being on treatment for low HDL-C (nicotinic acid); (iv) Elevated fasting glucose (FPG) ≥ 5.6 mmol/l (100 mg/dl) or on treatment for diabetes; (v) Elevated waist circumference (WC) according to population and country specific guidelines. We used a WC ≥ 90cm in both males and females, as a recent validation study showed this to be the optimal WC cut-off in mixed ancestry individuals (Matsha et al., 2013).

2.4 Procedures

Participants attended two to three study visits, each lasting around 3 hours. Diagnostic, clinical and participant-administered measures were completed at the first visit. The second visit occurred within 24-96 hours of the first visit and involved the physical procedures and additional lifestyle measures. Assessments were conducted in English or Afrikaans, depending on the language preference of participants. Psychiatrists and physicians with experience in psychiatry conducted diagnostic and clinical assessments. Research nurses performed the physical procedures and personally administered the participant-administered measures to all participants, to aid in comprehension and to accommodate for variation in reading level.



Hair sample not obtained (n = 278)		
Reason	PT	CT
Did not consent	14	26
Hair too short	104	91
Did not attend visit	13	11
Hairstyle prohibiting sampling	7	9
Not specified	1	2
Total	139	139

Excluded from analysis (n = 134)		
Reason	PT	CT
Scalp steroid use	1	2
Systemic steroid use	6	9
Pregnancy[a]	3	0
Significant medical comorbidity	10	1
Substance use disorder	19	5
Serious psychiatric disorder	5	1
Current psychiatric disorder[b]	-	26
Current psychiatric treatment[b]	-	5
Lifetime PTSD[b]	-	1
Trauma unexposed[b]	-	11
Pilot sample	1	12
Male	1	1
PTSD severity[c]	11	1
HCC not detectable	1	0
Outlier 3SD	0	1
Total	58	76

Figure 1 Flow diagram demonstrating inclusion and exclusion of participant

[a] Current pregnancy or pregnancy within prior 3 months
[b] Exclusion criteria only applicable to controls
[c] Exclusion based on CAPS severity criterion (PT severity < 23; CT severity ≥ 23)
CAPS, Clinician-Administered PTSD Scale; CT, control; HCC, hair cortisol concentrations; PT, PTSD patient; PTSD, posttraumatic stress disorder; SD, Standard deviation; TEC, trauma exposed control

2.4.1 Physical measurements

Physical measurements were conducted in a consistent method in accordance with the World Health Organization Stepwise approach to chronic disease risk factor surveillance (WHO STEPS) instrument, a standardised instrument designed for non-communicable disease surveillance (World Health Organization, 2005). The following physical parameters were included in analysis: (i) mean systolic (SBP) and diastolic blood pressure (DBP, mmHg); (ii) mean heartrate (HR, bpm); (iii) body mass index (BMI, kg/m^2); (iv) WC (cm); (v) hip circumference (HC, cm); and (vi) waist-to-hip ratio (WHR).

2.4.2 Blood samples for metabolic parameters

Venous blood samples were obtained following an overnight fast (at least 8 hours) and were analysed on the Cobas 6000 c501 Chemistry Analyser (Roche, Germany) at a commercial internationally accredited laboratory (Lancet laboratories) on the same day as sample collection. The following laboratory parameters were included in analysis: (i) FPG (mmol/l); (ii) trig (mmol/l); (iii) HDL-C (mmol/l); (iv) low-density lipoprotein cholesterol (LDL-C, mmol/l); (v) total cholesterol (TC, mmol/l); (vi) and glycated haemoglobin A1c (HbA1c, %). Values for TC > 5 mmol/l, LDL-C ≥ 3 mmol/l, and HbA1c ≥ 6.5% were considered to be elevated (Klug et al., 2018; SEMDSA Type 2 Diabetes Guidelines Expert Committee., 2017).

2.4.3 Hair sampling

Hair samples from the posterior vertex scalp were cut with fine scissors. Samples were secured, placed into aluminium foil, labelled and sealed inside an envelope and stored in dark containers at room temperature.

2.4.4 Hair analysis

Hair analyses were performed at the TU Dresden laboratory (Prof. Clemens Kirschbaum). Samples were analysed in two batches. A pilot sample comprising the first 21 hair samples obtained were sent for analysis in September 2014. The remaining samples were sent for analysis in December 2017. Samples were analysed using an established liquid chromatography-tandem mass spectrometry (LC-MS/MS) protocol (Gao et al., 2013). The proximal 3cm of the hair segments were used for analysis,

representing cortisol secretion for the prior 3 months based on an accepted growth rate of 1cm per month (Wennig, 2000).

2.5 Measures

2.5.1 Demographic questionnaire

Demographic variables included in this manuscript are self-identified gender (male or female), age in years (calculated as date of birth subtracted from date of assessment), highest level of education (HLOE, defined according to whether secondary education was completed), and employment status (defined as being employed for the greater part of the prior 12 months).

2.5.2 Medical history questionnaires

Variables from the medical questionnaires included in this manuscript were: (i) steroid use in the prior 6 months (split according to whether any systemic steroids were used (oral or parenteral), topical steroids where used on the scalp, and any other topical steroid use (dermatologic, inhaled, nasal), (ii) hormonal contraceptive use in prior 6 months (including oral contraceptives and other formulations, such as injectable contraceptives), (iii) whether the woman was currently breastfeeding, (iv) history of previous PTSD, (v) current psychiatric medication use, (vi) known CVD [angina, myocardial infarct or cerebrovascular accident (CVA)], (vii) statin use, (viii) aspirin use, (ix) any other current medical conditions, and (iv) self-reported tobacco and alcohol use in the prior six months.

2.5.3 The Clinician Administered Posttraumatic Stress Disorder Scale for DSM–5 (CAPS-5) (F W Weathers et al., 2013)

The CAPS-5 was used to determine current (past month) PTSD diagnostic status and severity. The CAPS-5 is a structured diagnostic interview for PTSD based on DSM-5 diagnostic criteria for PTSD. Each DSM-5 corresponding item is rated based on the frequency and intensity of the symptoms on a 5-point Likert score (0 = absent, 1 = mild/subthreshold, 2 = moderate/threshold, 3 = severe/markedly elevated, and 4 = extreme/incapacitating). A symptom is considered present if it is rated ‘2’ or higher, reflecting a minimum intensity of ‘clearly present’ and a minimum frequency of twice a month or 20-30% of the time. Symptom severity scores are computed by adding the severity scores for the items corresponding to each DSM-5 cluster and a total symptom severity score by adding the severity scores

for all 20 items (range 0 – 80). Initial psychometric evaluations of the CAPS-5 demonstrated sound psychometric properties for both PTSD diagnostic status and severity scores (Frank W. Weathers et al., 2018). Internal consistency in this sample was good (Cronbach's α: Cluster B = 0.820, Cluster C = 0.720, Cluster D = 0.897, Cluster E = 0.821, full scale = 0.950).

2.5.4 The Life Events Checklist for DSM-5 (LEC-5) (Frank W Weathers et al., 2013)

The LEC-5 was developed concurrently with the CAPS-5 to assess lifetime exposure to potentially traumatic events and is administered prior to the CAPS-5. The LEC-5 assesses for exposure to 16 types of potentially traumatic events and an additional item for any other stressful events. For each event type, an individual indicates if they were exposed to the event in one or more ways, including if it happened to them personally, if they witnessed it, learned about it happening to a close family member or friend, or if they were exposed to it as part of their job. The worst event, or index trauma, as reported on the LEC-5 was used to assess for PTSD symptoms in the prior month with the CAPS-5. The LEC has demonstrated adequate psychometric properties in both clinical and non-clinical samples (Gray, Litz, Hsu, & Lombardo, 2004). Other trauma variables that are reported on include (i) the index trauma category/type (ii) trauma load, namely the total number of types (categories) of traumatic events endorsed (iii) months since the index trauma, and (iv) developmental stage when the index trauma occurred (≤ 18years, > 18 years).

2.5.5 The Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998)

The MINI is a short structured diagnostic interview used to diagnose psychiatric disorders based on DSM-IV and International Classification of Diseases (ICD-10) criteria. The MINI version 6.0 was used to determine the presence of any current and lifetime psychiatric disorders.

2.5.6 Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003)

We used the short form of the Childhood Trauma Questionnaire (CTQ) to assess for exposure to maltreatment during childhood and adolescence (before the age of 18 years). The CTQ is a 28-item measure with 25 clinical items divided into five scales (physical, sexual and emotional abuse, and physical and emotional neglect), and three validity items assessing for minimisation or denial. Each item is rated on a 5-point Likert scale (1 = never true; 2 = rarely true; 3 = sometimes true; 4 = often true;

5 = very often true). The severity scores obtained can be used as a dimensional measure of exposure to child abuse and neglect (scores ranging 5-25 for each subscale and 25 – 125 overall) or cut-scores (physical abuse ≥ 10, emotional abuse ≥ 13, sexual abuse ≥ 8, physical neglect ≥ 10 or emotional neglect ≥ 15) can be applied to determine the presence or absence of each type of abuse and neglect. In the original development and validation study, the CTQ demonstrated adequate psychometric properties. Internal consistency in this sample was good (Cronbach's α: Emotional abuse = 0.858, physical abuse = 0.877, sexual abuse = 0.938, emotional neglect = 0.865, physical neglect = 0.696; total scale = 0.936). We report on the total severity score and the number of types of childhood traumas (none, one, two or more).

2.5.7 The Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983)

We used the 10-item PSS (PSS-10) to measure self-perceived stress. Individuals rate to what extent they found their lives unpredictable, uncontrollable or overloaded in the prior month. Each item is rated on a 5-point Likert scale (0 = Never; 1 = Almost Never; 2 = Sometimes; 3 = Fairly often; 4 = Very often) with a total score ranging from 0 – 40, and higher scores indicate higher perceived stress. The PSS has been widely used and has demonstrated adequate psychometric properties in multiple settings and languages (Lee, 2012) and Cronbach's α in this sample was 0.87.

2.5.8 The WHO stepwise approach to chronic disease risk factor surveillance (WHO STEPS) (World Health Organization, 2005)

The level of physical activity was determined with the global physical activity questionnaire (GPAQ) in the WHO STEPS. The GPAQ assesses average physical activity in a week occurring across three domains, namely work, travel and recreation. Level of physical activity (high, moderate, and low) was determined according to the GPAQ guidelines (World Health Organization, 2005).

2.5.9 Hair questionnaire

A hair questionnaire, designed for this study, assessed hair characteristics and hair care practices. Hair related variables reported in this manuscript include (i) whether natural hair colour is black, (ii) whether hair had been chemically treated in the prior three months (including colouring, relaxing and perming), (iii) the frequency of hair washing (two or more times a week, once a week, less frequent than once a

week), (iv) whether any additional hair products had been used in the prior three months (such as hair styling and care products e.g. gels, oils and creams), and (v) average hours per day that hair was exposed to sunlight (less than one hour, 1-2 hours, more than 2 hours). Additional hair related factors included the season of sampling and the duration of sample storage (less than 1 year, 1-2 years, 2-3.5 years).

2.6 Statistical analysis

Sample size estimations were computed using means and standard deviations (SD) reported in other hair cortisol studies in PTSD and MetS samples (Stalder et al., 2013; Steudte et al., 2013), with α set at 0.05 and power at 90%. The sample size required to distinguish between PTSD patients and controls was 106 (53 in each group), and 292 (146 in each group) to distinguish between those with and without MetS. The overall sample size was, however, limited by recruitment of the parent study as well as by limitations to hair sampling, such as short hair length. Due to the small number of males with hair cortisol data (n = 3, 1.9%), the analysis was restricted to females only. As found in other studies, HCC were not normally distributed and were positively skewed (Clark, Osborne, Gallagher, & Watson, 2016). Utilising the box-cox family of power transformations (Osborne, 2010) HCC were reciprocal square root transformed to meet the assumption of normality. HCC were not detectable in one sample and we excluded one outlier with transformed HCC 3SD beyond the mean, resulting in a final sample of 216 participants (Figure 1). We compared the included and excluded sample with Pearson chi-square for categorical variables, and Mann Whitney tests for continuous variables. We compared the PTSD patients and TEC with Pearson chi-square for categorical variables and independent sample t-tests for continuous variables. There were very few missing data items and these were excluded from analysis listwise (missing items and final sample numbers reported in tables). To determine whether trauma severity (CTQ total score, number of types of traumas), PTSD caseness, total CAPS and domain severity scores, and self-perceived stress were associated with HCC we conducted unadjusted and adjusted linear regression models with HCC as the dependent variable. Covariates included in models were age, trauma related variables (CTQ total score, number of types of traumas, months since index trauma, developmental stage of trauma) and factors that remained significantly

associated with HCC when we controlled for age and PTSD caseness (education, employment status, natural hair colour, frequency of hair washing, chemical treatment of hair, duration of storage, and breastfeeding). Interaction effects between MetS and PTSD caseness were assessed by regressing MetS on HCC in unadjusted, and then in adjusted models that included potential confounders for HCC alongside PTSD caseness. We also examined for any interaction effects between PTSD caseness and MetS. In exploratory analysis we also examined for associations between the individual CVD risk variables and HCC as well as for interactions with PTSD caseness. Limited sensitivity and post-hoc analyses were conducted to allow for additional interpretation of the data. Data were analysed with SPSS for windows, version 25.0, all tests were 2-tailed and the level of significance was set at .05.

3 Results

3.1 Participants

Hair samples were obtained in 350 (55.7%) of the 628 participants included in the PTSD cohort and short hair length was the main factor limiting sampling. A further 134 participants were excluded from analysis, leaving a final sample of 216 participants; 110 patients and 106 TECs (see Figure 1 for details). The following factors differed significantly between included and excluded samples: Gender ($p < 0.001$), duration of storage ($p = 0.021$), tobacco use ($p = 0.031$), alcohol use ($p < 0.001$), total number of categories of traumas ($p = 0.016$), HDL-C criterion ($p = 0.004$), BMI ($p < 0.001$), WC ($p = 0.003$), and HC ($p < 0.001$). When we controlled for gender, these factors were no longer significantly different, excluding only alcohol use and the duration of storage.

Descriptive data of the sample are presented in Table 1. Fifteen (13.6%) of the PTSD patients had a lifetime diagnosis of PTSD based on history and 27 (24.5%) were on current psychiatric medications. Of the PTSD patients, 71 (64.5%) had a current comorbid CMD MINI diagnosis, 35.5% with comorbid MDD and 48.2% with a comorbid anxiety or related disorder. Factors that were significantly different between cases and TEC included age, hair product use, duration of storage, hormonal contraceptive use, and tobacco use. Trauma related variables, including childhood maltreatment and trauma load, also differed significantly between PTSD cases and TEC.

Table 1 Comparison of sociodemographic, hair related, behavioural, clinical, trauma and PTSD related variables between PTSD cases and trauma exposed controls

Variables	Patients	TEC	Test statistic	p-value
Total	110	106		
Socio-demographic				
Age *M(SD)*	40.8 (11.4)	46.9 (14.4)	t (199.7) = 3.45	0.001*
Secondary education complete n(%)			$\chi^2(1) = 0.39$	0.533
No	79 (71.8)	72 (67.9)		
Yes	31 (28.2)	34 (32.1)		
Employed n(%)			$\chi^2(1) = 1.12$	0.291
No	76 (69.1)	66 (62.3)		
Yes	34 (30.9)	40 (37.7)		
Hair related				
Natural hair colour black[a] n(%)			$\chi^2(1) = 0.00$	0.970
No	63 (57.8)	61 (57.5)		
Yes	46 (42.2)	45 (42.3)		
Hair chemically treated[a] n(%)			$\chi^2(1) = 0.03$	0.873
No	34 (31.2)	32 (30.2)		
Yes	75 (68.8)	74 (69.8)		
Frequency of hair washing[a] n(%)			$\chi^2(2) = 2.91$	0.233
≥ 2 times a week	30 (27.5)	31 (29.2)		
1 time a week	49 (45.0)	56 (52.8)		
< 1 time a week	30 (27.5)	19 (17.9)		
Add on hair products[a] n(%)			$\chi^2(1) = 3.88$	0.049*
No	44 (40.4)	57 (53.8)		
Yes	65 (59.6)	49 (46.2)		
Average hours in sun[b] n(%)			$\chi^2(2) = 1.23$	0.540
< 1 hour	46 (43.0)	44 (41.9)		
1-2 hours	38 (35.5)	32 (30.5)		
> 2 hours	23 (21.5)	29 (27.6)		
Season sampled n(%)			$\chi^2(3) = 7.78$	0.051
Summer	29 (26.4)	25 (23.6)		
Autumn	18 (16.4)	30 (28.3)		
Winter	30 (27.3)	33 (31.1)		
Spring	33 (30.0)	18 (17.0)		
Duration of storage n(%)			$\chi^2(2) = 11.14$	0.004*
Less than 1 year	12 (10.9)	22 (20.8)		

1 - 2 years	32 (29.1)	44 (41.5)		
2 – 3.5 years	66 (60.0)	40 (37.7)		
Clinical				
Hormonal contraceptive n(%)			$\chi^2(1) = 4.17$	0.041*
No	76 (69.1)	86 (81.1)		
Yes	34 (30.9)	20 (18.9)		
Currently breastfeeding[a] n(%)			$\chi^2(1) = 0.65$	0.420
No	101 (92.7)	101 (95.3)		
Yes	8 (7.3)	5 (4.7)		
Topical steroid use n(%)			$\chi^2(1) = 0.62$	0.430
No	102 (92.7)	101 (95.3)		
Yes	8 (7.3)	5 (4.7)		
Other medical conditions n(%)			$\chi^2(1) = 0.61$	0.434
No	43 (39.1)	47 (44.3)		
Yes	67 (60.9)	59 (55.7)		
Behavioural				
Tobacco use n(%)			$\chi^2(1) = 5.14$	0.023*
No	57 (51.8)	71 (67.0)		
Yes	53 (48.2)	35 (33.0)		
Alcohol use n(%)			$\chi^2(1) = 0.01$	0.917
No	62 (56.4)	59 (55.7)		
Yes	48 (43.6)	47 (44.3)		
Level of physical activity[a] n(%)			$\chi^2(2) = 2.12$	0.347
High	6 (5.5)	10 (9.4)		
Moderate	33 (30.3)	38 (35.8)		
Low	70 (64.2)	58 (54.7)		
Trauma and PTSD related				
Childhood maltreatment				
CTQ severity score *M(SD)*	61. 2 (22.0)	45.8 (17.5)	t (214) = -5.69	< 0.001*
Number of childhood traumas n(%)			$\chi^2(2) = 15.75$	< 0.001*
None	20 (18.2)	43 (40.6)		
One	23 (20.9)	24 (22.6)		
Two or more	67 (60.9)	39 (36.8)		
LEC trauma variables				
Total number of trauma categories *M(SD)*	7.9 (3.4)	5.7 (2.9)	t (214) = -5.21	< 0.001*
Personally experienced *M(SD)*	3.5 (1.7)	2.1 (1.6)	t (214) = -6.36	< 0.001*
Witnessed *M(SD)*	2.0 (1.9)	1.5 (1.6)	t (214) = -1.97	0.050
Learned about *M(SD)*	2.4 (1.8)	2.0 (1.5)	t (214) = -1.38	0.170
As part of job *M(SD)*	0.07 (0.42)	0.02 (0.14)	t (132.4) = -1.27	0.206

Months since trauma *M(SD)*	124.8 (139.4)	143.4 (148.5)	t (214) = 0.95	0.343
Developmental stage index trauma n(%)			$\chi^2(1) = 2.76$	0.097
≤ 18 years	24 (21.8)	14 (13.2)		
> 18 years	86 (78.2)	92 (86.8)		
Index trauma main type n(%)			$\chi^2(10) = 27.72$	0.002*
Fire or explosion	1 (0.9)	2 (1.9)		
Transportation accident	3 (2.7)	11 (10.1)		
Other accident	1 (0.9)	0		
Physical assault	16 (14.5)	10 (9.4)		
Assault with a weapon	23 (20.9)	22 (20.8)		
Sexual assault	36 (32.7)	12 (11.3)		
Captivity	0	1 (0.9)		
Life-threatening illness or injury	2 (1.8)	4 (3.8)		
Sudden violent death	6 (5.5)	7 (6.6)		
Sudden unexpected loss of someone close	21 (19.1)	29 (27.4)		
Other event	1 (0.9)	8 (7.5)		
CAPS severity score				
Total severity score *M(SD)*	36.5 (8.4)	5.4 (5.7)	t (191.1) = -31.94	< 0.001*
Intrusion *M(SD)*	8.4 (3.0)	1.7 (2.3)	t (202.8) = -18.65	< 0.001*
Avoidance *M(SD)*	4.4 (1.3)	1.2 (1.7)	t (192.5) = -15.78	< 0.001*
Negative changes *M(SD)*	12.9 (4.2)	1.1 (2.1)	t (160.6) = -26.61	< 0.001*
Arousal *M(SD)*	10.8 (3.1)	1.4 (1.6)	t (165.5) = -28.27	< 0.001*
Perceived stress				
PSS total score *M(SD)*	27.2 (6.5)	17.0 (7.4)	t (214) = -10.78	<0.001*
HCC *Mdn(IQR)*	7.05 (4.08; 12.33)	4.15 (2.74; 7.23)	U=4228; Z=-3.49	< 0.001*
Transformed HCC *M(SD)*	1.28 (0.30)	1.15 (0.28)	t (214) = -3.21	0.002*

[a] One missing response on this item
[b] Four missing responses on this item
* $p < 0.05$
CAPS, Clinician Administered Posttraumatic Stress Disorder Scale; CTQ, Childhood Trauma Questionnaire; HCC, hair cortisol concentrations; Life Events Checklist (LEC); PSS, perceived stress scale; TEC, trauma exposed controls

Table 2 Trauma and PTSD related variables regressed on hair cortisol concentrations (HCC)

Variables	Simple linear regression model		Multiple linear regression[a]	
	B (95% CI)	p-value	B (95% CI)	p-value
Total number of types of traumas	0.01 (0.00; 0.02)	0.036*	0.00(-0.01; 0.01)	0.687
CTQ total severity score	0.00 (0.00; 0.00)	0.006*	0.00 (-0.00; 0.00)	0.187
PTSD caseness				
TEC	Ref		Ref	
Patient	0.13 (0.05; 0.20)	0.002*	0.09 (0.01; 0.18)[b]	0.033*
Total CAPS severity score	0.00 (0.00; 0.01)	<0.001*	0.00 (0.00; 0.01)[c]	0.005*
Intrusion	0.02 (0.01; 0.03)	<0.001*	0.02 (0.01; 0.03)	0.001*
Avoidance	0.03 (0.01; 0.04)	0.007*	0.02 (-0.00; 0.04)	0.060
Negative changes	0.01 (0.01; 0.02)	<0.001*	0.01 (0.00; 0.02)	0.009*
Arousal	0.01 (0.00; 0.02)	0.004*	0.01 (0.00; 0.02)	0.041*

[a] Controlled for age, education, employment status, natural hair colour, frequency of hair washing, chemical treatment of hair, duration of storage, breastfeeding, CTQ total score, number of types of traumas, months since index trauma, developmental stage of trauma, n = 214 (one participant with missing data for frequency of hair washing and chemical treatment of hair excluded and one participant with missing data on breastfeeding excluded)
[b]Model: F (15, 198) = 4.76 ($p < 0.001$*), Adj $R^2 = 0.209$
[c]Model: F (15, 198) = 5.05 ($p < 0.001$*), Adj $R^2 = 0.222$
* $p < 0.05$
CAPS, Clinician Administered Posttraumatic Stress Disorder Scale; CTQ, Childhood Trauma Questionnaire; HCC, hair cortisol concentrations; PTSD, posttraumatic stress disorder; TEC, trauma exposed control

3.2 PTSD, trauma and hair cortisol concentrations (Table 2)

Trauma related variables, total number of categories of traumas and CTQ severity, were significantly associated with HCC in unadjusted, but not in adjusted analyses. HCC were significantly higher (Cohen's d = 0.44) in PTSD cases than in TEC (Figure 2) in unadjusted and adjusted models. Total CAPS severity scores were significantly positively associated with HCC in unadjusted and adjusted analyses. PTSD symptom cluster severity scores were also significantly associated with HCC, in unadjusted and adjusted models, with the exception of avoidance severity that demonstrated a trend towards significance ($p = 0.060$) in the adjusted model. When symptom cluster severity scores were added to the model simultaneously, only intrusion severity (adj $B = 0.02$, 95% CI 0.00; 0.04, $p = 0.033$) remained significantly associated with HCC.

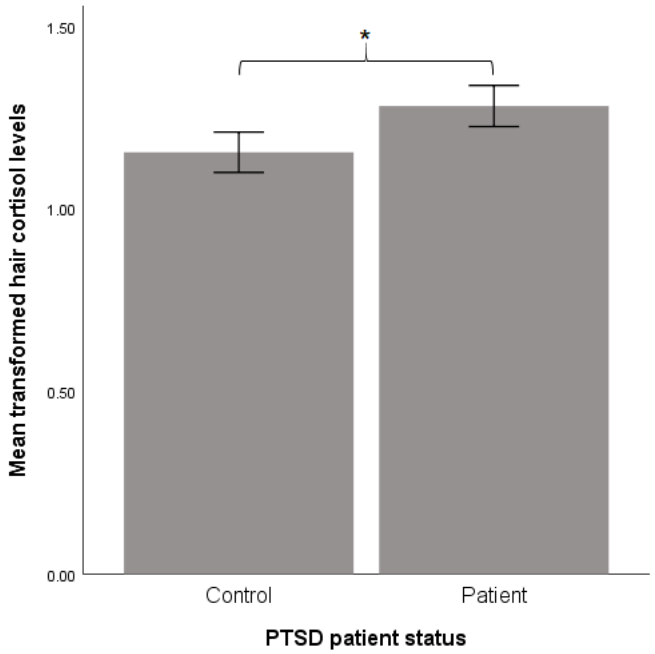


Figure 2 Hair cortisol concentrations (HCC) according to PTSD diagnosis

Bar chart demonstrating mean HCC according to PTSD patient status. HCC were significantly higher in PTSD patients than trauma exposed controls ($p = 0.002$)
*$p < 0.05$
Error bars ± 2SE

3.3 Self-perceived stress, PTSD and hair cortisol concentrations

PSS scores were significantly higher in PTSD patients than in TEC ($p < 0.001$) and PSS scores were significantly correlated with CAPS severity score ($r = 0.64$, $p < 0.001$). PSS scores were significantly associated with HCC in unadjusted ($B = 0.01$, 95% CI 0.0; 0.01, $p < 0.001$) and adjusted (adj $B = 0.01$, 95% CI 0.01; 0.02, $p = 0.029$) analyses. There were no significant interaction effects between PTSD caseness and PSS scores on HCC.

3.4 Cardiovascular disease risk factors, PTSD and hair cortisol concentrations

3.4.1 Metabolic syndrome, PTSD and hair cortisol concentrations (Table 3)

Rates of MetS were non-significantly higher ($p = 0.104$) in TEC than in PTSD patients. MetS was not significantly associated with HCC in unadjusted and adjusted analyses. Although the interaction between PTSD caseness and MetS was not significant, MetS was associated with higher HCC in TEC and with lower HCC in PTSD cases (Figure 3).

Table 3 PTSD and metabolic syndrome regressed on hair cortisol concentrations (HCC)

	Simple linear regression		Multiple linear regression[a]					
			Model 1		Model 2		Model 3	
	ß (95% CI)	p-value	ß (95% CI)	p-value	ß (95% CI)	p-value	ß (95% CI)	p-value
PTSD caseness								0.045*
TEC	Ref		Ref		Ref		Ref	
Patient	0.13 (0.05; 0.20)	0.002*	0.10 (0.02; 0.18)	0.010*	0.10 (0.02; 0.18)	0.010*	0.14 (0.05; 0.24)	0.002*
MetS								0.753
No	Ref				Ref		Ref	
Yes	-0.01 (-0.09; 0.08)	0.850			0.08 (-0.21; 0.37)	0.557	0.08 (-0.03; 0.19)	0.169
Patient*MetS							-0.13 (-0.28; 0.03)	0.101

[a] Controlled for age, education, employment status, natural hair colour, frequency of hair washing, chemical treatment of hair, duration of storage, breastfeeding, n = 214 (one participant with missing data for frequency of hair washing and chemical treatment of hair excluded and one participants with missing data on breastfeeding excluded)
Model 1 – Model without MetS: F (11, 202) = 6.31 ($p < 0.001$*), Adj $R^2 = 0.215$; n = 214
Model 2 – MetS added: F (12, 201) = 5.77 ($p < 0.001$*), Adj $R^2 = 0.212$, n = 214
Model 3 – Interaction between case status and MetS added: F (13, 201) = 5.58 ($p < 0.001$*), Adj $R^2 = 0.218$; n = 214
* $p < 0.05$
MetS, metabolic syndrome; ; TEC, trauma exposed control

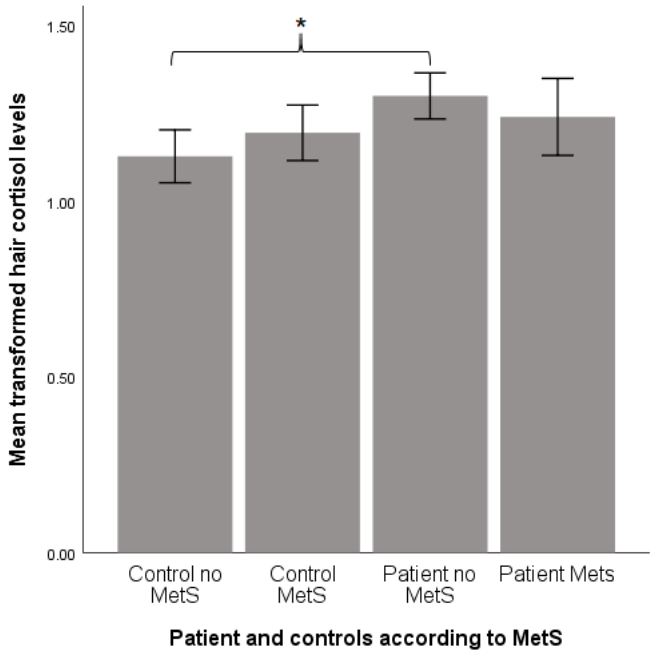


Figure 3 Hair cortisol concentrations (HCC) according to PTSD patient status and metabolic syndrome status

Bar chart demonstrating mean hair cortisol levels according to PTSD patient status and metabolic syndrome status (MetS). The model was significant (F (212, 3) = 4.22, p = 0.006). HCC were highest in PTSD patients without MetS, and were significantly higher than TEC without MetS (p = 0.003). There were no other significant differences between the groups based on Tukey HSD post hoc test.
*p < 0.05
Error bars ± 2SE

3.4.2 Individual cardiovascular disease risk factors, PTSD and hair cortisol concentrations (Supplementary Table 1)

CVD risk factors that differed between cases and TEC included antihypertensive medication use (p = 0.005), FPG (p = 0.029), FPG MetS criterion (p < 0.001) and HDL-C MetS criterion (p = 0.039). When we controlled for age, only FPG MetS criterion remained significantly different (p = 0.031). The only CVD risk factors that were significantly associated with HCC in unadjusted analyses and adjusted analyses were HDL-C levels and HDL-C MetS criterion (Figure 4), such that lower HDL-C levels were associated with increased HCC. The only significant interaction between PTSD patient status and CVD risk variables was demonstrated for WHR (adj B = -1.08, 95% CI -1.99; -0.17, p = 0.021) where WHR was significantly negatively associated with HCC in patients (adj B = -0.78, 95% CI -1.45; -0.09, p = 0.028), but not in TEC (adj B = 0.17, 95% CI -0.47; 0.81, p = 0.601).

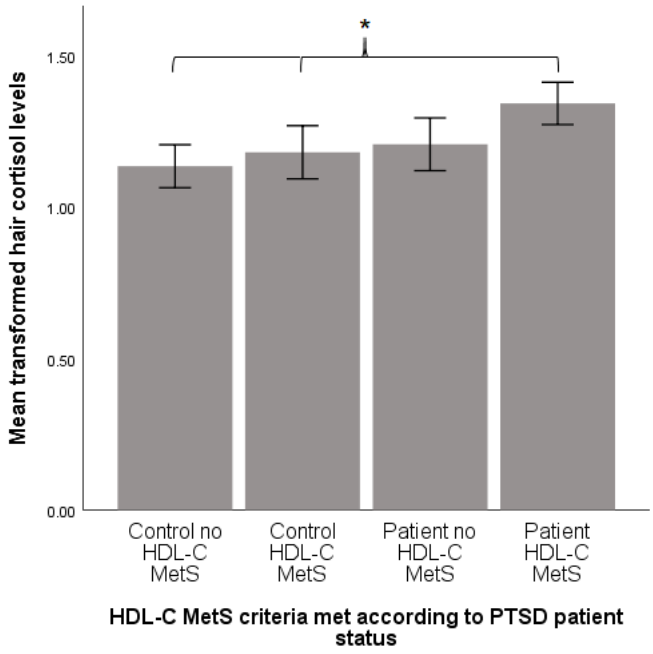


Figure 4 Hair cortisol concentrations (HCC) according to PTSD patient status and HDL-C criteria

Bar chart demonstrating mean hair cortisol levels according to PTSD patient status and HDL-C criteria. The model was significant (F (212, 3) = 5.75, $p = 0.001$). HCC were highest in PTSD patients with HDL-C criterion, and were significantly higher than TEC without HDL-C criterion ($p < 0.001$), TEC with HDL-C criterion ($p = 0.029$), and demonstrated a trend towards significance against PTSD patients without HDL-C criterion ($p = 0.068$). There were no other significant differences between the groups based on Tukey HSD post hoc test.
*$p < 0.05$
Error bars ± 2SE

3.5 Sensitivity analysis of common mental disorder comorbidity

To assess for the influence of CMD comorbidity we repeated the final models for PTSD patient status and total CAPS severity scores in the larger sample (n = 253; 119 PT and 134 TEC), including controls with CMD comorbidity and without applying the CAPS severity criteria. When MINI CMD comorbidity was added to the model both PTSD caseness (adj $B = 0.11$, 95% CI 0.02; 0.19, $p = 0.011$) and CAPS severity scores (adj $B = 0.00$, 95% CI 0.00; 0.01, $p = 0.009$) remained significantly associated with HCC and CMD comorbidity was not associated with HCC. Results remained similar when MINI MDD and MINI anxiety or related disorder were added to the model individually and simultaneously.

4 Discussion

We demonstrated that in South African mixed ancestry females, HCC were significantly higher in PTSD patients than in TEC. Furthermore, HCC were also significantly associated with PTSD severity, demonstrating a directly proportional relationship. The association between HCC and PTSD variables remained significant when potential confounders were controlled for, including trauma and CMD comorbidity. We did not demonstrate an association between HCC and MetS, nor an association with PTSD and MetS comorbidity. Individual CVD risk variables were on the whole not associated with HCC, with the exception of a negative association with HDL-C and HDL-C MetS criterion, and WHR, in PTSD cases only.

Largely, the literature demonstrates lower baseline cortisol levels in PTSD patients than in controls (Meewisse et al., 2007; Morris et al., 2012; Schumacher et al., 2019). We found the converse in our sample, with higher HCC in PTSD patients than in TEC. Our findings can plausibly be explained by the context in which our study was conducted. Our sample had very high rates of trauma exposure. Participants were exposed to an average of seven different types of trauma and more than two thirds (70.8%) had experienced some form of childhood abuse or maltreatment. Our results thus align with the general observation that in high trauma or high stress environments, PTSD patients demonstrate greater baseline cortisol output (Miller et al., 2007; Steudte-Schmiedgen et al., 2016). An interpretation of other studies examining the association between PTSD and HCC is to some extent aligned with this model. Studies conducted in low stress environments in the main have demonstrated negative associations between PTSD parameters and HCC (Steudte et al., 2013; Straub, Klaubert, Schmiedgen, Kirschbaum, & Goldbeck, 2017). In other samples with ongoing stressors, such as displaced populations (Dajani, Hadfield, van Uum, Greff, & Panter-Brick, 2018; Steudte et al., 2011) and enlisted soldiers (Groer, Kane, Williams, & Duffy, 2015) PTSD has been associated with increased HCC. This pattern, is however, not consistently demonstrated (Luo et al., 2012; van Zuiden et al., 2019) and what constitutes ongoing stress is to some degree a matter of interpretation. Other, unknown factors may also be contributing to the differing patterns of association observed between HCC and PTSD related outcomes. Our sample had very high comorbidity rates of MDD and anxiety, and a

systematic review demonstrated that afternoon cortisol levels were lower in PTSD patients without, and higher in PTSD patients with, comorbid MDD compared with trauma unexposed controls (Morris et al., 2012).

Similar to a recent meta-analysis (Khoury et al., 2019) we demonstrated a small positive association between trauma severity indicators (both trauma load and childhood maltreatment) and HCC. However, these associations were no longer significant when we controlled for potential confounders. Furthermore, whereas previous studies have suggested that the association between HCC and PTSD appears to be primarily trauma related (Khoury et al., 2019; Steudte-Schmiedgen et al., 2016), our results demonstrated that HCC were associated with the PTSD clinical phenotype. It is, however, difficult to accurately define and measure trauma severity in a sample with complex and repeated trauma histories, such as the sample reported on here. We also included a measure of self-perceived stress to evaluate for associations between self-perceived stress and HCC within the context of PTSD. PTSD patients reported higher self-perceived stress than TEC and self-perceived stress was also significantly associated with CAPS severity scores. PSS scores were significantly associated with HCC, providing further support that the psychological stress experienced by PTSD sufferers is associated with higher HCC.

We did not demonstrate a significant association between HCC and MetS, nor an association with PTSD and MetS comorbidity. Although not significant, MetS was associated with HCC in opposite directions in PTSD patients and TEC. Other studies utilising HCC tend to demonstrate higher HCC in relation to MetS (Kuehl et al., 2015; Stalder et al., 2013). However, a study in HIV positive individuals found a negative association between HCC and MetS (Langerak et al., 2015). The authors postulated that the contradictory findings in HIV patients could be related to cortisol hypersensitivity, where systemic levels of cortisol are low due to enhanced negative feedback. An analogous effect may be present in the PTSD patients with MetS. This similar pattern was also demonstrated for WHR in our sample, where WHR was significantly negatively associated with HCC in patients, but non-significantly positively associated with HCC in TEC. The direction of association between WHR and HCC in TEC aligned with what has generally been observed (Stalder et al., 2017). Of note, individual MetS related

factors were not associated with HCC in the same direction; meeting HDL-C and BP criteria were associated with higher HCC, whereas meeting FPG, Trig, and WC criteria were associated with lower HCC. The association between MetS and HPA-axis functioning appears to be of a smaller magnitude and more complex and may have been obscured by the stronger influence of trauma characteristics and other PTSD related factors in our sample.

The only MetS factor significantly associated with HCC was HDL-C, both continuous HDL-C levels and whether levels were below the HDL-C criterion threshold. Though some studies have not found an association between HLD-C and cortisol (de Vries, Mocking, Assies, Schene, & Olff, 2017), other studies have similarly demonstrated a negative association between cortisol levels and HDL-C (Fraser et al., 1999; Stalder et al., 2013). Conversely, a study in a male PTSD sample demonstrated that plasma cortisol levels were significantly positively correlated with HDL-C in PTSD patients (Blessing et al., 2017). HDL-C has anti-inflammatory, antioxidant, antithrombotic and anti-apoptotic functions and is a known independent risk factor for CVD (März et al., 2017). Cortisol and HDL-C are potentially linked together in different pathways. Cortisol affects lipid metabolism and may thus influence HDL-C levels and HDL-C acts as a cholesterol donor for steroidogenesis in the adrenal gland (Atogo-Asse et al., 2012; März et al., 2017). HDL-C appears to play a particular role in cortisol production in response to ACTH stimulation and under stressful conditions (Atogo-Asse et al., 2012).

High rates (45.9%) of low HDL-C levels have also been demonstrated in PTSD (Rosenbaum et al., 2015). In our sample, HDL-C levels were also significantly lower in PTSD patients than in TEC, although the association did not remain significant once we controlled for age. There, however, appeared to be a cumulative effect between PTSD caseness and HDL-C, such that HCC were highest in PTSD patients with low HDL-C (Figure 4). A study in baboons demonstrated a similar pattern, where subordinate males who experienced more social stress had significantly lower HDL-C than dominant males, and HDL-C and basal cortisol levels were inversely associated (Sapolskyf & Mott, 1987). Further evidence for unique stress related contributions to metabolic derangements were demonstrated in a study where diurnal stress-related cortisol release was significantly inversely associated with HDL-C, only in the presence of a blunted diurnal cortisol pattern, suggesting longer-

term HPA-dysfunction alongside increased stress sensitivity (Rosmond, Dallman, & Bjorntorp, 1998). HDL-C may thus be one of the factors linking increased CVD risk in PTSD patients to associated HPA-axis dysregulation. HDL-C can also be influenced by other factors such as genetic variation, smoking, physical activity, obesity, diet, alcohol consumption, and statin use (März et al., 2017). When we controlled for statin, tobacco and alcohol use, and physical activity in our study, results were unchanged.

4.1 Strengths and limitations

Our study includes one of the largest investigations of the association between PTSD and HCC to date. Our sample was, however, underpowered to detect significant associations between HCC and MetS. This was a cross-sectional study where findings of associations are not indicative of cause-effect relationships. A major limitation in our study was the requirement that scalp hair length had to be 3cm or longer and consequently many participants, particularly males, were excluded from participation. Our sample was limited to a single race classification and sex (female) and these results cannot be directly extrapolated to other populations. For instance, gender may influence the association between cortisol and metabolic parameters. One study in PTSD found that LDL-C was positively associated with morning plasma cortisol in men, but negatively associated with cortisol in women (de Vries et al., 2017). They furthermore demonstrated a significant interaction between PTSD patient status and sex, such that triglycerides were negatively associated with cortisol in male PTSD patients, but were positively associated with triglycerides in female patients with PTSD, as well as in controls (de Vries et al., 2017).

Our sample, however, is unique in representing a geographic and cultural setting where there is a considerable and varied trauma burden alongside notable socio-economic strain. Future studies in diverse populations and utilising other more viable methods of hair sampling will assist in contextualising these findings. We controlled for a variety of trauma related variables, including childhood maltreatment, trauma load, and time since index trauma, thus allowing us to partly distinguish PTSD related effects from trauma and adversity effects. As the pattern of cortisol dysregulation appears to change over time following trauma exposure (Miller et al., 2007; Steudte-Schmiedgen et al., 2016), time since trauma exposure could also influence basal cortisol levels

observed in relation to PTSD. We controlled for duration since the index trauma, but could not account for the influence of other, more recent or ongoing, trauma exposures. Furthermore, we could not assess for the independent effects of trauma exposure as we did not have a sufficiently large trauma-unexposed sample to include for comparison. We did not investigate the effects of specific trauma or maltreatment type on the association between PTSD and HCC and future studies examining these relationships in more depth are warranted. Our sample had high CMD comorbidity and PTSD patients with CMD comorbidity had significantly more severe illness ($p < 0.001$), and thus we could not meaningfully conduct analyses with PTSD patients without CMD comorbidity. PTSD is known to have high CMD comorbidity, for instance a meta-analysis reported that 52% of patients with PTSD had comorbid MDD (Rytwinski, Scur, Feeny, & Youngstrom, 2013). Including CMD comorbidity arguably reflects more real world clinical presentations of PTSD.

4.2 Conclusions

This study provides evidence of a chronically dysregulated neuroendocrine mediated stress response in PTSD. In our sample HCC appeared to be associated with the clinical phenotype, rather than with trauma exposure, per se. Our study is one of the few studies that have examined the association between PTSD, HPA-axis dysfunction and CVD risk. Similar to Blessing et al, 2017 we did not find a clear link between PTSD, CVD risk and cortisol levels. We did, however, demonstrate a significant association between HDL-C and cortisol levels, although the direction of association was opposite to that found in Blessing et al., 2017, which could potentially be related to gender effects. Other studies have suggested that potential links between PTSD, HPA-axis function, CVD risk and inflammation exist (Gocan, Rohr, Bachg, Schindler, & Rohr, 2012), although the exact nature of how these factors intersect remain to be determined. Including other markers of HPA-axis function and of inflammation could assist in better delineating pathways linking PTSD, HPA-axis dysregulation and CVD risk. Genomic analyses and longitudinal studies could also assist in identifying shared genetic risk factors and in determining cause and effect pathways.

5 Supplementary materials

Supplementary Table 1 Cardiovascular disease risk factors in relation to PTSD caseness and hair cortisol concentrations (HCC)

	CVD risk variables according to PTSD caseness		Simple linear regression		Multiple linear regression[a]	
	Patients	TEC	ß (95% CI)	p-value	ß (95% CI)	p-value
Total	110	106				
MetS variables						
MetS n(%)						
No	77 (70.0)	63 (59.4)	Ref		Ref	
Yes	33 (30.0)	43 (40.6)	-0.01 (-0.09; 0.08)	0.850	0.01 (-0.07; 0.10)[b]	0.723
Number of criteria met *M(SD)*	1.9 (1.3)	2.1 (1.3)	0.01 (-0.02; 0.04)	0.634	0.01 (-0.02; 0.04)	0.406
BP criterion n(%)						
No	52 (47.3)	45 (42.5)	Ref		Ref	
Yes	58 (52.7)	61 (57.5)	0.05 (-0.03; 0.13)	0.247	0.02 (-0.01; 0.05)	0.306
On antihypertensive rx n(%)						
No	83 (75.5)	61 (57.5)	Ref		Ref	
Yes	27 (24.5)	45 (42.5)	0.02 (-0.07; 0.10)	0.665	0.04 (-0.05; 0.14)	0.382
SBP (mmHg) *M(SD)*	126.7 (19.1)	127.5 (19.4)	0.00 (-0.00; 0.00)	0.801	0.00 (-0.00; 0.00)	0.920
DBP (mmHg) *M(SD)*	80.9 (12.2)	79.3 (10.9)	0.00 (-0.00; 0.01)	0.126	0.00 (-0.00; 0.01)	0.276
BP over threshold n(%)						
No	59 (53.6)	56 (52.8)	Ref		Ref	
Yes	51 (46.4)	50 (47.2)	0.03 (-0.05; 0.11)	0.508	0.00 (-0.07; 0.08)	0.942
FPG criterion[b] n(%)						
No	89 (80.9)	58 (54.7)	Ref		Ref	
Yes	21 (19.1)	48 (45.3)	-0.04 (-0.12; 0.05)	0.389	0.01 (-0.07; 0.10)	0.762
On diabetes rx n(%)						
No	103 (93.6)	95 (87.7)	Ref		Ref	
Yes	7 (6.4)	13 (12.3)	0.10 (-0.04; 0.24)	0.151	0.07 (-0.06; 0.20)	0.307
FPG (mmol/l) *M(SD)*	5.46 (2.19)	6.25 (3.06)	0.01 (-0.01; 0.02)	0.203	0.01 (-0.01; 0.02)	0.398
HDL-C criterion[c] n(%)						
No	51 (46.4)	64 (60.4)				
Yes	59 (53.6)	42 (39.6)	0.11 (0.03; 0.19)	0.007*	0.10 (0.02; 0.17)	0.010*
HDL-C (mmol/l) *M(SD)*	1.35 (0.40)	1.43 (0.37)	-0.11 (-0.21; -0.00)	0.043*	-0.11 (-0.20; -0.01)	0.030*
Trig criterion[d] n(%)						
No	95 (86.4)	88 (83.0)	Ref		Ref	
Yes	15 (13.6)	18 (17.0)	-0.05 (-0.16; 0.06)	0.374	-0.02 (-0.12; 0.09)	0.725
Trig (mmol/l) *M(SD)*	1.16 (0.49)	1.20 (0.61)	-0.01 (-0.09; 0.06)	0.729	0.02 (-0.05; 0.09)	0.630
WC criterion n(%)						
No	54 (49.1)	53 (50.0)	Ref		Ref	

Yes	56 (50.9)	53 (50.0)	-0.05 (-0.13; 0.03)	0.251	-0.04 (-0.11; 0.04)	0.343
WC (cm) *M(SD)*	92.2 (17.1)	93.1 (16.1)	-0.00 (-0.00; 0.00)	0.155	-0.00 (-0.00; 0.00)	0.309
Other CVD risk variables						
Physical parameters						
BMI (kg/m^2) *M(SD)*	31.0 (7.8)	32.2 (8.4)	-0.00 (-0.01; 0.00)	0.279	-0.00 (-0.01; 0.00)	0.737
HC (cm) *M(SD)*	108.9 (15.9)	110.4 (15.0)	-0.00 (-0.00; 0.00)	0.152	0.00 (-0.00; 0.00)	0.705
WHR *M(SD)*	0.84 (0.08)	0.84 (0.08)	-0.17 (-0.67; 0.32)	0.493	-0.33 (-0.78; 0.12)	0.152
HR (bpm) *M(SD)*	75.9 (11.8)	73.7 (11.1)	0.00 (-0.00; 0.01)	0.295	0.00 (-0.00; 0.00)	0.821
Laboratory parameters						
TC (mmol/l) *M(SD)*	4.84 (1.01)	4.95 (1.02)	-0.03 (-0.07; 0.07)	0.099	-0.02 (-0.06; 0.02)	0.389
TC > 5 mmol/l n(%)						
No	47 (42.7)	47 (44.3)	Ref		Ref	
Yes	63 (57.3)	59 (54.1)	0.06 (-0.02; 0.14)	0.136	0.03 (-0.04; 0.11)	0.400
LDL-C (mmol/l) *M(SD)*	2.97 (0.89)	2.97 (0.93)	-0.02 (-0.06; 0.02)	0.369	-0.00 (-0.04; 0.04)	0.885
LDL-C >= 3 mmol/l n(%)						
No	50 (45.5)	46 (43.4)	Ref		Ref	
Yes	60 (54.5)	60 (55.6)	0.02 (-0.06; 0.10)	0.600	0.01 (-0.07; 0.08)	0.890
HbA1c[e]	5.62 (1.06)	5.97 (1.52)	0.02 (-0.01; 0.05)	0.242	0.01 (-0.02; 0.04)	0.371
HbA1c >= 6.5% n(%)						
No	100 (52.4)	91 (86.7)	Ref		Ref	
Yes	10 (9.1)	14 (13.3)	-0.06 (-0.19; 0.07)	0.364	-0.03 (-0.16; 0.09)	0.578
CVD history variables						
Known CVD n(%)						
No	106 (96.4)	99 (93.4)	Ref		Ref	
Yes	4 (3.6)	7 (6.6)	-0.09 (-0.27; 0.09)	0.337	-0.04 (-0.21; 0.13)	0.644
On statins n(%)						
No	94 (85.5)	77 (72.6)	Ref		Ref	
Yes	16 (14.5)	29 (27.4)	0.01 (-0.10; 0.10)	0.920	0.05 (-0.06; 0.15)	0.381
On aspirin n(%)						
No	101 (91.8)	86 (81.1)	Ref		Ref	
Yes	9 (8.2)	20 (18.9)	-0.07 (-0.19; 0.04)	0.218	-0.06 (-0.18; 0.05)	0.288

[a] Controlled for age, education, employment status, natural hair colour, frequency of hair washing, chemical treatment of hair, duration of storage, breastfeeding, PTSD patients status, n = 214 (one participant with missing data for frequency of hair washing and chemical treatment of hair excluded and one participants with missing data on breastfeeding excluded)
[b] All participants who met the glucose criterion had glucose level above the threshold
[c] No participants were receiving treatment to increase HDL-C
[d] No participants were receiving treatment for hypertriglyceridemia
[e] One missing value on this item
* $p < 0.05$
BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; DBP, diastolic blood pressure; HbA1c, Glycated haemoglobin; HC, hip circumference; HDL-C, high-density lipoprotein cholesterol; HR, heart rate; FPG, fasting plasma glucose; LDL-C, Low density lipoprotein cholesterol; MetS, metabolic syndrome; Ref, reference category; rx, treatment; SBP, systolic blood pressure; TC, total cholesterol; trig; serum triglyceride; WC, waist circumference; WHR waist-to-hip ratio

6 References

Ahmadi, N., Hajsadeghi, F., Mirshkarlo, H. B., Budoff, M., Yehuda, R., & Ebrahimi, R. (2011). Post-traumatic stress disorder, coronary atherosclerosis, and mortality. *American Journal of Cardiology*, *108*(1), 29–33. https://doi.org/10.1016/j.amjcard.2011.02.340

Alberti, K. G., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., … Smith Jr, S. C. (2009). Harmonizing the Metabolic Syndrome A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International A. *Circulation*, *120*(16), 1640–1645. https://doi.org/10.1161/CIRCULATIONAHA.109.192644 [doi]

Anagnostis, P., Athyros, V. G., Tziomalos, K., Karagiannis, A., & Mikhailidis, D. P. (2009). The pathogenetic role of cortisol in the metabolic syndrome: a hypothesis. *Journal of Clinical Endocrinology & Metabolism*, *94*(8), 2692–2701. https://doi.org/10.1210/jc.2009-0370

Atogo-Asse, F. E., Vincent, R. P., Hughes, S. A., Auzinger, G., Le Roux, C. W., Wendon, J., & Bernal, W. (2012). High density lipoprotein in patients with liver failure; Relation to sepsis, adrenal function and outcome of illness. *Liver International*, *32*(1), 128–136. https://doi.org/10.1111/j.1478-3231.2011.02657.x

Bartoli, F., Carra, G., Crocamo, C., Carretta, D., & Clerici, M. (2013). Metabolic Syndrome in People Suffering from Posttraumatic Stress Disorder: A Systematic Review and Meta-Analysis. *Metabolic Syndrome and Related Disorders*, *11*(5), 301–308. https://doi.org/10.1089/met.2013.0010

Bernstein, D. P., Stein, J. A., Newcomb, M. D., Walker, E., Pogge, D., Ahluvalia, T., … Zule, W. (2003). Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse & Neglect*, *27*(2), 169–190. https://doi.org/10.1016/S0145-2134(02)00541-0

Blessing, E. M., Reus, V., Mellon, S. H., Wolkowitz, O. M., Flory, J. D., Bierer, L., … Marmar, C. R. (2017). Biological predictors of insulin resistance associated with posttraumatic stress disorder in

young military veterans. *Psychoneuroendocrinology*, *82*(April), 91–97. https://doi.org/10.1016/j.psyneuen.2017.04.016

Clark, J. E., Osborne, J. W., Gallagher, P., & Watson, S. (2016). A simple method for optimising transformation of non-parametric data: an illustration by reference to cortisol assays. *Human Psychopharmacology: Clinical and Experimental*, *31*(4), 259–267. https://doi.org/10.1002/hup.2528

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A Global Measure of Perceived Stress. *Journal of Health and Social Behavior*, *24*(4), 385–396. https://doi.org/10.1016/j.socscimed.2016.08.021

Dajani, R., Hadfield, K., van Uum, S., Greff, M., & Panter-Brick, C. (2018). Hair cortisol concentrations in war-affected adolescents: A prospective intervention trial. *Psychoneuroendocrinology*, *89*(December 2017), 138–146. https://doi.org/10.1016/j.psyneuen.2017.12.012

de Vries, G. J., Mocking, R., Assies, J., Schene, A., & Olff, M. (2017). Plasma lipoproteins in posttraumatic stress disorder patients compared to healthy controls and their associations with the HPA- and HPT-axis. *Psychoneuroendocrinology*, *86*(September), 209–217. https://doi.org/10.1016/j.psyneuen.2017.09.020

Farr, O., Sloan, D., Keane, T. M., & Mantzoros, C. S. (2014). Stress-and PTSD-associated obesity and metabolic dysfunction: a growing problem requiring further research and novel treatments. *Metabolism*, *63*(12), 1463–1468. https://doi.org/https://doi.org/10.1016/j.metabol.2014.08.009

Fraser, R., Ingram, M. C., Anderson, N. H., Morrison, C., Davies, E., & Connell, J. M. C. (1999). Cortisol effects on body mass, blood pressure, and cholesterol in the general population. *Hypertension*, *33*(6), 1364–1368. https://doi.org/https://doi.org/10.1161/01.HYP.33.6.1364

Gao, W., Stalder, T., Foley, P., Rauh, M., Deng, H., & Kirschbaum, C. (2013). Quantitative analysis of steroid hormones in human hair using a column-switching LC–APCI–MS/MS assay. *Journal of Chromatography B*, *928*, 1–8. https://doi.org/10.1016/j.jchromb.2013.03.008

Gocan, A. G., Rohr, U. D., Bachg, D., Schindler, A. E., & Rohr, U. D. (2012). Balancing steroidal

hormone cascade in treatment-resistant veteran soldiers with PTSD using a fermented soy product (FSWW08): A pilot study. *Hormone Molecular Biology and Clinical Investigation*, *10*(3), 301–314. https://doi.org/10.1515/hmbci-2011-0135

Gray, M. J., Litz, B. T., Hsu, J. L., & Lombardo, T. W. (2004). Psychometric properties of the life events checklist. *Assessment*, *11*(4), 330–341. https://doi.org/10.1177/1073191104269954

Groer, M. W., Kane, B., Williams, S. N., & Duffy, A. (2015). Relationship of PTSD symptoms with combat exposure, stress, and inflammation in American soldiers. *Biological Research for Nursing*, *17*(3), 303–310. https://doi.org/10.1177/1099800414544949

Khoury, J. E., Bosquet Enlow, M., Plamondon, A., & Lyons-Ruth, K. (2019). The association between adversity and hair cortisol levels in humans: A meta-analysis. *Psychoneuroendocrinology*, *103*(January), 104–117. https://doi.org/10.1016/j.psyneuen.2019.01.009

Klug, E. Q., Raal, F. J., Marais, A., Taskinen, M. R., Dalby, A. J., Schamroth, C., … Webb, D. A. (2018). South African dyslipidaemia guideline consensus statement: 2018 update A joint statement from the South African Heart Association (SA Heart) and the Lipid and Atherosclerosis Society of Southern Africa (LASSA). *South African Family Practice*, *108*(11b), 973–1000. https://doi.org/10.7196/SAMJ.2018.v108i11.13383

Kubzansky, L. D., Koenen, K., & Spiro, A. (2007). Prospective study of posttraumatic stress disorder symptoms and coronary heart disease in the normative aging study. *Archives of General Psychiatry*, *64*(1), 109–116. https://doi.org/10.1001/archpsyc.64.1.109

Kuehl, L. K., Hinkelmann, K., Muhtz, C., Dettenborn, L., Wingenfeld, K., Spitzer, C., … Otte, C. (2015). Hair cortisol and cortisol awakening response are associated with criteria of the metabolic syndrome in opposite directions. *Psychoneuroendocrinology*, *51*, 365–370. https://doi.org/10.1016/j.psyneuen.2014.09.012

Langerak, T., van den Dries, L. W. J., Wester, V. L., Staufenbiel, S. M., Manenschijn, L., van Rossum, E. F. C., & van Gorp, E. C. M. (2015). The relation between long-term cortisol levels and the

metabolic syndrome in HIV-infected patients. *Clinical Endocrinology*, *83*(2), 167–172. https://doi.org/10.1111/cen.12790

Lee, E. H. (2012). Review of the psychometric evidence of the perceived stress scale. *Asian Nursing Research*, *6*(4), 121–127. https://doi.org/10.1016/j.anr.2012.08.004

Luo, H., Hu, X., Liu, X. X. X. X., Ma, X., Guo, W., Qiu, C., … Li, T. (2012). Hair cortisol level as a biomarker for altered hypothalamic-pituitary- adrenal activity in female adolescents with posttraumatic stress disorder after the 2008 Wenchuan earthquake. *Biological Psychiatry*, *72*(1), 65–69. https://doi.org/10.1016/j.biopsych.2011.12.020

März, W., Kleber, M. E., Scharnagl, H., Speer, T., Zewinger, S., Ritsch, A., … Laufs, U. (2017). HDL cholesterol: reappraisal of its clinical relevance. *Clinical Research in Cardiology*, *106*(9), 663–675. https://doi.org/10.1007/s00392-017-1106-1

Matsha, T. E., Hassan, M. S., Hon, G. M., Soita, D. J., Kengne, A. P., & Erasmus, R. T. (2013). Derivation and validation of a waist circumference optimal cutoff for diagnosing metabolic syndrome in a South African mixed ancestry population. *International Journal of Cardiology*, *168*(3), 2954–2955. https://doi.org/https://doi.org/10.1016/j.ijcard.2013.03.150

Meewisse, M. L., Reitsma, J. B., de Vries, G. J., Gersons, B. P., & Olff, M. (2007). Cortisol and post-traumatic stress disorder in adults: systematic review and meta-analysis. *The British Journal of Psychiatry : The Journal of Mental Science*, *191*, 387–392. https://doi.org/191/5/387 [pii]

Michopoulos, V., Vester, A., & Neigh, G. (2016). Posttraumatic stress disorder: A metabolic disorder in disguise? *Experimental Neurology*, *284*, 220–229. https://doi.org/10.1016/j.expneurol.2016.05.038

Miller, G. E., Chen, E., & Zhou, E. S. (2007). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychological Bulletin*, *133*(1), 25–45. https://doi.org/10.1037/0033-2909.133.1.25

Morris, M. C., Compas, B. E. B. E., & Garber, J. (2012). Relations among posttraumatic stress

disorder, comorbid major depression, and HPA function: a systematic review and meta-analysis. *Clinical Psychology Review*, *32*(4), 301–315. https://doi.org/10.1016/j.cpr.2012.02.002.Relations

Osborne, J. W. (2010). Improving your data transformations : Applying the Box-Cox transformation. *Pratical Assessment, Research & Evaluation*, *15*(12), 1–9.

Rosenbaum, S., Stubbs, B., Ward, P. B., Steel, Z., Lederman, O., & Vancampfort, D. (2015). The prevalence and risk of metabolic syndrome and its components among people with posttraumatic stress disorder: A systematic review and meta-analysis. *Metabolism: Clinical and Experimental*, *64*(8), 926–933. https://doi.org/10.1016/j.metabol.2015.04.009

Rosmond, R., Dallman, M. F., & Bjorntorp, P. (1998). Stress-Related Cortisol Secretion in Men: Relationships with Abdominal Obesity and Endocrine, Metabolic and Hemodynamic Abnormalities. *Journal of Clinical Endocrinology & Metabolism*, *83*(6), 1853–1859. https://doi.org/https://doi.org/10.1210/jcem.83.6.4843

Rytwinski, N., Scur, M., Feeny, N. C. N., & Youngstrom, E. E. A. (2013). The co-occurrence of major depressive disorder among individuals with posttraumatic stress disorder: A meta-analysis. *Journal of Traumatic Stress*, *26*(3), 299–309. https://doi.org/https://doi.org/10.1002/jts.21814

Sapolskyf, R. M., & Mott, G. E. (1987). Social Subordinance in Wild Baboons Is Associated with Suppressed High Density Lipoprotein-Cholesterol Stress. *Endocrinology*, *121*(5), 1605–1610. https://doi.org/https://doi.org/10.1210/endo-121-5-1605

Schumacher, S., Niemeyer, H., Engel, S., Cwik, J. C., Laufer, S., Klusmann, H., & Knaevelsrud, C. (2019). HPA axis regulation in posttraumatic stress disorder: A meta-analysis focusing on potential moderators. *Neuroscience and Biobehavioral Reviews*, *100*(June 2018), 35–57. https://doi.org/10.1016/j.neubiorev.2019.02.005

SEMDSA Type 2 Diabetes Guidelines Expert Committee. (2017). The 2017 SEMDSA Guideline for the Management of Type 2 Diabetes. *Journal of Endocrinology, Metabolism and Diabetes of South Africa*, *22*(1), s1–s196. https://doi.org/10.1080/22201009.2012.10872276

Sheehan, D. V, Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., … Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry*, *59*(Suppl 20), 22–33. Retrieved from https://psycnet.apa.org/record/1998-03251-004

Stalder, T., Kirschbaum, C., Alexander, N., Bornstein, S. R., Gao, W., Miller, R., … Fischer, J. E. (2013). Cortisol in hair and the metabolic syndrome. *The Journal of Clinical Endocrinology & Metabolism*, *98*(6), 2573–2580. https://doi.org/10.1210/jc.2013-1056

Stalder, T., Steudte-Schmiedgen, S., Alexander, N., Klucken, T., Vater, A., Wichmann, S., … Miller, R. (2017). Stress-related and basic determinants of hair cortisol in humans: A meta-analysis. *Psychoneuroendocrinology*, *77*, 261–274. https://doi.org/10.1016/j.psyneuen.2016.12.017

Steudte-Schmiedgen, S., Kirschbaum, C., Alexander, N., & Stalder, T. (2016). An integrative model linking traumatization, cortisol dysregulation and posttraumatic stress disorder: Insight from recent hair cortisol findings. *Neuroscience and Biobehavioral Reviews*, *69*, 124–135. https://doi.org/10.1016/j.neubiorev.2016.07.015

Steudte, S., Kirschbaum, C., Gao, W., Alexander, N., Schönfeld, S., Hoyer, J. J., & Stalder, T. (2013). Hair cortisol as a biomarker of traumatization in healthy individuals and posttraumatic stress disorder patients. *Biological Psychiatry*, *74*(9), 639–646. https://doi.org/10.1016/j.biopsych.2013.03.011

Steudte, S., Kolassa, I.-T., Stalder, T., Pfeiffer, A., Kirschbaum, C., & Elbert, T. (2011). Increased cortisol concentrations in hair of severely traumatized Ugandan individuals with PTSD. *Psychoneuroendocrinology*, *36*(8), 1193–1200. https://doi.org/10.1016/j.psyneuen.2011.02.012

Straub, J., Klaubert, L. M., Schmiedgen, S., Kirschbaum, C., & Goldbeck, L. (2017). Hair cortisol in relation to acute and post-traumatic stress symptoms in children and adolescents. *Anxiety, Stress and Coping*, *30*(6), 661–670. https://doi.org/10.1080/10615806.2017.1355458

van Zuiden, M., Savas, M., Koch, S. B. J., Nawijn, L., Staufenbiel, S. M., Frijling, J. L., … Olff, M.

(2019). Associations Among Hair Cortisol Concentrations, Posttraumatic Stress Disorder Status, and Amygdala Reactivity to Negative Affective Stimuli in Female Police Officers. *Journal of Traumatic Stress*, *32*(2), 238–248. https://doi.org/10.1002/jts.22395

Weathers, F W, Blake, D. D., Schnurr, P. P., Kaloupek, D. G., Marx, B. P., & Keane, T. M. (2013). The clinician-administered PTSD scale for DSM-5 (CAPS-5). Retrieved from www.ptsd.va.gov.

Weathers, Frank W., Bovin, M. J., Lee, D. J., Sloan, D. M., Schnurr, P. P., Kaloupek, D. G., … Marx, B. P. (2018). The clinician-administered ptsd scale for DSM-5 (CAPS-5): Development and initial psychometric evaluation in military veterans. *Psychological Assessment*, *30*(3), 383–395. https://doi.org/10.1037/pas0000486

Weathers, Frank W, Blake, D. D., Schnurr, P. P., Kaloupek, D. G., Marx, B. P., & Keane, T. M. (2013). The Life Events Checklist for DSM-5 (LEC-5). Retrieved from www.ptsd.va.gov.

Wennig, R. (2000). Potential problems with the interpretation of hair analysis results. *Forensic Science International*, *107*(1), 5–12. https://doi.org/10.1016/S0379-0738(99)00146-2

Wolf, E. J., Bovin, M. J., Green, J. D., Mitchell, K. S., Stoop, T. B., Barretto, K. M., … Trachtenberg, F. (2016). Longitudinal associations between post-traumatic stress disorder and metabolic syndrome severity. *Psychological Medicine*, *46*(10), 1–12. https://doi.org/https://doi.org/10.1017/S0033291716000817

World Health Organization. (2005). *WHO STEPS surveillance manual: the WHO STEPwise approach to chronic disease risk factor surveillance*. Geneva: World Health Organization (WHO).

World Medical Association. (2013). World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA - Journal of the American Medical Association*, *310*(20), 2191–2194. https://doi.org/10.1001/jama.2013.281053

CHAPTER 3

Hair glucocorticoid levels in females with Parkinson's disease

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Hair glucocorticoid levels in females with Parkinson's disease

Abstract

Background: Parkinson's disease (PD) and metabolic syndrome (MetS) share certain pathophysiological pathways, including hypothalamic pituitary adrenal (HPA) axis dysfunction. Hair glucocorticoid (GC) levels reflect longer-term HPA axis function and can provide additional insights into the role of a dysregulated HPA-axis in PD and co-occurring cardiovascular disease (CVD) risk.

Objectives: In a case-control study of 56 females (25 PD patients and 31 controls) of mixed ancestry, aged between 45 and 78 years (M = 59.6, SD = 8.7) we examined the association of hair GC (cortisol and cortisone) levels with PD diagnosis, clinical features and PD-CVD risk (as defined by the MetS) co-occurrence.

Methods: Hair samples, representing a three-month retrospective window of GC levels, were collected and analysed utilizing liquid chromatography tandem mass spectrometry. Multivariate regression models were constructed with PD diagnostic status, clinical features and MetS comorbidity regressed on hair GC levels, adjusting for potential confounders.

Results: The prevalence of MetS was 56.0% in PD patients and 25.8% in controls. Hair cortisone (adj *B* = 5.31, 95% CI 1.88; 8.74, *p* = 0.003), but not hair cortisol levels (adj *B* = 0.04, 95% CI -0.13; 0.21, *p* = 0.646), were significantly higher (Cohen's d = 0.87) in PD patients than in controls. Non-motor symptoms of PD (e.g., mood, anhedonia and anxiety) were significantly associated with hair cortisone levels (adj *B* = 0.26, 95% CI 0.06; 0.46, *p* = 0.013). MetS was not associated with hair GC levels and there were no significant interactions between PD and MetS on hair GC levels. Hair cortisone levels were significantly positively associated with one of the components of MetS, waist-to-hip ratio (adj *B* = 21.64, 95% CI 4.04; 39.24, *p* = 0.017).

Conclusions: This study is the first study reporting on hair GC levels in PD. We found chronically increased cortisone, but not cortisol, levels in PD patients compared to controls. Furthermore, hair cortisone levels were significantly positively associated with PD symptoms related to mood, anhedonia

and anxiety. Hair GC levels were not associated with PD-MetS comorbidity in this sample. Hair cortisone levels may provide additional insights into HPA-axis dysfunction in PD.

Keywords: Parkinson's disease; hair cortisol levels; hair cortisone levels; metabolic syndrome; stress

1 Introduction

Parkinson's disease (PD) is primarily distinguished from other neurodegenerative diseases by the death of dopaminergic neurons in the substantia nigra and related motor symptoms, including bradykinesia, resting tremor and rigidity. Symptoms of PD extend beyond classic motor symptoms and include sleep disturbances, autonomic dysfunction, neuropsychiatric symptoms and cognitive impairment (Wirdefeldt, Adami, Cole, Trichopoulos, & Mandel, 2011). Genetic, environmental and aging processes combine to increase the risk for PD (Herrero, Estrada, Maatouk, & Vyas, 2015). Several pathways play a role in the pathophysiology of PD, including accumulation of toxic proteins (α-synuclein especially), mitochondrial dysfunction, oxidative stress, apoptosis and neuro-inflammation (Levy, Malagelada, & Greene, 2009).

Stress has also been postulated to play a role in PD onset and progression (Djamshidian & Lees, 2014; Smith, Castro, & Zigmond, 2002). PD symptoms can be triggered and exacerbated by stress in both animal models and clinical settings (Vyas et al., 2016). Excessive stress leading to chronically raised cortisol is linked to neurotoxicity and neurodegeneration of dopaminergic neurons (Djamshidian & Lees, 2014; Smith et al., 2002; Vyas et al., 2016). Glucocorticoids (GC) can contribute to neuronal loss through pathways involving excitotoxicity, mitochondrial dysfunction, synaptic dysfunction, apoptotic mechanisms and sensitization of neurons (Kibel & Drenjančević-Perić, 2008; Vyas et al., 2016). Stress and GC exposure also influence epigenetic processes, such as DNA methylation, and this may also play a role in PD pathophysiology (Du & Pang, 2015).

Conversely, there is also evidence suggesting that the pathology of PD influences HPA-axis function. Lewy bodies (LBs), eosinophilic inclusions containing α-synuclein are found not only in dopaminergic cells, but also in other cells throughout the body, including the adrenal glands and the hypothalamus (Du & Pang, 2015; Levy et al., 2009). Dopaminergic pathways regulate hypothalamic corticotrophin releasing factor (CRF) neurons and a study in children with growth hormone (GH) deficiency demonstrated that adrenocorticotropic hormone (ACTH) and cortisol levels increased after administration of levodopa, suggesting dopaminergic regulation of HPA-axis function (Marakaki,

Papadimitriou, Kleanthous, Papadopoulou, & Papadimitriou, 2015). HPA-axis dysfunction is likely to occur across different levels of the HPA-axis in PD (Du & Pang, 2015).

GC regulate inflammatory and immune processes and these are also pathways through which HPA-axis dysfunction and the pathophysiology of PD may be linked (Herrero et al., 2015; Nolan, Sullivan, & Toulouse, 2013). Chronically elevated cortisol can lead to glucocorticoid receptor (GR) insensitivity and consequent dysregulated immune and inflammatory processes contributing to neurodegeneration in PD (Herrero et al., 2015; Vyas et al., 2016). Downregulated GR expression in the substantia nigra and increased reactive microglia have been demonstrated in PD brains and animal models of PD (Du & Pang, 2015; Vyas et al., 2016). Additionally, other stressors and pro-inflammatory cytokines can increase the release of cortisol through different mechanisms and thus contribute to chronic activation of the HPA-axis (Herrero et al., 2015). Although various pathways have been suggested, the cause of HPA-axis dysfunction and its influence on the pathophysiology of PD remains to be determined (Herrero et al., 2015). There is, however, clear evidence of HPA-axis dysfunction and higher cortisol levels in PD, although the direction of association is altered in some studies (Du & Pang, 2015; Herrero et al., 2015; Soares, Pereira, Altmann, de Almeida, & Rieder, 2019). The lack of studies measuring longer-term cortisol release has also been highlighted as a limitation to current understanding (Soares et al., 2019).

Since GC were first quantified in hair, hair analyses have increasingly been utilised for measuring longer-term basal cortisol (Raul, Cirimele, Ludes, & Kintz, 2004). Measuring GC in hair allows for a retrospective calendar of cumulative glucocorticoid levels spanning months (Stalder et al., 2017). Hair cortisol levels (hairF) have been widely used as a marker of longer-term HPA-axis function across various settings and in multiple conditions (Stalder et al., 2017; Wosu, Valdimarsdóttir, Shields, Williams, & Williams, 2013). Measurement of hair cortisone levels (hairE) has also gained traction (Kuehl et al., 2015; Savas et al., 2019; Stalder et al., 2013). Cortisol is converted to cortisone by 11β-hydroxysteroid-dehydrogenase type 2 (11β-HSD2) and 11β-hydroxysteroid-dehydrogenase type 1 (11β-HSD1) converts cortisone to cortisol in adipose tissue and in the liver. In hair and saliva samples, levels of cortisone are higher than cortisol, with this ratio reversed in blood (Perogamvros, Keevil, Ray,

& Trainer, 2010; Raul et al., 2004). These differences have been postulated to be due to different corticosteroid-binding globulin (CBG) and albumin affinities for cortisol and cortisone and the different locations and functions of 11β-HSD. Use of analyses of hairE may provide a more complete picture of HPA-axis function (Stalder et al., 2013; Staufenbiel, Penninx, de Rijke, van den Akker, & van Rossum, 2015). Both hairF and HairE have demonstrated moderate test-retest correlations and similar intraindividual stability (Q. Zhang, Chen, Chen, Xu, & Deng, 2017). Hair GC have, to our knowledge, not been studied in PD, and there have been no published studies of cortisone levels in PD.

Neurodegenerative disorders, such as PD, also share pathways with cardiovascular disease (CVD) and metabolic syndrome (MetS). MetS denotes a cluster of risk factors for CVD and type 2 diabetes mellitus and risk factors include central obesity, hypertension, elevated fasting glucose, hypertriglyceridemia and low levels of high-density lipoprotein cholesterol (HDL-C) (Alberti et al., 2009). PD and CVD have been conceptualised as disorders involving accelerated aging processes (Baquer et al., 2009). Both PD and MetS are associated with dysregulated inflammation and increased oxidative stress and these have been suggested as potential pathways linking the two conditions (Bainbridge et al., 2017; P. Zhang & Tian, 2014). Similar to PD, HPA-axis dysfunction and altered GC levels have also been demonstrated in relation to MetS and CVD (Anagnostis, Athyros, Tziomalos, Karagiannis, & Mikhailidis, 2009; Stalder et al., 2013) and may theoretically also play a role in comorbidity. Although there are some pathways potentially linking PD and MetS, the relationship between the two disorders is not well understood. MetS has been associated with lower PD risk in some samples (Sääksjärvi, Knekt, Männistö, Lyytinen, & Heliövaara, 2015). However, recent studies in large cohorts have demonstrated that MetS and various CVD risk factors including obesity, insulin resistance and hyperhomocysteinemia are associated with increased risk for PD (Nam et al., 2018; P. Zhang & Tian, 2014). MetS has also been associated with poor outcomes in patients with PD, including worsened PD severity and increased risk for cognitive impairment and dementia (Bainbridge et al., 2017; P. Zhang & Tian, 2014).

Hair GC levels, reflecting chronic GC release, can potentially provide additional insights into the role of HPA-axis dysfunction in relation to PD and associated CVD risk. We sought to investigate, in a sample

of South African mixed ancestry adults, whether (i) hair GC levels (hairF and hairE) were associated with PD and clinical factors (self-perceived stress and PD symptoms); and (ii) there were interaction effects between PD and MetS (and individual CVD risk factors) on hair GC levels.

2 Methods

2.1 Study design

This was an ancillary neuroendocrine case-control study to a project titled 'Understanding the SHARED ROOTS of Neuropsychiatric Disorders and Modifiable Risk Factors for Cardiovascular Disease' or SHARED ROOTS (SR). SR was a cross-sectional matched case-control study in three neuropsychiatric disorder cohorts (PD, posttraumatic stress disorder and schizophrenia). Controls were group matched to patients with PD based on age, gender, and MetS status. Here we address the original main aims of the neuroendocrine study in the PD cohort.

2.1.1 Ethical aspects

This study was approved by the Health Research Ethics Committee at Stellenbosch University (HREC N13/08/115) and conducted according to principles of the seventh revision of the Declaration of Helsinki (World Medical Association, 2013). Written informed consent was obtained from all participants and a tiered consenting process was adhered to, allowing participants to opt-out of the hair sampling procedure. Participants were referred to their usual health care service providers for any medical or psychiatric problems warranting further investigation and treatment.

2.2 Setting

Participants were enrolled over 3 years from May 2014 until June 2017 in Cape Town, South Africa. We utilised a purposive sampling approach. PD patients were recruited through (i) an existing database of patients at the Movement Disorders clinic at our facility; (ii) advertisements placed at local secondary and primary health care facilities; (iii) referrals of patients at district hospitals and a neighbouring academic centre; and (iv) print, radio and web advertisements. Control participants were recruited through (i) print, radio and web advertisements; (ii) active recruitment within communities by a

registered nurse visiting various community centres; and (iii) word of mouth by participants already recruited.

2.3 Participants

The study sample was limited to individuals who self-identified as belonging to the mixed ancestry (coloured) ethnic group. The decision to limit the study to one ethnic group was chiefly to avoid the effects of population stratification on genomic analyses. All participants had to be 18 years and older, willing and able to provide informed consent and be able to read and write in English or Afrikaans (the predominant languages spoken within the Western Cape mixed ancestry population). Participants were excluded from hair sampling if they had hair length shorter than 3cm (see Figure 1 for a flow diagram of participants included in this study). Further exclusion criteria applied in this study were, (i) systemic or scalp steroid use, (ii) current pregnancy, or pregnancy within the prior three months, (iii) significant medical comorbidity (e.g., HIV, auto-immune disorders), (iv) current substance use disorders, as determined with the Mini International Neuropsychiatric Interview (MINI), (v) lifetime or current serious psychiatric disorder (a psychotic or bipolar disorder) as determined based on the psychiatric history or the MINI. Patients with PD were included if they had a UK Brain Bank clinical diagnosis (Hughes, Daniel, Kilford, & Lees, 1992) of idiopathic PD made by a neurologist and were excluded if they had a history of a significant head injury, exposure to neuroleptics, and atypical forms of PD. Exclusion criteria applied only to controls were current psychiatric disorder, as determined on history or the MINI and current psychotropic medication use.

MetS status was determined based on the harmonized Joint Interim Statement (JIS) criteria (Alberti et al., 2009), with the presence of any three of the following five risk factors required for a diagnosis of MetS: (i) Raised blood pressure (BP), systolic ≥ 130 and/or diastolic ≥ 85 mmHg, or being on antihypertensive treatment; (ii) Elevated triglycerides > 1.70 mmol/l (150 mg/dl) or being on treatment for hypertriglyceridemia; (iii) Reduced HDL-C, < 1.0 mmol/l (40mg/dl) in males, and < 1.3 mmol/l (50mg/dl) in females, or being on treatment for low HDL-C; (iv) Elevated fasting glucose ≥ 5.6 mmol/l (100 mg/dl) or on treatment for diabetes; (v) Elevated waist circumference according to population and country specific guidelines. We used a waist circumference ≥ 90cm in both males and females, as a

recent validation study in the Western Cape showed this to be the optimal WC cut-off in mixed ancestry individuals (Matsha et al., 2013).

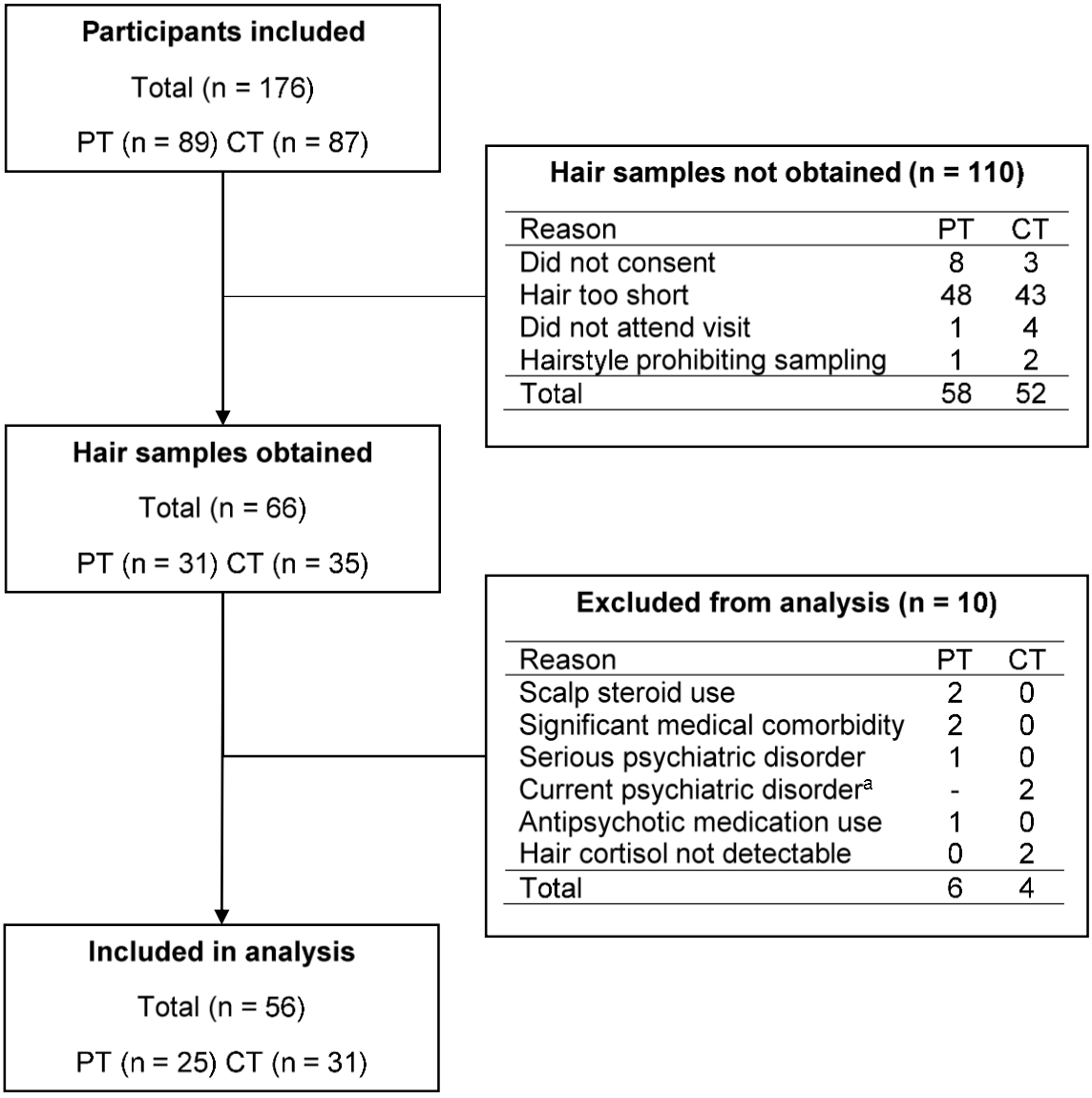

Figure 1 Flow diagram demonstrating inclusion and exclusion of participants

[a] Exclusion criteria only applicable to controls
CT, control; PT, patient

2.4 Procedures

Participants attended two to three study visits, each lasting around 3 hours. Diagnostic, clinical and participant-administered measures were completed at the first visit. The second visit occurred within 24-96 hours of the first visit and involved the physical procedures (e.g. fasting blood sampling, physical measurements) and additional lifestyle measures. Assessments were conducted in English or

Afrikaans, depending on the language preference of participants. Psychiatrists and physicians with experience in neuropsychiatry conducted diagnostic and clinical assessments. Research nurses performed the physical procedures and personally administered the participant-administered measures to all participants, to aid comprehension and to accommodate variation in reading level.

2.4.1 Physical measurements

Physical measurements were conducted in a consistent method in accordance with the WHO STEPwise approach to chronic disease risk factor surveillance (WHO STEPS) instrument, a standardised instrument designed for non-communicable disease surveillance (World Health Organization, 2005). The following physical parameters were included in this analysis: (i) mean systolic (SBP) and diastolic blood pressure (DBP, mmHg); (ii) mean heartrate (HR, bpm); (iii) body mass index (BMI, kg/m^2); (iv) waist circumference (WC, cm); (v) hip circumference (HC, cm); and (vi) waist-to-hip ratio (WHR).

2.4.2 Blood samples for metabolic parameters

Venous blood samples were obtained following an overnight fast (at least 8 hours) and were analysed on the Cobas 6000 c501 Chemistry Analyser (Roche, Germany) at a commercial internationally accredited laboratory (Lancet laboratories) on the same day as sample collection. The following laboratory parameters were included in analysis: (i) fasting plasma glucose (FPG, mmol/l); (ii) triglycerides (trig, mmol/l); (iii) high-density lipoprotein cholesterol (HDL-C, mmol/l); (iv) low-density lipoprotein cholesterol (LDL-C, mmol/l); (v) total cholesterol (TC, mmol/l); (vi) and glycated haemoglobin A1c (HbA1c, %). Values for TC > 5 mmol/l, LDL-C ≥3 mmol/l, and HbA1c ≥ 6.5% were considered to be elevated (Klug et al., 2018; SEMDSA Type 2 Diabetes Guidelines Expert Committee., 2017).

2.4.3 Hair sampling

Hair samples from the posterior vertex scalp were cut close to the scalp with fine scissors. Samples were secured, placed into aluminium foil, labelled and sealed inside an envelope and were stored in dark containers at room temperature.

2.4.4 Hair analysis

Hair analyses were performed at the TU Dresden laboratory (Prof Clemens Kirschbaum). Samples were analysed in two batches. A pilot sample comprising the first 21 hair samples obtained were sent for analysis in September 2014 and the remaining samples were sent in December 2017. Samples were analysed using an established liquid chromatography-tandem mass spectrometry (LC-MS/MS) protocol (Gao et al., 2013). LC-MS/MS is the benchmark (gold standard) method for hair GC determination and different laboratories using LC-MS/MS have demonstrated almost identical results (r = 0.98) (Gao, Kirschbaum, Grass, & Stalder, 2016; Russell et al., 2015; Stalder et al., 2012). The proximal 3cm of the hair segments were used for analysis, representing cortisol secretion for the prior 3 months based on an accepted growth rate of 1cm per month (Wennig, 2000).

2.5 Measures

2.5.1 Demographic questionnaire

Demographic details were collected with a demographic questionnaire. Demographic variables included in this manuscript are self-identified gender (male or female), age in years, highest level of education (HLOE, defined according to whether secondary education was completed), and employment status (defined as being employed for the greater part of the prior 12 months).

2.5.2 Medical history questionnaires

Medical and psychiatric histories were collected with comprehensive medical questionnaires. Clinical items reported on in this manuscript are: (i) steroid use in the prior 6 months (split according to whether any systemic steroids were used (oral or parenteral), topical steroids where used on the scalp, and any other topical steroid use (dermatologic, inhaled, nasal), (ii) hormonal contraceptive use in prior 6 months (including oral contraceptives and other formulations, such as implants and injectable contraceptives), (iii) PD disease duration, (iv) medications used for PD (v) current psychiatric medication use, (vi) known CVD [previous angina, myocardial infarct or cerebrovascular accident (CVA)], (vii) statin use, (viii) aspirin use, (ix) any other current medical conditions and (iv) self-reported tobacco and alcohol use in the prior six months. Details regarding medications used for PD were used to calculate levodopa equivalent daily doses (LED) (Tomlinson et al., 2010).

2.5.3 The Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998)

The MINI is a short structured diagnostic interview used to diagnose psychiatric disorders based on DSM-IV and International Classification of Diseases (ICD-10) criteria. The MINI version 6.0 was used to determine the presence of any current and lifetime psychiatric disorders.

2.5.4 The Unified Parkinson's Disease Rating Scale (UPDRS) (Fahn, Elton, & UPDRS program members, 1987)

Parkinson's disease severity was assessed with the UPDRS with patients in the ON-phase. We report scores for the three subscales (I – Mentation, behaviour and mood, range 1-16; II Activities of Daily Living, range 0 – 52; III – Motor examination, range 0 – 108) and total scores (range 1 – 176). Disease stage was determined with the modified Hoehn and Yahr (H&Y) scale (Goetz et al., 2004). The H&Y includes seven stages, with stages 1.0 - 3.0 reflecting mild to moderate disability and 4.0 - 5.0 severe disability.

2.5.5 The Non-motor Symptoms Scale (NMSS) (Chaudhuri et al., 2007)

Non-motor symptoms (NMS) of PD were assessed with the NMSS (Chaudhuri et al., 2007). The 30 items on the NMSS are scored by multiplying severity (0 - 3) and frequency (1 - 4), with total scores ranging between 0 – 360. The NMSS assesses for NMS in nine domains: cardiovascular (range 0-12), sleep/fatigue (range 0-24), mood/cognition (range 0-72), perceptual problems (range 0-18), attention/memory (range 0-18), gastrointestinal tract (range 0-18), urinary (range 0-18), sexual function (range 0-12), and miscellaneous (range 0-14). Cronbach's α in the PD cohort for the full scale was 0.91 and for the subscales ranged between 0.66 – 0.92.

2.5.6 The Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983)

We used the 10-item PSS (PSS-10) to measure self-perceived stress. Individuals rate to what extent they found their lives unpredictable, uncontrollable or overloaded in the prior month. Each item is rated on a 5-point Likert scale (0 = Never; 1 = Almost Never; 2 = Sometimes; 3 = Fairly often; 4 = Very often) with a total score ranging from 0 – 40, and higher scores indicate higher perceived stress. The PSS has been widely used and has demonstrated adequate psychometric properties in multiple settings and languages (Lee, 2012) and Cronbach's α in the PD cohort was 0.80.

2.5.7 The WHO STEPwise approach to chronic disease risk factor surveillance (WHO STEPS) (World Health Organization, 2005)

The level of physical activity was determined with the global physical activity questionnaire (GPAQ) in the WHO STEPS. The GPAQ assesses average physical activity in a week occurring across three domains, namely work, travel and recreation. Level of physical activity (high, moderate, and low) was determined according to the GPAQ guidelines (World Health Organization, 2005).

2.5.8 Hair questionnaire

Participants who provided hair samples were questioned about their hair characteristics and hair care practices. Hair related variables reported in this manuscript include (i) whether natural hair colour is black, (ii) whether hair had been chemically treated in the prior three months (including colouring, relaxing and perming), (iii) the frequency of hair washing (two or more times a week, once a week, less frequent than once a week), (iv) whether any additional hair products had been used in the prior three months (such as hair styling and care products e.g. gels, oils and creams), and (v) average hours per day that hair was exposed to sunlight (less than one hour, 1-2 hours, more than 2 hours). Additional hair related factors included the season sampled (based on the month the sample was obtained in), the duration of storage before sending the samples for analysis (less than 1 year, 1-2 years, 2-3.5 years) and the batch analysed (pilot, final).

2.6 Statistical analysis

Sample size estimations for the neuroendocrine study were based on hairF studies in PTSD and MetS samples (Stalder et al., 2013; Steudte et al., 2012) as no hairF studies had been done in PD. Overall sample size was also limited by recruitment of the parent study as well as by factors influencing hair sampling, such as short hair length. HairE levels were normally distributed whereas HairF levels were positively skewed (Clark, Osborne, Gallagher, & Watson, 2016; Miller & Plessow, 2013). Utilising the box-cox family of power transformations (Osborne, 2010) hairF levels were reciprocal square root transformed to meet the assumption of normality. There were no outliers (3SD), though we excluded two participants with undetectable hairF levels, resulting in a final sample of 56 participants (Figure 1).

We compared the PD patients and controls with Pearson chi-square for categorical variables, and independent sample t-tests for continuous variables. There were only two missing responses on the item 'average hours in sun' and these were excluded from analysis listwise (missing items and final sample numbers reported in tables). To determine whether hair GC levels were associated with PD patient status, PD disease features and perceived stress we conducted linear regression models with hair GC levels as the dependent variable. Covariates included in the models were age, batch analysed and factors that remained significantly associated with HairF (duration of sun exposure) and HairE (employment status and chemical treatment of hair) after controlling for age and PD diagnosis. To assess interaction effects between MetS and PD we first regressed MetS on hair GC levels in simple linear regression models, and then in models controlled for covariates alongside PD diagnosis. In exploratory analysis we also examined for associations between the individual CVD risk variables and HCC as well as for interactions with PD diagnosis. Limited sensitivity and post-hoc analyses were conducted to allow for additional interpretation of the data. Data were analysed with SPSS for windows, version 25.0, all tests were 2-tailed and the level of significance was set at .05.

3 Results

3.1 Participants

Of the 176 PD cohort participants, hair samples were obtained in 66 (37.5%) participants, a further 10 participants were excluded from analysis, leaving a final sample of 56 participants; 25 PD patients and 31 controls (see Figure 1 for details). Hair length shorter than 3cm was the main factor (82.7%) limiting sampling. Our analysis was limited to females as hair samples were only obtained in one (1.1%) male participant, a PD patient excluded due to antipsychotic use. The following factors differed significantly between included and excluded samples: Gender ($p < 0.001$), level of physical activity ($p = 0.028$), HLOE ($p = 0.045$), HDL-C ($p < 0.001$), BMI ($p = 0.017$), HC ($p < 0.001$), WHR ($p < 0.001$), TC ($p = 0.010$), TC within range ($p = 0.011$), HbA1c ($p = 0.040$), and current psychiatric medication use ($p = 0.030$). When we controlled for gender the only factor that remained significantly different between included and excluded samples was level of physical activity ($p = 0.033$).

Descriptive data of the sample are presented in Table 1. Factors that were significantly different between the patients and controls included age, hair chemically treated, hair product use, duration of storage, and tobacco use. Clinical factors in PD patients are described in Table 2.

Table 1 Comparison of sociodemographic, hair related, behavioural, and clinical characteristics between PD patients and controls

Variables	Patients	Controls	Test statistic	p-value
Total	25	31		
Socio-demographic				
Age *M(SD)*	64.5 (8.4)	55.7 (6.9)	t (54) = -4.35	<0.001*
Secondary education complete n(%)			$\chi^2(1) = 0.10$	0.623
No	22 (88.0)	27 (87.1)		
Yes	3 (12.0)	4 (12.9)		
Employed n(%)			$\chi^2(1) = 3.90$	0.052
No	24 (96.0)	24 (77.4)		
Yes	1 (4.0)	7 (22.6)		
Hair related				
Natural hair colour black n(%)			$\chi^2(1) = 3.34$	0.067
No	15 (60.0)	11 (35.5)		
Yes	10 (40.0)	20 (64.5)		
Hair chemically treated n(%)			$\chi^2(1) = 9.81$	0.002*
No	16 (64.0)	7 (22.6)		
Yes	9 (36.0)	24 (77.4)		
Frequency of hair washing n(%)			$\chi^2(2) = 1.87$	0.393
≥ 2 times a week	7 (28.0)	5 (16.1)		
1 time a week	12 (48.0)	14 (45.2)		
< 1 time a week	6 (24.0)	12 (38.7)		
Add on hair products n(%)			$\chi^2(1) = 4.42$	0.035*
No	19 (76.0)	15 (48.4)		
Yes	6 (24.0)	16 (51.6)		
Average hours in sun[a] n(%)		2	$\chi^2(2) = 3.17$	0.205
< 1 hour	18 (72.0)	14 (48.3)		
1-2 hours	5 (20.0)	10 (34.5)		
> 2 hours	2 (8.0)	5 (17.2)		
Season sampled n(%)			$\chi^2(3) = 3.60$	0.308
Summer	3 (12.0)	3 (9.7)		
Autumn	11 (44.0)	9 (29.0)		
Winter	7 (28.0)	7 (22.6)		
Spring	4 (16.0)	12 (75.0)		
Duration of storage n(%)			$\chi^2(2) = 25.64$	<0.001*
Less than 1 year	8 (32.0)	2 (6.5)		
1 - 2 years	13 (52.0)	3 (9.7)		
2 – 3.5 years	4 (16.0)	26 (83.9)		
Batch analysed n(%)			$\chi^2(1) = 2.32$	0.132
Pilot	5 (20.0)	2 (6.5)		
Final sample	20 (80.0)	29 (93.5)		
Clinical				
Hormonal contraceptive n(%)			$\chi^2(1) = 1.63$	0.309
No	25 (100)	29 (93.5)		
Yes	0 (0)	2 (6.5)		
Topical steroid use n(%)			$\chi^2(1) = 1.32$	0.237
No	21 (84.0)	29 (93.5)		
Yes	4 (16.0)	2 (6.5)		
Other medical conditions n(%)			$\chi^2(1) = 0.32$	0.571
No	11 (44.0)	16 (51.6)		
Yes	14 (56.0)	15 (48.4)		

Behavioural				
Tobacco use n(%)			$\chi^2(1) = 6.08$	0.013*
No	22 (88.0)	18 (58.1)		
Yes	3 (12.0)	13 (41.9)		
Alcohol use n(%)			$\chi^2(1) = 3.50$	0.056
No	21 (84.0)	19 (61.3)		
Yes	4 (16.0)	12 (38.7)		
Level of physical activity n(%)			$\chi^2(2) = 5.10$	0.078
High	0 (0)	2 (6.1)		
Moderate	3 (11.5)	10 (30.3)		
Low	23 (88.5)	21 (63.6)		
Perceived stress				
PSS total score *M(SD)*	15.2 (7.7)	12.3 (7.2)	t (54) = -1.43	0.159
Hair glucocorticoid levels				
HairF *Mdn(IQR)*	4.5 (2.7; 7.9)	3.9 (2.4; 8.7)	U=367; Z=-0.33	0.742
Transformed HairF *M(SD)*	1.14 (0.25)	1.11 (0.29)	t (54) = -0.43	0.667
HairE *M(SD)*	11.33 (6.96)	6.25 (4.50)	t (54) = -3.30	0.002*

[a] Two missing responses on this item
* $p < 0.05$
HairE, hair cortisone levels; HairF, hair cortisol levels; PSS, perceived stress scale

3.2 Parkinson's disease and hair glucocorticoids

HairF and hairE levels were significantly positively correlated with each other ($r = 0.39$, $p = 0.003$). HairF levels were not significantly different (Cohen's d = 0.12) between PD patients and controls (Figure 2a; Table 3) in unadjusted ($B = 0.03$, 95% CI -0.12; 0.18, $p = 0.667$) and adjusted models (adj $B = 0.04$, 95% CI -0.13; 0.21, $p = 0.646$). HairE levels were significantly higher (Cohen's d = 0.87) in PD patients than in controls (Figure 2b; Table 4) in unadjusted ($B = 5.08$, 95% CI 1.99; 8.17, $p = 0.045$) and adjusted models (adj $B = 5.31$, 95% CI 1.88; 8.74, $p = 0.003$).

3.3 Parkinson's disease clinical factors and hair glucocorticoids (Table 2)

None of the PD clinical factors were significantly associated with HairF levels. NMSS domain 3 (mood/cognition) and domain 8 (sexual function) scores were significantly positively associated with HairE levels. When we controlled for covariates (age, employment status and chemical treatment of hair) domain 8 scores (adj $B = 0.59$, 95% CI -4.72; 7.37, $p = 0.650$) were no longer significantly associated, but domain 3 scores (adj $B = 0.26$, 95% CI 0.06; 0.46, $p = 0.013$) remained significantly associated with HairE levels.

Table 2 Association between clinical variables and hair glucocorticoid levels in PD patients (n = 25)

Variables	***Descriptive statistics*** ***M (SD)***	**HairF** **ß (95% CI)**	**HairE** **ß (95% CI)**
Current CMD[a] n(%)			
No	16 (64.0)	Ref	Ref
Yes	9 (36.0)	-0.08 (-0.30; 0.13)	0.75 (-5.37; 6.87)
Current psychiatric medication[b] n(%)			
No	14 (56.0)	Ref	Ref
Yes	11 (44.0)	0.08 (-0.13; 0.29)	-1.46 (-7.35; 4.44)
Duration of PD (months)	65.8 (44.4)	-0.00 (-0.00; 0.00)	-0.02 (-0.09; 0.04)
LED[c]	600.6 (426.5)	0.00 (0.00; 0.00)	0.004 (-0.00; 0.01)
UPDRS total score	54.5 (19.3)	-0.00 (-0.01; 0.00)	0.06 (-0.08; 0.20)
UPDRS I	3.9 (2.1)	0.02 (-0.03; 0.07)	0.53 (-0.73; 1.80)
UPDRS II	13.1 (6.2)	-0.00 (-0.02; 0.02)	0.10 (-0.34; 0.54)
UPDRS III	37.5 (13.2)	-0.00 (-0.01; 0.01)	0.09 (-0.11; 0.30)
H&Y disability	2.9 (0.9)	0.02 (-0.11; 0.14)	0.84 (-2.35; 4.02)
NMSS total score	53.0 (37.6)	0.00 (-0.00; 0.00)	0.05 (-0.02; 0.12)
Cardiovascular (range 0-12)	4.2 (5.9)	0.00 (-0.02; 0.02)	-0.01 (-0.48; 0.46)
Sleep/fatigue (range 0-24)	10.8 (7.7)	-0.00 (-0.02; 0.01)	0.07 (-0.33; 0.46)
Mood/cognition (range 0-72)	7.6 (10.9)	0.01 (-0.01; 0.02)	0.30 (0.08; 0.52)*
Perceptual problems (range 0-18)	0.9 (2.2)	-0.03 (-0.08; 0.03)	0.37 (-1.08; 1.82)
Attention/memory (range 0-18)	5.3 (6.6)	-0.01 (-0.02; 0.01)	-0.23 (-0.64; 0.17)
Gastrointestinal tract (range 0-18)	5.2 (6.5)	0.00 (-0.01; 0.02)	0.14 (-0.28; 0.56)
Urinary (range 0-18)	9.4 (10.5)	0.00 (-0.01; 0.01)	0.18 (-0.12; 0.48)
Sexual function (range 0-12)	1.2 (2.9)	0.02 (-0.02; 0.05)	0.90 (0.05; 1.75)*
Miscellaneous (range 0-14)	8.4 (7.6)	0.00 (-0.01; 0.02)	0.16 (-0.20; 0.52)

[a] One (4.0%) with comorbid major depressive disorder (MDD) and 8 (32.0%) with a comorbid anxiety disorder; 3 (12.0%) with agoraphobia, 2 (8.0%) with generalised anxiety disorder (GAD) and 5 (20.0%) with social anxiety disorder (related to PD symptoms).
[b]Five (20.0%) were on benzodiazepines (BZD), 6 (24.0%) were on therapeutic antidepressants and 4 (16.0%) on low dose low dose amitriptyline.
[c]Twenty-three (92.0%) of the PD patients were on levodopa, 7 (28.0%) were on dopamine agonists, 12 (48.0%) were taking other medications for PD, including amantadine (n = 2, 8.0%), anticholinergics (n = 10, 40.0%), and beta-blockers (n = 1, 4.0%).
* $p < 0.05$
CMD, common mental disorder; HairE, hair cortisone levels; HairF, hair cortisol levels; H&Y, Hoehn and Yahr scale; LED, levodopa equivalent daily doses; NMSS, Non-motor symptoms scale; PD, Parkinson's disease; UPDRS, Unified Parkinson's Disease Rating Scale

Table 3 Parkinson's disease and metabolic syndrome regressed on hair cortisol levels (hairF)

	Simple linear regression		Multiple linear regression[a]					
			Model 1		Model 2		Model 3	
	ß (95% CI)	p-value	ß (95% CI)	p-value	ß (95% CI)	p-value	ß (95% CI)	p-value
Case status								0.629
Control	Ref		Ref		Ref		Ref	
Patient	0.03 (-0.12; 0.18)	0.667	0.04 (-0.13; 0.21)	0.646	0.04 (-0.13; 0.21)	0.658	0.00 (-0.24; 0.24)	0.996
MetS								0.946
No	Ref				Ref		Ref	
Yes	0.06 (-0.09; 0.21)	0.418			0.00 (-0.16; 0.16)	0.992	-0.05 (-0.31; 0.21)	0.710
Patient*MetS							0.09 (-0.27; 0.44)	0.630

[a] Multivariate adjusted model (adjusted for age, batch analysed and duration of sun exposure); n = 54; two participants with missing data for 'average hours in sun' excluded
Model 1 – Model without MetS: F (5, 48) = 2.55 (p = 0.040*), Adj R^2 = 0.127; n = 54
Model 2 – MetS added: F (6, 47) = 2.08 (p = 0.074), Adj R^2 = 0.109; n = 54
Model 3 – Interaction between case status and MetS added: F (7, 47) = 1.79 (p = 0.113), Adj R^2 = 0.094; n = 54
* p < 0.05
MetS, metabolic syndrome

Table 4 Parkinson's disease and metabolic syndrome regressed on hair cortisone levels (hairE)

	Simple linear regression		Multiple linear regression[a]					
			Model 1		Model 2		Model 3	
	ß (95% CI)	p-value	ß (95% CI)	p-value	ß (95% CI)	p-value	ß (95% CI)	p-value
Case status								0.010*
Control	Ref		Ref		Ref		Ref	
Patient	5.08 (1.99; 8.17)	0.045*	5.31 (1.88; 8.74)	0.003*	5.12 (1.60; 8.64)	0.005*	6.72 (2.36; 11.07)	0.003*
MetS								0.464
No	Ref				Ref		Ref	
Yes	2.96 (-0.39; 6.31)	0.082			0.87 (-2.25; 3.98)	0.579	2.78 (-1.61; 7.17)	0.210
Patient*MetS							-3.98 (-10.46; 2.50)	0.223

[a] Multivariate adjusted model (adjusted for age, employment status and chemical treatment of hair), n = 56
Model 1 – Model without MetS: F (5, 50) = 6.41 (p < 0.001*), Adj R^2 = 0.294; n = 56
Model 2 – MetS added: F (6, 49) = 4.64 (p = 0.001*), Adj R^2 = 0.284; n = 56
Model 3 – Interaction between case status and MetS added: F (7, 48) = 4.24 (p = 0.001*), Adj R^2 = 0.292; n = 56
* p < 0.05
MetS, metabolic syndrome

3.4 Self-perceived stress, Parkinson's disease and hair glucocorticoids

In analyses controlling for age, PSS scores were significantly higher in PD patients than in controls (OR = 1.14; 95% CI 1.03; 1.27, *p* = 0.015), although this effect was not evident in unadjusted analyses (p = 0.159). Concerning hair GCs, in unadjusted analyses PSS scores were not significantly associated with hairF (*B* = -0.00, 95% CI -0.01; 0.01, *p* = 0.547), and were significantly positively associated with hairE (*B* = 0.23, 95% CI 0.01; 0.45, *p* = 0.038). When we controlled for covariates PSS scores were not significantly associated with hairF (adj *B* = -0.01, 95% CI -0.02; 0.00, *p* = 0.259) nor hairE (*B* = 0.10, 95% CI -0.12; 0.32, *p* = 0.385). There were no significant interaction effects between PD diagnosis and PSS scores.

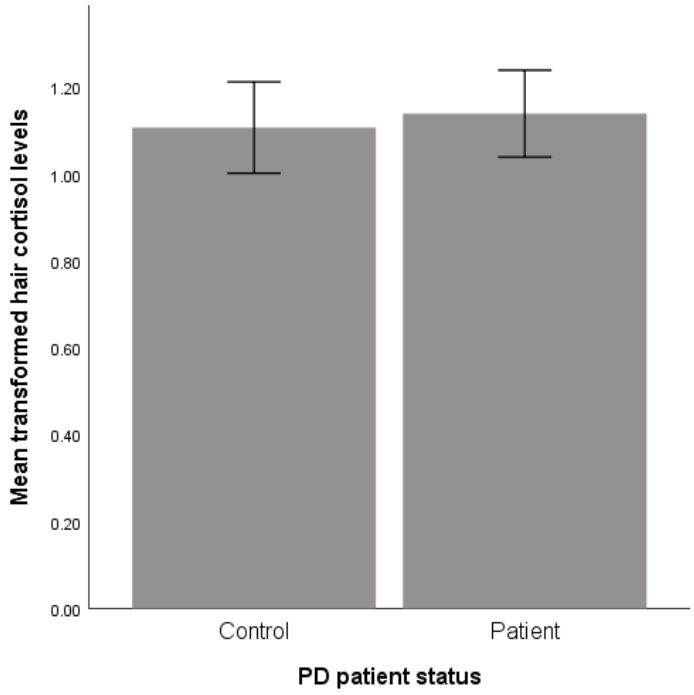

Figure 2a Hair cortisol (hairF) levels according to PD patient status

Bar chart demonstrating mean hairF according to PD patient status. HairF levels were not significantly higher in PD patients than controls (*p* = 0.667)
*$p < 0.05$
Error bars ± 2SE

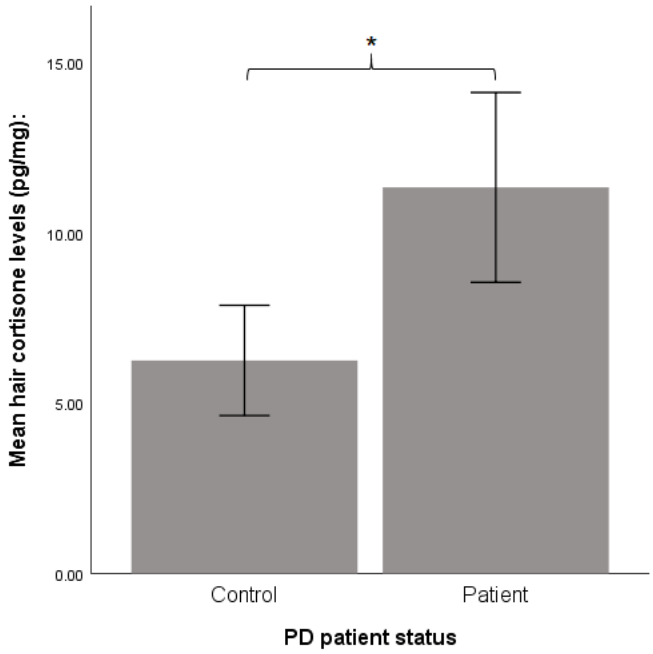

Figure 2b Hair cortisone (hairE) levels according to PD patient status

Bar chart demonstrating mean HairE levels according to PD patient status. HairE levels were significantly higher in PD patients than controls ($p = 0.002$)
*$p < 0.05$
Error bars ± 2SE

3.5 Cardiovascular disease risk factors, Parkinson's disease and hair glucocorticoids

3.5.1 Metabolic syndrome, Parkinson's disease and hair glucocorticoids

Rates of MetS were significantly higher ($p = 0.021$) in PD patients (56.0%) than in controls (25.8%), but when we controlled for age there was only a trend towards significance ($p = 0.077$). MetS was not significantly associated with hairF in unadjusted ($B = 0.06$, 95% CI -0.09; 0.21, $p = 0.418$) and adjusted (adj $B = 0.00$, 95% CI -0.16; 0.16, $p = 0.992$) models and there were no significant interactions between PD and MetS (Figure 3a; Table 3). HairE levels were higher in those with MetS than in those without, showing a trend towards significance in unadjusted ($B = 2.96$, 95% CI -0.39; 6.31, $p = 0.082$) analysis, but were non-significant in adjusted (adj $B = 0.87$, 95% CI -2.25; 3.98, $p = 0.579$) analysis and there was no interaction between PD diagnosis and MetS. HairF levels remained significantly higher in patients without MetS than controls without MetS and there were no significant interactions between PD and MetS (Figure 3b; Table 4).

3.5.2 Individual cardiovascular disease risk factors, Parkinson's disease and hair glucocorticoids (Supplementary table 1)

CVD risk factors that differed significantly between patients and controls included the number of MetS criteria ($p = 0.041$), BP MetS criterion ($p = 0.013$), FPG MetS criterion ($p = 0.038$), and antihypertensive medication ($p < 0.001$), statin ($p < 0.001$) and aspirin use ($p = 0.005$). When we controlled for age, BP MetS criterion ($p = 0.040$), antihypertensive medication ($p = 0.001$), and aspirin use ($p = 0.044$) remained significantly different. None of the individual CVD risk factors were significantly associated with HairF in unadjusted or adjusted analyses. The CVD risk factors that were significantly associated with HairE in unadjusted analyses were the number of MetS criteria ($B = 1.43$, 95% CI 0.04; 2.82, $p = 0.045$), antihypertensive medication use ($B = 3.50$, 95% CI 0.24; 6.77, $p = 0.036$), WHR ($B = 26.74$, 95% CI 7.52; 45.95, $p = 0.007$), known CVD ($B = 7.86$, 95% CI 0.70; 15.02, $p = 0.032$), and aspirin use ($B = 4.77$, 95% CI 1.11; 8,43, $p = 0.012$) and there was a trend toward significance demonstrated for HDL-C ($B = -3.06$, 95% CI -6.67; 0.55, $p = 0.095$). When we controlled for covariates WHR remained significant (adj $B = 21.64$, 95% CI 4.04; 39.24, $p = 0.017$) and HDL-C still showed a trend towards significance (adj $B = -2.80$, 95% CI -6.09; 0.50, $p = 0.095$). There were no significant interactions between PD diagnosis and any of the CVD variables on HairF or HairE.

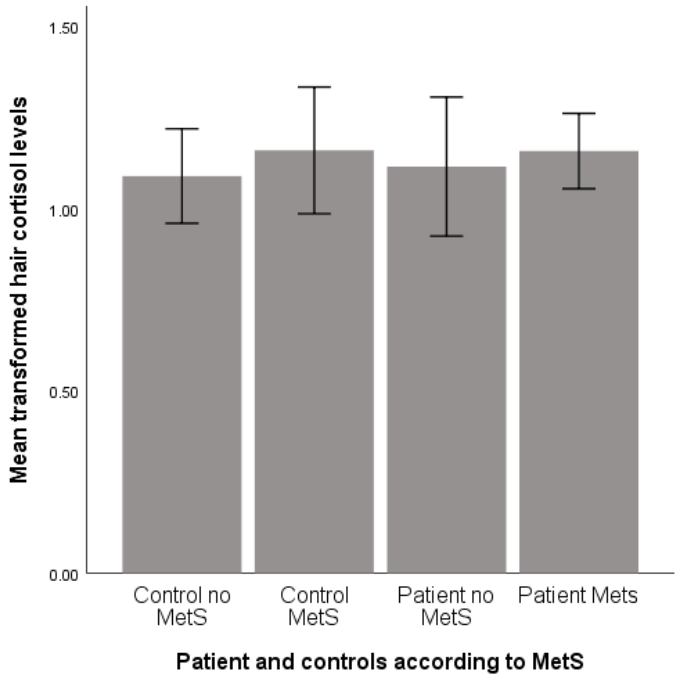

Figure 3a Hair cortisol (hairF) levels according to PD patient status and metabolic syndrome status

Bar chart demonstrating mean hairF levels according to PD patient status and metabolic syndrome status (MetS). The model was not significant ($F(52, 3) = 0.24$, $p = 0.871$) and there were no significant differences between the groups based on Tukey HSD post hoc test.
*$p < 0.05$
Error bars ± 2SE

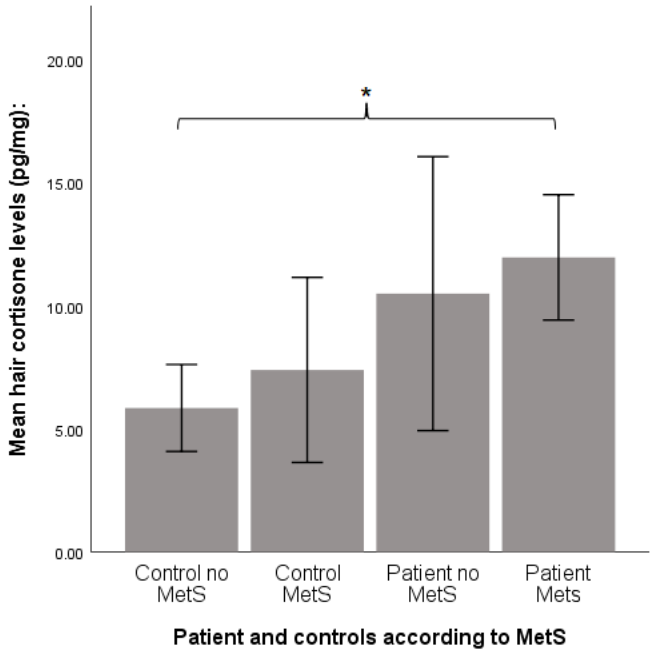

Figure 3b Hair cortisone (hairE) levels according to PD patient status and metabolic syndrome status

Bar chart demonstrating mean HairE levels according to PD patient status and metabolic syndrome status (MetS). The model was significant ($F(52, 3) = 3.82$, $p = 0.015$) and HairE were highest in PD patients with MetS, and were significantly higher than controls without MetS ($p = 0.015$). There were no other significant differences between the groups based on Tukey HSD post hoc test.
*$p < 0.05$
Error bars ± 2SE

4 Discussion

In a sample of South African mixed ancestry females we demonstrated significantly higher hairE, but not hairF levels, in PD patients than in controls. Furthermore increased severity of NMS of PD related to depression, anhedonia and anxiety were significantly positively associated with hairE levels. We did not demonstrate a significant association between hairF or hairE levels and MetS, nor with PD-MetS comorbidity. None of the individual CVD risk variables were associated with hairF levels, whereas one of the more robust predictors of CVD risk, WHR, was positively associated with hairE levels.

HairE, but not hairF levels, were significantly higher in PD patients than in controls. However, similar to other studies, hairF and hairE levels were significantly positively correlated (Kuehl et al., 2015; Savas et al., 2019; Stalder et al., 2013). Generally studies demonstrate higher cortisol levels in PD patients than controls (Soares et al., 2019). Although no studies have directly examined cortisone in PD, most

studies in PD have used an immunoassay measurement which is associated with increased cross-reactivity with cortisone (Perogamvros et al., 2010). Furthermore, most studies have measured total cortisol in blood and not free cortisol, which is considered to be the biologically active form of cortisol (Perogamvros et al., 2010). Cortisone in different tissues may provide a better reflection of free cortisol, as for instance, salivary cortisone demonstrated the strongest correlation with free serum cortisol in one study, stronger than total serum cortisol and salivary cortisol (Perogamvros et al., 2010). HairE also appears to hold certain benefits to HairF. For instance, one study found diagnostic accuracy for Cushing's syndrome was significantly better for HairE than HairF (Savas et al., 2019). HairF may also be influenced by additional factors, as evidenced by greater variability, with higher outlying values found with hairF than hairE (Stalder et al., 2013; Staufenbiel et al., 2015). Other studies have also found stronger associations with hairE than hairF and variables studied, including stress related variables (Davison, Singh, & McFarlane, 2019; Stalder et al., 2013), and may explain, in part, why significant associations were demonstrated for hairE and not hairF. Furthermore, PD patients in our sample were on treatment and studies have demonstrated that cortisol levels are higher in untreated PD patients, and conversely, that cortisol levels decrease in PD patients on treatment with dopaminergic drugs (Müller & Muhlack, 2007; Müller, Welnic, & Muhlack, 2007; Stypuła, Kunert-Radek, Stępień, Żylińska, & Pawlikowski, 1996) and in PD patients receiving sub thalamic nucleus deep brain stimulation (STN DBS) (Nováková et al., 2011; Růžička et al., 2015; Seifried et al., 2013). Thus in our study, treatment may have contributed to the non-significant results demonstrated for hairF. We, however, did not find a directly proportional association between LED and hairF or hairE levels.

A measure of self-perceived stress was included to assess whether altered hair GC levels could partly be explained by increased stress experienced by PD patients. When we controlled for age, PD patients were found to have higher perceived stress scores than controls. Perceived stress was also significantly positively associated with hairE, but not hairF. When controlled for covariates this association was no longer significant and is likely explained by higher hairE levels in PD patients than in controls. Perceived stress has not been extensively studied in PD and there is limited evidence that psychosocial stress confers increased risk for PD (Du & Pang, 2015). However, animal models show

that stress increases severity of parkinsonistic symptoms (Du & Pang, 2015). Although not significant, our results suggest that PD patients experience higher levels of stress that may play a role in GC dysregulation. Additionally, the NMS of PD involving mood, anxiety and anhedonia were significantly positively associated with hairE, suggesting a dysregulated HPA-axis in PD patients may be related to NMS of PD. One study that demonstrated significantly higher salivary cortisol levels in PD patients than in controls also found that cortisol levels were significantly correlated with anxiety and depression severity and another found that reductions in cortisol levels were correlated with improvements in depression scores following STN DBS (Medeiros Costa et al., 2019; Seifried et al., 2013). Cell loss in PD extends beyond the substantia nigra and include the locus ceruleus, dorsal raphe nuclei, nucleus basalis of Meynert and postganglionic sympathetic neurons (Levy et al., 2009). HPA-axis dysfunction in PD may also be related to these other systems as reduced serotonergic and opioid induced cortisol release have been demonstrated in male PD patients (Volpi et al., 1997, 1994).

Motor symptoms of PD and disease stage were not associated with hair GC levels. Other studies have similarly demonstrated no association between cortisol levels and motor symptoms or H&Y stage (Charlett et al., 2009; Djamshidian et al., 2011). Although findings have been mixed, with one study demonstrating positive correlations between cortisol and UPDRS scores (Håglin & Bäckman, 2016), whilst another finding an inverse association between UPDRS scores and overall cortisol release following levodopa intake (Müller & Muhlack, 2007).

MetS was not significantly associated with hairF or hairE, with only a trend toward significance demonstrated for higher hairE levels in MetS in unadjusted analysis. There were also no significant interactions between PD and MetS. The small sample size of this study is a limitation considering that other studies in larger samples have demonstrated significantly higher hairF and hairE levels in individuals with MetS (Kuehl et al., 2015; Stalder et al., 2013). The lack of a significant finding may also be ascribed to the fact that the individual MetS factors demonstrated different directions of association with GCs, suggesting the association between hair GCs and MetS may be more complex. None of the individual CVD risk factors were associated with hairF, whereas WHR was significantly positively associated with hairE and HDL-C was inversely associated with hairE, showing a trend toward

significance. These associations between hairE and WHR and HDL-C have also been demonstrated in one other study utilising hairE (Stalder et al., 2013), although HDL-C was not associated with hairE in another study (Wester et al., 2017). The direction of association between hair GCs and HDL-C and WHR are consistent with what has generally been observed for studies evaluating cortisol (Fraser et al., 1999; Stalder et al., 2013, 2017). Similar to our study other studies have also found that HairE demonstrated stronger associations with CVD risk markers than HairF (Stalder et al., 2013), further supporting the utility of including hairE as a measurement of HPA-axis function.

4.1 Strengths and limitations

As far as we are aware, this is the first study to examine hair GC levels in PD. Hair analyses were conducted with LC-MS/MS which is the gold standard method for hair GC determination (Russell et al., 2015), whereas most studies examining basal cortisol levels in PD have used immunoassays. However, our sample may have been underpowered to detect significant associations, particularly between hair GC levels and MetS. Our sample size was greatly reduced due to the requirement that hair length be 3cm or longer and this resulted in the exclusion of almost all male participants. Restriction to a single ethnicity and sex limits the generalisability of our results. HPA axis function can differ according to sex with different patterns of association demonstrated. For instance, one study found that women, and in particular, obese women, had a higher cortisol to cortisone conversion rate than men (Vierhapper, Heinze, & Nowotny, 2007). It has also been shown that hairE, but not hairF, levels were significantly higher in Australian indigenous women than in non-indigenous women and the reverse was demonstrated for men (Davison et al., 2019). Due to the cross-sectional nature, cause-effect relationships cannot be inferred. As this was an exploratory study of hair GC in PD we did not control for multiple comparisons. We included PD patients with CMD comorbidity, which may have influenced our results, however CMD comorbidity and psychiatric medication use was not associated with hair GC levels.

4.2 Conclusions

This study adds to the evidence of a dysregulated HPA-axis in PD. Though hair cortisol levels were not significantly higher in PD patients than in controls, hair cortisone levels were. Our study, along with

other studies, found that hair cortisone levels demonstrate stronger associations and show less variability than hair cortisol levels, and hair cortisone levels thus exhibit utility as an additional marker of HPA-axis function. Non-motor symptoms of PD related to mood and anxiety were also positively associated with hair cortisone levels, suggesting a role for HPA-axis dysregulation in the NMS of PD. Hair GC levels were not significantly associated with MetS, although hair cortisone was associated with WHR. Future studies, in larger samples, and representing both genders, will assist with delineating the role of stress and of longer-term HPA-axis dysfunction in PD patients and in the comorbidity of PD and MetS. As inflammatory pathways also play a role in PD and CVD and are linked to HPA-axis function, including markers of inflammation alongside HPA-axis function may provide additional insights into the pathways involved.

5 Supplementary materials

Supplementary Table 1 Cardiovascular disease risk factors in relation to PD patient status and hair glucocorticoid levels

	CVD risk variables according to PD groups		Hair F	Hair E
	PD Patients	**Controls**	**ß (95% CI)**	**ß (95% CI)**
Total	25	31		
MetS variables				
MetS n(%)				
No	11 (44.0)	23 (74.2)	Ref	Ref
Yes	14 (56.0)	8 (25.8)	0.06 (-0.09; 0.21)	2.96 (-0.39; 6.31)
Number of criteria met *M(SD)*	2.5 (1.2)	1.8 (1.1)	0.01 (-0.05; 0.08)	1.43 (0.04; 2.82)*
BP criterion n(%)				
No	4 (16.0)	15 (48.4)	Ref	
Yes	21 (84.0)	16 (51.6)	-0.09 (-0.25; 0.06)	1.26 (-2.28; 4.80)
On antihypertensive rx n(%)				
No	7 (28.0)	25 (80.6)	Ref	Ref
Yes	18 (72.0)	6 (19.4)	0.03 (-0.12; 0.18)	3.50 (0.24; 6.77)*
SBP (mmHg) *M(SD)*	133.0 (25.6)	128.6 (20.5)	0.00 (-0.00; 0.00)	0.00 (-0.07; 0.08_
DBP (mmHg) *M(SD)*	77.1 (13.9)	80.5 (14.9)	0.00 (-0.00; 0.01)	0.01 (-0.11; 0.13)
BP over threshold n(%)				
No	12 (48.0)	16 (51.6)	Ref	Ref
Yes	13 (52.0)	15 (48.4)	-0.09 (-0.24; 0.05)	-0.63 (-3.99; 2.73)
FPG criterion n(%)				
No	10 (40.0)	21 (67.7)	Ref	Ref
Yes	15 (60.0)	10 (32.3)	0.05 (-0.10; 0.20)	2.60 (-0.71; 5.91)
On diabetes rx n(%)				
No	18 (72.0)	29 (93.5)	Ref	Ref
Yes	7 (28.0)	2 (6.5)	0.06 (-0.14; 0.26)	2.92 (-1.59; 7.43)
FPG (mmol/l) *M(SD)*	6.10 (1.79)	6.03 (2.76)	0.00 (-0.03; 0.04)	0.09 (-0.63; 0.81)
FPG ≥ 5.6 mmol/l n(%)				
No	11 (44.0)	21 (67.7)	Ref	Ref
Yes	14 (56.0)	10 (32.3)	0.04 (-0.10; 0.19)	2.36 (-0.98; 5.69)
HDL-C criterion[a] n(%)				
No	18 (72.0)	23 (74.2)	Ref	Ref
Yes	7 (28.0)	8 (25.8)	0.02 (-0.15; 0.19)	3.05 (-0.66; 6.76)
HDL-C (mmol/l) *M(SD)*	1.69 (0.53)	1.51 (0.39)	-0.06 (-0.22; -0.10)	-3.06 (-6.67; 0.55)
Trig criterion[b] n(%)				
No	21 (84.0)	25 (80.6)	Ref	Ref
Yes	4 (16.0)	6 (19.4)	-0.04 (-0.24; 0.15)	-0.23 (-4.62; 4.17)

Trig (mmol/l) *M(SD)*	1.17 (0.45)	1.25 (0.41)	-0.09 (-0.27; 0.08)	1.06 (-2.94; 5.07)
WC criterion n(%)				
No	10 (40.0)	14 (45.2)	Ref	Ref
Yes	15 (60.0)	17 (54.8)	0.11 (-0.04; 0.25)	1.83 (-1.54; 5.19)
WC (cm) *M(SD)*	89.7 (13.1)	94.1 (13.9)	-0.00 (-0.01; 0.01)	0.04 (-0.08; 0.17)
Other CVD risk variables				
Physical parameters				
BMI (kg/m^2) *M(SD)*	28.5 (5.0)	30.3 (6.5)	-0.00 (-0.01; 0.01)	-0.02 (-0.31; 0.27)
HC (cm) *M(SD)*	104.9 (10.8)	110.0 (13.2)	-0.00 (-0.01; 0.01)	-0.09 (-0.23; 0.05)
WHR *M(SD)*	0.85 (0.09)	0.86 (0.08)	0.02 (-0.88; 0.92)	26.74 (7.52; 45.95)*
HR (bpm) *M(SD)*	77.3 (12.5)	75.0 (8.9)	0.00 (-0.00; 0.01)	0.12 (-0.03; 0.28)
Laboratory parameters				
TC (mmol/l) *M(SD)*	5.37 (1.03)	5.43 (1.03)	-0.05 (-0.12; 0.02)	-0.92 (-2.56; 0.72)
TC > 5 mmol/l n(%)				
No	15 (60.0)	21 (67.7)	Ref	Ref
Yes	10 (40.0)	10 (32.3)	0.12 (-0.03; 0.27)	1.79 (-1.69; 5.26)
LDL-C (mmol/l) *M(SD)*	3.14 (0.96)	3.35 (1.03)	-0.03 (-0.11; 0.04)	-0.41 (-2.12; 1.29)
LDL-C >= 3 mmol/l n(%)				
No	17 (68.0)	19 (61.3)	Ref	Ref
Yes	8 (32.0)	12 (38.7)	-0.00 (-0.17; 0.14)	-0.78 (-4.29; 2.73)
HbA1c	6.15 (1.12)	6.22 (1.35)	-0.01 (-0.07; 0.05)	-0.26 (-1.63; 1.11)
HbA1c >= 6.5% n(%)				
No	18 (72.0)	25 (80.6)	Ref	Ref
Yes	7 (28.0)	6 (19.4)	0.01 (-0.16; 0.19)	0.66 (-3.32; 4.64)
CVD clinical variables				
Known CVD n(%)				
No	22 (88.0)	31 (100)	Ref	Ref
Yes	3 (12.0)	0 (0)	0.12 (-0.20; 0.45)	7.86 (0.70; 15.02)*
On statins n(%)				
No	13 (52.0)	31 (100)	Ref	Ref
Yes	12 (48.0)	0 (0)	-0.08 (-0.25; 0.10)	1.51 (-2.57; 5.59)
On aspirin n(%)				
No	14 (56.0)	28 (90.3)	Ref	Ref
Yes	11 (44.0)	3 (9.7)	0.06 (-0.11; 0.23)	4.77 (1.11; 8.43)*

[a] No participants were receiving treatment to increase HDL-C
[b] No participants were receiving treatment for hypertriglyceridemia
* $p < 0.05$
BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; DBP, diastolic blood pressure; HbA1c, Glycated haemoglobin; HC, hip circumference; HDL-C, high-density lipoprotein cholesterol; HR, heart rate; FPG, fasting plasma glucose; LDL-C, Low density lipoprotein cholesterol; MetS, metabolic syndrome; Ref, reference category; rx, treatment; SBP, systolic blood pressure; TC, total cholesterol; trig; serum triglyceride; WC, waist circumference; WHR waist-to-hip ratio

6 References

Alberti, K. G., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., … Smith Jr, S. C. (2009). Harmonizing the Metabolic Syndrome A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International A. *Circulation*, *120*(16), 1640–1645. https://doi.org/10.1161/CIRCULATIONAHA.109.192644 [doi]

Anagnostis, P., Athyros, V. G., Tziomalos, K., Karagiannis, A., & Mikhailidis, D. P. (2009). The pathogenetic role of cortisol in the metabolic syndrome: a hypothesis. *Journal of Clinical Endocrinology & Metabolism*, *94*(8), 2692–2701. https://doi.org/10.1210/jc.2009-0370

Bainbridge, J., Boyd, J., Cambi, F., Christine, C., Dhall, R., Leehey, M., … Zhang, Y. (2017). Association of metabolic syndrome and change in Unified Parkinson Disease Rating Scale scores. *Neurology*, *89*(17), 1789–1794. https://doi.org/https://doi.org/10.1212/WNL.0000000000004572

Baquer, N. Z., Taha, A., Kumar, P., McLean, P., Cowsik, S. M., Kale, R. K., … Sharma, D. (2009). A metabolic and functional overview of brain aging linked to neurological disorders. *Biogerontology*, *10*(4), 377–413. https://doi.org/10.1007/s10522-009-9226-2

Charlett, A., Dobbs, R. J., Purkiss, A. G., Wrighe, D. J., Peterson, D. W., Weller, C., & Dobbs, S. M. (2009). Cortisol is higher in parkinsonism and associated with gait deficit. *Acta Neurologica Scandinavica*, *97*(2), 77–85. https://doi.org/10.1111/j.1600-0404.1998.tb00614.x

Chaudhuri, K. R., Martinez- Martin, P., Brown, R. G., Sethi, K., Stocchi, F., Odin, P., … MacMahon, D. (2007). The metric properties of a novel non- motor symptoms scale for Parkinson's disease: Results from an international pilot study. *Movement Disorders*, *22*(13), 1901–1911. https://doi.org/https://doi.org/10.1002/mds.21596

Clark, J. E., Osborne, J. W., Gallagher, P., & Watson, S. (2016). A simple method for optimising transformation of non-parametric data: an illustration by reference to cortisol assays. *Human*

Psychopharmacology: Clinical and Experimental, *31*(4), 259–267. https://doi.org/10.1002/hup.2528

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A Global Measure of Perceived Stress. *Journal of Health and Social Behavior*, *24*(4), 385–396. https://doi.org/10.1016/j.socscimed.2016.08.021

Davison, B., Singh, G. R., & McFarlane, J. (2019). Hair cortisol and cortisone as markers of stress in indigenous and non-indigenous young adults. *Stress*, *22*(2), 210–220. https://doi.org/10.1080/10253890.2018.1543395

Djamshidian, A., & Lees, A. J. (2014). Can stress trigger Parkinson's disease? *Journal of Neurology, Neurosurgery and Psychiatry*, *85*(8), 879–882. https://doi.org/10.1136/jnnp-2013-305911

Djamshidian, A., O'Sullivan, S. S., Papadopoulos, A., Bassett, P., Shaw, K., Averbeck, B. B., & Lees, A. (2011). Salivary cortisol levels in Parkinson's disease and its correlation to risk behaviour. *Journal of Neurology, Neurosurgery and Psychiatry*, *82*(10), 1107–1111. https://doi.org/10.1136/jnnp.2011.245746

Du, X., & Pang, T. Y. (2015). Is dysregulation of the HPA-axis a core pathophysiology mediating co-morbid depression in neurodegenerative diseases? *Frontiers in Psychiatry*, *6*(MAR), 1–33. https://doi.org/10.3389/fpsyt.2015.00032

Fahn, S., Elton, R., & UPDRS program members. (1987). Unified Parkinsons disease rating scale. In Fahn S, Marsden CD, Goldstein M, Calne DB (Ed.), *Recent developments in Parkinsons disease, vol 2.* (pp. 153–163). Florham Park, NJ: Macmillan Healthcare Information.

Fraser, R., Ingram, M. C., Anderson, N. H., Morrison, C., Davies, E., & Connell, J. M. C. (1999). Cortisol effects on body mass, blood pressure, and cholesterol in the general population. *Hypertension*, *33*(6), 1364–1368. https://doi.org/https://doi.org/10.1161/01.HYP.33.6.1364

Gao, W., Kirschbaum, C., Grass, J., & Stalder, T. (2016). LC–MS based analysis of endogenous steroid hormones in human hair. *Journal of Steroid Biochemistry and Molecular Biology*, *162*, 92–99. https://doi.org/10.1016/j.jsbmb.2015.12.022

Gao, W., Stalder, T., Foley, P., Rauh, M., Deng, H., & Kirschbaum, C. (2013). Quantitative analysis of steroid hormones in human hair using a column-switching LC–APCI–MS/MS assay. *Journal of Chromatography B*, *928*, 1–8. https://doi.org/10.1016/j.jchromb.2013.03.008

Goetz, C. G., Poewe, W., Rascol, O., Sampaio, C., Stebbins, G. T., Counsell, C., … Seidl, L. (2004). Movement Disorder Society Task Force report on the Hoehn and Yahr staging scale: Status and recommendations. *Movement Disorders*, *19*(9), 1020–1028. https://doi.org/10.1002/mds.20213

Håglin, L., & Bäckman, L. (2016). Covariation between plasma phosphate and daytime cortisol in early Parkinson's disease. *Brain and Behavior*, *6*(12), 1–7. https://doi.org/10.1002/brb3.556

Herrero, M.-T., Estrada, C., Maatouk, L., & Vyas, S. (2015). Inflammation in Parkinson's disease: role of glucocorticoids. *Frontiers in Neuroanatomy*, *9*(April), 1–12. https://doi.org/10.3389/fnana.2015.00032

Hughes, A., Daniel, S., Kilford, L., & Lees, A. (1992). Accuracy of clinical diagnosis of idiopathic Parkinson's disease: A clinico-pathological study of 100 cases. *Journal of Neurology Neurosurgery and Psychiatry*, *55*(3), 181–184. https://doi.org/10.1136/jnnp.55.3.181

Kibel, A., & Drenjančević-Perić, I. (2008). Impact of glucocorticoids and chronic stress on progression of Parkinson's disease. *Medical Hypotheses*, *71*(6), 952–956. https://doi.org/10.1016/j.mehy.2008.06.036

Klug, E. Q., Raal, F. J., Marais, A., Taskinen, M. R., Dalby, A. J., Schamroth, C., … Webb, D. A. (2018). South African dyslipidaemia guideline consensus statement: 2018 update A joint statement from the South African Heart Association (SA Heart) and the Lipid and Atherosclerosis Society of Southern Africa (LASSA). *South African Family Practice*, *108*(11b), 973–1000. https://doi.org/10.7196/SAMJ.2018.v108i11.13383

Kuehl, L. K., Hinkelmann, K., Muhtz, C., Dettenborn, L., Wingenfeld, K., Spitzer, C., … Otte, C. (2015). Hair cortisol and cortisol awakening response are associated with criteria of the metabolic syndrome in opposite directions. *Psychoneuroendocrinology*, *51*, 365–370.

https://doi.org/10.1016/j.psyneuen.2014.09.012

Lee, E. H. (2012). Review of the psychometric evidence of the perceived stress scale. *Asian Nursing Research*, *6*(4), 121–127. https://doi.org/10.1016/j.anr.2012.08.004

Levy, O. A., Malagelada, C., & Greene, L. A. (2009). Cell death pathways in Parkinson's disease: Proximal triggers, distal effectors, and final steps. *Apoptosis*, *14*(4), 478–500. https://doi.org/10.1007/s10495-008-0309-3

Marakaki, C., Papadimitriou, D. T., Kleanthous, K., Papadopoulou, A., & Papadimitriou, A. (2015). L-Dopa Stimulates Cortisol Secretion through Adrenocorticotropic Hormone Release in Short Children. *Hormone Research in Paediatrics*, *84*(5), 319–322. https://doi.org/10.1159/000439532

Matsha, T. E., Hassan, M. S., Hon, G. M., Soita, D. J., Kengne, A. P., & Erasmus, R. T. (2013). Derivation and validation of a waist circumference optimal cutoff for diagnosing metabolic syndrome in a South African mixed ancestry population. *International Journal of Cardiology*, *168*(3), 2954–2955. https://doi.org/https://doi.org/10.1016/j.ijcard.2013.03.150

Medeiros Costa, C., de Oliveira, G. L., Sousa Fonseca, A. C., de Carvalho Lana, R., Cunha Polese, J., & Pereira Pernambuco, A. (2019). Levels of cortisol and neurotrophic factor brain-derived in Parkinson's disease. *Neuroscience Letters*, *708*(May), 134359. https://doi.org/10.1016/j.neulet.2019.134359

Miller, R., & Plessow, F. (2013). Transformation techniques for cross-sectional and longitudinal endocrine data: Application to salivary cortisol concentrations. *Psychoneuroendocrinology*, *38*(6), 941–946. https://doi.org/10.1016/j.psyneuen.2012.09.013

Müller, T., & Muhlack, S. (2007). Acute levodopa intake and associated cortisol decrease in patients with Parkinson disease. *Clinical Neuropharmacology*, *30*(2), 101–106. https://doi.org/10.1097/01.WNF.0000240954.72186.91

Müller, T., Welnic, J., & Muhlack, S. (2007). Acute levodopa administration reduces cortisol release in patients with Parkinson's disease. *Journal of Neural Transmission*, *114*(3), 347–350.

https://doi.org/10.1007/s00702-006-0552-0

Nam, G. E., Kim, S. M., Han, K., Kim, N. H., Chung, H. S., Kim, J. W., … Choi, K. M. (2018). Metabolic syndrome and risk of Parkinson disease: A nationwide cohort study. *PLoS Medicine*, *15*(8), 1–15. https://doi.org/10.1371/journal.pmed.1002640

Nolan, Y. M., Sullivan, A. M., & Toulouse, A. (2013). Parkinson's disease in the nuclear age of neuroinflammation. *Trends in Molecular Medicine*, *19*(3), 187–196. https://doi.org/10.1016/j.molmed.2012.12.003

Nováková, L., Haluzík, M., Jech, R., Urgošík, D., Růžička, F., & Růžička, E. (2011). Hormonal regulators of food intake and weight gain in Parkinson's disease after subthalamic nucleus stimulation. *Neuroendocrinology Letters*, *32*(4), 437–441.

Osborne, J. W. (2010). Improving your data transformations : Applying the Box-Cox transformation. *Pratical Assessment, Research & Evaluation*, *15*(12), 1–9.

Perogamvros, I., Keevil, B. G., Ray, D. W., & Trainer, P. J. (2010). Salivary cortisone is a potential biomarker for serum free cortisol. *Journal of Clinical Endocrinology and Metabolism*, *95*(11), 4951–4958. https://doi.org/10.1210/jc.2010-1215

Raul, J.-S. S., Cirimele, V., Ludes, B., & Kintz, P. (2004). Detection of physiological concentrations of cortisol and cortisone in human hair. *Clinical Biochemistry*, *37*(12), 1105–1111. https://doi.org/10.1016/j.clinbiochem.2004.02.010

Russell, E., Kirschbaum, C., Laudenslager, M. L., Stalder, T., Rijke, Y. De, van Rossum, E. F. C., … Koren, G. (2015). Toward Standardization of Hair Cortisol Measurement : Results of the First International Interlaboratory Round Robin. *Therapeutic Drug Monitoring*, *37*(1), 71–75. https://doi.org/10.1097/FTD.0000000000000148

Růžička, F., Jech, R., Nováková, L., Urgošík, D., Bezdíček, O., Vymazal, J., & Růžička, E. (2015). Chronic stress-like syndrome as a consequence of medial site subthalamic stimulation in Parkinson's disease. *Psychoneuroendocrinology*, *52*(1), 302–310.

https://doi.org/10.1016/j.psyneuen.2014.12.001

Sääksjärvi, K., Knekt, P., Männistö, S., Lyytinen, J., & Heliövaara, M. (2015). Prospective study on the components of metabolic syndrome and the incidence of Parkinson's disease. *Parkinsonism and Related Disorders*, *21*(10), 1148–1155. https://doi.org/10.1016/j.parkreldis.2015.07.017

Savas, M., Wester, V., de Rijke, Y. B., Rubinstein, G., Zopp, S., Dorst, K., … van Rossum, E. F. C. (2019). Hair glucocorticoids as biomarker for endogenous Cushing's syndrome: validation in two independent cohorts. *Neuroendocrinology*. https://doi.org/10.1159/000498886

Seifried, C., Boehncke, S., Heinzmann, J., Baudrexel, S., Weise, L., Gasser, T., … Hilker, R. (2013). Diurnal variation of hypothalamic function and chronic subthalamic nucleus stimulation in parkinson's disease. *Neuroendocrinology*, *97*(3), 283–290. https://doi.org/10.1159/000343808

SEMDSA Type 2 Diabetes Guidelines Expert Committee. (2017). The 2017 SEMDSA Guideline for the Management of Type 2 Diabetes. *Journal of Endocrinology, Metabolism and Diabetes of South Africa*, *22*(1), s1–s196. https://doi.org/10.1080/22201009.2012.10872276

Sheehan, D. V, Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., … Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry*, *59*(Suppl 20), 22–33. Retrieved from https://psycnet.apa.org/record/1998-03251-004

Smith, A. D., Castro, S. L., & Zigmond, M. J. (2002). Stress-induced Parkinson's disease: a working hypothesis. *Physiology & Behavior*, *77*(4), 527–531. https://doi.org/10.1016/S0031-9384(02)00939-3

Soares, N. M., Pereira, G. M., Altmann, V., de Almeida, R. M. M., & Rieder, C. R. M. (2019). Cortisol levels, motor, cognitive and behavioral symptoms in Parkinson's disease: a systematic review. *Journal of Neural Transmission*, *126*(3), 219–232. https://doi.org/10.1007/s00702-018-1947-4

Stalder, T., Kirschbaum, C., Alexander, N., Bornstein, S. R., Gao, W., Miller, R., … Fischer, J. E. (2013). Cortisol in hair and the metabolic syndrome. *The Journal of Clinical Endocrinology &*

Metabolism, *98*(6), 2573–2580. https://doi.org/10.1210/jc.2013-1056

Stalder, T., Steudte-Schmiedgen, S., Alexander, N., Klucken, T., Vater, A., Wichmann, S., … Miller, R. (2017). Stress-related and basic determinants of hair cortisol in humans: A meta-analysis. *Psychoneuroendocrinology*, *77*, 261–274. https://doi.org/10.1016/j.psyneuen.2016.12.017

Stalder, T., Steudte, S., Alexander, N., Miller, R., Gao, W., Dettenborn, L., & Kirschbaum, C. (2012). Cortisol in hair, body mass index and stress-related measures. *Biological Psychology*, *90*(3), 218–223. https://doi.org/10.1016/j.biopsycho.2012.03.010

Staufenbiel, S. M., Penninx, B. W. J. H., de Rijke, Y. B., van den Akker, E. L. T., & van Rossum, E. F. C. (2015). Determinants of hair cortisol and hair cortisone concentrations in adults. *Psychoneuroendocrinology*, *60*, 182–194. https://doi.org/10.1016/j.psyneuen.2015.06.011

Steudte, S., Kirschbaum, C., Gao, W., Schoenfeld, S., Hoyer, J., & Stalder, T. (2012). Glucocorticoids in hair as biomarkers of traumatization in healthy individuals and PTSD patients. *European Journal of Psychotraumatology*, *3*(1). https://doi.org/10.3402/ejpt.v3i0.19472

Stypuła, G., Kunert-Radek, J., Stępień, H., Żylińska, K., & Pawlikowski, M. (1996). Evaluation of interleukins, ACTH, cortisol and prolactin concentrations in the blood of patients with Parkinson's disease. *Neuroimmunomodulation*, *3*(2–3), 131–134. https://doi.org/https://doi.org/10.1159/000097237

Vierhapper, H., Heinze, G., & Nowotny, P. (2007). Sex-specific difference in the interconversion of cortisol and cortisone in men and women. *Obesity*, *15*(4), 820–824. https://doi.org/10.1038/oby.2007.592

Volpi, R., Caffarra, P., Boni, S., Scaglioni, A., L, M., Saginario, A., … Coiro, V. (1997). ACTH/cortisol involvement in the serotonergic disorder affecting the parkinsonian brain. *Neuropsychobiology*, *35*(2), 73–78. https://doi.org/10.1159/000119394

Volpi, R., Caffarra, P., Scaglioni, A., Maestri, D., Chiodera, P., & Coiro, V. (1994). Restoration of ACTH/cortisol and LH responses to naloxone by chronic dopaminergic treatment in Parkinson's

disease. *Journal of Neural Transmission - Parkinson's Disease and Dementia Section*, *7*(1), 1–11. https://doi.org/10.1007/BF02252658

Vyas, S., Rodrigues, A. J., Silva, J. M., Tronche, F., Almeida, O. F. X., Sousa, N., & Sotiropoulos, I. (2016). Chronic stress and glucocorticoids: From neuronal plasticity to neurodegeneration. *Neural Plasticity*, *2016*. https://doi.org/10.1155/2016/6391686

Wennig, R. (2000). Potential problems with the interpretation of hair analysis results. *Forensic Science International*, *107*(1), 5–12. https://doi.org/10.1016/S0379-0738(99)00146-2

Wester, V. L., Noppe, G., Savas, M., van den Akker, E. L. T., de Rijke, Y. B., & van Rossum, E. F. C. (2017). Hair analysis reveals subtle HPA axis suppression associated with use of local corticosteroids : The Lifelines cohort study. *Psychoneuroendocrinology*, *80*, 1–6. https://doi.org/10.1016/j.psyneuen.2017.02.024

Wirdefeldt, K., Adami, H., Cole, P., Trichopoulos, D., & Mandel, J. (2011). Epidemiology and etiology of Parkinson's disease: a review of the evidence. *European Journal of Epidemiology*, *26*(Suppl 1), 0–58. https://doi.org/10.1007/s10654-011-9581-6

World Health Organization. (2005). *WHO STEPS surveillance manual: the WHO STEPwise approach to chronic disease risk factor surveillance*. Geneva: World Health Organization (WHO).

World Medical Association. (2013). World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA - Journal of the American Medical Association*, *310*(20), 2191–2194. https://doi.org/10.1001/jama.2013.281053

Wosu, A. C., Valdimarsdóttir, U., Shields, A. E., Williams, D. R., & Williams, M. A. (2013). Correlates of cortisol in human hair: Implications for epidemiologic studies on health effects of chronic stress. *Annals of Epidemiology*, *23*(12), 797–811. https://doi.org/10.1016/j.annepidem.2013.09.006

Zhang, P., & Tian, B. (2014). Metabolic syndrome: An important risk factor for Parkinson's disease. *Oxidative Medicine and Cellular Longevity*, *2014*. https://doi.org/10.1155/2014/729194

Zhang, Q., Chen, Z., Chen, S., Xu, Y., & Deng, H. (2017). Intraindividual stability of cortisol and

cortisone and the ratio of cortisol to cortisone in saliva, urine and hair. *Steroids*, *118*, 61–67. https://doi.org/10.1016/j.steroids.2016.12.008

CHAPTER 4

Hair cortisol levels in schizophrenia and metabolic syndrome

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Hair cortisol levels in schizophrenia and metabolic syndrome

Abstract

Background: Individuals with schizophrenia demonstrate higher rates of metabolic syndrome (MetS) and cardiovascular disease (CVD) than the general population. Hypothalamic pituitary adrenal (HPA) axis dysfunction has been demonstrated in relation to both schizophrenia and MetS and may be aetiologically linked to CVD risk in schizophrenia. Few studies have, however, directly investigated these links. Hair cortisol concentrations (HCC) reflect longer-term HPA axis function and can provide additional insights into the role of the HPA-axis in schizophrenia and co-occurring CVD risk.

Objectives: In a case-control study of 16 patients with schizophrenia (11 first episode psychosis [FEP] and 5 chronic) and 21 controls aged between 23 and 32 years (M = 28.0, SD = 6.5) we investigated whether HCC were associated with schizophrenia diagnostic status, clinical features and with CVD risk in schizophrenia (as defined by MetS co-occurrence). As a secondary aim we also assessed the longitudinal trajectory of HCC in FEP patients.

Methods: Hair samples, representing a three-month retrospective window of cortisol, were collected and analysed utilizing liquid chromatography tandem mass spectrometry. Multivariate regression models were constructed with schizophrenia diagnostic status, clinical features and MetS comorbidity regressed on HCC, adjusting for potential confounders. A mixed models analysis was conducted to assess whether HCC changed between baseline and month-12 in FEP patients as compared to controls and in relation to MetS.

Results: At baseline the prevalence of MetS was 25.0% in patients with schizophrenia and 9.5% in controls. At 12 months, 3 of 5 FEP patients and 3 of the 6 controls, for whom HCC were available, had MetS. At baseline HCC were significantly lower (Cohen's d = 0.88) in patients with schizophrenia than in controls (adj *B* = -0.27, 95% CI -0.48; -0.06, *p* = 0.014) and when limited to FEP patients remained significantly lower (Cohen's d = 0.97) in FEP patients than in controls (adj *B* = -0.28, 95% CI -0.53; -0.03, *p* = 0.030). A mixed models analyses revealed a trend to significance (adj *B* = 0.33, 95% CI -0.07;

0.73, *p* = 0.097) for increased HCC from baseline to month-12 in FEP patients. Domains of symptom severity in schizophrenia were not associated with HCC, but better patient insight (*adj B* = -0.07, 95% CI -0.13; -0.01, *p* = 0.037) was inversely associated with HCC. MetS was not associated with HCC at baseline, but HCC increased significantly from baseline to month-12 in those with MetS (adj *B* = 0.47, 95% CI 0.04; 0.90, *p* = 0.037) and MetS was positively associated with HCC at month-12 follow-up (adj *B* = 0.41, 95% CI 0.02; 0.80, *p* = 0.041). There were no significant interactions between schizophrenia diagnosis and MetS on HCC.

Conclusions: Contrary to the majority of the literature we demonstrated lower HCC in schizophrenia patients, and in particular in FEP patients, than controls. In a subgroup of schizophrenia patients acute psychosis may be associated with a blunted HPA-axis with lower total cortisol output. MetS was associated with an increase in HCC and higher HCC at month-12 and elevated cortisol levels found in schizophrenia studies may be related to increased rates of MetS in schizophrenia patients.

Keywords: Schizophrenia; hair cortisol concentrations; metabolic syndrome; stress

1 Introduction

Schizophrenia is a severe and disabling mental disorder (Laursen, Nordentoft, & Mortensen, 2014). Individuals with schizophrenia have a shorter life-span and higher mortality rates than the general population (Hjorthøj, Stürup, McGrath, & Nordentoft, 2017; Laursen et al., 2014; Piotrowski et al., 2017). Although unnatural causes, such as suicide, contribute to the increased mortality risk, natural causes, and in particular cardiovascular disease (CVD), are the leading causes of mortality in patients with schizophrenia (Penninx & Lange, 2018; Piotrowski et al., 2017).

Meta-analyses have demonstrated that individuals with schizophrenia have increased rates of CVD, with a pooled prevalence of 11.8% (Correll et al., 2017). Furthermore, patients with schizophrenia have significantly higher rates of individual CVD risk factors, including abdominal obesity (OR = 4.4), low high-density lipoprotein cholesterol (HDL-C. OR = 2.4), hypertriglyceridemia (OR = 2.7), diabetes (OR – 2.0) and hypertension (OR = 1.4) (Vancampfort et al., 2013). Individuals with schizophrenia also have higher rates of the metabolic syndrome (MetS, OR = 2.4), with an overall prevalence of 32.5% in schizophrenia and 13.0% in first episode psychosis (FEP) (Mitchell et al., 2013; Vancampfort et al., 2013). MetS denotes a cluster of risk factors for CVD and type 2 diabetes mellitus, including central obesity, hypertension, elevated fasting glucose, hypertriglyceridemia and low levels of HDL-C (Alberti et al., 2009). Although CVD risk is higher in medicated chronic schizophrenia patients there is evidence of increased CVD risk factors in FEP and drug free patients, as well as in at-risk groups, such as ultra-high-risk groups and family members of patients with schizophrenia (Bradley & Dinan, 2010; Penninx & Lange, 2018; Petrikis et al., 2015; Spelman, Walsh, Sharifi, Collins, & Thakore, 2007).

Various factors may contribute to increased CVD risk in patients with schizophrenia. Factors that have been associated with increased MetS risk in meta-analyses include antipsychotic medication use, duration of illness, age and body mass index (BMI) (Mitchell et al., 2013; Penninx & Lange, 2018; Vancampfort et al., 2015, 2013). Lifestyle factors, such as smoking, alcohol and substance use, physical inactivity and poor nutrition also play a role (Carney, Cotter, Bradshaw, Firth, & Yung, 2016; De Hert, Detraux, & Vancampfort, 2018; Penninx & Lange, 2018). Despite higher rates of CVD, individuals with schizophrenia also have decreased access to and receipt of adequate medical care

(Laursen et al., 2014; Penninx & Lange, 2018; Walker, McGee, & Drus, 2015). The pathophysiology linking MetS and schizophrenia is not well understood, although there is evidence of shared pathways, including inflammation, mitochondrial dysfunction, oxidative stress, accelerated aging, epigenetic factors and shared genetic risk (Laursen et al., 2014; Malan-Müller et al., 2016; Penninx & Lange, 2018; Vancampfort et al., 2015).

A dysfunctional hypothalamic pituitary adrenal (HPA) axis has also been postulated to play a role in CVD morbidity in schizophrenia (Bradley & Dinan, 2010; De Hert et al., 2018; Penninx & Lange, 2018). Systematic reviews and meta-analyses demonstrate that patients with schizophrenia exhibit a dysregulated HPA-axis, with higher basal cortisol levels, a blunted cortisol awakening response (CAR) and a blunted cortisol stress response (Berger et al., 2016; Borges, Gayer-Anderson, & Mondelli, 2013; Bradley & Dinan, 2010; Gajsak, Gelemanovic, Kuzman, & Puljak, 2017; Girshkin, Matheson, Shepherd, & Green, 2014; Hubbard & Miller, 2019; Zorn et al., 2017). Chronically elevated cortisol could increase CVD risk in patients with schizophrenia due to consequent effects such as central obesity, lipid abnormalities, and insulin resistance (Bradley & Dinan, 2010; Penninx & Lange, 2018). The HPA-axis also regulates inflammatory and immune function and this can be another pathway through which a dysfunctional HPA-axis in patients with schizophrenia can contribute to increased CVD risk (Bradley & Dinan, 2010; Penninx & Lange, 2018). Although a dysregulated HPA-axis has been postulated to play a role in CVD in patients with schizophrenia, limited studies have investigated the links between schizophrenia, HPA-axis function and CVD risk. A study in FEP, drug naïve, schizophrenia patients found that patients had significantly higher fasting plasma glucose (FPG), insulin and cortisol levels than controls (Spelman et al., 2007). Although the authors did not report on correlations between metabolic measures and cortisol levels the results suggested synergistic effects related to metabolic risk and cortisol levels.

Although systematic reviews tend to demonstrate higher basal cortisol levels in schizophrenia, most studies have utilised measures reflecting acute or short-term cortisol release (Borges et al., 2013; Gajsak et al., 2017). In recent years measuring cortisol levels in hair has been established as a useful marker of longer-term HPA-axis function (Stalder et al., 2017; Wosu, Valdimarsdóttir, Shields, Williams,

& Williams, 2013). Studies are also increasingly using hair cortisol concentrations (HCC) to evaluate HPA-axis function in patients with schizophrenia (Aas et al., 2019; Andrade et al., 2016; Streit et al., 2016; Touskova et al., 2018). There are certain advantages to using HCC; hair sampling is non-invasive and samples can be stored and transported at room temperature, HCC are less influenced by acute factors such as the stress of sampling or diurnal variations, and provide a retrospective window of HPA-axis function spanning months (Stalder & Kirschbaum, 2012; Stalder et al., 2017). This is particularly useful when studying HPA-axis function in FEP drug-naive patients as HCC reflect average cortisol levels for the illness period prior to antipsychotic treatment initiation.

HCC, reflecting chronic cortisol release, can potentially provide additional insights into the role of HPA-axis dysfunction in relation to schizophrenia and associated CVD risk. Our objectives were thus in a sample of South African adults (i) to determine whether schizophrenia diagnostic status (in FEP and chronic schizophrenia patients) and clinical factors (schizophrenia disease related and self-perceived stress) were associated with HCC; (ii) to investigate for any interactions between schizophrenia and MetS (as well as individual CVD risk factors) on HCC (iii) to assess temporal change in HCC in FEP schizophrenia patients.

2 Methods

2.1 Study design

This is a neuroendocrine ancillary study to 'Understanding the SHARED ROOTS of Neuropsychiatric Disorders and Modifiable Risk Factors for Cardiovascular Disease' or SHARED ROOTS (SR). The SR study was a matched case-control study in three neuropsychiatric disease cohorts (schizophrenia, Parkinson's disease and posttraumatic stress disorder). Controls were matched to the schizophrenia cohort based on age, gender, and MetS status. The schizophrenia cohort consisted of two arms, a FEP arm and a chronic schizophrenia arm. The FEP patients were newly initiated on open-label flupenthixol decanoate (a depot antipsychotic) and the chronic cohort included patients with schizophrenia who had participated in a previous open-label flupenthixol decanoate study (Chiliza et

al., 2016). There was a longitudinal component to the FEP cohort and both patients and controls returned for follow-up assessments 12 months post baseline.

This study was approved by the Health Research Ethics Committee at Stellenbosch University (HREC N13/08/115) and conducted according to the principles of the seventh revision of the Declaration of Helsinki (World Medical Association, 2013). Written informed consent was obtained from all participants and a tiered consenting process was adhered to, allowing participants to opt-out of the hair sampling procedure. Participants were referred to their usual health care service providers for any medical or psychiatric problems that warranted further investigation and treatment.

2.2 Setting

Participants were enrolled over 3 years from May 2014 until June 2017 in Cape Town, South Africa. We utilised a purposive sampling approach and participants for all cohorts were recruited using multi-pronged strategies. FEP schizophrenia patients were recruited from general and psychiatric hospitals and community health centres (CHCs) within the study catchment area following their first psychotic episode. Control participants were recruited through (i) print, radio and web advertisements; (ii) active recruitment within communities by a registered nurse; and (iii) word of mouth by participants already recruited. The chronic schizophrenia cohort was contacted and invited to participate in SR.

2.3 Participants

All participants had to be 18 years and older, willing and able to provide informed consent and able to read and write in English or Afrikaans. Participants were excluded from hair sampling if they had hair length shorter than 3cm (see Figure 1 for a flow diagram of schizophrenia cohort participants included in this study). Patients with a diagnosis of schizophrenia, schizophreniform, or schizoaffective disorder based on the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (SCID-IV) (First, Spitzer, Gibbon, & Williams, 1997) and without intellectual disability were eligible for inclusion. FEP, but not chronic, schizophrenia patients were excluded if they had received prior antipsychotic treatment (barring the few days prior to inclusion in the study). Further exclusion criteria applied in this study were, (i) systemic or scalp steroid use, (ii) current pregnancy, or pregnancy within the prior three months, and (iii) significant medical comorbidity (e.g., HIV, auto-

immune disorders). Exclusion criteria applied only to controls were lifetime or current serious psychiatric disorder (psychotic disorder or bipolar disorder) or the presence of any current psychiatric disorder as based on the history, the Mini International Neuropsychiatric Interview (MINI) or current psychiatric medication use. Patients with schizophrenia were permitted to have psychiatric comorbidity (substance use disorders (SUDs) and common mental disorders (CMDs) provided the primary diagnosis, based on diagnostic interview with the SCID and clinician assessment, was schizophrenia, schizophreniform, or schizoaffective disorder and not a substance induced psychotic disorder or a mood disorder with psychotic features.

MetS status was determined based on the harmonized Joint Interim Statement (JIS) criteria (Alberti et al., 2009), with the presence any three of the following five risk factors required for a diagnosis of MetS: (i) Raised blood pressure (BP), systolic ≥ 130 and/or diastolic ≥ 85 mmHg, or being on antihypertensive treatment; (ii) Elevated triglycerides > 1.70 mmol/l (150 mg/dl) or being on treatment for hypertriglyceridemia; (iii) Reduced HDL-C, < 1.0 mmol/l (40mg/dl) in males, and < 1.3 mmol/l (50mg/dl) in females, or being on treatment for low HDL-C; (iv) Elevated fasting glucose ≥ 5.6 mmol/l (100 mg/dl) or on treatment for diabetes; (v) Elevated waist circumference according to population and country specific guidelines. We used a waist circumference ≥ 90cm in both males and females, as a recent validation study in the Western Cape showed this to be the optimal WC cut-off in the source population (Matsha et al., 2013).

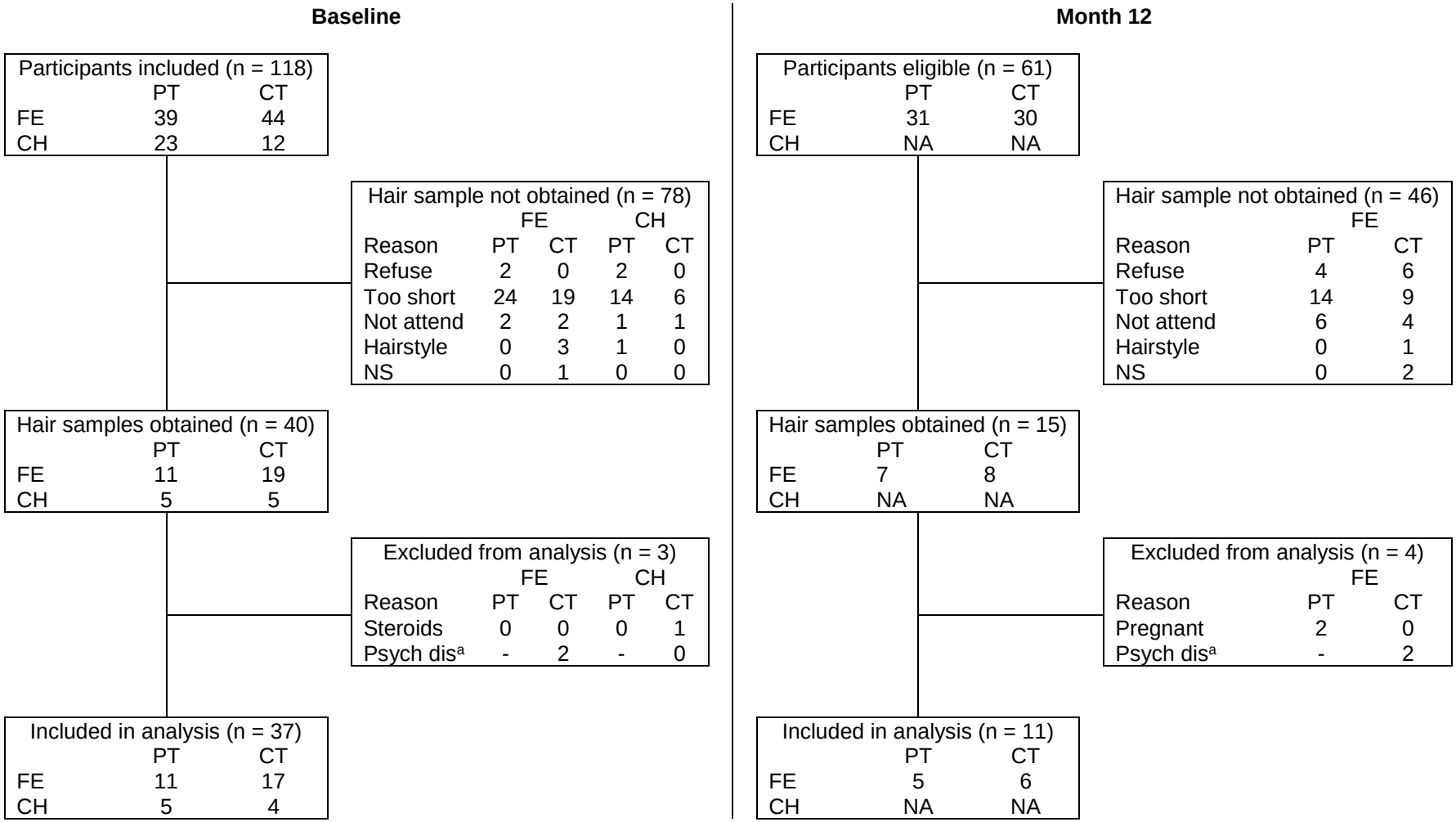

Figure 1 Flow diagram demonstrating inclusion and exclusion of participants

[a] Exclusion criteria only applicable to controls

CH, Chronic; CT, control; FE, first episode; hairstyle, hairstyle prohibiting sampling; NA, not applicable; NS, not specified; Refuse, did not provide consent for hair sampling; PT, patient; Psych dis, psychiatric disorder; steroids, systemic steroid use; too short, hair shorter than 3cm

2.4 Procedures

Participants in SR attended two to three study visits, each lasting around 3 hours. Diagnostic, clinical and participant-administered measures were completed at the first visit. The second visit occurred within 24-96 hours of the first visit and involved physical procedures (e.g. fasting blood sampling, physical measurements) and additional lifestyle measures. Assessments were conducted in English or Afrikaans, depending on the language preference of participants. Psychiatrists conducted diagnostic and clinical assessments. Research nurses performed physical procedures and personally administered the participant-administered measures to all participants, to aid comprehension and to accommodate variations in reading level. All measures performed at baseline were repeated at 12-month follow-up assessment (with the exception of measures that were not appropriate for repeated administration).

2.4.1 Physical measurements

Physical measurements were conducted consistently and methodically in accordance with the WHO stepwise approach to chronic disease risk factor surveillance (WHO STEPS) instrument, a standardised instrument designed for non-communicable disease surveillance (World Health Organization, 2005). The following physical parameters were included in analysis: (i) mean systolic (SBP) and diastolic (DBP) blood pressure (mmHg); (ii) mean heart rate (HR, bpm); (iii) body mass index (BMI, kg/m^2); (iv) waist circumference (WC, cm); (v) hip circumference (HC, cm); and (vi) waist-to-hip ratio (WHR).

2.4.2 Blood samples for metabolic parameters

Venous blood samples were obtained following an overnight fast (at least 8 hours). Samples were analysed on the Cobas 6000 c501 Chemistry Analyser (Roche, Germany) at a commercial internationally accredited laboratory (Lancet laboratories) on the same day as sample collection. The following laboratory parameters were included in analysis: (i) fasting plasma glucose (FPG, mmol/l); (ii) triglycerides (trig, mmol/l); (iii) HDL-C (mmol/l); (iv) low-density lipoprotein cholesterol (LDL-C, mmol/l); (v) total cholesterol (TC, mmol/l); (vi) and glycated haemoglobin A1c (HbA1c, %). Values for TC > 5

mmol/l, LDL-C >= 3 mmol/l, and HbA1c >= 6.5% were considered to be elevated (Klug et al., 2018; SEMDSA Type 2 Diabetes Guidelines Expert Committee., 2017).

2.4.3 Hair sampling

Hair samples from the posterior vertex scalp were cut close to the scalp with fine scissors. Samples were secured, placed into aluminium foil, labelled and sealed inside an envelope. All samples were stored in dark containers at room temperature. In both the FEP and chronic cohort hair sampling was done at baseline and in the FEP cohort again at 12 months follow-up.

2.4.4 Hair analysis

Samples were sent for analysis at the TU Dresden laboratory (Prof Clemens Kirschbaum) in December 2017 and were analysed using an established liquid chromatography-tandem mass spectrometry (LC-MS/MS) protocol (Gao et al., 2013). LC-MS/MS is the benchmark (gold standard) method for hair cortisol determination and different laboratories using LC-MS/MS have demonstrated almost identical results (r = 0.98) (Russell et al., 2015). The proximal 3cm of the hair segments were used for analysis, representing cortisol secretion for the prior 3 months based on an accepted growth rate of 1cm per month (Wennig, 2000).

2.5 Measures

2.5.1 Demographic questionnaire

Demographic details were collected with a demographic questionnaire. Demographic variables included in this manuscript are self-identified gender (male or female), self-identified ethnicity (mixed ancestry [coloured] or other), age in years, highest level of education (HLOE, defined according to whether secondary education was completed), and employment status (defined as being employed for the greater part of the prior 12 months).

2.5.2 Medical history questionnaires

Medical and psychiatric histories were collected with comprehensive medical questionnaires. Clinical items reported on in this manuscript are: (i) steroid use in the prior 6 months (split according to whether any systemic steroids were used (oral or parenteral), topical steroids where used on the scalp, and any

other topical steroid use (dermatologic, inhaled, nasal), (ii) hormonal contraceptive use in prior 6 months (including oral contraceptives and other formulations, such as implants and injectable contraceptives), (iii) current breastfeeding in women, (iv) current psychiatric medication use, (v) statin use, (vi) aspirin use, (vii) any other current medical conditions, and (viii) self-reported tobacco, alcohol, and illicit substance use in the prior six months.

2.5.3 The Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998)

The MINI version 6.0 was used to determine the presence of any current and lifetime psychiatric disorders. The MINI is a short structured diagnostic interview used to diagnose psychiatric disorders based on Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and International Classification of Diseases (ICD-10) criteria.

2.5.4 Schizophrenia clinical assessments

Symptom severity in schizophrenia patients was assessed with the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). We report on the total score (range 30 – 210, Cronbach's α = 0.94); positive scale (7 – 49, Cronbach's α = 0.86), negative scale (7 – 49, Cronbach's α = 0.95), and general psychopathology scale (16 – 112, Cronbach's α = 0.89). Putative remission status was determined based on established criteria (symptom severity rated mild or less on PANSS items P1-3, N1, N4, N6, G5 and G9) (Andreasen et al., 2005), but without the time criterion as we could not establish whether symptoms had been below the threshold for at least 6 months. Functional impairment was rated with the Social and Occupational Functioning Assessment Scale (SOFAS) (Association, 2000) and the Clinical Global Impression (CGI-S) scale (Guy, 1976) was applied to provide a global impression of mental illness severity. We assessed insight into illness with the Birchwood Insight Scale (BIS) (Birchwood et al., 1994). The BIS assesses for three factors related to insight; awareness of illness, need for treatment and attribution of symptoms. The scale contains 8 items with total scores ranging from 0-12, with higher scores reflecting better insight. Cronbach's α in this sample was 0.67. We also report on the time since onset of psychosis, time since diagnosis and duration of untreated psychosis (DUP).

2.5.5 The Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983)

We used the 10-item PSS (PSS-10) to measure self-perceived stress. Individuals rate to what extent they found their lives unpredictable, uncontrollable or overloaded in the prior month. Each item is rated on a 5-point Likert scale (0 = Never; 1 = Almost Never; 2 = Sometimes; 3 = Fairly often; 4 = Very often) with a total score ranging from 0 – 40, and higher scores indicate higher perceived stress. The PSS has been widely used and has demonstrated adequate psychometric properties in multiple settings and languages (Lee, 2012) and Cronbach's α in the schizophrenia cohort was 0.87.

2.5.6 The WHO stepwise approach to chronic disease risk factor surveillance (WHO STEPS) (World Health Organization, 2005)

The level of physical activity was determined with the global physical activity questionnaire (GPAQ) in the WHO STEPS. The GPAQ assesses average physical activity for a week occurring across three domains, namely work, travel and recreation. Level of physical activity (high, moderate, and low) was determined according to the GPAQ guidelines (World Health Organization, 2005).

2.5.7 Hair questionnaire

Participants who provided hair samples were questioned about their hair characteristics and hair care practices. Hair related variables reported in this manuscript include (i) whether natural hair colour is black, (ii) whether hair had been chemically treated in the prior three months (including colouring, relaxing and perming), (iii) the frequency of hair washing (two or more times a week, once a week, less frequent than once a week), (iv) whether any additional hair products had been used in the prior three months (such as hair styling and care products e.g. gels, oils and creams), and (v) average hours per day that hair was exposed to sunlight (less than one hour, 1-2 hours, more than 2 hours). Additional hair related factors included the season sampled (based on the month the sample was obtained in) and the duration of storage before sending the samples for analysis (less than 1 year, 1-2 years, 2-3.5 years).

2.6 Statistical analysis

Sample size estimations for the neuroendocrine study were based on hair cortisol studies in PTSD and MetS samples (Stalder et al., 2013; Steudte et al., 2012) as no studies had been done in schizophrenia

at the point that the study was conceptualised. The overall sample size was, however, limited by recruitment of the parent study as well as by factors influencing hair sampling, such as short hair length. HCC were not normally distributed and were positively skewed (Clark, Osborne, Gallagher, & Watson, 2016; Miller & Plessow, 2013). Utilising the box-cox family of power transformations (Osborne, 2010) HCC were reciprocal square root transformed to meet the assumption of normality. There were no outliers 3SD beyond the mean. We compared the included and excluded sample with Pearson chi-square for categorical variables, and Mann Whitney tests for continuous variables. We compared the schizophrenia patients and controls with Pearson chi-square or Fisher's exact tests for categorical variables, and independent sample t-tests for continuous variables. To determine whether HCC were associated with a diagnosis of schizophrenia, schizophrenia symptom severity, and self-perceived stress we conducted simple and multiple linear regression models with HCC as the dependent variable. Covariates included in models were age and sex. We did not include other possible covariates for HCC due to the small sample size and as none of the factors remained significantly associated with HCC once we controlled for age, sex and case-control status. To assess whether HCC was associated with putative remission status we conducted an exploratory analysis by combining the data pertaining to HCC and remission status for the chronic patients at baseline with the FEP patients at month-12 follow-up. Interaction effects between MetS and schizophrenia diagnosis were assessed by regressing MetS on HCC in unadjusted, and then in adjusted models that included covariates alongside schizophrenia diagnosis. We examined for any interaction effects between schizophrenia diagnostic status and MetS. In exploratory analysis we also examined for associations between the individual CVD risk variables and HCC as well as for interactions with schizophrenia. To assess for change in HCC over time between FEP patients and controls and those with and without MetS we used a linear mixed model, with restricted maximum likelihood and an unstructured covariance matrix. To assess for overall change in HCC over time we first included only the events (baseline, month-12), we then included case status and the interaction between case status and events, finally we included MetS and the interaction between MetS and events as fixed effects. To account for within subject correlations we included the participant ID as well as the intercept as random effects. Data were

analysed with SPSS for windows, version 25.0, all tests were 2-tailed and the level of significance was set at .05.

3 Results

3.1 Participants

At baseline hair samples were obtained in 40 (33.9%) of the 118 participants included in the schizophrenia cohort, a further 3 participants were excluded from analysis, leaving a final sample of 37 participants; 16 patients and 21 controls (see Figure 1 for details). Hair length shorter than 3cm was the main factor (80.8%) limiting sampling. The following factors differed significantly between included and excluded samples: gender ($p < 0.001$), HLOE ($p = 0.009$), HDL-C ($p = 0.033$), trig ($p = 0.033$), and WHR ($p = 0.019$). When we controlled for gender the only factor that remained significantly different between included and excluded samples was HLOE ($p = 0.033$), such that the included sample had a higher percentage of individuals who had completed secondary education than the excluded sample.

At month-12 follow-up hair samples were obtained in 15 (29.4) of the 51 FEP participants who returned for follow-up assessments, a further 4 participants were excluded from analysis, leaving a final sample of 11 participants; 5 FEP patients and 6 controls (see Figure 1 for details). The only factor that differed between FEP cohort participants who returned for month-12 assessments as compared to those who did not was that participants who returned were more likely to be female ($p = 0.006$).

Descriptive data of the sample are presented in Table 1. Factors that were significantly different between the patients and controls, included ethnicity ($p = 0.010$), employment status ($p = 0.039$) and illicit substance use ($p = 0.010$). Schizophrenia disease related factors are presented in Table 2. At baseline the clinical variables that differed between FEP and chronic patients were time since psychosis onset ($p = 0.011$), time since diagnosis ($p = 0.002$), PANSS total ($p = 0.018$) and positive scale ($p = 0.001$) scores, SOFAS score ($p = 0.045$), CGI-S ($p = 0.001$) and antipsychotic treatment ($p = 0.003$). PANSS total ($p = 0.043$) and positive scale ($p = 0.043$) scores changed significantly from baseline to month-12 in patients with FEP who provided samples at month-12.

Table 1 Comparison of sociodemographic, hair related, behavioural, and clinical factors between schizophrenia patients and controls

Variables	Patients	Controls	Test statistic	p-value
Total	16	21		
Cohort n(%)			$\chi^2(1) = 0.74$	0.458
First episode	11 (68.8)	17 (81.0)		
Chronic	5 (31.3)	4 (19.0)		
Socio-demographic				
Age *M(SD)*	30.1 (6.9)	26.4 (5.8)	t (35) = -1.77	0.085
Female n(%)			$\chi^2(1) = 1.60$	0.371
No	4 (25.0)	2 (9.5)		
Yes	12 (75.0)	19 (90.5)		
Mixed ancestry ethnicity n(%)			$\chi^2(1) = 7.59$	0.010*
No	11 (68.8)	21 (100.0)		
Yes	5 (31.3)	0 (0.0)		
Secondary education complete n(%)			$\chi^2(1) = 0.12$	0.729
No	7 (43.8)	8 (38.1)		
Yes	9 (56.3)	13 (61.9)		
Employed n(%)			$\chi^2(1) = 4.26$	0.039*
No	10 (62.5)	6 (28.6)		
Yes	6 (37.5)	15 (71.4)		
Hair related				
Natural hair colour black n(%)			$\chi^2(1) = 0.19$	0.705
No	12 (75.0)	17 (81.0)		
Yes	4 (25.0)	4 (19.0)		
Hair chemically treated n(%)			$\chi^2(1) = 0.27$	0.603
No	9 (56.3)	10 (47.6)		
Yes	7 (43.8)	11 (52.4)		
Frequency of hair washing n(%)			$\chi^2(2) = 2.35$	0.309
≥ 2 times a week	8 (50.0)	7 (33.3)		
1 time a week	7 (43.8)	9 (42.9)		
< 1 time a week	1 (6.3)	5 (23.8)		
Add on hair products n(%)			$\chi^2(1) = 1.21$	0.272
No	9 (56.3)	8 (38.1)		
Yes	7 (43.8)	13 (61.9)		
Average hours in sun n(%)			$\chi^2(2) = 0.77$	0.679
< 1 hour	8 (50.0)	11 (52.4)		
1-2 hours	4 (25.0)	3 (14.3)		

> 2 hours	4 (25.0)	7 (33.3)		
Season sampled n(%)			$\chi^2(3) = 3.14$	0.371
Summer	5 (31.3)	6 (28.6)		
Autumn	1 (6.3)	6 (28.6)		
Winter	6 (37.5)	5 (23.8)		
Spring	4 (25.0)	4 (19.0)		
Duration of storage n(%)			$\chi^2(2) = 2.33$	0.311
Less than 1 year	1 (6.3)	5 (23.8)		
1 - 2 years	8 (50.0)	10 (47.6)		
2 – 3.5 years	7 (43.8)	6 (28.6)		
Clinical				
Hormonal contraceptive n(%)			$\chi^2(1) = 3.03$	0.128
No	10 (83.3)	10 (52.6)		
Yes	2 (16.7)	9 (47.4)		
Currently breastfeeding n(%)			$\chi^2(1) = 2.10$	0.265
No	12 (100.0)	16 (84.2)		
Yes	0 (0.0)	3 (15.8)		
Topical steroid use n(%)			$\chi^2(1) = 0.04$	1.000
No	15 (93.8)	20 (95.2)		
Yes	1 (6.3)	1 (4.8)		
Other medical conditions n(%)			$\chi^2(1) = 1.27$	0.315
No	11 (75.0)	12 (57.1)		
Yes	4 (25.0)	9 (42.9)		
Behavioural				
Tobacco use n(%)			$\chi^2(1) = 3.11$	0.078
No	6 (37.5)	14 (66.7)		
Yes	10 (62.5)	8 (33.3)		
Alcohol use n(%)			$\chi^2(1) = 0.00$	0.957
No	7 (43.8)	9 (42.9)		
Yes	9 (56.3)	12 (57.1)		
Illicit substance use[a] n(%)			$\chi^2(1) = 7.59$	0.010*
No	11 (68.8)	21 (100.0)		
Yes	5 (31.3)	0 (0.0)		
Level of physical activity n(%)			$\chi^2(2) = 1.10$	0.576
High	3 (18.8)	6 (28.6)		
Moderate	8 (50.0)	6 (33.3)		
Low	5 (31.3)	8 (38.1)		
Perceived stress				
PSS total score *M(SD)*	24.5 (8.1)	15.1 (6.2)	t (35) = -3.98	<0.001*

Hair glucocorticoid levels				
HCC *Mdn(IQR)*	7.0 (3.3; 18.7)	22.1 (11.0; 31.8)	U=81; Z=-2.67	0.008*
Transformed HCC *M(SD)*	1.25 (0.31)	1.51 (0.28)	t (35) = 2.75	0.009*

[a] 2 using cannabis, 1 using methamphetamine, 1 using cannabis and methamphetamine, and 1 using cannabis and methaqualone
* $p < 0.05$
HCC, hair cortisol concentration; PSS, perceived stress scale

Table 2 Association between clinical variables and hair cortisol concentrations in schizophrenia patients

Variables	**Baseline FEP**	**Chronic**	**Follow-up FEP**	**Baseline comparison Test statistic**	**p-value**	**HCC ß (95% CI)**
	n = 11	**n = 5**	**n = 5**			
Age at first psychosis	25.8 (4.1)	26.8 (6.7)		t (14) = -0.35	0.732	-0.01 (-0.04; 0.03)
Months since psychosis onset *Mdn (IQR)*	3.8 (3.1; 37.6)	104 (80; 106)		U=71; Z=-2.55	0.011*	0.00 (-0.00; 0.00)
Months since diagnosis *Mdn (IQR)*	0.23 (0.20; 0.49)	77 (93; 102)		U=66; Z=-3.14	0.002*	0.00 (-0.00; 0.01)
DUP (months) *Mdn (IQR)*	2.7 (3.6; 37.4)	4.9 (1.7; 9.0)		U=36; Z=-0.74	0.461	-0.01 (-0.01; 0.00)
PANSS total score *M (SD)*	84.8 (23.2)	55.4 (9.9)	57.6 (10.1)[a]	t (14) = 2.69	0.018*	0.00 (-0.01; 0.01)
Total P score *M (SD)*	23.8 (5.0)	12.2 (4.6)	10.2 (2.9)[a]	t (14) = 4.42	0.001*	-0.01 (-0.04; 0.01)
Total N score *M (SD)*	22.6 (8.4)	17.6 (3.9)	20.2 (5.1)	t (14) = 1.24	0.234	0.01 (-0.02; 0.03)
Total G score *M (SD)*	38.5 (13.6)	25.6 (5.3)	27.2 (9.3)	t (14) = 2.01	0.064	0.00 (-0.01; 0.02)
BIS *M (SD)*	7.5 (2.9)	6.4 (2.1)	5.5 (2.9)	t (14) = 0.72	0.483	-0.06 (-0.12; -0.01)*
SOFAS *M (SD)*	44.2 (9.2)	57.8 (15.7)	53.2 (12.4)	t (14) = -2.20	0.045*	-0.00 (-0.02; 0.01)
CGI-S *M (SD)*	4.9 (0.5)[b]	3.2 (1.1)[c]	3.0 (0.7)[d]	t (14) = 4.27	0.001*	-0.02 (-0.18; 0.14)
Antipsychotic treatment				$\chi^2(2) = 11.7$	0.003*	
Flupenthixol decanoate - initiated	11 (100.0)	1 (20.0)[e]	0 (0.0)			0.13 (-0.35; 0.61)
Flupenthixol decanoate - ongoing	0 (0.0)	2 (40.0)	5 (100.0)			0.53 (-0.09; 1.16)
Other	0 (0.0)	2 (40.0)[f]	0 (0.0)			Ref
Other psychiatric medications				$\chi^2(1) = 4.75$	0.063	
No	1 (9.1)	3 (60.0)	2 (40.0)			Ref
Yes	10 (90.9)[g]	2 (40.0)[h]	3 (60.0)[i]			-0.05 (-0.45; 0.34)
Psychiatric comorbidity n(%)				$\chi^2(1) = 2.42$	0.245	
No	7 (72.7)	5 (100.0)	4 (80.0)			Ref
Yes	4 (27.3)[j]	0 (0.0)	1 (20.0)[k]			0.09 (-0.30; 0.48)

* $p < 0.05$
[a] Significant change ($p < 0.05$) from baseline based on Wilcoxon signed rank test
[b] 2 (18.2%) moderately ill, 8 (72.7%) markedly ill, 1 (9.1%) severely ill
[c] 1 (20%) borderline mentally ill, 3 (60.0%) mildly ill, 1 (20.0%) markedly ill
[d] 1 (20%) borderline mentally ill, 3 (60.0%) mildly ill, 1 (20.0%) markedly ill

[e] flupenthixol decanoate was restarted in 1 chronic schizophrenia patient
[f] 1 receiving risperidone depot and 1 receiving clozapine and amisulpiride
[g] 9 (81.8%) were on short-term benzodiazepines (BZD) and 2 (18.2%) on anticholinergics
[h] 2 (40.0%) were on antidepressants, 1 (20.0%) on lithium, and 1 (20.0%) on anticholinergics
[i] 2 (40.0%) on antidepressants, 1 (20.0%) on lithium, 2 (40.0%) on anticholinergics.
[j] Diagnosed based on the MINI; 2 (12.5%) with a comorbid substance use disorder; 1 (16.3%) with panic disorder, agoraphobia and generalised anxiety disorder (GAD) and 1 (6.3%) with social anxiety disorder and a substance use disorder
[k] 1 with MDD and agoraphobia

BIS, Birchwood Insight Scale; CGI, Clinical Global Impression; CMD, common mental disorder; DUP, duration of untreated psychosis; FEP, first episode; HCC, hair cortisol concentration; MINI, the Mini International Neuropsychiatric Interview; PANSS, the Positive and Negative Syndrome Scale; SOFAS, Social and Occupational Functioning Assessment Scale; SUD, substance use disorder;

Table 3 Schizophrenia and metabolic syndrome regressed on hair cortisol concentrations (HCC)

	Simple linear regression		**Multiple linear regression[a]**					
			Model 1		**Model 2**		**Model 3**	
	ß (95% CI)	**p-value**	**ß (95% CI)**	**p-value**	**ß (95% CI)**	**p-value**	**ß (95% CI)**	**p-value**
Case status								0.020*
Control	Ref		Ref		Ref		Ref	
Patient	-0.27 (-0.47; -0.07)	0.009*	-0.27 (-0.48; -0.06)	0.014*	-0.27 (-0.48; -0.06)	0.014*	-0.24 (-0.48; -0.00)	0.048*
MetS								0.434
No	Ref				Ref		Ref	
Yes	0.04 (-0.26; 0.33)	0.806			0.08 (-0.21; 0.37)	0.557	0.23 (-0.25; 0.71)	0.337
Patient*MetS							-0.22 (-0.81; 0.36)	0.443

[a] Multivariate adjusted model (adjusted for age and sex); n = 37
Model 1 – Model without MetS: F (3, 33) = 3.21 (p = 0.036*), Adj R^2 = 0.156; n = 37
Model 2 – MetS added: F (4, 32) = 2.45 (p = 0.066), Adj R^2 = 0.139; n = 37
Model 3 – Interaction between case status and MetS added: F (5, 31) = 2.06 (p = 0.098), Adj R^2 = 0.128; n = 37
* p < 0.05
MetS, metabolic syndrome

3.2 Schizophrenia and hair cortisol concentrations

The HCC for cases and controls at each time-point are illustrated in Figure 2. At baseline HCC were significantly lower in patient than controls (Cohen's d = 0.88) in unadjusted (*B* = -0.27, 95% CI -0.47; -0.07, *p* = 0.009) and adjusted analyses (adj *B* = -0.27, 95% CI -0.48; -0.06, *p* = 0.014) (Table 3). When analysed according to cohort, patients with FEP also had significantly lower HCC than controls (Cohen's d = 0.97) in unadjusted (*B* = -0.30, 95% CI -0.54; -0.06, *p* = 0.017) and adjusted analyses (adj *B* = -0.28, 95% CI -0.53; -0.03, *p* = 0.030) (Figure 3a). Exploratory analysis examining HCC in relation to putative remission status revealed that non-remitters (n = 5) had significantly lower HCC (*B* = -0.44, 95% CI -0.80; -0.07, *p* = 0.025) than remitters (n = 5) and when compared to controls non-remitters (n = 5), but not remitters (n = 5), had significantly lower HCC than controls (*B* = -0.41, 95% CI -6.69; -0.13, *p* = 0.006) (Figure 3b).

Overall sample

	Baseline	
	PT	**CT**
Total	n = 16	n = 21
HCC	1.25 (0.31)*	1.51 (0.28)*
FEP	n = 11	n = 17
HCC	1.19 (0.30)*	1.48 (0.30)*
CHR	n = 5	n = 4
HCC	1.37 (0.31)	1.64 (0.17)

	Month-12	
	PT	**CT**
Total	n = 5	n = 6
HCC	1.27 (0.37)	1.27 (0.30)
FEP	n = 5	n = 6
HCC	1.27 (0.37)	1.27 (0.30)

HCC for baseline and month-12

	Baseline	
	PT	**CT**
FEP	n = 4	n = 5
HCC	1.10 (0.22)*	1.51 (0.26)*

	Month-12	
	PT	**CT**
FEP	n = 4	n = 5
HCC	1.22 (0.41)	1.34 (0.27)

Figure 2 Hair cortisol concentrations according to diagnostic groups and events

Figure illustrating HCC *M*(*SD*) according to diagnostic groups and events. The only significant differences were between patients and controls at baseline. HCC were available for both baseline and month-12 in 4 FEP patients and 4 controls.
* significant difference between patients and controls, p < 0.05
CHR, Chronic; CT, control; FEP, first episode; HCC, hair cortisol concentration, PT, patient

3.2.1 Schizophrenia clinical variables and hair cortisol concentrations (Table 2)

Although not significant HCC were inversely associated with PANSS positive symptoms scores and positively associated with negative, general and total symptom scores. The BIS insight score was significantly associated with HCC at baseline in unadjusted (*B* = -0.06, 95% CI -0.12; -0.01, *p* = 0.028) and adjusted (*adj B* = -0.07, 95% CI -0.13; -0.01, *p* = 0.037) analyses.

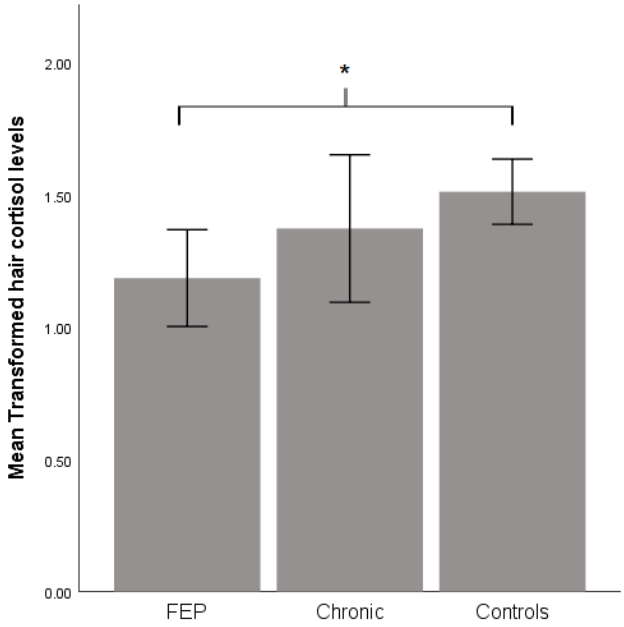

Figure 3a Baseline hair cortisol concentrations (HCC) according to diagnostic status

Bar chart demonstrating mean baseline HCC levels according to diagnostic status. The model was significant (F(34, 2) = 4.5, *p* = 0.018) and HCC were lowest in first episode psychosis (FEP) patients and were significantly lower than controls (*p* = 0.013). There were no other significant differences between the groups based on Tukey HSD post hoc test.
*$p < 0.05$
Error bars ± 2SE

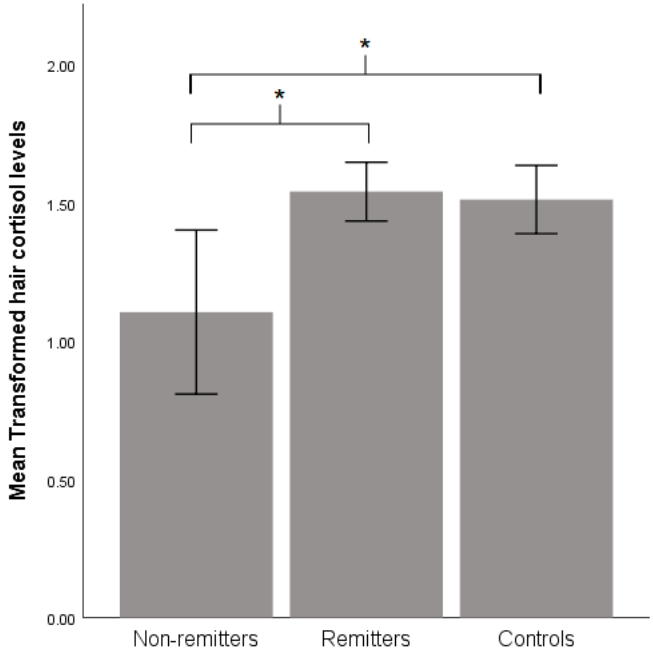

Figure 3b Hair cortisol concentrations (HCC) according to putative remission status

Bar chart demonstrating mean HCC levels according to putative patient remission status and controls. The model was significant (F(28, 2) = 4.8, p = 0.016) and HCC were lowest in non-remitters (n = 5) and were significantly lower than controls (n = 22, p = 0.015) and putative remitters (n = 5, p = 0.045). There were no other significant differences between the groups based on Tukey HSD post hoc test.
*p < 0.05
Error bars ± 2SE

3.3 Self-perceived stress, schizophrenia and hair cortisol concentrations

PSS scores were significantly higher in schizophrenia patients than in controls (p < 0.001) at baseline, but not at follow-up (p = 0.667) in the FEP participants who provided hair samples at month-12. PSS scores were not significantly associated with HCC in unadjusted (B = -0.01, 95% CI -0.02; 0.01, p = 0.449) and adjusted (adj B = 0.01, 95% CI -0.01; 0.02, p = 0.527) analyses at baseline. PSS scores were significantly associated with HCC at month-12 follow-up in unadjusted analysis (B = -0.02, 95% CI -0.04; -0.00, p = 0.022) and demonstrated a trend toward significance in adjusted analysis (adj B = -0.02, 95% CI -0.04; 0.00, p = 0.054). There were no significant interaction effects between schizophrenia diagnosis and PSS scores at baseline or month-12 follow-up.

3.4 Cardiovascular disease risk factors, schizophrenia and hair cortisol concentrations

3.4.1 Metabolic syndrome, schizophrenia and hair cortisol concentrations

Rates of MetS were non-significantly higher in schizophrenia patients than in controls ($p = 0.371$), and two FEP and two chronic schizophrenia patients had MetS. MetS was not significantly associated with HCC in unadjusted ($B = 0.04$, 95% CI -0.26; 0.33, $p = 0.806$) and adjusted (adj $B = 0.08$, 95% CI -0.21; 0.37, $p = 0.557$) analyses at baseline (Table 3). The interaction between schizophrenia and MetS was not significant (adj $B = -0.22$, 95% CI -0.81; 0.36, $p = 0.443$). In FEP participants who provided hair samples at month-12, MetS was present in 3 of the patients and 3 of the controls and HCC were significantly higher in those with MetS in unadjusted ($B = 0.42$, 95% CI 0.10; 0.75, $p = 0.017$) and adjusted (adj $B = 0.41$, 95% CI 0.02; 0.80, $p = 0.041$) analyses. The interaction between schizophrenia and MetS was not significant (adj $B = -0.10$, 95% CI -1.09; 0.89, $p = 0.806$) at month-12.

3.5 Individual cardiovascular disease risk factors, schizophrenia and hair cortisol concentrations (Supplementary table 1)

CVD risk factors that differed significantly between patients and controls included triglycerides ($p = 0.033$), WHR ($p = 0.008$) and HR ($p = 0.004$). When we controlled for age and gender only HR ($p = 0.026$) remained significantly different. None of the individual CVD risk factors were significantly associated with HCC in unadjusted or adjusted analyses and there were no significant interactions between schizophrenia and any of the CVD variables on HCC. HR demonstrated a trend toward significance in adjusted analyses (adj $B = 0.01$, 95% CI 0.00; 0.01, $p = 0.054$).

At month-12 follow-up HDL-C MetS criterion was associated with HCC in unadjusted ($B = 0.42$, 95% CI 0.10; 0.75, $p = 0.017$) and adjusted (adj $B = 0.41$, 95% CI 0.02; 0.80, $p = 0.041$) analyses. HDL-C was also associated with HCC in unadjusted ($B = -0.71$, 95% CI -1.20; -0.23, $p = 0.008$) and adjusted (adj $B = -0.70$, 95% CI -1.28; -0.12, $p = 0.024$) analyses. Number of MetS criteria showed a trend towards significance in unadjusted ($B = 0.13$, 95% CI -0.01; 0.26, $p = 0.058$) analyses, but was not significant in adjusted (adj $B = 0.12$, 95% CI -0.04; 0.28, $p = 0.120$) analyses.

Table 4 Fixed Effects of schizophrenia diagnosis and metabolic syndrome on change in hair cortisol concentrations (HCC) from baseline to month-12

	ß (95% CI)	S.E	df	t	p-value
Events					0.840
Baseline	Ref				
Month-12	-0.40 (-0.67; -0.12)	-0.12	9.49	-3.23	0.010*
Case status					0.257
Control	Ref				
Patient	-0.27 (-0.52; -0.03)	0.12	25.93	-2.27	0.032*
Month-12*patient	0.29 (-0.06; 0.63)	0.15	8.53	1.90	0.091
MetS					0.419
No	Ref				
Yes	-0.13 (-0.50; 0.23)	0.17	15.07	0.79	0.443
Month-12*MetS	0.47 (0.04; 0.90)	0.19	9.92	2.41	0.037*

-2 RLL = 21.15, n =30
* p < 0.05
MetS, metabolic syndrome

3.6 Schizophrenia, MetS and temporal change in HCC

HCC did not change significantly overall between baseline and month-12 (*B* = -0.07, 95% CI -0.27; 0.13, *p* = 0.443). When case status and the interaction between case status and events were added to the model there were no overall effects demonstrated for events (*p* = 0.649) nor case status (*p* = 0.389). HCC were significantly lower in patients than controls at baseline (*adj B* = -0.28, 95% CI -0.51; -0.04, *p* = 0.022) and did not change significantly from baseline to month-12 in controls (*adj B* = -0.21, 95% CI -0.48; 0.06, *p* = 0.116). The interaction between events and case status demonstrated a trend to significance (*adj B* = 0.33, 95% CI -0.07; 0.73, *p* = 0.097), such that HCC increased in patients from baseline to month-12. The results of the final model with MetS and the interaction between MetS and events are included in Table 4. The final model illustrated that in controls HCC decreased significantly from baseline to month-12 (*p* = 0.010), HCC were also significantly lower in patients than controls at baseline (*p* = 0.032), again there was a trend to significance demonstrated (*p* = 0.091) for HCC increasing in patients from baseline to month-12 (Figure 4a). HCC did not differ significantly between those with and without MetS at baseline (*p* = 0.443), but the interaction between MetS and events was significant, such that HCC increased significantly (*p* = 0.037) from baseline to month-12 in those with MetS (Figure 4b). Adding an interaction between case status and MetS and case status, MetS and events did not improve the model and these terms were not associated with HCC.

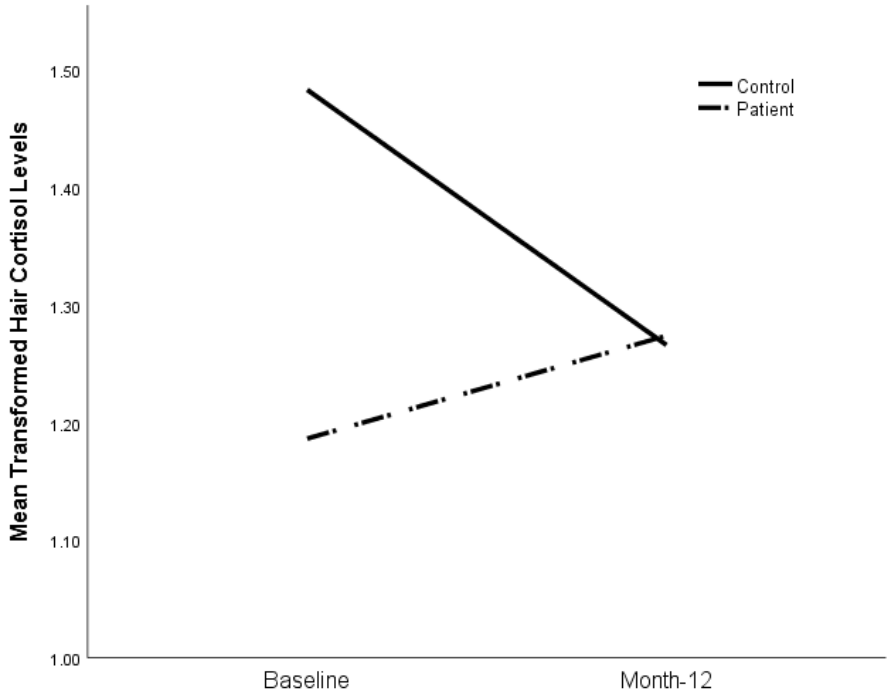

Figure 4a Change in hair cortisol concentrations (HCC) between patients and controls from baseline to month-12 follow-up

Figure illustrating the change in mean HCC between FEP patients and controls between baseline and month-12 follow-up. HCC increased from baseline to month-12 in FEP patients, demonstrating a trend to significance (*adj B* = 0.33, 95% CI -0.07; 0.73, *p* = 0.097).

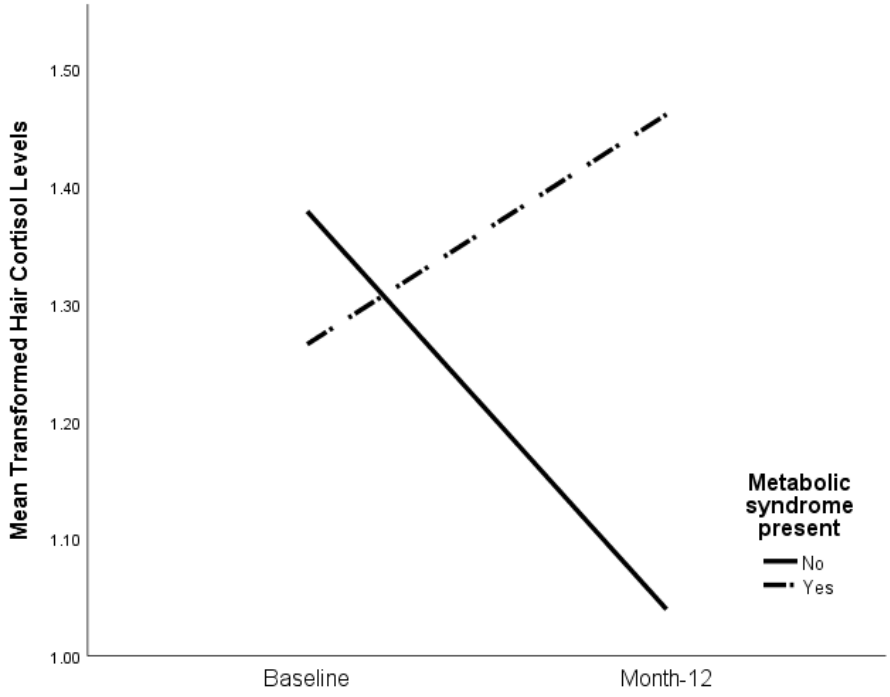

Figure 4b Change in hair cortisol concentrations (HCC) in relation to metabolic syndrome (MetS) from baseline to month-12 follow-up

Figure illustrating the change in mean HCC between those with and without MetS baseline and month-12 follow-up. HCC increased significantly from baseline to month-12 in those with MetS (*adj B* = 0.47, 95% CI 0.04 0.90, *p* = 0.037).

4 Discussion

We demonstrated that in a sample of South African adults HCC were significantly lower in patients with schizophrenia, and in particular FEP patients, than controls. HCC were not significantly associated with schizophrenia symptom severity domains, but were significantly inversely associated with patient insight scores. Neither MetS, nor individual CVD risk factors were associated with HCC at baseline, but MetS and HDL-C were associated with HCC at month-12 follow-up, with higher HCC in individuals with MetS and low HDL-C. HCC increased in FEP patients from baseline to month-12, demonstrating a trend towards significance and increased significantly from baseline to month-12 in those with MetS.

In our sample HCC were significantly lower in schizophrenia patients, and in particular in FEP patients. Our results do not align with the majority of studies that have demonstrated higher basal cortisol levels in schizophrenia, and in FEP patients, than in controls (Borges et al., 2013; Bradley & Dinan, 2010; Gajsak et al., 2017; Girshkin et al., 2014; Hubbard & Miller, 2019). Although most studies demonstrate

higher basal cortisol levels, or non-significant results, some have also found lower basal cortisol levels in schizophrenia patients than in controls. Of note, in one systematic review, the studies demonstrating lower cortisol levels in schizophrenia patients were largely in newly admitted drug free patients which resembles our FEP sample (Bradley & Dinan, 2010). Other recent studies have also demonstrated lower basal cortisol levels in FEP patients than in controls (Mondelli et al., 2015; Seitz et al., 2019). We also found that HCC were lower in non-remitted patients than in putatively remitted patients and controls, suggesting lower HCC were related to symptomatic status. Similarly, Mondelli et al., 2015 found that the CAR was significantly lower in FEP treatment non-responders than in responders (Mondelli et al., 2015). Furthermore, a recent meta-analysis revealed that salivary cortisol levels were higher in individuals at ultra-high risk (UHR) for psychosis, but not in FEP patients, than controls (Chaumette et al., 2016). Taken together these results suggest that though basal cortisol levels may generally be higher in patients with schizophrenia and individuals at risk for psychosis there may be a subgroup of patients, and in particular FEP and symptomatic patients, who exhibit a blunted HPA-axis with overall lower cortisol output.

Current meta-analyses and systematic reviews have not included cortisol measures in hair which may reflect a different aspect of HPA-axis functioning than acute measures. There have recently been other studies investigating HCC in schizophrenia samples. Contrary to our results another study in a FEP sample reported significantly higher HCC in FEP patients than in controls (Andrade et al., 2016). Their FEP sample also included patients with mood disorders with psychotic features and the inclusion of mood disorders may have contributed to higher HCC in the FEP patients. For instance, another study examining HCC in schizophrenia and bipolar disorder, found that HCC were significantly higher in individuals with bipolar disorder than in patients with schizophrenia and controls (Streit et al., 2016). They, along with another study (Aas et al., 2019) found that HCC did not differ between schizophrenia patients and controls. Thus results across studies have not been consistent and this could be related to sample characteristics, for instance two of the studies sourced controls from university/clinic staff and students (Andrade et al., 2016; Streit et al., 2016) and others were largely in patients with chronic schizophrenia (Aas et al., 2019; Streit et al., 2016). The inverse association demonstrated between

schizophrenia patients and HCC may also still align with studies demonstrating higher short-term cortisol levels in patients with schizophrenia as studies have demonstrated a dissociation between HCC and acute measures of cortisol. For instance, one study in older adults found that higher HCC were associated with better cognitive performance, whereas higher diurnal salivary cortisol levels were associated with poorer cognitive performance, thus suggesting HCC may reflect different aspects of HPA-axis function as compared to acute measures (Pulopulos et al., 2014).

Although HCC increased in FEP patients following treatment initiation this effect only demonstrated a trend towards significance. Another study that included acute inpatients with schizophrenia who were followed-up at 3 and 6 months found that HCC did not change significantly over the three time-points (Streit et al., 2016). We found that HCC were not associated with schizophrenia symptom severity domains, but were significantly lower in patients with better insight. Another study utilising HCC in females with FEP similarly found that HCC were not significantly associated with positive, negative or general psychopathology scores on the PANSS and were associated with insight, however they demonstrated a positive correlation between insight (self-reflection) and HCC (Touskova et al., 2018). Contrary to our results another study found that HCC were positively correlated with the PANSS general psychopathology scale (Streit et al., 2016).

A measure of self-perceived stress was included to assess whether HCC were associated with increased stress experienced by patients with schizophrenia. Patients reported significantly greater self-perceived stress at baseline than controls, but not in those who provided hair samples at month-12 follow-up. Self-perceived stress was not associated with HCC at baseline, but was inversely associated with HCC at month-12 follow-up, demonstrating a trend towards significance in adjusted analysis. The association between self-perceived stress and HCC is contrary to what one would expect with higher self-perceived stress associated with greater cortisol output, although systematic reviews and meta-analyses generally report mixed results (Stalder et al., 2017; Wosu et al., 2013). These results may suggest that within this sample lower HCC may represent stress related HPA-axis dysfunction.

MetS was not significantly associated with HCC at baseline, but was significantly associated with HCC at month-12 follow-up and HCC increased from baseline to month-12 in those with MetS. The direction

of association between MetS and HCC aligns with what has been demonstrated in other HCC studies (Kuehl et al., 2015; Stalder et al., 2013). Although few studies have investigated cortisol levels in relation to metabolic risk in schizophrenia they tend to demonstrate a lack of significant associations between CVD risk factors and cortisol levels. A study including patients with psychotic disorders, individuals at high risk for psychotic disorders and controls, found that plasma and salivary cortisol levels were not significantly different between the groups and that cortisol levels were not associated with CVD risk factors, physical activity or dietary intake patterns (Manzanares et al., 2014). They only demonstrated a significant positive association between salivary cortisol levels and intake of saturated fats in the high risk group. Another study in patients with bipolar disorder and schizophrenia found that there was no association between morning serum cortisol levels and MetS (Vuksan-Cúsa, Săgud, Mihaljevic-Peleš, Jaksĭć, & Jakovljević, 2014). The authors postulated that the psychiatric disorders themselves or medication prescribed may have a larger influence on cortisol levels than MetS. A similar explanation may also apply to our sample where at baseline the more prominent effects associated with psychosis potentially obscured smaller effects related to metabolic factors. This explanation is supported by the stronger association demonstrated for MetS, and individual CVD risk factors, with HCC at month-12 than HCC at baseline and that MetS was associated with an increase in HCC from baseline to month-12. Many of the studies demonstrating higher HCC in patients with schizophrenia do not take comorbid medical disorders, and in particular MetS, into consideration (Gajsak et al., 2017). Considering that MetS is associated with higher basal cortisol levels and that schizophrenia patients, particularly those on treatment have increased rates of MetS (Mitchell et al., 2013; Vancampfort et al., 2013) this may be an influential confounding factor in higher basal cortisol levels demonstrated in schizophrenia patients. HDL-C was also not significantly associated with HCC at baseline, but significantly inversely associated with HCC at 12-months follow-up. Though some studies have not demonstrated significant associations between HDL-C and HCC (Wester et al., 2017) others have also found significant inverse associations (Stalder et al., 2013). A study evaluating metformin effects on the metabolic profile of schizophrenia patients on clozapine found that in the placebo group there was a significant negative correlation between change in HDL-C and change in fasting serum cortisol further suggesting a link between cortisol levels and HDL-C (Carrizo et al., 2009).

4.1 Strengths and limitations

A major limitation of our study was the small sample size, this was secondary to difficulty recruiting schizophrenia patients that qualified for inclusion in SR and a large proportion of participants were excluded from hair sampling due to short hair length. Future studies in larger samples that use hair-sampling methods that will allow for broader inclusion, such as shorter hair length, are required to expand on our findings. We also allowed for psychiatric comorbidity and concomitant medication in our schizophrenia sample which may have influenced our results, although neither were significantly associated with HCC. Due to the exploratory nature of our study we did not control for multiple comparisons. The exploratory analyses pertaining to putative remission status is limited as we could not establish that symptoms had been in remission for at least six months.

Strengths include that the control participants were recruited from the same communities as our patients and were group matched to patients. Our FEP sample were all newly initiated on flupenthixol decanoate treatment and thus HCC would not be influenced by antipsychotic use at baseline and at follow-up the FEP patients were in receipt of the same depot antipsychotic treatment. Hair analyses were conducted with LC-MS/MS which is the gold standard method for hair glucocorticoid determination (Russell et al., 2015).

4.2 Conclusions

We demonstrated that HCC were significantly lower in schizophrenia patients, and in particular in FEP patients, than in controls. We also found that HCC were lower in non-remitted patients and that HCC increased following 12 months of flupenthixol decanoate treatment, demonstrating a trend towards significance. Although individuals at high risk for psychosis and with schizophrenia have generally been found to exhibit higher basal cortisol levels than controls our results suggest that the nature of HPA-axis dysfunction may be altered during periods of active psychosis. It may be that a generally overburdened HPA-axis stress system becomes decompensated during acute illness episodes, although further work is required to confirm this theory. Furthermore, other studies have demonstrated a dissociation between HCC and acute cortisol measures (Pulopulos et al., 2014) and thus further studies incorporating both HCC and acute cortisol measures are required to investigate whether HCC may

reflect a different aspect of HPA-axis function in schizophrenia. We also demonstrated that HCC increased from baseline to month-12 in those with MetS and that MetS was associated with increased HCC at follow-up and thus comorbid metabolic risk may contribute to higher cortisol levels observed in schizophrenia samples. Longitudinal studies in larger samples investigating the predictive utility of HCC as a biomarker of combined schizophrenia-CVD risk and treatment outcome (clinical response and adverse effects), and incorporating inflammatory markers and genetic/epigenetic markers of HPA-axis function are required to delineate the potential pathways involved.

5 Supplementary materials

Supplementary Table 1 Cardiovascular disease risk factors in relation to schizophrenia diagnosis and hair cortisol concentrations (HCC)

Variables	Descriptive data		Simple linear regression		Multiple linear regression[a]	
	Patient	Control	ß (95% CI)	p-value	ß (95% CI)	p-value
Total	n = 16	n = 21				
MetS variables						
MetS n(%)						
No	12 (75.0)	19 (90.5)	Ref		Ref	
Yes	4 (25.0)	2 (9.5)	0.04 (-0.26; 0.33)	0.806	0.08 (-0.21; 0.37)	0.557
Number of criteria met *M(SD)*	1.2 (1.3)	1.0 (1.1)	0.02 (-0.07; 0.12)	0.629	0.03 (-0.06; 0.12)	0.517
BP criterion[b] n(%)						
No	9 (56.3)	12 (57.1)	Ref		Ref	
Yes	7 (43.8)	9 (42.9)	0.11 (-0.10; 0.33)	0.302	0.11 (-0.09; 0.31)	0.263
On antihypertensive rx n(%)						
No	16 (100.0)	20 (95.2)	#		#	
Yes	0 (0.0)	1 (4.8)				
SBP (mmHg) *M(SD)*	129.0 (17.5)	124.2 (11.6)	-0.00 (-0.01; 0.01)	0.647	0.00 (-0.01; 0.01)	0.943
DBP (mmHg) *M(SD)*	81.5 (15.1)	82.6 (8.6)	0.00 (-0.01; 0.01)	0.766	-0.00 (-0.01; 0.01)	0.858
FPG criterion[c] n(%)						
No	15 (93.8)	18 (85.7)	Ref		Ref	
Yes	1 (6.3)	3 (14.3)	-0.19 (-0.53; 0.16)	0.278	-0.24 (-0.55; 0.08)	0.137
On diabetes Rx n(%)						
No	16 (100.0)	20 (95.2)	#		#	
Yes	0 (0.0)	1 (4.8)				
FPG (mmol/l) *M(SD)*	5.08 (0.93)	4.87 (0.64)	-0.06 (-0.20; 0.08)	0.416	-0.03 (-0.17; 0.10)	0.606
HDL-C criterion[d] n(%)						
No	11 (68.8)	17 (81.0)				
Yes	5 (31.3)	4 (19.0)	0.17 (-0.08; 0.42)	0.167	0.19 (-0.04; 0.43)	0.104
HDL-C (mmol/l) *M(SD)*	1.45 (0.41)	1.60 (0.48)	-0.09 (-0.33; 0.15)	0.434	-0.14 (-0.36; 0.09)	0.229
Trig criterion[e] n(%)						
No	15 (93.8)	20 (95.2)	#		#	
Yes	1 (6.3)	1 (4.8)				
Trig (mmol/l) *M(SD)*	1.01 (0.36)	0.76 (0.32)	0.00 (-0.31; 0.31)	0.998	0.12 (-0.20; 0.45)	0.443
WC criterion n(%)						
No	11 (68.8)	18 (85.7)	Ref		Ref	

Yes	5 (31.3)	3 (14.3)	-0.08 (-0.35; 0.18)	0.516	-0.04 (-0.30; 0.22)	0.744
WC (cm) *M(SD)*	85.2 (14.1)	78.3 (12.4)	-0.00 (-0.01; 0.01)	0.630	0.00 (-0.01; 0.01)	0.933
Other CVD risk variables						
Physical parameters						
BMI (kg/m^2) *M(SD)*	25.6 (5.6)	25.9 (6.5)	0.00 (-0.02; 0.02)	0.915	-0.00 (-0.02; 0.02)	0.915
HC (cm) *M(SD)*	94.6 (8.9)	93.3 (11.8)	0.00 (-0.01; 0.01)	0.704	0.00 (-0.01; 0.01)	0.736
WHR *M(SD)*	0.90 (0.08)	0.84 (0.05)	-1.14 (-2.70; 0.42)	0.148	-0.26 (-2.11; 1.59)	0.778
HR (bpm) *M(SD)*	93.2 (17.2)	78.4 (11.9)	0.00 (-0.01; 0.01)	0.712	0.01 (0.00; 0.01)	0.054
Laboratory parameters						
TC (mmol/l) *M(SD)*	4.50 (0.91)	4.38 (0.82)	-0.10 (-0.22; 0.03)	0.130	-0.08 (-0.20; 0.03)	0.157
TC > 5 mmol/l n(%)						
No	10 (62.5)	16 (76.2)	Ref		Ref	
Yes	6 (37.5)	5 (23.8)	0.05 (-0.19; 0.29)	0.678	0.01 (-0.21; 0.23)	0.932
LDL-C (mmol/l) *M(SD)*	2.58 (0.69)	2.44 (0.65)	-0.11 (-0.27; 0.05)	0.162	-0.09 (-0.24; 0.06)	0.234
LDL-C >= 3 mmol/l n(%)						
No	11 (68.8)	19 (90.5)	Ref		Ref	
Yes	5 (31.3)	2 (9.5)	0.16 (-0.12; 0.43)	0.253	0.09 (-0.19; 0.37)	0.515
HbA1c	5.34 (0.37)	5.18 (0.62)	0.06 (-0.15; 0.26)	0.594	0.06 (-0.15; 0.27)	0.578
HbA1c >= 6.5% n(%)						
No	16 (100.0)	20 (95.2)	#		#	
Yes	0 (0.0)	1 (4.8)				
CVD history variables						
On statins n(%)						
No	15 (93.8)	20 (95.2)	#		#	
Yes	1 (6.3)	1 (4.8)				
On aspirin n(%)						
No	16 (100.0)	20 (95.2)	#		#	
Yes	0 (0.0)	1 (4.8)				

[a] Controlled for age, sex, and schizophrenia diagnostic status
[b] All participants who met the blood pressure criterion had blood pressure above the threshold
[c] All participants who met the glucose criterion had glucose levels above the threshold
[d] No participants were receiving treatment to increase HDL-C
[e] No participants were receiving treatment for hypertriglyceridemia
* $p < 0.05$
# Too few observations to conduct analyses
BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; DBP, diastolic blood pressure; HbA1c, Glycated haemoglobin; HC, hip circumference; HDL-C, high-density lipoprotein cholesterol; HR, heart rate; FPG, fasting plasma glucose; LDL-C, Low density lipoprotein cholesterol; MetS, metabolic syndrome; Ref, reference category; rx, treatment; SBP, systolic blood pressure; TC, total cholesterol; trig; serum triglyceride; WC, waist circumference; WHR waist-to-hip ratio

6 References

Aas, M., Pizzagalli, D. A., Laskemoen, J. F., Reponen, E. J., Ueland, T., Melle, I., … Andreassen, O. A. (2019). Elevated hair cortisol is associated with childhood maltreatment and cognitive impairment in schizophrenia and in bipolar disorders. *Schizophrenia Research*, (xxxx), 1–7. https://doi.org/10.1016/j.schres.2019.01.011

Alberti, K. G., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., … Smith Jr, S. C. (2009). Harmonizing the Metabolic Syndrome A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International A. *Circulation*, *120*(16), 1640–1645. https://doi.org/10.1161/CIRCULATIONAHA.109.192644 [doi]

Andrade, E. H., Rizzo, L. B., Noto, C., Ota, V. K., Gadelha, A., Daruy-Filho, L., … Brietzke, E. (2016). Hair cortisol in drug-naïve first-episode individuals with psychosis. *Revista Brasileira de Psiquiatria*, *38*(1), 11–16. https://doi.org/10.1590/1516-4446-2014-1634

Andreasen, N. C., Carpenter, W. T., Kane, J. M., Lasser, R. A., Marder, S. R., & Weinberger, D. R. (2005). Remission in Schizophrenia: Proposed Criteria and Rationale for Consensus. *American Journal of Psychiatry*, *162*(3), 441–449. https://doi.org/10.1176/appi.ajp.162.3.441

Association, A. P. (2000). Social and Occupational Functioning Assessment Scale (SOFAS). In *Diagnostic and Statistical Manual of Mental Disorders* (pp. 817–818). American Psychiatric Association, Washington, DC.

Berger, M., Kraeuter, A. K., Romanik, D., Malouf, P., Amminger, G. P., & Sarnyai, Z. (2016). Cortisol awakening response in patients with psychosis: Systematic review and meta-analysis. *Neuroscience and Biobehavioral Reviews*, *68*(January 2018), 157–166. https://doi.org/10.1016/j.neubiorev.2016.05.027

Birchwood, M., Smith, J., Drury, V., Healy, J., Macmillan, F., & Slade, M. (1994). A self- report Insight Scale for psychosis: reliability, validity and sensitivity to change. *Acta Psychiatrica Scandinavica*,

89(1), 62–67.

Borges, S., Gayer-Anderson, C., & Mondelli, V. (2013). A systematic review of the activity of the hypothalamic-pituitary-adrenal axis in first episode psychosis. *Psychoneuroendocrinology*, *38*(5), 603–611. https://doi.org/10.1016/j.psyneuen.2012.12.025

Bradley, A. J., & Dinan, T. G. (2010). A systematic review of hypothalamic-pituitary-adrenal axis function in schizophrenia: implications for mortality. *Journal of Psychopharmacology (Oxford, England)*, *24*(4 Suppl), 91–118. https://doi.org/10.1177/1359786810385491; 10.1177/1359786810385491

Carney, R., Cotter, J., Bradshaw, T., Firth, J., & Yung, A. R. (2016). Cardiometabolic risk factors in young people at ultra-high risk for psychosis: A systematic review and meta-analysis. *Schizophrenia Research*, *170*(2–3), 290–300. https://doi.org/10.1016/j.schres.2016.01.010

Carrizo, E., Fernández, V., Connell, L., Sandia, I., Prieto, D., Mogollón, J., … Baptista, T. (2009). Extended release metformin for metabolic control assistance during prolonged clozapine administration: A 14 week, double-blind, parallel group, placebo-controlled study. *Schizophrenia Research*, *113*(1), 19–26. https://doi.org/10.1016/j.schres.2009.05.007

Chaumette, B., Kebir, O., Mam-Lam-Fook, C., Morvan, Y., Bourgin, J., Godsil, B. P., … Krebs, M. O. (2016). Salivary cortisol in early psychosis: New findings and meta-analysis. *Psychoneuroendocrinology*, *63*, 262–270. https://doi.org/10.1016/j.psyneuen.2015.10.007

Chiliza, B., Ojagbemi, A., Esan, O., Asmal, L., Oosthuizen, P., Kidd, M., … Emsley, R. (2016). Combining depot antipsychotic with an assertive monitoring programme for treating first-episode schizophrenia in a resource-constrained setting. *Early Intervention in Psychiatry*, *10*(1), 54–62. https://doi.org/10.1111/eip.12141

Clark, J. E., Osborne, J. W., Gallagher, P., & Watson, S. (2016). A simple method for optimising transformation of non-parametric data: an illustration by reference to cortisol assays. *Human Psychopharmacology: Clinical and Experimental*, *31*(4), 259–267.

https://doi.org/10.1002/hup.2528

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A Global Measure of Perceived Stress. *Journal of Health and Social Behavior*, *24*(4), 385–396. https://doi.org/10.1016/j.socscimed.2016.08.021

Correll, C. U., Solmi, M., Veronese, N., Bortolato, B., Rosson, S., Santonastaso, P., … Stubbs, B. (2017). Prevalence, incidence and mortality from cardiovascular disease in patients with pooled and specific severe mental illness: a large-scale meta-analysis of 3,211,768 patients and 113,383,368 controls. *World Psychiatry*, *16*(2), 163–180. https://doi.org/10.1002/wps.20420

De Hert, M., Detraux, J., & Vancampfort, D. (2018). The intriguing relationship between coronary heart disease and mental disorders. *Dialogues in Clinical Neuroscience*, *20*(1), 31–40.

First, M. B., Spitzer, R. L., Gibbon, M., & Williams, J. B. W. (1997). *Structured Clinical Interview for DSM-IV Axis I Disorders, Clinical Version (SCID-CV)*. Washington, DC: American Psychiatric Press.

Gajsak, L. R., Gelemanovic, A., Kuzman, M. R., & Puljak, L. (2017). Impact of stress response in development of first-episode psychosis in schizophrenia: An overview of systematic reviews. *Psychiatria Danubina*, *29*(1), 14–23.

Gao, W., Stalder, T., Foley, P., Rauh, M., Deng, H., & Kirschbaum, C. (2013). Quantitative analysis of steroid hormones in human hair using a column-switching LC–APCI–MS/MS assay. *Journal of Chromatography B*, *928*, 1–8. https://doi.org/10.1016/j.jchromb.2013.03.008

Girshkin, L., Matheson, S. L., Shepherd, A. M., & Green, M. J. (2014). Morning cortisol levels in schizophrenia and bipolar disorder: A meta-analysis. *Psychoneuroendocrinology*, *49*(1), 187–206. https://doi.org/10.1016/j.psyneuen.2014.07.013

Guy, W. (1976). Clinical global impression scale. *The ECDEU Assessment Manual for Psychopharmacology-Revised.Volume DHEW Publ No ADM 76*, *338*, 218–222.

Hjorthøj, C., Stürup, A. E., McGrath, J. J., & Nordentoft, M. (2017). Years of potential life lost and life expectancy in schizophrenia: a systematic review and meta-analysis. *The Lancet Psychiatry*,

4(4), 295–301. https://doi.org/10.1016/S2215-0366(17)30078-0

Hubbard, D. B., & Miller, B. J. (2019). Meta-analysis of blood cortisol levels in individuals with first-episode psychosis. *Psychoneuroendocrinology*, *104*(September 2018), 269–275. https://doi.org/10.1016/j.psyneuen.2019.03.014

Klug, E. Q., Raal, F. J., Marais, A., Taskinen, M. R., Dalby, A. J., Schamroth, C., … Webb, D. A. (2018). South African dyslipidaemia guideline consensus statement: 2018 update A joint statement from the South African Heart Association (SA Heart) and the Lipid and Atherosclerosis Society of Southern Africa (LASSA). *South African Family Practice*, *108*(11b), 973–1000. https://doi.org/10.7196/SAMJ.2018.v108i11.13383

Kuehl, L. K., Hinkelmann, K., Muhtz, C., Dettenborn, L., Wingenfeld, K., Spitzer, C., … Otte, C. (2015). Hair cortisol and cortisol awakening response are associated with criteria of the metabolic syndrome in opposite directions. *Psychoneuroendocrinology*, *51*, 365–370. https://doi.org/10.1016/j.psyneuen.2014.09.012

Laursen, T. M., Nordentoft, M., & Mortensen, P. B. (2014). Excess Early Mortality in Schizophrenia. *Annual Review of Clinical Psychology*, *10*(1), 425–448. https://doi.org/10.1146/annurev-clinpsy-032813-153657

Lee, E. H. (2012). Review of the psychometric evidence of the perceived stress scale. *Asian Nursing Research*, *6*(4), 121–127. https://doi.org/10.1016/j.anr.2012.08.004

Malan-Müller, S., Kilian, S., Van Den Heuvel, L. L., Bardien, S., Asmal, L., Warnich, L., … Seedat, S. (2016). A systematic review of genetic variants associated with metabolic syndrome in patients with schizophrenia. *Schizophrenia Research*, *170*(1). https://doi.org/10.1016/j.schres.2015.11.011

Manzanares, N., Monseny, R., Ortega, L., Montalvo, I., Franch, J., Gutiérrez-Zotes, A., … Labad, J. (2014). Unhealthy lifestyle in early psychoses: The role of life stress and the hypothalamic-pituitary-adrenal axis. *Psychoneuroendocrinology*, *39*(1), 1–10. https://doi.org/10.1016/j.psyneuen.2013.09.023

Matsha, T. E., Hassan, M. S., Hon, G. M., Soita, D. J., Kengne, A. P., & Erasmus, R. T. (2013). Derivation and validation of a waist circumference optimal cutoff for diagnosing metabolic syndrome in a South African mixed ancestry population. *International Journal of Cardiology*, *168*(3), 2954–2955. https://doi.org/https://doi.org/10.1016/j.ijcard.2013.03.150

Miller, R., & Plessow, F. (2013). Transformation techniques for cross-sectional and longitudinal endocrine data: Application to salivary cortisol concentrations. *Psychoneuroendocrinology*, *38*(6), 941–946. https://doi.org/10.1016/j.psyneuen.2012.09.013

Mitchell, A. J., Vancampfort, D., Sweers, K., Van Winkel, R., Yu, W., & De Hert, M. (2013). Prevalence of metabolic syndrome and metabolic abnormalities in schizophrenia and related disorders-a systematic review and meta-analysis. *Schizophrenia Bulletin*, *39*(2), 306–318. https://doi.org/10.1093/schbul/sbr148

Mondelli, V., Ciufolini, S., Murri, M. B., Bonaccorso, S., Di Forti, M., Giordano, A., … Dazzan, P. (2015). Cortisol and inflammatory biomarkers predict poor treatment response in first episode psychosis. *Schizophrenia Bulletin*, *41*(5), 1162–1170. https://doi.org/10.1093/schbul/sbv028

Osborne, J. W. (2010). Improving your data transformations : Applying the Box-Cox transformation. *Pratical Assessment, Research & Evaluation*, *15*(12), 1–9.

Penninx, B. W. J. H., & Lange, S. M. M. (2018). Metabolic syndrome in psychiatric patients: Overview, mechanisms, and implications. *Dialogues in Clinical Neuroscience*, *20*(1), 63–73.

Petrikis, P., Tigas, S., Tzallas, A. T., Papadopoulos, I., Skapinakis, P., & Mavreas, V. (2015). Parameters of glucose and lipid metabolism at the fasted state in drug-naïve first-episode patients with psychosis: Evidence for insulin resistance. *Psychiatry Research*, *229*(3), 901–904. https://doi.org/10.1016/j.psychres.2015.07.041

Piotrowski, P., Gondek, T. M., Królicka-Deręgowska, A., Misiak, B., Adamowski, T., & Kiejna, A. (2017). Causes of mortality in schizophrenia: An updated review of European studies. *Psychiatria Danubina*, *29*(2), 108–120. https://doi.org/10.24869/psyd.2017.108

Pulopulos, M. M., Hidalgo, V., Almela, M., Puig-Perez, S., Villada, C., & Salvador, A. (2014). Hair cortisol and cognitive performance in healthy older people. *Psychoneuroendocrinology*, *44*, 100–111. https://doi.org/10.1016/j.psyneuen.2014.03.002

Russell, E., Kirschbaum, C., Laudenslager, M. L., Stalder, T., Rijke, Y. De, van Rossum, E. F. C., … Koren, G. (2015). Toward Standardization of Hair Cortisol Measurement : Results of the First International Interlaboratory Round Robin. *Therapeutic Drug Monitoring*, *37*(1), 71–75. https://doi.org/10.1097/FTD.0000000000000148

Seitz, R., Vracotas, N., Bechard-Evans, L., King, S., Abadi, S., Joober, R., … Pruessner, M. (2019). The Trier Social Stress Test in first episode psychosis patients: Impact of perceived stress, protective factors and childhood trauma. *Psychoneuroendocrinology*, *105*(July 2018), 155–163. https://doi.org/10.1016/j.psyneuen.2019.01.010

SEMDSA Type 2 Diabetes Guidelines Expert Committee. (2017). The 2017 SEMDSA Guideline for the Management of Type 2 Diabetes. *Journal of Endocrinology, Metabolism and Diabetes of South Africa*, *22*(1), s1–s196. https://doi.org/10.1080/22201009.2012.10872276

Sheehan, D. V, Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., … Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry*, *59*(Suppl 20), 22–33. Retrieved from https://psycnet.apa.org/record/1998-03251-004

Spelman, L. M., Walsh, P. I., Sharifi, N., Collins, P., & Thakore, J. H. (2007). Impaired glucose tolerance in first-episode drug-naïve patients with schizophrenia. *Diabetic Medicine*, *24*(5), 481–485. https://doi.org/10.1111/j.1464-5491.2007.02092.x

Stalder, T., & Kirschbaum, C. (2012). Analysis of cortisol in hair–State of the art and future directions. *Brain, Behavior, and Immunity*, *26*(7), 1019–1029. https://doi.org/10.1016/j.bbi.2012.02.002

Stalder, T., Kirschbaum, C., Alexander, N., Bornstein, S. R., Gao, W., Miller, R., … Fischer, J. E. (2013). Cortisol in hair and the metabolic syndrome. *The Journal of Clinical Endocrinology &*

Metabolism, *98*(6), 2573–2580. https://doi.org/10.1210/jc.2013-1056

Stalder, T., Steudte-Schmiedgen, S., Alexander, N., Klucken, T., Vater, A., Wichmann, S., … Miller, R. (2017). Stress-related and basic determinants of hair cortisol in humans: A meta-analysis. *Psychoneuroendocrinology*, *77*, 261–274. https://doi.org/10.1016/j.psyneuen.2016.12.017

Steudte, S., Kirschbaum, C., Gao, W., Schoenfeld, S., Hoyer, J., & Stalder, T. (2012). Glucocorticoids in hair as biomarkers of traumatization in healthy individuals and PTSD patients. *European Journal of Psychotraumatology*, *3*(1). https://doi.org/10.3402/ejpt.v3i0.19472

Streit, F., Memic, A., Hasandedić, L., Rietschel, L., Frank, J., Lang, M., … Rietschel, M. (2016). Perceived stress and hair cortisol: Differences in bipolar disorder and schizophrenia. *Psychoneuroendocrinology*, *69*, 26–34. https://doi.org/10.1016/j.psyneuen.2016.03.010

Touskova, T. P., Bob, P., Pec, O., Mishara, A., Vanickova, Z., Raboch, J., & Lysaker, P. (2018). Insight and cortisol responses in women with first episode psychosis. *Schizophrenia Research*, *201*, 428–429. https://doi.org/10.1016/j.schres.2018.06.002

Vancampfort, D., Stubbs, B., Mitchell, A. J., De Hert, M., Wampers, M., Ward, P. B., … Correll, C. U. (2015). Risk of metabolic syndrome and its components in people with schizophrenia and related psychotic disorders, bipolar disorder and major depressive disorder: A systematic review and meta-analysis. *World Psychiatry*, *14*(3), 339–347. https://doi.org/10.1002/wps.20252

Vancampfort, D., Wampers, M., Mitchell, A. J., Correll, C. U., De Herdt, A., Probst, M., & De Hert, M. (2013). A meta-analysis of cardio-metabolic abnormalities in drug naïve, first-episode and multi-episode patients with schizophrenia versus general population controls. *World Psychiatry*, *12*(3), 240–250. https://doi.org/10.1002/wps.20069

Vuksan-Cúsa, B., Săgud, M., Mihaljevic-Peleš, A., Jaksĭć, N., & Jakovljević, M. (2014). Metabolic syndrome and cortisol/dheas ratio in patients with bipolar disorder and schizophrenia. *Psychiatria Danubina*, *26*(2), 187–189.

Walker, E. R., McGee, R. E., & Drus, B. G. (2015). Mortality in Mental Disorders and Global Disease

Burden Implications: A Systematic Review and Meta-analysis. *JAMA Psychiatry*, *72*(4), 334–341. https://doi.org/10.1001/jamapsychiatry.2014.2502

Wennig, R. (2000). Potential problems with the interpretation of hair analysis results. *Forensic Science International*, *107*(1), 5–12. https://doi.org/10.1016/S0379-0738(99)00146-2

Wester, V. L., Noppe, G., Savas, M., van den Akker, E. L. T., de Rijke, Y. B., & van Rossum, E. F. C. (2017). Hair analysis reveals subtle HPA axis suppression associated with use of local corticosteroids : The Lifelines cohort study. *Psychoneuroendocrinology*, *80*, 1–6. https://doi.org/10.1016/j.psyneuen.2017.02.024

World Health Organization. (2005). *WHO STEPS surveillance manual: the WHO STEPwise approach to chronic disease risk factor surveillance*. Geneva: World Health Organization (WHO).

World Medical Association. (2013). World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA - Journal of the American Medical Association*, *310*(20), 2191–2194. https://doi.org/10.1001/jama.2013.281053

Wosu, A. C., Valdimarsdóttir, U., Shields, A. E., Williams, D. R., & Williams, M. A. (2013). Correlates of cortisol in human hair: Implications for epidemiologic studies on health effects of chronic stress. *Annals of Epidemiology*, *23*(12), 797–811. https://doi.org/10.1016/j.annepidem.2013.09.006

Zorn, J. V., Schür, R. R., Boks, M. P., Kahn, R. S., Joëls, M., & Vinkers, C. H. (2017). Cortisol stress reactivity across psychiatric disorders: A systematic review and meta-analysis. *Psychoneuroendocrinology*, *77*, 25–36. https://doi.org/10.1016/j.psyneuen.2016.11.036

CHAPTER 5

Hair cortisol as a neuroendocrine biomarker to evaluate the impact of chronic stress on the interaction between neuropsychiatric disorders and metabolic syndrome in females

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Hair cortisol as a neuroendocrine biomarker to evaluate the impact of chronic stress on the interaction between neuropsychiatric disorders and metabolic syndrome in females

Abstract

Background: Rates of metabolic syndrome (MetS) and cardiovascular disease (CVD) are higher in individuals with neuropsychiatric disorders (NPDs) compared to the general population, suggesting that there are shared pathophysiological pathways underlying the increased CVD risk in NPDs. Hypothalamic pituitary adrenal (HPA) axis dysfunction has also been observed in a variety of NPDs and in MetS and may be one of the shared explanatory pathways for the co-occurrence.

Objectives: In a case-control study of NPDs evaluating the factors that contribute to increased risk for CVD, our aims were to (i) compare HCC across three NPDs (posttraumatic stress disorder [PTSD], Parkinson's disease and schizophrenia); (ii) investigate the interaction effects of NPDs and MetS on HCC.

Methods: In 354 females of mixed ancestry (PTSD: 139 patients, 128 controls: Parkinson's disease: 25 patients, 31 controls, schizophrenia: 11 patients ,19 controls), aged between 18 – 79 years, hair samples, representing a three-month retrospective window of cortisol levels, were obtained and analysed utilizing liquid chromatography tandem mass spectrometry. We constructed multivariate linear regression models to evaluate whether NPDs and MetS comorbidity were associated with HCC (reciprocal square root transformed) controlling for potential confounders.

Results: The overall prevalence of MetS was 33.1% with the highest prevalence in Parkinson's disease patients (56.0%) and the lowest in schizophrenia controls (10.5%). HCC were significantly higher in NPD patients than in controls ($p = 0.027$) overall. HCC did not differ significantly between the three NPD patient groups. Although HCC differed significantly among the three cohorts ($p < 0.001$) overall, with highest HCC demonstrated in the schizophrenia cohort, followed by the PTSD and

Parkinson's disease cohorts. MetS was not significantly associated with HCC ($p = 0.285$) and there were no significant interactions between NPDs and MetS on HCC ($p = 0.111$).

Conclusions: Overall patients had higher HCC than controls, although patterns differed within the individual cohorts. HCC did not differ significantly between the three patient groups, but differed across the three cohorts overall (patients and controls). The presence of MetS was not associated with HCC. Due to significant differences between the cohorts, within cohort case-control investigations may provide a better reflection of patterns of HPA-axis dysregulation than cross-cohort comparisons of HCC.

Keywords: Neuropsychiatric disorders; post-traumatic stress disorder; Parkinson's disease; schizophrenia; hair cortisol concentrations; metabolic syndrome

1 Introduction

Individuals with neuropsychiatric disorders (NPDs) have elevated mortality rates and lower life expectancy than the general population (Penninx & Lange, 2018; Walker, McGee, & Drus, 2015). Although the elevated risk can partly be ascribed to unnatural causes it is largely due to natural causes and cardiovascular disease (CVD) in particular (Correll et al., 2017; De Hert, Detraux, & Vancampfort, 2018; Penninx & Lange, 2018; Walker et al., 2015). Both CVD and NPDs have a major impact on society and in the global burden of disease study CVD and psychiatric disorders were the largest contributors to disability-adjusted life years (DALYs, 11.9%) and years lived with disability (YLDs, 22.9%), respectively (Whiteford et al., 2013). Metabolic syndrome (MetS) constitutes a cluster of risk factors for CVD and allows for earlier identification, and possible earlier intervention, of individuals with increased risk for CVD (Alberti et al., 2009; Penninx & Lange, 2018). MetS has also been associated with poorer outcomes in psychiatric conditions, including increased chronicity and treatment resistance (Penninx & Lange, 2018). Increased rates of MetS and CVD are found across a number of different NPDs as compared to the general population, suggesting shared mechanisms may be playing a role (Bradley & Dinan, 2010; Penninx & Lange, 2018; Vancampfort et al., 2015).

Although individuals with mental disorders have higher rates of CVD and death due to CVD the pathophysiology linking CVD and mental disorder is not well understood (Correll et al., 2017; De Hert et al., 2018; Vancampfort et al., 2015). Potential pathways involved include inflammation, autonomic nervous system dysfunction, mitochondrial dysfunction, oxidative stress, genes with pleiotropic effects and epigenetic mechanisms (De Hert et al., 2018; Vancampfort et al., 2015). HPA-axis dysfunction has also been demonstrated both in NPDs and in CVD and may represent a shared pathway contributing to the increase in CVD in NPDs (Bradley & Dinan, 2010; Penninx & Lange, 2018). A dysregulated HPA-axis can lead to central obesity, metabolic derangements, insulin resistance and inflammation (De Hert et al., 2018).

Various biological markers, including cortisol, show non-specificity for NPDs (Boksa, 2013). A meta-analysis investigating prognostic biomarkers across disorders found that cortisol was elevated across disorders and effect sizes were similar for schizophrenia, bipolar disorder and major depressive

disorder (MDD) (Pinto, Moulin, & Amaral, 2017). There are, however, also differences in the alterations of HPA-axis across NPDs, for instance one meta-analysis demonstrated a blunted cortisol stress response in patients with schizophrenia, but not in MDD, as compared to controls (Ciufolini, Dazzan, Kempton, Pariante, & Mondelli, 2014). Our understanding of the role of the HPA-axis in different NPDs, as well as in relation to CVD risk, is limited by the lack of studies directly comparing HPA-axis function between different disorders. In fact the number of studies comparing biomarkers across disorders have been decreasing over time (Pinto et al., 2017). There are only a few meta-analyses of studies of HPA-axis functioning in different NPDs, and these have mostly focused on schizophrenia and mood disorders (Ciufolini et al., 2014; Pinto et al., 2017). Although these meta-analyses allow for comparison of NPDs versus controls they have by and large not directly compared cortisol levels between disorders, likely due to the paucity of studies that have compared cortisol levels between disorders. The lack of studies performing cross-disorder investigations limits our ability to more clearly distinguish shared versus specific pathophysiological pathways involved in NPDs and the co-occurrence of CVD.

In three cohorts of NPDs (posttraumatic stress disorder [PTSD], Parkinson's disease and schizophrenia) we first investigated whether hair cortisol concentrations (HCC), as a marker of long-term HPA-axis function, were associated with each of the aforementioned NPDs compared to controls (Chapters 2 - 4). Second, we investigated interaction effects of each NPD and MetS on HCC. For each of the three NPDs, there is evidence of adverse CVD related outcomes. Increased rates of MetS, CVD and associated increased mortality risk have been demonstrated in both PTSD and schizophrenia (Ahmadi et al., 2011; Bartoli, Carra, Crocamo, Carretta, & Clerici, 2013; Correll et al., 2017; Kubzansky, Koenen, & Spiro, 2007; Mitchell et al., 2013; Rosenbaum et al., 2015; Vancampfort et al., 2013). MetS is also associated with worsened outcomes in patients with Parkinson's disease and there is some evidence that MetS can increase the risk of developing Parkinson's disease (Bainbridge et al., 2017; Nam et al., 2018; Zhang & Tian, 2014). There is also evidence for a dysregulated HPA-axis in all three NPDs. Largely elevated basal cortisol levels were observed in patients with Parkinson's disease and schizophrenia as compared to controls (Borges, Gayer-Anderson, & Mondelli, 2013; Bradley & Dinan, 2010; Du & Pang, 2015; Gajsak, Gelemanovic, Kuzman, & Puljak, 2017; Girshkin, Matheson,

Shepherd, & Green, 2014; Herrero, Estrada, Maatouk, & Vyas, 2015; Hubbard & Miller, 2019; Soares, Pereira, Altmann, de Almeida, & Rieder, 2019), and lower cortisol levels in patients with PTSD than in controls (Meewisse, Reitsma, de Vries, Gersons, & Olff, 2007; Morris, Compas, & Garber, 2012; Schumacher et al., 2019).

Our results in the three individual cohorts demonstrated that HCC were significantly higher in PTSD patients than trauma exposed controls (TEC, Chapter 2), were non-significantly higher in Parkinson's disease patients than controls (Chapter 3) and were significantly lower in schizophrenia patients than controls (Chapter 4). We thus observed different patterns in the three NPDs. MetS was not associated with HCC in any of our cohorts and there were no interactions between NPDs and MetS. In this study we, firstly, aim to extrapolate those results by directly comparing HCC among the three NPDs. Secondly, we investigate whether there is an interaction effect between NPDs overall and MetS on HCC. Finally, we examine whether self-perceived stress, as a cross-disorder measure of psychological stress, is associated with HCC overall and investigate whether there are interactions between self perceived stress, NPD diagnosis, cohort and MetS on HCC.

2 Methods

2.1 Study design

This study is a neuroendocrine ancillary study to 'Understanding the SHARED ROOTS of Neuropsychiatric Disorders and Modifiable Risk Factors for Cardiovascular Disease' or SHARED ROOTS (SR). SR was a cross-sectional matched case-control study investigating the factors that contribute to comorbidity of NPDs and MetS utilising a multi-omics approach. Participants were enrolled from May 2014 until June 2017 in Cape Town, South Africa and controls were matched to the three NPD patient groups based on age, gender, and MetS status. We utilised a purposive sampling approach and participants for each of the cohorts were recruited utilising multi-pronged strategies. We have previously reported the methods for each of the individual NPD cohorts (Chapters 2-4) and here provide an overview of pertinent methods.

This study was approved by the Health Research Ethics Committee at Stellenbosch University (HREC N13/08/115) and conducted according to principles of the seventh revision of the Declaration of Helsinki (World Medical Association, 2013). A tiered consenting process was adhered to, allowing participants to opt-out of the hair sampling procedure.

2.2 Participants

Participants were mixed ancestry (coloured) adults, aged 18 years and older, who provided hair samples for neuroendocrine analyses. Patients with a UK Brain Bank clinical diagnosis (Hughes, Daniel, Kilford, & Lees, 1992) of idiopathic Parkinson's disease, a diagnosis of schizophrenia based on the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (SCID-IV) (First, Spitzer, Gibbon, & Williams, 1997), and meeting the Diagnostic and Statistical Manual of Mental Disorders (DSM–5) criteria of PTSD according to the diagnostic evaluation with the Clinician Administered Posttraumatic Stress Disorder Scale for DSM–5 (CAPS-5) were included as patients in the three respective cohorts. For this study patients were permitted to have common mental disorder (CMD) and substance use disorder (SUD) comorbidity as long as the primary diagnosis, based on diagnostic interview and clinician assessment, was not deemed to be a substance induced disorder. Patients with psychiatric comorbidity were included to optimise the sample size, particularly of the schizophrenia and PTSD cohorts. Further exclusion criteria applied in this study were systemic or scalp steroid use, current pregnancy, or pregnancy within the prior three months, and significant medical comorbidity (e.g. cancer, HIV, auto-immune disorders). Exclusion criteria applied only to controls were lifetime or current serious psychiatric disorder (a psychotic or bipolar disorder) or the presence of a possible current psychiatric disorder as based on the history, the Mini International Neuropsychiatric Interview (MINI) or current psychotropic medication use.

MetS was determined based on the harmonized Joint Interim Statement (JIS) criteria (Alberti et al., 2009), with the presence of any three of the following five risk factors required for a diagnosis of MetS: (i) Raised blood pressure (BP), systolic ≥ 130 and/or diastolic ≥ 85 mmHg, or being on antihypertensive treatment; (ii) Elevated triglycerides (trig) > 1.70 mmol/l (150 mg/dl) or being on treatment for hypertriglyceridemia; (iii) Reduced high-density lipoprotein cholesterol (HDL-C), < 1.0 mmol/l (40mg/dl)

in males, and < 1.3 mmol/l (50mg/dl) in females, or being on treatment for low HDL-C; (iv) Elevated fasting glucose (FPG) ≥ 5.6 mmol/l (100 mg/dl) or on treatment for diabetes; (v) Elevated waist circumference (WC) according to population and country specific guidelines. We used a WC ≥ 90cm in both males and females, as a recent validation study showed this to be the optimal WC cut-off in mixed ancestry individuals (Matsha et al., 2013).

2.3 Procedures

Diagnostic, clinical, participant-administered assessments and physical procedures were conducted by psychiatrists, physicians and nurses across two study visits 24-96 hours apart. Physical measurements were conducted in a consistent method in accordance with the WHO stepwise approach to chronic disease risk factor surveillance (WHO STEPS) instrument, a standardised instrument designed for non-communicable disease surveillance (World Health Organization, 2005). Venous blood samples were obtained following an overnight fast (at least 8 hours) and were analysed on the Cobas 6000 c501 Chemistry Analyser (Roche, Germany) at a commercial internationally accredited laboratory (Lancet laboratories) on the same day as sample collection. Hair samples were cut from the posterior vertex scalp and were secured, placed into aluminium foil, labelled and sealed inside an envelope and stored in dark containers at room temperature. Hair analyses using an established liquid chromatography-tandem mass spectrometry (LC-MS/MS) protocol (Gao et al., 2013) were performed at the TU Dresden laboratory (Prof Clemens Kirschbaum). Samples were analysed in two batches; a pilot sample (n = 21) analysed in September 2014 and the remaining samples in December 2017. The proximal 3cm of the hair segments were used for analysis, representing cortisol secretion for the prior 3 months based on an accepted growth rate of 1cm per month (Wennig, 2000).

2.4 Measures

2.4.1 Demographic questionnaire

Demographic variables included in this manuscript were self-identified gender (male or female), age in years (calculated as date of birth subtracted from date of assessment), highest level of education (HLOE, defined according to whether secondary education was completed), and employment status (defined as being employed for the greater part of the prior 12 months).

2.4.2 Medical history questionnaires

Variables from the medical questionnaires included in this manuscript were: (i) steroid use in the prior 6 months [split according to whether any systemic steroids were used (oral or parenteral), topical steroids where used on the scalp, and any other topical steroid use (dermatologic, inhaled, nasal)], (ii) hormonal contraceptive use in prior 6 months (including oral contraceptives and other formulations, such as injectable contraceptives), (iii) whether the woman was currently breastfeeding, (iv) current psychiatric medication use, (v) any other current medical conditions, and (vi) self-reported tobacco , alcohol, and illicit substance use in the prior six months.

2.4.3 The Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998)

The MINI version 6.0, a short structured diagnostic interview, was used to diagnose current and lifetime psychiatric disorders based on DSM-IV and International Classification of Diseases (ICD-10) criteria.

2.4.4 The Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983)

The 10-item PSS (PSS-10) was used to measure self-perceived stress with higher scores (range 0 – 40) indicating higher perceived stress. The PSS has been widely used and has demonstrated adequate psychometric properties in multiple settings and languages (Lee, 2012).

2.4.5 The WHO STEPwise approach to chronic disease risk factor surveillance (World Health Organization, 2005)

The level of physical activity (high, moderate, and low) was determined with the global physical activity questionnaire (GPAQ) in the WHO STEPS.

2.4.6 Hair questionnaire

A hair questionnaire, designed for this study, assessed hair characteristics and hair care practices. Hair related variables reported in this manuscript include (i) whether natural hair colour is black, (ii) whether hair had been chemically treated in the prior three months (including colouring, relaxing and perming), (iii) the frequency of hair washing (two or more times a week, once a week, less frequent than once a week), (iv) whether any additional hair products had been used in the prior three months (such as hair styling and care products e.g. gels, oils and creams), and (v) average hours per day that hair was

exposed to sunlight (less than one hour, 1-2 hours, more than 2 hours). Additional hair related factors included the season of sampling, the duration of sample storage (less than 1 year, 1-2 years, 2-3.5 years) and the batch analysed (pilot, final).

2.5 Statistical analysis

The sample size required to distinguish between those with and without MetS was 292 based on a priori sample size estimations computed using means and standard deviations (SD) reported in other hair cortisol studies (Stalder et al., 2013; Steudte et al., 2012). Due to the small number of males (n = 6) with hair cortisol data, the analysis was restricted to females only. HCC were reciprocal square root transformed to meet the assumption of normality. We compared descriptive factors across the three cohorts with Pearson chi-square for categorical variables and ANOVA for continuous variables. There were very few missing data items and these were excluded from analysis listwise (missing items and final sample numbers reported in tables). We regressed NPD cohort (PTSD, Parkinson's disease, and schizophrenia), NPD diagnosis (patient or control) and MetS on HCC in unadjusted and adjusted linear regression models. Covariates were age and factors that remained significantly associated with HCC when we controlled for age, NPD cohort and NPD status (HLOE, natural hair colour, frequency of hair washing, chemical treatment of hair, and breastfeeding). We assessed for interactions between having a NPD and MetS. To assess for differences between the NPD patients groups we added an interaction term between NPD cohort and NPD diagnosis to the model. To assess whether self-perceived stress is associated with HCC we regressed PSS scores on HCC in unadjusted and adjusted analyses and investigated for any interactions between PSS scores, NPD status, NPD cohort and MetS on HCC. To directly compare HCC between the three NPD patient groups we regressed NPD patient group on HCC, in unadjusted and adjusted regression models, that were limited to the patients only. Data were analysed with SPSS for windows, version 25.0, all tests were 2-tailed and the level of significance was set at .05.

3 Results

3.1 Participants

Each of the cohorts have been described previously (Chapters 2 - 4), but brief descriptive data are presented in Table 1 for the three NPD cohorts. Numerous factors differed between the patients and controls across the three cohorts, including age, HLOE, employment status, natural hair colour, chemical treatment of hair, additional hair product use, season sampled, duration of storage, batch analysed, hormonal contraceptive use, MetS, tobacco use, alcohol use, illicit substance use, and level of physical activity. Between the three NPD patient groups the rates of current comorbid CMDs and psychiatric medication use also differed significantly.

3.2 Neuropsychiatric disorders and hair cortisol concentrations (Table 2)

HCC were significantly higher in patients than controls in both unadjusted ($p = 0.023$) and adjusted ($p = 0.027$) analyses. NPD cohort was significantly associated with HCC in unadjusted and adjusted analyses ($p < 0.001$). HCC were significantly higher in the schizophrenia cohort than the PTSD cohort in unadjusted and adjusted analyses ($p < 0.001$) and were significantly lower in the Parkinson's disease cohort than the PTSD cohort in unadjusted analysis ($p = 0.043$) and demonstrated a trend towards significance in adjusted analysis ($p = 0.055$). When the interaction term between NPD cohort and NPD status was added, NPD cohort ($p < 0.001$) and the interaction term ($p = 0.013$) were significantly associated with HCC, but NPD status was not significantly ($p = 0.549$) associated with HCC overall. HCC were higher in PTSD patients than Parkinson's disease patients, demonstrating a trend towards significance ($p = 0.057$), and were not significantly different between PTSD patients and schizophrenia patients ($p = 0.399$). HCC were significantly higher in PTSD patients than PTSD controls ($p = 0.003$), there was a significant interaction between schizophrenia cohort and controls ($p = 0.004$), such that in the schizophrenia cohort controls had significantly higher HCC than patients, a significant interaction was not demonstrated between Parkinson's disease cohort and controls ($p = 0.409$) (Figure 1).

When we limited analyses to the patients only NPD group demonstrated a trend to significance in unadjusted analysis ($p = 0.091$), but was no longer significantly associated with HCC in adjusted

analysis (p = 0.789) (Supplementary Table 1). HCC were significantly higher in PTSD patients than Parkinson's disease patients in unadjusted (p = 0.033), but not adjusted (p = 0.543) analyses and there were no differences between PTSD and schizophrenia patients in unadjusted or adjusted analyses (p = 0.792). A post-hoc Tukey test demonstrated that HCC were higher in PTSD patients than Parkinson's disease patients, demonstrating a trend towards significance (p = 0.084), but there were no other significant differences between the groups. When we limited the analysis to NPD patients without psychiatric comorbidity NPD group was not associated with HCC in unadjusted (p = 0.253) and adjusted (p = 0.127) analyses (Supplementary Table 2). Although HCC were not significantly lower in PTSD patients than in schizophrenia patients in unadjusted analysis (p = 0.261), this was significant in the adjusted analysis (p = 0.046). HCC were not significantly different between patients with PTSD and patients with Parkinson's disease in both unadjusted (p = 0.328) and adjusted (p = 0.848) analyses. There were no differences between the groups based on a post-hoc Tukey test.

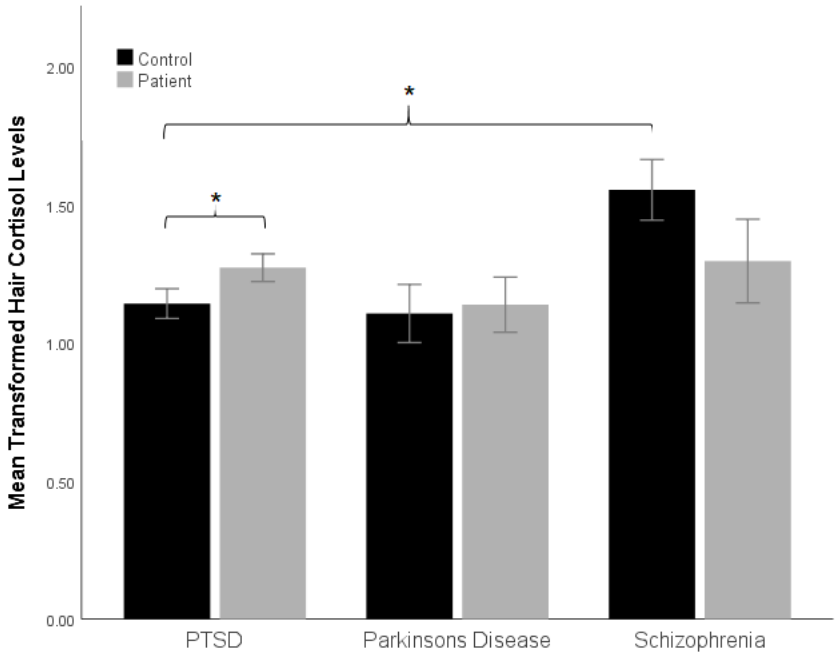

Figure 1 Hair cortisol concentrations (HCC) according to cohort and case status

Bar chart demonstrating mean HCC levels according to cohort and case status. The model was significant (F (348, 5) = 9.11, p < 0.001). HCC were significantly higher in schizophrenia controls than all other groups ($p \leq 0.001$), excluding schizophrenia patients (p = 0.187). PTSD patients had significantly higher HCC than PTSD controls (p = 0.004). There were no other significant differences between the groups based on Tukey HSD post hoc test.
*p < 0.05
Error bars ± 2SE

Table 1 Description and comparison of sociodemographic, hair related, behavioural, and clinical factors between patients and controls across the three cohorts

Variables	Total	PTSD		Parkinson's disease		Schizophrenia		Test statistic	p-value
		Patients	Controls	Patients	Controls	Patients	Controls		
Total	354	139	129	25	31	11	19		
Socio-demographic									
Age *M(SD)*	44.5 (14.7)	40.7 (11.6)	45.9 (14.8)	64.5 (8.4)	55.7 (6.9)	28.3 (7.1)	26.8 (5.8)	F(5, 348) = 34.0	<0.001*
Secondary education complete n(%)								$\chi^2(5) = 19.8$	0.001*
No	246 (69.5)	100 (71.9)	84 (65.1)	22 (88.0)	27 (87.1)	5 (45.5)	8 (42.1)		
Yes	108 (30.5)	39 (28.1)	45 (34.9)	3 (12.0)	4 (12.9)	6 (54.5)	11 (57.9)		
Employed n(%)								$\chi^2(5) = 27.0$	<0.001*
No	245 (69.2)	97 (69.8)	86 (66.7)	24 (96.0)	24 (77.4)	9 (81.8)	5 (26.3)		
Yes	109 (30.8)	42 (30.2)	43 (33.3)	1 (4.0)	7 (22.6)	2 (18.2)	14 (73.7)		
Hair related									
Natural hair colour black[a] n(%)								$\chi^2(5) = 12.46$	0.029*
No	203 (57.5)	79 (57.2)	74 (57.4)	15 (60.0)	11 (35.5)	9 (81.8)	15 (78.9)		
Yes	150 (42.5)	59 (42.8)	55 (42.6)	10 (40.0)	20 (64.5)	2 (18.2)	4 (21.1)		
Hair chemically treated[a] n(%)								$\chi^2(5) = 15.65$	0.008*
No	111 (31.4)	39 (28.3)	37 (28.7)	16 (64.0)	7 (22.6)	4 (36.4)	8 (42.1)		
Yes	242 (68.6)	99 (71.7)	92 (71.3)	9 (36.0)	24 (77.4)	7 (63.6)	11 (57.9)		
Frequency of hair washing[a] n(%)								$\chi^2(10) = 9.58$	0.478
≥ 2 times a week	93 (26.3)	36 (26.1)	36 (27.9)	7 (28.0)	5 (16.1)	4 (36.4)	5 (26.3)		
1 time a week	169 (47.9)	60 (43.5)	68 (52.7)	12 (48.0)	14 (45.2)	6 (54.5)	9 (47.4)		
< 1 time a week	91 (25.8)	42 (30.4)	25 (19.4)	6 (24.0)	12 (38.7)	1 (9.1)	5 (26.3)		
Add on hair products[a] n(%)								$\chi^2(5) = 11.83$	0.037*
No	178 (50.4)	60 (43.5)	71 (55.0)	19 (76.0)	15 (48.4)	6 (54.5)	7 (36.8)		
Yes	175 (49.6)	78 (56.5)	58 (45.0)	6 (24.0)	16 (51.6)	5 (45.5)	12 (63.2)		
Average hours in sun[b] n(%)								$\chi^2(10) = 12.92$	0.228
< 1 hour	162 (46.6)	59 (43.4)	54 (42.2)	18 (72.0)	14 (48.3)	7 (63.6)	10 (52.6)		
1-2 hours	106 (30.5)	46 (33.8)	40 (31.3)	5 (20.0)	10 (34.5)	2 (18.2)	3 (15.8)		
> 2 hours	80 (23.0)	31 (22.8)	34 (26.6)	2 (8.0)	5 (17.2)	2 (18.2)	6 (31.6)		
Season sampled n(%)								$\chi^2(15) = 28.92$	0.016*
Summer	76 (21.5)	34 (24.5)	27 (20.9)	3 (12.0)	3 (9.7)	3 (27.3)	6 (31.6)		
Autumn	85 (24.0)	22 (15.8)	38 (29.5)	11 (44.0)	9 (29.0)	1 (9.1)	4 (21.1)		
Winter	102 (28.8)	36 (25.9)	43 (33.3)	7 (28.0)	7 (22.6)	4 (36.4)	5 (26.3)		
Spring	91 (25.7)	47 (33.8)	21 (16.3)	4 (16.0)	12 (75.0)	3 (27.3)	4 (21.1)		
Duration of storage n(%)								$\chi^2(10) = 48.7$	<0.001*
Less than 1 year	66 (18.6)	17 (12.2)	34 (26.4)	8 (32.0)	2 (6.5)	1 (9.1)	4 (21.1)		
1 - 2 years	116 (32.8)	38 (27.3)	46 (35.7)	13 (52.0)	3 (9.7)	6 (54.5)	10 (52.6)		
2 – 3.5 years	172 (48.6)	84 (60.4)	49 (38.0)	4 (16.0)	26 (83.9)	4 (36.4)	5 (26.3)		

Batch analysed n(%)								$\chi^2(5) = 21.07$	0.001*
Pilot	16 (4.5)	1 (0.7)	8 (6.2)	5 (20.0)	2 (6.5)	0 (0.0)	0 (0.0)		
Final sample	338 (95.5)	138 (99.3)	121 (93.8)	20 (80.0)	29 (93.5)	11 (100.0)	19 (100.0)		
Clinical									
Hormonal contraceptive n(%)								$\chi^2(5) = 24.33$	<0.001*
No	270 (76.3)	95 (68.3)	102 (79.1)	25 (100)	29 (93.5)	9 (81.8)	10 (52.6)		
Yes	84 (23.7)	44 (31.7)	27 (20.9)	0 (0)	2 (6.5)	2 (18.2)	9 (47.4)		
Currently breastfeeding[a] n(%)								$\chi^2(5) = 9.80$	0.081
No	335 (94.9)	128 (92.8)	124 (96.1)	25 (100.0)	31 (100.0)	11 (100.0)	16 (84.2)		
Yes	18 (5.1)	10 (7.2)	5 (3.9)	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.8)		
Topical steroid use n(%)								$\chi^2(5) = 5.46$	0.363
No	331 (93.5)	129 (92.8)	124 (96.1)	21 (84.0)	29 (93.5)	10 (90.9)	18 (94.7)		
Yes	23 (6.5)	10 (7.2)	5 (3.9)	4 (16.0)	2 (6.5)	1 (9.1)	1 (5.3)		
Metabolic syndrome n(%)								$\chi^2(5) = 13.42$	0.020*
No	237 (66.9)	98 (70.5)	80 (62.0)	11 (44.0)	23 (74.2)	8 (72.7)	17 (89.5)		
Yes	117 (33.1)	41 (29.5)	49 (38.0)	14 (56.0)	8 (25.8)	3 (27.3)	2 (10.5)		
Other medical conditions n(%)								$\chi^2(5) = 10.00$	0.075
No	160 (45.2)	54 (38.8)	59 (45.7)	11 (44.0)	16 (51.6)	9 (81.8)	11 (57.9)		
Yes	194 (54.8)	85 (61.2)	70 (54.3)	14 (56.0)	15 (48.4)	2 (18.2)	8 (42.1)		
Behavioural									
Tobacco use n(%)								$\chi^2(5) = 20.94$	0.001*
No	212 (59.9)	66 (47.5)	87 (67.4)	22 (88.0)	18 (58.1)	6 (54.5)	13 (68.4)		
Yes	142 (40.1)	73 (52.5)	42 (32.6)	3 (12.0)	13 (41.9)	5 (45.5)	6 (31.6)		
Alcohol use n(%)								$\chi^2(5) = 12.73$	0.026*
No	192 (54.2)	70 (50.4)	69 (53.5)	21 (84.0)	19 (61.3)	6 (54.5)	7 (36.8)		
Yes	162 (45.8)	69 (49.6)	60 (46.5)	4 (16.0)	12 (38.7)	5 (45.5)	12 (63.2)		
Illicit substance use[c] n(%)								$\chi^2(5) = 29.42$	<0.001*
No	337 (95.2)	130 (93.5)	125 (96.9)	25 (100.0)	31 (100.0)	7 (63.6)	19 (100.0)		
Yes	17 (4.8)	9 (6.5)	4 (3.1)	0 (0.0)	0 (0.0)	4 (36.4)	0 (0.0)		
Level of physical activity[a] n(%)								$\chi^2(5) = 25.97$	0.004*
High	26 (7.4)	8 (5.8)	11 (8.5)	0 (0)	2 (6.1)	1 (9.1)	5 (26.3)		
Moderate	109 (30.9)	41 (29.7)	44 (34.1)	3 (11.5)	10 (30.3)	6 (54.5)	6 (31.6)		
Low	218 (61.8)	89 (64.5)	74 (57.4)	23 (88.5)	21 (63.6)	4 (36.4)	8 (42.1)		
Perceived stress scores *M(SD)*	20.5 (8.9)	27.0 (6.4)	16.9 (7.4)	15.2 (7.7)	12.3 (7.2)	24.8 (9.4)	15.6 (6.0)	F(5, 348) = 44.8	<0.001*
Psychiatric comorbidity									
Common mental disorder[d] n(%)								$\chi^2(2) = 14.7$	0.001*
No	74 (42.3)	49 (35.3)	NA	16 (64.0)	NA	9 (81.8)	NA		
Yes	101 (57.7)	90 (64.7)		9 (36.0)		2 (18.2)			
Substance use disorder[d] n(%)								$\chi^2(2) = 4.17$	0.124
No	154 (88.0)	120 (86.3)	NA	25 (100.0) (64.0)	NA	9 (81.8)	NA		
Yes	21 (12.0)	19 (13.7)		0 (0.0)		2 (18.2)			

Current psychiatric medication n(%)								$\chi^2(2) = 29.6$	<0.001
No	120 (68.6)	106 (76.3)	NA	14 (56.0)	NA	0 (0.0)	NA		
Yes	55 (31.4)	33 (23.7)		11 (44.0)		11 (100.0)			
Transformed hair cortisol levels *M(SD)*	1.22 (0.31)	1.27 (0.30)	1.14 (0.31)	1.14 (0.25)	1.11 (0.29)	1.30 (0.25)	1.56 (0.24)	F(5, 348) = 9.11	<0.001*

[a] One missing response on this item
[b] Six missing responses on this item
[c] 14 using cannabis, 8 using methamphetamine and 2 using methaqualone
[d] Determined based on the Mini International Neuropsychiatric Interview (MINI)
* p < 0.05

Table 2 Cohort, case status and metabolic syndrome regressed on hair cortisol concentrations (HCC)

	Simple linear regression		**Multiple linear regression[a]**					
			Model 1		**Model 2**		**Model 3**	
	ß (95% CI)	**p-value**	**ß (95% CI)**	**p-value**	**ß (95% CI)**	**p-value**	**ß (95% CI)**	**p-value**
Cohort		<0.001*		<0.001*		<0.001*		<0.001*
PTSD	Ref		Ref		Ref		Ref	
SCZ	0.25 (0.14; 0.36)	<0.001*	0.28 (0.16; 0.39)	<0.001*	0.08 (-0.10; 0.26)	0.399	-0.28 (-0.40; -0.17)	<0.001*
PD	-0.09 (-0.18; -0.00)	0.043*	-0.09 (-0.18; 0.00)	0.055	-0.13 (-0.27; 0.00)	0.057	-0.36 (-0.51; -0.21)	<0.001*
NPD status						0.549		0.114
Control	-0.08 (-0.14; -0.01)	0.023*	-0.07 (-0.13; -0.01)	0.027*	-0.11 (-0.18; -0.04)	0.003*	-0.10 (-0.18; -0.03)	0.006*
Patient	Ref		Ref		Ref		Ref	
MetS								0.272
No	Ref		Ref		Ref		Ref	
Yes	-0.01 (-0.08; 0.06)	0.761	0.04 (-0.03; 0.10)	0.285	0.04 (-0.02; 0.11)	0.210	-0.02 (-0.11; 0.08)	0.756
Cohort*NPD status						0.013*		
SCZ*Control					0.33 (0.11; 0.56)	0.004*		
PD*Control					0.07 (-0.10; 0.25)	0.409		
Control*MetS							0.10 (-0.02; 0.23)	0.111

[a] Multivariate adjusted model (adjusted for age, education, natural hair colour, frequency of hair washing, chemical treatment of hair, and breastfeeding), n = 352; (one participant with missing data related to hair care practices and one participant with missing data on breastfeeding excluded)
Model 1 – Model without interaction terms: F (11, 340) = 7.10 (p < 0.001*), Adj R^2 = 0.160
Model 2 – Interaction between cohort and case status added: F (13, 338) = 6.80 (p < 0.001*), Adj R^2 = 0.177
Model 3 – Interaction between case status and MetS added: F (12, 339) = 6.75 (p < 0.001*), Adj R^2 = 0.164
HCC, hair cortisol concentrations; MetS, metabolic syndrome; PD, Parkinson's disease; PTSD, posttraumatic stress disorder; NPD, neuropsychiatric disorder; SCZ, schizophrenia

3.3 Metabolic syndrome, neuropsychiatric disorder status and hair cortisol concentrations (Table 2)

Rates of MetS differed significantly across the groups and were highest in the Parkinson's disease patients and lowest in the schizophrenia controls. HCC were not significantly associated with MetS in unadjusted (p = 0.761) and adjusted (p = 0.285) analyses. When the interaction between NPDs and MetS was added to the model neither NPDs (p = 0.114) nor MetS (p = 0.272) were significantly associated with HCC overall. Patients without MetS demonstrated significantly higher HCC than controls without MetS (p = 0.006), HCC did not differ significantly between patients with and without MetS (p = 0.756), and the interaction between NPDs and MetS was not significant (p = 0.111) (Figure 2).

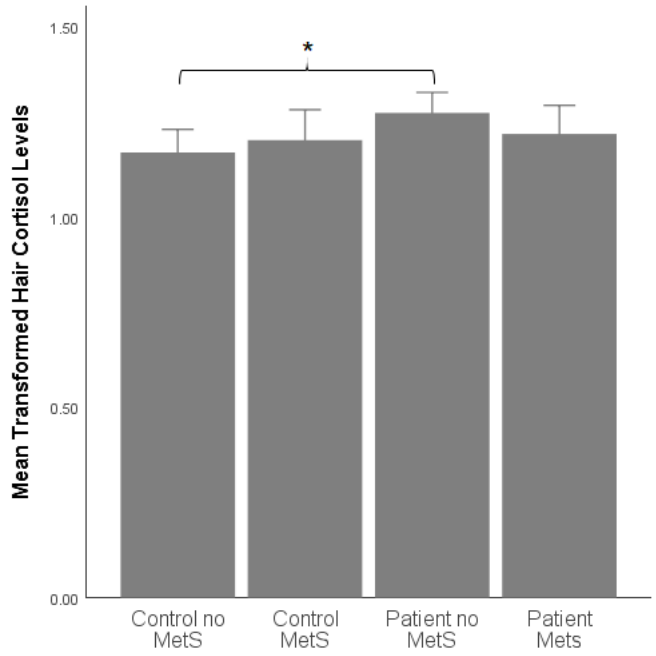

Figure 2 Hair cortisol concentrations (HCC) according to case status and metabolic syndrome

Bar chart demonstrating mean HCC levels according to NPD case status and metabolic syndrome (MetS). The model demonstrated a trend towards significance (F (353, 3) = 2.31, p = 0.076) and HCC were significantly higher in patients without MetS than controls without MetS (p = 0.048). There were no other significant differences between the groups based on Tukey HSD post hoc test.
*p < 0.05
Error bars ± 2SE

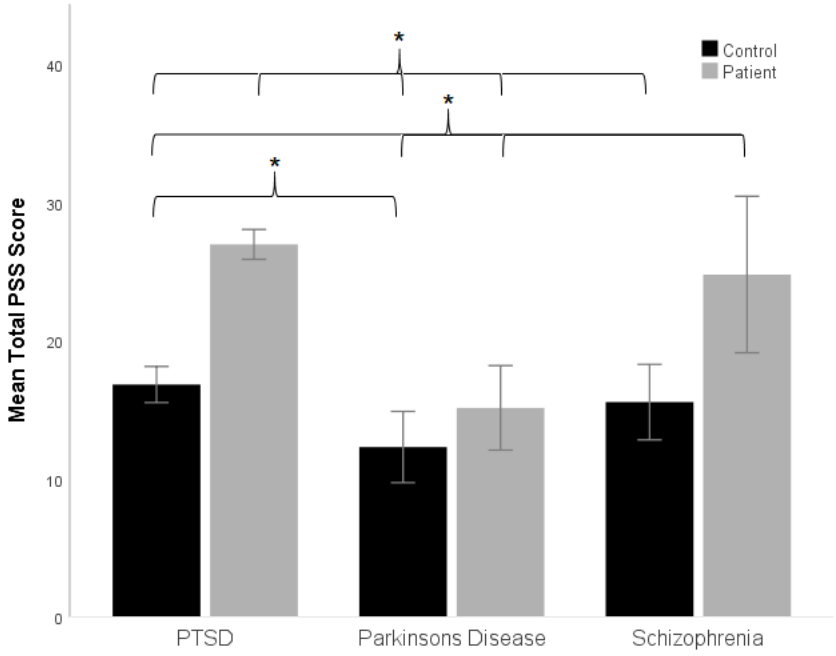

Figure 3 Perceived stress scale (PSS) scores according to cohort and case status

Bar chart demonstrating mean PSS scores according to cohort and case status. The model was significant (F (348, 5) = 44.79, p < 0.001) and a post-hoc Tukey test revealed that PSS scores were highest in PTSD patients and were significantly higher (p < 0.001) than all the other groups, excluding schizophrenia patients. Schizophrenia patients also demonstrated significantly higher PSS scores (p < 0.001) than all the other groups and PTSD controls had significantly higher PSS scores than Parkinson's disease controls (p = 0.017).
*p < 0.05
Error bars ± 2SE

3.4 Self-perceived stress, neuropsychiatric disorder status and hair cortisol concentrations

PSS scores differed significantly between the six groups (Table 1; Figure 3) and a post-hoc Tukey test revealed that PSS scores were highest in PTSD patients and were significantly higher ($p < 0.001$) than all the other groups, excluding schizophrenia patients. Schizophrenia patients also demonstrated significantly higher PSS scores ($p < 0.001$) than all the other groups and PTSD controls had significantly higher PSS scores than Parkinson's disease controls ($p = 0.017$). PSS scores were significantly positively associated with HCC in unadjusted analysis ($B = 0.01$, 95% CI 0.00; 0.01, $p = 0.002$), but not in adjusted analysis (*adj* $B = 0.00$, 95% CI -0.00; 0.01, $p = 0.210$). There were no significant interactions between PSS scores and case status on HCC ($p = 0.986$). There was a trend towards significance demonstrated for the interaction between cohort and PSS scores ($p = 0.079$), such that PSS scores were significantly positively associated with HCC in the PTSD cohort (*adj* $B =$

0.01, 95% CI 0.00; 0.01, $p = 0.037$), and were inversely associated with HCC in the Parkinson's disease (*adj B* = -0.01, 95% CI -0.02; 0.00, $p = 0.132$) and schizophrenia (*adj B* = -0.01, 95% CI -0.03; 0.00, $p = 0.066$) cohorts, demonstrating a trend towards significance in the latter cohort. There were no significant interactions between PSS scores and MetS on HCC ($p = 0.490$) and no other interaction terms added were significant.

4 Discussion

Our objectives, in a sample of South African mixed ancestry females were to compare HCC between three NPDs and investigate interactions between NPDs and MetS on HCC. HCC were significantly higher in NPD patients than controls overall. We also found that HCC differed significantly across the three cohorts overall, with highest HCC in the schizophrenia cohort, followed by the PTSD and Parkinson's disease cohorts. With respect to differences between the three NPD patient groups, when mental disorder comorbidity was included HCC were significantly higher in PTSD than Parkinson's disease patients, but only in unadjusted analysis. When we excluded patients with mental disorder comorbidity HCC were significantly higher in schizophrenia patients than PTSD patients in the adjusted model. MetS was not significantly associated with HCC and there were no interactions between NPDs and MetS.

HCC were significantly higher in patients with NPDs than controls overall, suggesting HPA-axis dysfunction occurs across NPDs. These results are, however, likely influenced by the larger sample size of the PTSD cohort. When the interaction between cohort and NPD diagnosis was included HCC remained significantly higher in PTSD patients than PTSD controls and the direction of association was reversed for the schizophrenia cohort, with higher HCC in the controls than the patients. These results align with those demonstrated in the individual cohorts (Chapters 2 - 4). Thus although we demonstrated opposite directions of association for PTSD and schizophrenia as compared to controls to what has generally been observed (Gajsak et al., 2017; Girshkin et al., 2014; Hubbard & Miller, 2019; Meewisse et al., 2007; Morris et al., 2012; Schumacher et al., 2019) the consistency in outcomes provide support for the veracity of the results found in the individual NPD cohorts.

PTSD patients demonstrated the highest self-perceived stress scores, followed by schizophrenia patients and scores for both these groups were significantly higher than all the other groups. Perceived stress scores were significantly positively associated with HCC in the PTSD cohort and were inversely associated with HCC in the schizophrenia cohort, demonstrating a trend towards significance. These results further suggest that the relationship between the psychological experience of stress and HCC is reversed in these two cohorts, and that in the context of PTSD stress may be associated with increased cortisol output and in schizophrenia with a blunted HPA-axis.

The differences observed in HCC among the three NPD cohorts may not be surprising as the groups also differed according to various factors. HCC decreased in the cohorts according to the average age of the groups, which aligns with the inverse association demonstrated between age and HCC in our control sample (Chapter 6). The pattern observed in the three patient groups mirrored that of the cohorts overall, with highest HCC in the schizophrenia patients, followed by the PTSD patients and lowest HCC in the Parkinson's disease patients. In the analysis where the total sample was included HCC were higher in PTSD patients than Parkinson's disease patients, demonstrating a trend towards significance. When analyses were limited to the patient groups and psychiatric comorbidity was permitted HCC were significantly lower in Parkinson's disease patients than PTSD patients, but only in unadjusted analysis. When patients with psychiatric comorbidity were excluded, HCC were significantly higher in schizophrenia patients than PTSD patients in the adjusted model. The sample size was, however, significantly reduced once patients with psychiatric comorbidity were excluded. There thus appear to be some weak differences between the patient groups. Considering that HCC differed significantly among the three cohorts overall and the cohorts differed significantly in various respects direct comparisons between the patient groups may not accurately reflect how HCC differ across the three NPDs. For instance, although HCC were higher in the schizophrenia patients than the PTSD patients, the direction of association between their respective control groups was reversed. Thus higher HCC in schizophrenia patients than PTSD patients, and in PTSD patients than Parkinson's disease patients may, for instance, reflect higher HCC in younger cohorts than older cohorts, rather than true differences between the patient groups. Taken together our results suggest that due to the prominent

differences between the three NPD cohorts that case-control comparisons within the NPD cohorts likely provide more valid estimates than comparisons across the NPD patient groups.

Similar to the analyses according to the individual cohorts, HCC were not significantly associated with MetS, nor with NPD and MetS comorbidity. The association patterns followed those demonstrated for the PTSD cohort (Chapter 2), which again may be ascribed to the larger sample size of the PTSD cohort, with non-significantly lower HCC in patients with MetS than those without. Based on analyses of the individual cohorts we found that MetS was non-significantly associated with higher HCC across all the groups, excluding only the PTSD patients. If we excluded PTSD patients from the total sample we demonstrated a trend towards significance ($p = 0.072$) for higher HCC in individuals with MetS. Although our total sample was sufficiently powered to examine associations between MetS and HCC once we excluded PTSD patients our power to demonstrate significant associations was reduced and may explain why we only found a trend towards significance. Thus for all the groups, excluding PTSD patients, our results align with what is generally demonstrated in terms of higher HCC in the context of MetS (Kuehl et al., 2015; Stalder et al., 2013). The altered pattern demonstrated in our PTSD cases may possibly be due to enhanced HPA-axis negative feedback sensitivity secondary to a dual burden of PTSD and MetS (Langerak et al., 2015). Our results also highlight the importance of adjusting for metabolic factors in studies assessing for associations between NPDs and cortisol levels. Considering the high comorbidity of MetS in NPDs (Bradley & Dinan, 2010; Penninx & Lange, 2018; Vancampfort et al., 2015), some of the cortisol related effects observed may be secondary to MetS rather than be specific to the NPDs studied. However, we generally observed that the effects related to NPD phenotype were larger, particularly for PTSD and schizophrenia, than the effects related to MetS. Furthermore, in our Parkinson's disease patients we observed significant associations between non-motor symptoms pertaining to depression and anhedonia and HCC (Chapter 3). Our results suggest that clinical features related to mental disorder are associated with chronic stress as reflected by long-term HPA-axis dysfunction and that these effects are more prominent than those secondary to CVD risk.

4.1 Strengths and limitations

There are limitations pertaining to the study, such as the cross-sectional nature of the study, the hair length requirement resulting in exclusion of many participants and the study being limited to a single ethnicity and gender that have been discussed in the individual cohort manuscripts. Here we focus the discussion on strengths and limitations particular to the combined analysis conducted here. Although the aim of the study was to obtain equivalent sample sizes for the three cohorts, there were various factors influencing recruitment of patients with Parkinson's disease and schizophrenia and thus the samples included across the three cohorts differed in size. The three cohorts also differed in various other respects, and of particular importance according to age. This can partly be ascribed to the epidemiology of the three NPDs, schizophrenia usually has its onset in late-adolescence and early adulthood, PTSD can occur at any age, but is observed particularly in younger adults and Parkinson's disease is usually observed in older adults and increases in prevalence with age (American Psychiatric Association [APA], 2013; Pringsheim, Jette, Frolkis, & Steeves, 2014). The differences in age occurrence make it difficult to disentangle age related effects from those specific to the different NPDs.

Although our study design allowed estimations within the individual NPDs, there were aspects of the study design that limit these investigations across NPD diagnoses. Some of these factors, such as the differing epidemiology of the disorders, may not be possible to fully control for. However, certain study design features can be adapted to allow for better equivalence across groups. Future studies, aiming to compare factors across disorders, should consider methods to increase comparability across groups, such as considerations pertaining to age, stage of illness and treatment received. However, even considering the limitations to cross-disorder comparisons in this study, the analyses across cohorts still provided additional insights to those limited to the individual NPD cohorts and thus emphasize the utility of conducting studies across disorders. Furthermore, although the cohorts differed in certain respects the participants in the different cohorts completed the same measures and assessments were conducted in a uniform way thus ensuring consistent methodology across cohorts.

4.2 Conclusions

There are few studies directly comparing cortisol levels (HPA-axis function) between different NPDs. HCC were significantly higher in patients with NPDs than controls overall, although this varied between the cohorts. When the NPD patient groups were directly compared, there were some significant findings, that differed depending on whether psychiatric comorbidity was included. We also found that HCC differed significantly between the three cohorts overall and that various factors differed among the groups, thus suggesting the cohorts were not directly comparable. Our results suggest that evaluating case-control effects within the individual cohorts likely provide better estimates of patterns of HPA-axis dysregulation present in each of the NPDs than direct cross-disorder comparisons. We also observed that overall, excluding PTSD patients demonstrating an inverse pattern, HCC were higher in individuals with MetS, demonstrating a trend towards significance. The altered pattern demonstrated for PTSD patients suggests that associations between HPA-axis dysfunction and MetS within NPDs may not be universal and warrants further investigation. Although our study had several limitations influencing cross-cohort comparisons, our results still highlight the benefit of including different NPDs within the same study. To accurately determine shared versus unique pathways involved in the pathophysiology of NPDs and associated CVD risk more studies are required that directly compare effects across NPDs. To better estimate the association between NPDs, HPA-axis dysfunction and CVD risk longitudinal studies are required that can evaluate how these systems interact over time and thus provide better estimates of cause and effect pathways.

5 Supplementary materials

Supplementary Table 1 NPD patient groups regressed on hair cortisol concentrations (HCC), psychiatric comorbidity included

	Simple linear regression		Multiple linear regression[a]	
	ß (95% CI)	**p-value**	**ß (95% CI)**	**p-value**
Cohort		0.091		0.789
PTSD	Ref		Ref	
SCZ	0.02 (-0.15; 0.20)	0.792	0.03 (-0.15; 0.22)	0.792
PD	-0.13 (-0.36; -0.01)	0.033*	-0.05 (-0.21; 0.11)	0.543

[a] Multivariate adjusted model (adjusted for age, education, natural hair colour, frequency of hair washing, chemical treatment of hair, breastfeeding, and MetS), n = 174; (one participant with missing data on breastfeeding excluded)
Simple linear regression model: F (2, 172) = 2.43 (p = 0.091), Adj R^2 = 0.016
Multiple linear regression model: F (10, 162) = 2.80 (p = 0.003*), Adj R^2 = 0.095
HCC, hair cortisol concentrations; MetS, metabolic syndrome; PD, Parkinson's disease; PTSD, posttraumatic stress disorder; NPD, neuropsychiatric disorder; SCZ, schizophrenia

Supplementary Table 2 NPD patient groups regressed on hair cortisol concentrations (HCC), psychiatric comorbidity excluded

	Simple linear regression		Multiple linear regression[a]	
	ß (95% CI)	**p-value**	**ß (95% CI)**	**p-value**
Cohort		0.253		0.127
PTSD	Ref		Ref	
SCZ	0.13 (-0.10; 0.35)	0.261	0.23 (0.00; 0.46)	0.046*
PD	-0.08 (-0.26; 0.09)	0.328	0.22 (-0.21; 0.25)	0.848

[a] Multivariate adjusted model (adjusted for age, education, natural hair colour, frequency of hair washing, chemical treatment of hair, breastfeeding, and MetS), n = 69
Simple linear regression model: F (2, 66) = 1.41 (p = 0.253), Adj R^2 = 0.012
Multiple linear regression model: F (10, 58) = 2.75 (p = 0.008*), Adj R^2 = 0.204
HCC, hair cortisol concentrations; MetS, metabolic syndrome; PD, Parkinson's disease; PTSD, posttraumatic stress disorder; NPD, neuropsychiatric disorder; SCZ, schizophrenia

6 References

Ahmadi, N., Hajsadeghi, F., Mirshkarlo, H. B., Budoff, M., Yehuda, R., & Ebrahimi, R. (2011). Post-traumatic stress disorder, coronary atherosclerosis, and mortality. *American Journal of Cardiology*, *108*(1), 29–33. https://doi.org/10.1016/j.amjcard.2011.02.340

Alberti, K. G., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., … Smith Jr, S. C. (2009). Harmonizing the Metabolic Syndrome A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International A. *Circulation*,

120(16), 1640–1645. https://doi.org/10.1161/CIRCULATIONAHA.109.192644 [doi]

American Psychiatric Association [APA]. (2013). *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5®)*. Arlington, VA.

Bainbridge, J., Boyd, J., Cambi, F., Christine, C., Dhall, R., Leehey, M., … Zhang, Y. (2017). Association of metabolic syndrome and change in Unified Parkinson Disease Rating Scale scores. *Neurology*, *89*(17), 1789–1794. https://doi.org/https://doi.org/10.1212/WNL.0000000000004572

Bartoli, F., Carra, G., Crocamo, C., Carretta, D., & Clerici, M. (2013). Metabolic Syndrome in People Suffering from Posttraumatic Stress Disorder: A Systematic Review and Meta-Analysis. *Metabolic Syndrome and Related Disorders*, *11*(5), 301–308. https://doi.org/10.1089/met.2013.0010

Boksa, P. (2013). A way forward for research on biomarkers for psychiatric disorders. *Journal of Psychiatry and Neuroscience*, *38*(2), 75–77. https://doi.org/10.1503/jpn.130018

Borges, S., Gayer-Anderson, C., & Mondelli, V. (2013). A systematic review of the activity of the hypothalamic-pituitary-adrenal axis in first episode psychosis. *Psychoneuroendocrinology*, *38*(5), 603–611. https://doi.org/10.1016/j.psyneuen.2012.12.025

Bradley, A. J., & Dinan, T. G. (2010). A systematic review of hypothalamic-pituitary-adrenal axis function in schizophrenia: implications for mortality. *Journal of Psychopharmacology (Oxford, England)*, *24*(4 Suppl), 91–118. https://doi.org/10.1177/1359786810385491; 10.1177/1359786810385491

Ciufolini, S., Dazzan, P., Kempton, M. J., Pariante, C., & Mondelli, V. (2014). HPA axis response to social stress is attenuated in schizophrenia but normal in depression: Evidence from a meta-analysis of existing studies. *Neuroscience and Biobehavioral Reviews*, *47*, 359–368. https://doi.org/10.1016/j.neubiorev.2014.09.004

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A Global Measure of Perceived Stress. *Journal of*

Health and Social Behavior, *24*(4), 385–396. https://doi.org/10.1016/j.socscimed.2016.08.021

Correll, C. U., Solmi, M., Veronese, N., Bortolato, B., Rosson, S., Santonastaso, P., … Stubbs, B. (2017). Prevalence, incidence and mortality from cardiovascular disease in patients with pooled and specific severe mental illness: a large-scale meta-analysis of 3,211,768 patients and 113,383,368 controls. *World Psychiatry*, *16*(2), 163–180. https://doi.org/10.1002/wps.20420

De Hert, M., Detraux, J., & Vancampfort, D. (2018). The intriguing relationship between coronary heart disease and mental disorders. *Dialogues in Clinical Neuroscience*, *20*(1), 31–40.

Du, X., & Pang, T. Y. (2015). Is dysregulation of the HPA-axis a core pathophysiology mediating co-morbid depression in neurodegenerative diseases? *Frontiers in Psychiatry*, *6*(MAR), 1–33. https://doi.org/10.3389/fpsyt.2015.00032

First, M. B., Spitzer, R. L., Gibbon, M., & Williams, J. B. W. (1997). *Structured Clinical Interview for DSM-IV Axis I Disorders, Clinical Version (SCID-CV)*. Washington, DC: American Psychiatric Press.

Gajsak, L. R., Gelemanovic, A., Kuzman, M. R., & Puljak, L. (2017). Impact of stress response in development of first-episode psychosis in schizophrenia: An overview of systematic reviews. *Psychiatria Danubina*, *29*(1), 14–23.

Gao, W., Stalder, T., Foley, P., Rauh, M., Deng, H., & Kirschbaum, C. (2013). Quantitative analysis of steroid hormones in human hair using a column-switching LC–APCI–MS/MS assay. *Journal of Chromatography B*, *928*, 1–8. https://doi.org/10.1016/j.jchromb.2013.03.008

Girshkin, L., Matheson, S. L., Shepherd, A. M., & Green, M. J. (2014). Morning cortisol levels in schizophrenia and bipolar disorder: A meta-analysis. *Psychoneuroendocrinology*, *49*(1), 187–206. https://doi.org/10.1016/j.psyneuen.2014.07.013

Herrero, M.-T., Estrada, C., Maatouk, L., & Vyas, S. (2015). Inflammation in Parkinson's disease: role of glucocorticoids. *Frontiers in Neuroanatomy*, *9*(April), 1–12. https://doi.org/10.3389/fnana.2015.00032

Hubbard, D. B., & Miller, B. J. (2019). Meta-analysis of blood cortisol levels in individuals with first-episode psychosis. *Psychoneuroendocrinology*, *104*(September 2018), 269–275. https://doi.org/10.1016/j.psyneuen.2019.03.014

Hughes, A., Daniel, S., Kilford, L., & Lees, A. (1992). Accuracy of clinical diagnosis of idiopathic Parkinson's disease: A clinico-pathological study of 100 cases. *Journal of Neurology Neurosurgery and Psychiatry*, *55*(3), 181–184. https://doi.org/10.1136/jnnp.55.3.181

Kubzansky, L. D., Koenen, K., & Spiro, A. (2007). Prospective study of posttraumatic stress disorder symptoms and coronary heart disease in the normative aging study. *Archives of General Psychiatry*, *64*(1), 109–116. https://doi.org/10.1001/archpsyc.64.1.109

Kuehl, L. K., Hinkelmann, K., Muhtz, C., Dettenborn, L., Wingenfeld, K., Spitzer, C., … Otte, C. (2015). Hair cortisol and cortisol awakening response are associated with criteria of the metabolic syndrome in opposite directions. *Psychoneuroendocrinology*, *51*, 365–370. https://doi.org/10.1016/j.psyneuen.2014.09.012

Langerak, T., van den Dries, L. W. J., Wester, V. L., Staufenbiel, S. M., Manenschijn, L., van Rossum, E. F. C., & van Gorp, E. C. M. (2015). The relation between long-term cortisol levels and the metabolic syndrome in HIV-infected patients. *Clinical Endocrinology*, *83*(2), 167–172. https://doi.org/10.1111/cen.12790

Lee, E. H. (2012). Review of the psychometric evidence of the perceived stress scale. *Asian Nursing Research*, *6*(4), 121–127. https://doi.org/10.1016/j.anr.2012.08.004

Matsha, T. E., Hassan, M. S., Hon, G. M., Soita, D. J., Kengne, A. P., & Erasmus, R. T. (2013). Derivation and validation of a waist circumference optimal cutoff for diagnosing metabolic syndrome in a South African mixed ancestry population. *International Journal of Cardiology*, *168*(3), 2954–2955. https://doi.org/https://doi.org/10.1016/j.ijcard.2013.03.150

Meewisse, M. L., Reitsma, J. B., de Vries, G. J., Gersons, B. P., & Olff, M. (2007). Cortisol and post-traumatic stress disorder in adults: systematic review and meta-analysis. *The British Journal of*

Psychiatry : The Journal of Mental Science, *191*, 387–392. https://doi.org/191/5/387 [pii]

Mitchell, A. J., Vancampfort, D., Sweers, K., Van Winkel, R., Yu, W., & De Hert, M. (2013). Prevalence of metabolic syndrome and metabolic abnormalities in schizophrenia and related disorders-a systematic review and meta-analysis. *Schizophrenia Bulletin*, *39*(2), 306–318. https://doi.org/10.1093/schbul/sbr148

Morris, M. C., Compas, B. E. B. E., & Garber, J. (2012). Relations among posttraumatic stress disorder, comorbid major depression, and HPA function: a systematic review and meta-analysis. *Clinical Psychology Review*, *32*(4), 301–315. https://doi.org/10.1016/j.cpr.2012.02.002.Relations

Nam, G. E., Kim, S. M., Han, K., Kim, N. H., Chung, H. S., Kim, J. W., … Choi, K. M. (2018). Metabolic syndrome and risk of Parkinson disease: A nationwide cohort study. *PLoS Medicine*, *15*(8), 1–15. https://doi.org/10.1371/journal.pmed.1002640

Penninx, B. W. J. H., & Lange, S. M. M. (2018). Metabolic syndrome in psychiatric patients: Overview, mechanisms, and implications. *Dialogues in Clinical Neuroscience*, *20*(1), 63–73.

Pinto, J. V., Moulin, T. C., & Amaral, O. B. (2017). On the transdiagnostic nature of peripheral biomarkers in major psychiatric disorders: A systematic review. *Neuroscience and Biobehavioral Reviews*, *83*(21), 97–108. https://doi.org/10.1016/j.neubiorev.2017.10.001

Pringsheim, T., Jette, N., Frolkis, A., & Steeves, T. D. L. (2014). The prevalence of Parkinson's disease: A systematic review and meta-analysis. *Movement Disorders*, *29*(13), 1583–1590. https://doi.org/10.1002/mds.25945

Rosenbaum, S., Stubbs, B., Ward, P. B., Steel, Z., Lederman, O., & Vancampfort, D. (2015). The prevalence and risk of metabolic syndrome and its components among people with posttraumatic stress disorder: A systematic review and meta-analysis. *Metabolism: Clinical and Experimental*, *64*(8), 926–933. https://doi.org/10.1016/j.metabol.2015.04.009

Schumacher, S., Niemeyer, H., Engel, S., Cwik, J. C., Laufer, S., Klusmann, H., & Knaevelsrud, C. (2019). HPA axis regulation in posttraumatic stress disorder: A meta-analysis focusing on

potential moderators. *Neuroscience and Biobehavioral Reviews*, *100*(June 2018), 35–57. https://doi.org/10.1016/j.neubiorev.2019.02.005

Sheehan, D. V, Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., … Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry*, *59*(Suppl 20), 22–33. Retrieved from https://psycnet.apa.org/record/1998-03251-004

Soares, N. M., Pereira, G. M., Altmann, V., de Almeida, R. M. M., & Rieder, C. R. M. (2019). Cortisol levels, motor, cognitive and behavioral symptoms in Parkinson's disease: a systematic review. *Journal of Neural Transmission*, *126*(3), 219–232. https://doi.org/10.1007/s00702-018-1947-4

Stalder, T., Kirschbaum, C., Alexander, N., Bornstein, S. R., Gao, W., Miller, R., … Fischer, J. E. (2013). Cortisol in hair and the metabolic syndrome. *The Journal of Clinical Endocrinology & Metabolism*, *98*(6), 2573–2580. https://doi.org/10.1210/jc.2013-1056

Steudte, S., Kirschbaum, C., Gao, W., Schoenfeld, S., Hoyer, J., & Stalder, T. (2012). Glucocorticoids in hair as biomarkers of traumatization in healthy individuals and PTSD patients. *European Journal of Psychotraumatology*, *3*(1). https://doi.org/10.3402/ejpt.v3i0.19472

Vancampfort, D., Stubbs, B., Mitchell, A. J., De Hert, M., Wampers, M., Ward, P. B., … Correll, C. U. (2015). Risk of metabolic syndrome and its components in people with schizophrenia and related psychotic disorders, bipolar disorder and major depressive disorder: A systematic review and meta-analysis. *World Psychiatry*, *14*(3), 339–347. https://doi.org/10.1002/wps.20252

Vancampfort, D., Wampers, M., Mitchell, A. J., Correll, C. U., De Herdt, A., Probst, M., & De Hert, M. (2013). A meta-analysis of cardio-metabolic abnormalities in drug naïve, first-episode and multi-episode patients with schizophrenia versus general population controls. *World Psychiatry*, *12*(3), 240–250. https://doi.org/10.1002/wps.20069

Walker, E. R., McGee, R. E., & Drus, B. G. (2015). Mortality in Mental Disorders and Global Disease Burden Implications: A Systematic Review and Meta-analysis. *JAMA Psychiatry*, *72*(4), 334–341.

https://doi.org/10.1001/jamapsychiatry.2014.2502

Wennig, R. (2000). Potential problems with the interpretation of hair analysis results. *Forensic Science International*, *107*(1), 5–12. https://doi.org/10.1016/S0379-0738(99)00146-2

Whiteford, H. A., Degenhardt, L., Rehm, J., Baxter, A. J., Ferrari, A. J., Erskine, H. E., … Johns, N. (2013). Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *The Lancet*, *382*(9904), 1575–1586. https://doi.org/https://doi.org/10.1016/S0140-6736(13)61611-6

World Health Organization. (2005). *WHO STEPS surveillance manual: the WHO STEPwise approach to chronic disease risk factor surveillance*. Geneva: World Health Organization (WHO).

World Medical Association. (2013). World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA - Journal of the American Medical Association*, *310*(20), 2191–2194. https://doi.org/10.1001/jama.2013.281053

Zhang, P., & Tian, B. (2014). Metabolic syndrome: An important risk factor for Parkinson's disease. *Oxidative Medicine and Cellular Longevity*, *2014*. https://doi.org/10.1155/2014/729194

CHAPTER 6

Hair cortisol as a biomarker of stress and resilience in South African mixed ancestry females

(*manuscript accepted)*

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Hair cortisol as a biomarker of stress and resilience in South African mixed ancestry females

Abstract

Background: Hair cortisol concentrations (HCC) are increasingly used as a biomarker of stress, however limited research exists regarding the relationship between HCC and protective factors, such as resilience. Additionally, studies measuring HCC need to account for possible confounders, and these factors have not been examined in sufficiently diverse settings.

Objectives: Our objectives were to identify determinants of HCC in a sample of mixed ancestry adults and investigate the association of HCC with measures of self-perceived stress and resilience.

Methods: Our sample comprised 164 females (mean age 46.5 years, *SD* = 15.0), self-identifying as mixed ancestry, who were control participants in a cross sectional case-control study (SHARED ROOTS), conducted in Cape Town, South Africa from May 2014 until June 2017. We examined which socio-demographic, hair related, clinical and behavioural factors were associated with HCC in both unadjusted and adjusted linear regression models. Furthermore, the relationship of HCC with self-perceived stress and resilience scores were also examined.

Results: HCC (*Mdn* 4.4 pg/ml; *IQR* 2.8; 11.4) were significantly positively associated with hair product use and breastfeeding, and significantly negatively associated with age, level of education, duration of sun exposure, duration of storage, and demonstrated a trend towards significance with frequency of hair washing, in adjusted models. HCC were inversely associated with CD-RISC scores (*adj β* = -0.179, *p* = 0.012) scores but were not significantly associated with PSS scores (*adj β* = -0.001, *p* = 0.989).

Conclusions: We identified specific determinants of HCC in our sample, including the first indication that sun exposure has an effect on HCC under naturalistic conditions. These potential confounders need to be controlled for in the design and analysis of future studies. HCC may be a biomarker of

resilience to stress, rather than perceived stress. Further research measuring HCC in more diverse settings and populations and including constructs related to resilience are needed.

Keywords: Hair cortisol concentrations; determinants; self-perceived stress; resilience

1 Introduction

One of the central pathways involved in the physiological response to stress is the hypothalamic-pituitary-adrenal (HPA) axis, with many of the ensuing effects of stress facilitated through cortisol. Cortisol has thus long been investigated as a stress biomarker in various contexts (Chrousos, 2009). Utilising cortisol as a biomarker is, however, complicated by the diverse components that can influence cortisol secretion, such as diurnal fluctuations, physical activity, food intake and substance use (Stalder & Kirschbaum, 2012; Wosu, Valdimarsdóttir, Shields, Williams, & Williams, 2013). Additionally, traditional sampling methods used, such as blood, saliva and urine, are more suited to estimating acute cortisol levels (Stalder & Kirschbaum, 2012). The measurement of hair cortisol concentrations (HCC) provides distinct advantages over traditional cortisol measurement techniques, potentially making it the ideal measure of longer-term HPA axis function. HCC are assumed to capture cumulative cortisol secretion over periods of several months (Stalder & Kirschbaum, 2012). Hair sampling is non-invasive, inexpensive, less influenced by sampling and environmental factors, and samples can be stored at room temperature (Wosu et al., 2013). Although hair cortisol appears to be a robust measure, there are also confounding factors that need to be accounted for when HCC are investigated as a marker of stress.

Considering that the neuroendocrine effects demonstrated in relation to stress have generally been of small magnitude, it is important to identify confounders that can potentially obscure the relationship between stress and cortisol (Staufenbiel, Penninx, de Rijke, van den Akker, & van Rossum, 2015). To this end, previous studies have been conducted to determine the socio-demographic, hair related, behavioral and clinical factors that may be potential confounders (Abell et al., 2016; Dettenborn, Tietze, Kirschbaum, & Stalder, 2012; Feller et al., 2014; Garcia-Leon et al., 2018; Staufenbiel et al., 2015). Meta-analyses and systematic reviews have also sought to identify the most relevant factors by aggregating the results of individual studies (Gray et al., 2018; Stalder et al., 2017; Wosu et al., 2013). Some key relationships that have been identified include higher HCC in males, positive associations with age and anthropometry, and the impact of hair care practices, such as decreased HCC with increased hair washing frequency and in chemically treated hair (Stalder et al., 2017; Wosu et al.,

2013). Raised HCC have also been found in relation to physical or psychological stressors, such as shift work, unemployment, caregiving stress and chronic pain (Stalder et al., 2017; Wosu et al., 2013). HCC have further been investigated as a correlate of psychometric measures of stress, with self-perceived stress being the most frequently utilised construct (Stalder et al., 2017). Although there have been some significant findings, meta-analyses and systematic reviews have largely found that HCC are not associated with self-perceived stress (Stalder et al., 2017; Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, 2013; Wosu et al., 2013). Authors have suggested that the heterogeneous results may be related to the complexity in classifying and evaluating stress (Gray et al., 2018).

To understand the stress response more broadly, it is also important to consider factors that may be protective. Generally, more is known about the biological factors related to risk and pathology than biological factors related to protection and resilience (Bowes & Jaffee, 2013). Resilience is commonly defined as the dynamic process whereby an individual demonstrates positive adaption following adversity or stressful situations (Luthar, Cicchetti, & Becker, 2000). Although not all results converge, protective factors, such as positive affect and resilience, have generally been associated with lower cortisol levels (Dockray & Steptoe, 2010; Steptoe, Dockray, & Wardle, 2009). For instance, a study in caregivers of people with autism found that caregiver resilience was significantly negatively correlated with the cortisol awakening response (CAR). Furthermore, cortisol levels were significantly higher in the low resilience group as compared to the medium and high resilience groups (Ruiz-Robledillo, De Andrés-García, Pérez-Blasco, González-Bono, & Moya-Albiol, 2014). In contrast to self-perceived stress, the relationship between HCC and resilience has not been extensively examined. A study in 80 university students found that HCC were significantly negatively correlated with resilience scores (Garcia-Leon et al., 2018). Similarly, a study in 27 adolescents demonstrated that HCC were significantly inversely associated with dispositional optimism (Milam, Slaughter, Verma, & McConnell, 2014), while a pilot study in 40 students found that HCC were non-significantly negatively correlated with resilience (Ullmann et al., 2016).

Thus, increased efforts are required to understand the biological processes underpinning resilience (Bowes & Jaffee, 2013). HCC may provide additional insights into the complex relationship between

stress, resilience and HPA-axis functioning (Gray et al., 2018). A major limitation of the existing body of research examining hair cortisol as a biomarker of stress, is the lack of geographic, ethnic and cultural diversity in samples that have been studied (Wosu et al., 2013). Previous studies seeking to identify the basic determinants of HCC have largely been conducted in samples representative of European and Caucasian populations (Abell et al., 2016; Dettenborn et al., 2012; Feller et al., 2014; Garcia-Leon et al., 2018; Staufenbiel et al., 2015). Investigations have revealed that HCC can vary significantly between ethnic groups (Abell et al., 2016; Schreier et al., 2016). These differences may be due to biological factors, haircare practices, socio-economic- and contextual- factors (Abell et al., 2016; Wosu et al., 2013). Data gleaned from diverse populations can serve to enhance the design, interpretation and understanding of stress-related hair cortisol biomarker studies (Wosu et al., 2013).

We sought to examine whether HCC was a potential biomarker of stress and/or resilience in a South African mixed ancestry sample. As no studies utilising hair cortisol have been conducted in this population we also wanted to identify the determinants of HCC in a sample drawn from this cultural and ethnic group. Identifying potential confounders of HCC is a necessary first step in investigating associations between HCC and clinical variables of interest in a specific population. Identification of variables that influence HCC can also inform future research utilising HCC in representative populations. The variables (or determinants) were selected based on those that have been found to be associated with HCC in other settings. We adopted a similar approach to other studies that have examined the basic determinants of HCC. We selectively chose a set of variables, each representing sociodemographic, hair related, clinical and behavioural features, that we considered to be of relevance based on prior research and the specific features of our sample.

2 Methods

2.1 Study design

The objectives addressed here are part of the original aims of a study examining the role of chronic stress, as mediated through long-term HPA-axis dysfunction (HCC), in the interaction between neuropsychiatric disorders (NPDs) and metabolic syndrome (MetS). This study is a neuroendocrine

ancillary study to 'Understanding the SHARED ROOTS of Neuropsychiatric Disorders and Modifiable Risk Factors for Cardiovascular Disease' or the SHARED ROOTS project. The main aim of the SHARED ROOTS (SR) study is to determine the factors that contribute to comorbidity of NPDs and MetS, as a marker of cardiovascular disease (CVD) risk. This research question was investigated in three cohorts of NPDs, namely posttraumatic stress disorder (PTSD), schizophrenia and Parkinson's disease. The SR study was a cross-sectional matched case-control study. The current aims are addressed in the control sample included in SR.

2.2 Ethical aspects

This study was approved by the Health Research Ethics Committee at Stellenbosch University (HREC N13/08/115) and conducted according to ethical guidelines and principles of the seventh revision of the Declaration of Helsinki (World Medical Association, 2013). A tiered informed consent process was adhered to, allowing participants to opt-out of the hair sampling procedure. Participants were referred to their usual health care service providers for any medical or psychiatric problems warranting further investigation and treatment.

2.3 Setting

Participants were assessed over 3 years from May 2014 until June 2017. The neuroendocrine ancillary study was conceived prior to initiating recruitment and thus all participants were eligible for inclusion. We utilised a purposive sampling approach and participants were recruited utilising multi-pronged strategies. Control participants were recruited through (i) print, radio and web advertisements; (ii) active recruitment within communities by a registered nurse; and (iii) word of mouth by participants already recruited. Participants were evaluated at two research sites located in Cape Town, Western Cape, South Africa.

2.4 Participants

The SR study sample was limited to individuals who self-identified as belonging to the mixed ancestry (coloured) ethnic group. The decision to limit the study to one ethnic group was to avoid the effects of population stratification on genomic analyses. All participants had to be 18 years and older, willing and able to provide informed consent and be able to read and write in English or Afrikaans (the

predominant languages spoken within the Western Cape mixed ancestry population). Participants were excluded from hair sampling if they had hair length shorter than 3cm. Hair samples were obtained in 241 (51.9%) of the 464 control participants included in SR and the main factor limiting sampling was short hair length (see Figure 1 for a flow-diagram of control participants included in this study). Further exclusions applied to this sample were (i) any current psychiatric disorder as determined by history, diagnostic interview or current psychotropic medication use, (ii) significant medical morbidity (such as cancer, auto-immune disorders or chronic infections), (iii) systemic or scalp steroid medication use, (iv) current pregnancy or pregnancy within the prior three months. These exclusions were applied as we wanted our sample to reflect generally healthy adults without significant psychiatric or medical morbidity.

2.5 Procedures

Participants attended two to three study visits, each lasting around 3 hours. At the first visit, diagnostic, clinical measures and participant-administered measures were completed. The second visit occurred within 24-96 hours of the first visit and entailed physical procedures and administration of additional lifestyle measures. Assessments were conducted in English or Afrikaans, depending on the language preference of participants. Psychiatrists and physicians with experience in psychiatry conducted diagnostic and clinical assessments. Research nurses performed the physical procedures and personally administered the participant-administered measures to aid comprehension and to accommodate the variation in reading level.

2.5.1 Hair sampling

About 20mg (3mm diameter) samples of hair were cut with scissors from the posterior vertex scalp. Samples were secured, placed into aluminium foil, labelled and sealed inside an envelope. All the samples were stored in dark containers at room temperature.

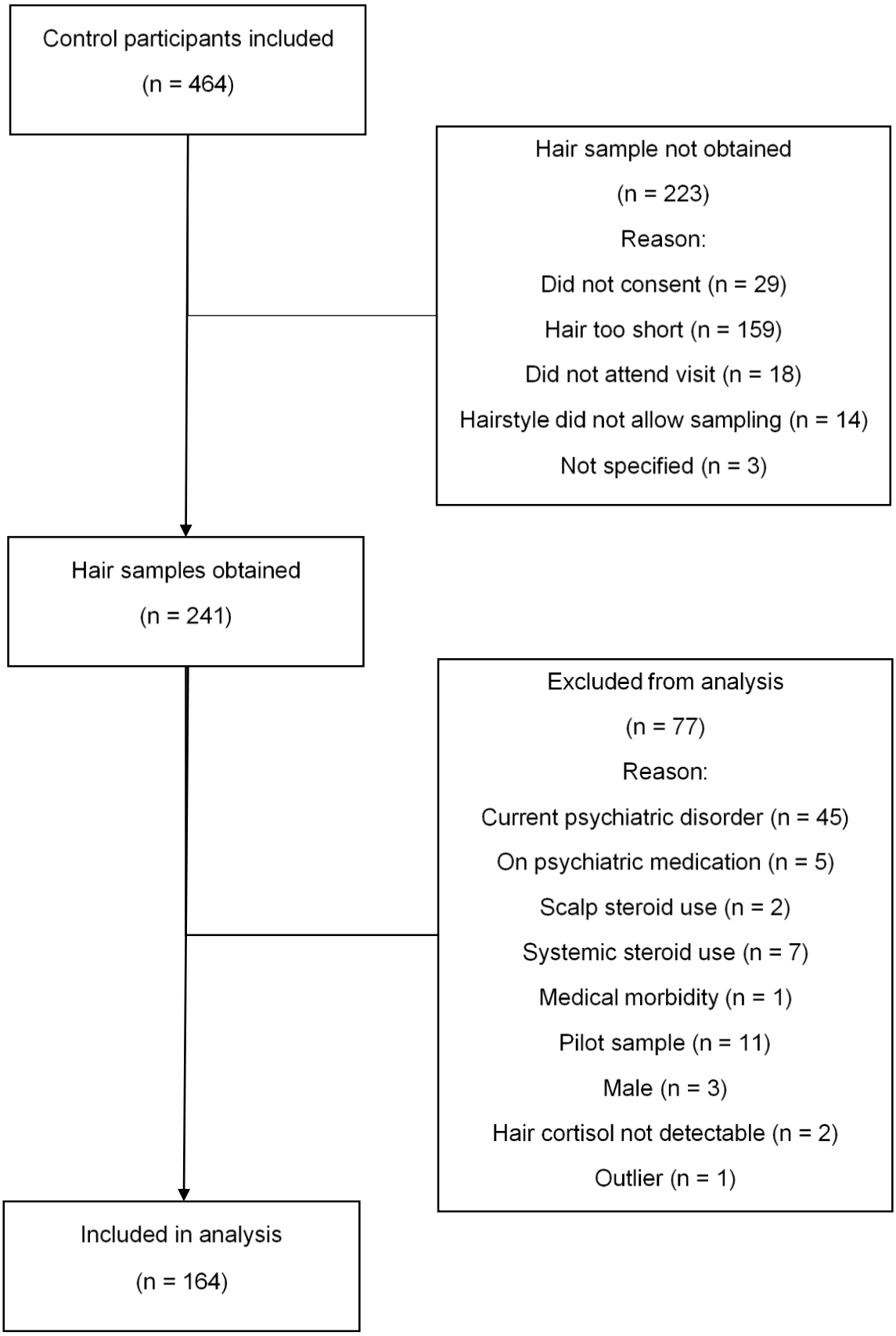

Figure 1 Flow diagram demonstrating inclusion and exclusion of control participants

2.5.2 Hair analysis

Hair analyses were performed at the TU Dresden laboratory (Prof. Clemens Kirschbaum). Samples were analysed in two batches. A pilot sample comprising the first 21 hair samples obtained were sent for analysis in September 2014. The remaining samples were sent for analysis in December 2017. Samples were analysed using an established liquid chromatography-tandem mass spectrometry (LC-MS/MS) protocol (Gao et al., 2013). The proximal 3cm of the hair segments were used for analysis, representing cortisol secretion for the prior 3 months based on an accepted growth rate of 1cm per month (Wennig, 2000).

2.6 Measures

2.6.1 Demographic questionnaire

Demographic details were collected with a demographic questionnaire. Variables included in this manuscript are self-identified gender (male or female), age in years, highest level of education (whether secondary education was completed), employment status (being employed for the greater part of the prior 12 months), and monthly household income (refused/don't know, less than ZAR3000, ZAR3000 – ZAR6000, more than ZAR6000).

2.6.2 Medical history questionnaires

Medical and psychiatric histories were collected with comprehensive medical questionnaires. Clinical items reported on in this manuscript are: (i) steroid use in the prior 6 months (split according to systemic steroids (oral or parenteral), topical steroids used on the scalp, and any other topical steroid use (dermatologic, inhaled, nasal), (ii) hormonal contraceptive use in prior 6 months (including oral contraceptives and other formulations, such as injectable contraceptives), (iii) whether a woman was currently breastfeeding, and (iv) self-reported tobacco and alcohol use in the prior six months.

2.6.3 The Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998)

The MINI is a short structured diagnostic interview developed to diagnose psychiatric disorders based on Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and International Classification of Diseases (ICD-10) criteria. The MINI is administered by trained clinicians and takes about 15 minutes to complete. The MINI version 6.0 was utilised to evaluate for current and lifetime psychiatric disorders.

Participants with any current or major lifetime (psychotic, bipolar) disorders were excluded from this analysis.

2.6.4 The WHO STEPwise approach to chronic disease risk factor surveillance (WHO STEPS) (World Health Organization, 2005)

Height (cm) and weight (kg) were measured according to the WHO STEPS and were used to determine body mass index (BMI, kg/m2). The level of physical activity was determined with the global physical activity questionnaire (GPAQ) in the WHO STEPS. The GPAQ assesses average physical activity in a week occurring in three domains, namely work, travel and recreation. Level of physical activity (high, moderate, and low) was determined according to the GPAQ guidelines (World Health Organization, 2005).

2.6.5 The Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983)

We used the 10-item PSS (PSS-10) to measure self-perceived stress. Individuals rate to what extent they found their lives unpredictable, uncontrollable or overloaded in the prior month. Each item is rated on a 5-point Likert scale (0 = Never; 1 = Almost Never; 2 = Sometimes; 3 = Fairly often; 4 = Very often) with a total score ranging from 0 – 40, and higher scores indicate higher perceived stress. The PSS has been widely used and has demonstrated adequate psychometric properties in multiple settings and languages (Lee, 2012) and Cronbach's α in this sample was 0.83. The PSS has been the most widely used measure of self-perceived stress in hair cortisol research (Stalder et al., 2017).

2.6.6 Connor-Davidson Resilience Scale (CD-RISC) (Connor & Davidson, 2003)

The CD-RISC is a 25-item self-rated scale to measure resilience, conceptualised as stress-coping ability in the prior month. Items are rated on a 5-point Likert scale (0 = not true at all; 1 = rarely true; 2 = sometimes true; 3 = often true; 4 = true nearly all of the time), and scores range from 0-100, with higher scores reflecting greater resilience. The CD-RISC has been translated into many languages and studied in a variety of populations and a methodological review of resilience scales identified the CD-RISC as one of the three scales demonstrating the best psychometric properties (Windle, Bennett, & Noyes, 2011). Cronbach's α in this sample was 0.93.

2.6.7 Hair questionnaire

Participants who provided hair samples were questioned about their hair characteristics and hair care practices. Hair related variables reported in this manuscript include (i) natural hair colour (black versus any other colour), (ii) whether hair had been chemically treated in the prior three months (including colouring, relaxing and perming), (iii) the frequency of hair washing (2 or more times a week, once a week, less than once a week), (iv) whether any additional hair products had been used in the prior three months (such as hair styling and care products e.g. gels, oils and creams, excluding products used during hair washing [see Supplementary Table 1]), and (v) average hours per day that hair was exposed to sunlight (less than one hour, 1-2 hours, more than 2 hours). Additional hair related factors derived from the date sampling occurred included the season sampled (based on the month the sample was obtained in) and the duration of storage before sending the samples for analysis (less than 1 year, 1-2 years, 2-3.5 years).

2.7 Statistical analysis

A priori sample size estimations were computed based on the main outcomes of the neuroendocrine study. Post-hoc power analysis using G*Power 3.1.9.4 (Faul, Erdfelder, Buchner, & Lang, 2009) with the effect size determined based on a linear regression model including all the variables simultaneously (*adj* R^2 = 0.182, *n* = 161, df = 28, α = 0.05) provided an estimated power of 99% in this sample. Due to the small number of males with hair cortisol data available (n = 3) we decided to limit our analysis to females only to allow us to consider female specific variables. As found in other studies, HCC were not normally distributed and were positively skewed (Clark, Osborne, Gallagher, & Watson, 2016). We utilised the box-cox family of power transformations to find the optimal normalising transformation, utilising the approach and syntax outlined in Osborne, 2010 (Osborne, 2010). The transformation that best reduced skewness was a reciprocal square root transformation and the transformed HCC met the assumption of normality. We excluded two samples where HCC were not detectable and one outlier with transformed HCC 3SD beyond the mean (3955.5 pg/mg), resulting in a final sample of 164 participants (Figure 1). We compared the included and excluded sample with Pearson chi-square or Fisher's exact statistic for categorical variables, and Mann-Whitney U tests for continuous variables. To

identify the factors associated with HCC in our sample we first conducted simple linear regression with each variable, then multiple linear regression controlling for age and other variables found to be significantly associated with HCC in simple linear regression. The only missing data were three missing responses on the item 'average hours per day that hair was exposed to sunlight' and these were excluded from analysis listwise, resulting in a sample size for the adjusted models of 161. We constructed similar regression models for PSS and CD-RISC total scores, again controlling for the same variables in adjusted models. We also assessed for interactions effects between PSS and CD-RISC scores and interaction effects between these and all other variables. For ease of interpretation, reverse transformed geometric means of HCC are also reported. Limited sensitivity and post-hoc analyses were conducted to allow for additional interpretation of the data. Data were analysed with SPSS for windows, version 25.0, all tests were 2-tailed and the level of significance was set at .05.

3 Results

3.1 Participants

Hair samples were obtained in 241 (51.9%) of the 464 control participants enrolled. A further 77 participants who provided hair samples were excluded from analysis, resulting in a final sample of 164 participants (see Figure 1 for details). A comparison of the included (n=164) and excluded participants (n=300) are detailed in Table 1. When we controlled for gender differences in BMI, tobacco use, alcohol use and level of physical activity were no longer significant between included and excluded groups. The duration of storage was no longer significant when we controlled for batch effects and this could be explained by the shorter duration of storage ($p < 0.001$) of the pilot sample. The mean age of participants was 46.5 years, ranging from 18 to 79 years and the mean HCC was 16.0 pg/mg (*SD* 54.3). The mean PSS score was 16.0 (*SD* 7.6) and the mean CD-RISC score was 83.1 (*SD* 14.4). Further descriptive data are presented in Tables 1 and 2.

Table 1 Comparison of included and excluded participants

Variables	Included Number (%)	Excluded Number (%)	Test statistic	p-value
Total sample	164 (100)	300 (100)		
Socio-demographic				
Age	48.5 (33.2, 58.5)[a]	45.3 (33.2, 56.2)[a]	*U*=23037; *Z*=-1.13	0.258
Gender			$\chi^2(1) = 117.63$	< 0.001*
Male	0	153 (49.0)		
Female	147 (100)	164 (51.0)		
Secondary education complete			$\chi^2(1) = 0.81$	0.397
No	110 (67.1)	188 (62.9)		
Yes	54 (32.9)	111 (37.1)		
Employed			$\chi^2(1) = 0.72$	0.367
No	104 (63.4)	177 (59.4)		
Yes	60 (36.6)	121 (40.6)		
Monthly income			$\chi^2(3) = 2.90$	0.408
Refused/don't know	6 (3.7)	16 (5.4)		
< ZAR3000	51 (31.1)	100 (33.6)		
ZAR3000 – ZAR6000	52 (31.7)	74 (24.8)		
> ZAR6000	55 (33.5)	108 (36.2)		
Hair related[b]				
Natural hair colour black			$\chi^2(1) = 0.23$	0.634
No	89 (54.3)	46 (57.5)		
Yes	75 (45.7)	34 (42.5)		
Hair chemically treated			$\chi^2(1) = 0.10$	0.750
No	50 (30.5)	26 (32.5)		
Yes	114 (69.5)	54 (67.5)		
Frequency of hair washing			$\chi^2(2) = 2.59$	0.273
≥ 2 times a week	44 (26.8)	16 (20.0)		
1 time a week	81 (49.4)	38 (47.5)		
< 1 time a week	39 (23.8)	26 (32.5)		
Add on hair products			$\chi^2(1) = 3.36$	0.067
No	84 (51.2)	31 (38.8)		
Yes	80 (48.8)	49 (61.3)		
Average hours in sun			$\chi^2(2) = 0.02$	0.989
< 1 hour	74 (45.7)	36 (46.2)		
1-2 hours	46 (28.4)	23 (29.5)		

> 2 hours	39 (24.1)	19 (24.4)		
Season sampled			$\chi^2(3) = 0.77$	0.857
Summer	33 (20.1)	15 (19.5)		
Autumn	45 (27.4)	24 (31.2)		
Winter	49 (29.9)	24 (31.2)		
Spring	37 (22.6)	14 (18.2)		
Duration of storage			$\chi^2(2) = 12.3$	0.002*
< 1 year	28 (17.1)	29 (37.7)		
1 - 2 years	58 (35.4)	21 (27.3)		
2 – 3.5 years	78 (47.6)	27 (35.1)		
Clinical				
Hormonal contraceptive			$\chi^2(1) = 0.02$	0.891
No	133 (81.1)	125 (81.7)		
Yes	31 (18.9)	28 (18.3)		
Currently breastfeeding			$\chi^2(1) = 0.47$	0.494
No	157 (95.9)	142 (94.0)		
Yes	7 (4.3)	9 (6.0)		
Topical steroid use			$\chi^2(1) = 0.90$	0.264
No	157 (95.7)	258 (93.1)		
Yes	7 (4.3)	19 (6.9)		
Body mass index (BMI)	30.0 (25.8, 35.6)[a]	27.2 (22.7, 33.2)[a]	*U*=18380; *Z*=-3.62	<0.001*
Behavioural				
Tobacco use			$\chi^2(1) = 5.55$	0.019*
No	110 (67.1)	167 (55.9)		
Yes	54 (32.9)	132 (44.1)		
Alcohol use			$\chi^2(1) = 8.54$	0.003*
No	91 (55.5)	124 (41.3)		
Yes	73 (44.5)	176 (58.7)		
Level of physical activity			$\chi^2(2) = 7.08$	0.029*
High	16 (9.8)	54 (19.1)		
Moderate	56 (34.1)	91 (32.3)		
Low	92 (56.1)	137 (48.6)		
HCC (pg/mg)	4.4 (2.8, 11.4)[a]	5.5 (3.0, 9.0)[a]	*U*=5847.5; *Z*=-0.92	0.355
Perceived stress scores	16.0 (11.0, 21.0)[a]	17.0 (10.0, 23.0)[a]	*U*=18380; *Z*=-3.62	0.218
Resilience scores	86.0 (73.0, 95.8)[a]	87.0 (73.8, 95.0)[a]	*U*=18380; *Z*=-3.62	0.826

[a] Median and interquartile range
[b] Data on hair related features were only collected in participants who provided hair samples
* P < 0.05
HCC, hair cortisol concentrations

Table 2 Regression analyses of factors associated with hair cortisol concentrations (HCC)

Variables	Number (%)	Simple linear regression			Multiple linear regression[a]	
		Mean HCC[b]	ß	p-value	ß	p-value
Total sample	164 (100)	4.93				
Socio-demographic						
Age	46.5 (15.0)[c]		-0.115	0.143	-0.177	0.032*
Secondary education complete						
No	110 (67.1)	5.44	Ref		Ref	
Yes	54 (32.9)	4.05	-0.154	0.049*	-0.160	0.040*
Employed						
No	104 (63.4)	4.73	Ref		Ref	
Yes	60 (36.6)	5.29	0.059	0.451	0.046	0.558
Monthly income				0.839		0.901
Refused/don't know	6 (3.7)	4.44	-0.033	0.807	0.021	0.779
< ZAR3000	51 (31.1)	5.39	0.033	0.585	0.034	0.699
ZAR3000 – ZAR6000	52 (31.7)	4.61	-0.020	0.742	-0.026	0.759
> ZAR6000	55 (33.5)	4.89	Ref		Ref	
Hair related						
Natural hair colour black						
No[d]	89 (54.3)	4.78	Ref		Ref	
Yes	75 (45.7)	5.21	0.036	0.643	0.031	0.682
Hair chemically treated						
No	50 (30.5)	5.95	Ref		Ref	
Yes	114 (69.5)	4.55	-0.134	0.087	-0.014	0.854
Frequency of hair washing				0.238		0.078
≥ 2 times a week	44 (26.8)	4.88	-0.106	0.278	-0.094	0.326
1 time a week	81 (49.4)	4.49	-0.166	0.091	-0.182	0.047*
< 1 time a week	39 (23.8)	6.10	Ref		Ref	
Add on hair products						
No	84 (51.2)	4.32			Ref	
Yes	80 (48.8)	5.68	0.151	0.054	0.164	0.025*
Average hours in sun[e]				0.012*		0.033*
< 1 hour	74 (45.7)	5.24	Ref		Ref	
1-2 hours	46 (28.4)	3.64	-0.186	0.027*	-0.163	0.035*
> 2 hours	39 (24.1)	6.37	0.089	0.284	0.056	0.477
Season sampled				0.788		0.754
Summer	33 (20.1)	5.24	0.071	0.465	0.057	0.558

Autumn	45 (27.4)	5.36	0.090	0.371	0.046	0.648
Winter	49 (29.9)	4.72	0.029	0.774	0.101	0.282
Spring	37 (22.6)	4.46	Ref		Ref	
Duration of storage				0.001*		<0.001*
< 1 year	28 (17.1)	8.10	Ref		Ref	
1 - 2 years	58 (35.4)	5.45	-0.192	0.073	-0.260	0.011*
2 – 3.5 years	78 (47.6)	3.90	-0.388	< 0.001*	-0.461	< 0.001*
Clinical						
Hormonal contraceptive						
No	133 (81.1)	4.70	Ref		Ref	
Yes	31 (18.9)	6.04	0.106	0.178	-0.009	0.909
Currently breastfeeding						
No	157 (95.9)	4.69	Ref		Ref	
Yes	7 (4.3)	18.7	0.252	0.001*	0.186	0.013*
Topical steroid use						
No	157 (95.7)	4.86	Ref		Ref	
Yes	7 (4.3)	6.90	0.075	0.340	0.072	0.323
Body mass index (BMI)	31.2 (8.2)[c]		-0.059	0.457	-0.049	0.523
Behavioural						
Tobacco use						
No	110 (67.1)	4.88	Ref		Ref	
Yes	54 (32.9)	5.03	0.016	0.837	0.057	0.440
Alcohol use						
No	91 (55.5)	4.87	Ref		Ref	
Yes	73 (44.5)	5.00	0.014	0.858	-0.006	0.940
Level of physical activity				0.693		0.863
High	16 (9.8)	5.90	0.058	0.471	0.054	0.478
Moderate	56 (34.1)	4.71	-0.022	0.782	0.021	0.781
Low	92 (56.1)	4.91	Ref		Ref	
Perceived stress score	15.9 (7.6)[c]		0.125	0.111	-0.001	0.989
Resilience score	83.1 (14.4)[c]		-0.213	0.006*	-0.179	0.012*

[a] Multivariate adjusted model (adjusted for age, education, average hours in the sun, duration of storage, breastfeeding), n = 161; The multivariate model including age and the variables significantly associated with HCC in unadjusted analyses was significant (F (7,153) = 6.46, p < .001), with an R^2 of 0.228.
[b] Reverse transformed geometric means
[c] Mean and standard deviation
[d] 87 (53.0%) had brown hair, 1 (0.6%) had blonde and 1 (0.6%) red hair colour
[e] Three responses were missing for this item, n = 161
* p-value < 0.05
HCC, hair cortisol concentrations; NA, not applicable; Ref, Reference category

3.2 Determinants of hair cortisol concentrations (HCC) (Table 2)

3.2.1 Socio-demographic

HCC decreased with increasing age, although this association was only significant in the adjusted model ($p = 0.032$). HCC were significantly lower in those who had completed secondary education compared to those who had not in unadjusted ($p = 0.049$) and adjusted ($p = 0.040$) models. Employment status and income were not significantly associated with HCC in any of the models.

3.2.2 Hair related

Hair washing frequency was not associated with HCC overall, though HCC were significantly lower in those who washed their hair once a week compared to those washing their hair less frequently in the adjusted ($p = 0.047$) model. HCC were significantly higher in those using additional hair products in the adjusted model ($p = 0.025$), with a trend towards significance in the unadjusted ($p = 0.054$) model. When analysed by hair product category in post-hoc analysis, HCC were significantly higher in those who only used hair treatment products ($\beta = 0.166$, $p = 0.045$), but not those who used only hair styling products ($\beta = 0.092$, $p = 0.248$) or both treatment and styling products ($\beta = 0.068$, $p = 0.410$), compared to those who did not use any additional hair products (see Supplementary Table 1 for hair product types). Duration of sun exposure was significantly associated with HCC in both unadjusted ($p = 0.012$) and adjusted ($p = 0.033$) models. HCC were significantly lower in those reporting 1-2 hours of sun exposure per day than in those reporting less than 1 hour of sun exposure in unadjusted ($p = 0.027$) and adjusted ($p = 0.035$) models. However, HCC were non-significantly higher in those reporting more than 2 hours of sun exposure per day as compared to those with less than 1 hour of sun exposure. HCC were significantly associated with duration of storage in unadjusted ($p = 0.001$) and adjusted models ($p < 0.001$). HCC decreased with longer duration of storage and this relationship was significant in unadjusted and adjusted ($p < 0.001$) models when 2-3.5 years of storage were compared to less than 1 year, and significant in the adjusted model ($p = 0.011$) when 1-2 years of storage where compared to less than 1 year of storage. There was also a negative correlation between HCC and duration of storage (r_s (164) = -0.277, $p < 0.001$) in post-hoc analysis. Sensitivity analysis including the pilot sample (n=11) showed HCC were significantly higher (*adj* $\beta = 0.147$, $p = 0.039$) in

the final batch than in the pilot batch and duration of storage remained significantly associated with HCC ($p < 0.001$) when we controlled for batch. HCC were not significantly associated with natural hair colour, chemically treated hair, and season sampled in any models.

3.2.3 Clinical factors

HCC were significantly higher in women who were breastfeeding in unadjusted ($p = 0.001$) and adjusted ($p = 0.013$) models. HCC were not significantly associated with hormonal contraceptive use, topical steroid use, and BMI in any models. On further examination a cubic curve better represented the association between BMI and HCC (R^2 change = 0.036, $p = .054$) although the model only demonstrated a trend to significance (F (3,160) = 2.17, $p = .094$). When we assessed according to BMI categories, HCC were lowest in the overweight group (BMI = 25.0 – 29.9, $n = 45$, mean HCC = 4.13) and were significantly lower than the normal weight group (BMI = 18.5 – 24.9, $n = 34$, mean HCC = 6.43, $p = 0.035$), but not the obese group (BMI ≥ 30, $n = 82$, mean HCC = 4.74, $p = 0.405$). HCC were also not significantly associated with systemic steroid use (*adj* $\beta = 0.034$, $p = 0.623$) or with any steroid use (systemic and topical combined, *adj* $\beta = 0.071$, $p = 0.296$) in sensitivity analysis including those with systemic steroid use ($n = 168$).

3.2.4 Behavioural factors

HCC were not associated with tobacco or alcohol use, nor with level of physical activity, in any of the models.

3.2.5 Stress and resilience

PSS scores were significantly inversely correlated with CDRISC scores ($r_s(164) = -0.386$, $p < 0.001$). HCC increased with increased severity of perceived stress but there was no significant relationship demonstrated in any of the models. HCC were significantly inversely associated with CD-RISC scores (Figure 2) in unadjusted ($p = 0.006$) and adjusted models ($p = 0.012$). Neither PSS, nor CD-RISC scores demonstrated significant curvilinear associations with HCC when quadratic and cubic terms were added to the models. There was a significant interaction demonstrated between CD-RISC scores and level of education ($p = 0.023$), such that CD-RISC scores remained significantly inversely associated with HCC in those who had not completed secondary education (*adj* $\beta = -0.282$, $p = 0.002$)

and was not significantly associated with HCC in those who had (*adj β* = 0.076, *p* = 0.572). There were no other significant interactions demonstrated for PSS or CD-RISC scores and the other variables investigated. In sensitivity analysis including participants with common mental disorders (*n* = 198), the results remained unchanged with no significant association demonstrated between PSS scores and HCC (*adj β* = 0.014, *p* = 0.847) and an inverse significant association demonstrated between CD-RISC scores and HCC (*adj β* = -0.179, *p* = 0.008).

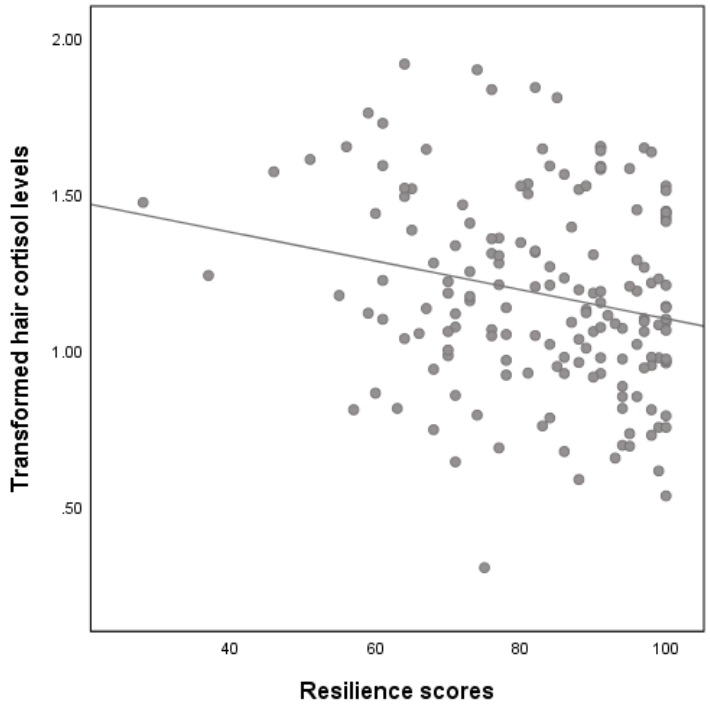

Figure 2 Scatterplot demonstrating significant inverse relationship between hair cortisol concentrations (HCC) and resilience scores
(*F* (1,163) = 7.71, *p* = .006), with an R^2 of 0.319

4 Discussion

We investigated the relationship between HCC and key factors previously demonstrated to influence HCC, in a sample of South African mixed ancestry females. HCC were significantly associated with age, level of education, hair product use, duration of sun exposure, duration of storage and breastfeeding, and demonstrated a trend towards significance with frequency of hair washing, in adjusted models. Additionally, we demonstrated that HCC were significantly inversely associated with resilience scores, but were not significantly associated with self-perceived stress scores. HCC were not significantly associated with natural hair colour, chemically treated hair, season sampled, hormonal contraceptive use, topical steroid use, tobacco use, alcohol use, level of physical activity, BMI, and employment status in any of the models.

We demonstrated an inverse relationship between age and HCC, which is contrary to what has been found overall, with HCC increasing with advancing age in adults (Stalder et al., 2017; Wosu et al., 2013). Although some studies have not found HCC to be associated with level of education (Kuehl et al., 2015; Staufenbiel et al., 2015), our results align with other studies that have demonstrated higher HCC in individuals with less formal education (Orta et al., 2018; Wosu et al., 2013). An earlier systematic review suggested that HCC are increased in unemployed individuals (Wosu et al., 2013), but we did not demonstrate a relationship between employment status and HCC, although our results may have been influenced by the high rate of unemployment (63.4%). We also did not demonstrate an association between monthly income and HCC, though some studies have demonstrated higher HCC in lower income groups (Serwinski, Salavecz, Kirschbaum, & Steptoe, 2016; Ursache, Merz, Melvin, Meyer, & Noble, 2017).

Unlike other studies, we did not demonstrate higher HCC in individuals with black hair colour (Abell et al., 2016; Staufenbiel et al., 2015). This relationship is, however, usually found when black hair is compared to blonde hair and 98.8% of our sample had either black or brown hair. Contrary to a recent meta-analysis (Stalder et al., 2017) we did not demonstrate a significant association between chemically treated hair and HCC. Lower HCC have been associated with an increased frequency of hair washing (Stalder et al., 2017) and HCC demonstrated a trend towards significance with frequency

of hair washing in our sample, with lowest levels reported in those washing their hair once a week than in those washing their hair less frequently. We also found that individuals who reported using any additional hair products on their hair had higher HCC and this effect was strongest for those who only used hair treatment products. As has previously been suggested, more frequent hair washing may lead to a washout effect (Staufenbiel et al., 2015), and chemical treatments of hair may damage the hair structure further contributing to this washout effect (Cooper, Kronstrand, & Kintz, 2012). We postulate that using additional hair products, particularly treatment products, may form a protective layer over hair, thus slowing degradation and washout effects.

Similar to other studies (Abell et al., 2016; Steudte-Schmiedgen et al., 2017; Wikenius et al., 2016) we demonstrated a significant decline in hair cortisol with increased storage time and we also found that HCC differed significantly according to the batch analysed in sensitivity analysis. Most studies do not report on the duration of storage nor the batch analysed and our results indicate that these factors should be considered in study design and analyses.

In experimental paradigms exposure to ultraviolet (UV) radiation have been associated with reductions in HCC (Grass et al., 2016; Wester, van der Wulp, Koper, de Rijke, & van Rossum, 2016). We included two variables generally considered to be associated with UV exposure, namely season sampled and the average daily duration of sunlight exposure. According to our knowledge, our study is the first to demonstrate a significant association between HCC and duration of sun exposure under naturalistic conditions. HCC were significantly lower in individuals reporting 1-2 hours of sun exposure per day, as compared to those reporting less than one hour of sun exposure per day, however, HCC were highest in those reporting more than 2 hours of sun exposure per day. Duration of sun exposure per day may also be linked to factors other than UV exposure, such as increased sweating, which has been associated with increased HCC in other samples (Wester et al., 2017), although experimental work did not find evidence for the acute effects of sweating on HCC (Grass et al., 2016).

Similar to findings from other studies, hormonal contraceptive use did not appear to be a major factor influencing HCC in our sample (Stalder et al., 2017; Wosu et al., 2013). We, however, demonstrated increased HCC in breastfeeding women and this effect was independent of pregnancy, as we

excluded women who had delivered within the prior three months and post-hoc analysis demonstrated that results remained unchanged when duration since last pregnancy was included in the model. Breastfeeding was also not associated with any socio-economic factors (e.g. education, employment and income), suggesting breastfeeding may influence the HPA-axis directly. Although some studies specifically exclude breastfeeding women (Jackson, Kirschbaum, & Steptoe, 2017), most studies do not account for women who may be breastfeeding. Although preliminary, our results suggest breastfeeding may have a significant effect on HPA-axis function. Similar to other studies, neither topical, nor systemic steroid use were associated with HCC in our sample (Schreier et al., 2016; Wester et al., 2017). Synthetic steroids can decrease endogenous production of cortisol and cause cross-reactivity (Raul, Cirimele, Ludes, & Kintz, 2004), although the latter is less likely with LC-MS/MS than with immunoassay methods (Abell et al., 2016; Russell et al., 2015). Although a large epidemiological study also utilising LC-MS/MS demonstrated significantly higher HCC in relation to topical and systemic steroid use (Abell et al., 2016), suggesting our sample may have been under powered to detect a significant effect. To assess for effects of anthropometry we included BMI. Overwhelmingly research has demonstrated a positive relationship between HCC and BMI (Abell et al., 2016; Stalder et al., 2017). Although not significant, our results suggest a more complex curvilinear (cubic) relationship between BMI and HCC with lowest levels in the overweight group and levels increasing as BMI changes in both directions. Importantly, our sample likely had an overrepresentation of obese individuals as 50% of our sample had a BMI falling within the obese category. This was likely due to the sampling strategy used, where efforts were directed towards recruiting more participants with possible MetS.

In alignment with the majority of previous research (Stalder et al., 2013; Staufenbiel et al., 2015), neither tobacco use nor alcohol use was associated with HCC in our sample. Of note, we excluded individuals with substance use disorders and studies have reported altered HCC in relation to alcohol use disorders (Muehlhan et al., 2018; Stalder et al., 2010). We demonstrated no clear relationship between level of physical activity and HCC in our sample. Although some studies have similarly not demonstrated a relationship between physical activity and HCC (Stalder et al., 2013; Staufenbiel et al.,

2015), other studies have found higher HCC in relation to higher levels of physical activity (Garcia-Leon et al., 2018; Ullmann et al., 2016). The small number of individuals reporting high levels of physical activity likely affected our results. Males reported higher rates of tobacco use, alcohol use and higher levels of physical activity, thus a sample representing both genders might have exhibited altered relationships.

We demonstrated a non-significant positive association with perceived stress scores and a significant negative association with resilience scores and HCC. Furthermore, similar to other studies, perceived stress scores and resilience scores were inversely correlated (García-León, Pérez-Mármol, Gonzalez-Pérez, García-Ríos, & Peralta-Ramírez, 2019). Our results align with a model whereby increased perceived stress is associated with increased long-term cortisol levels and resilience with lower long-term cortisol levels. Generally, the extant literature demonstrates the lack of a significant relationship between self-perceived stress and HCC (Stalder et al., 2017; Staufenbiel et al., 2013; Wosu et al., 2013) and this may be related to the way self-perceived stress is conceptualised and measured (Gray et al., 2018). Our results, in conjunction with other recent studies (García-León et al., 2019; Milam et al., 2014), suggest that HCC may be a biomarker of stress resilience. It is also apparent that a unidimensional approach to assessing psychological constructs related to stress does not adequately capture the picture and that additional insights can be gleaned by incorporating assessments of resilience and other protective factors. Although sensitivity analysis revealed that resilience scores remained significantly associated with HCC when individuals with common mental disorders were included the relationship between resilience and HCC warrants further investigation as research suggests the interaction between resilience and HPA-axis function may vary according to psychopathology and environmental exposures. For instance, in a sample of low-income children, lower morning saliva cortisol levels were associated with higher resilience in non-maltreated children, but not in maltreated children (Cicchetti & Rogosh, 2007). We also demonstrated a significant interaction between level of education and resilience on HCC, suggesting that resilience was primarily associated with lower HCC in those who had not completed secondary education.

A review of biomarkers for resilience identified salivary cortisol as a potentially suitable biomarker due to the demonstrated relationship, and saliva samples being fairly simple, cost-effective, and non-invasive to collect and analyse (Walker, Pfingst, Carnevali, Sgoifo, & Nalivaiko, 2017). Based on the results from our study and other recent studies (García-León et al., 2019; Milam et al., 2014), as well as the simplicity of collecting and storing hair samples, HCC may be even better suited. There are, however, also important limitations influencing the use of hair samples. The major factor limiting sampling in our study was the requirement that scalp hair had to be 3cm or longer. This contributed to the exclusion of the majority of males. The scalp hair length requirement has been demonstrated to lead to disproportionate exclusion of males and individuals of specific cultural and ethnic groups in other settings as well (Kalmakis, Meyer, Chiodo, & Leung, 2015; Pacella, Hruska, Steudte-Schmiedgen, George, & Delahanty, 2017; Simmons et al., 2016). Investigators have instituted approaches to circumvent this limitation, such as to use hair samples of shorter length and to shave hair from the forearm in military samples (Groer, Kane, Williams, & Duffy, 2015; Mewes, Reich, Skoluda, Seele, & Nater, 2017). Although the ideal method of sampling has not been established, it is clear that methods need to take cognisance of cultural, sex based and ethnic practices related to hairstyling and hair care.

4.1 Strengths and limitations

Our study is cross-sectional and we cannot comment on any cause-effect relationships. Moreover, although post-hoc analyses demonstrated overall adequate power, our sample may still have been underpowered to detect specific associations. Similar to other studies examining the determinants of HCC (Abell et al., 2016; Dettenborn et al., 2012; Feller et al., 2014; Garcia-Leon et al., 2018; Staufenbiel et al., 2015) and due to the exploratory nature of our study, we did not control for multiple comparisons. We utilised a purposive sampling approach and thus our sample does not reflect a random selection and results obtained must be viewed in this light. Furthermore, our sample was limited to a single ethnic group and sex. Notwithstanding, our sample appeared to be representative of South African mixed-ancestry women in terms of education and employment status, with the percentage that had completed secondary education (32.9%) roughly equivalent to the national average (30.0%), and if we excluded individuals aged 65 years and older the percentage who were

employed (40.8%) was also roughly equivalent to the national average (42.3%) (Statistics South Africa, 2013). Investigation and validation of the factors identified in this study in larger community based cohorts with broader ethnic and cultural representation would be a prudent next step. However, our study is one of the first to examine the basic determinants of HCC in a sample representing a different geographic, ethnic and cultural setting than in most studies conducted thus far. In this context, our study can be considered as a starting point to guide further research in more diverse groups. Our study is also one of the first studies to include measures of resilience alongside measures of self-perceived stress and to demonstrate the importance of doing so.

4.2 Conclusions

The main determinants of HCC in our sample of mixed ancestry females included age, level of education, hair product use, duration of sun exposure per day, duration of sample storage, batch analysed and breastfeeding. These factors need to be taken into consideration in the design, implementation and analysis of future studies. Future studies are also required to replicate and further interrogate the novel associations demonstrated between hair product use, breastfeeding, duration of sun exposure and HCC. Hair sampling procedures also need to be adapted to allow for equivalent inclusion of participants of different sexes and ethnic groups. We demonstrated a significant inverse association between HCC and resilience scores, alongside a non-significant positive association between self-perceived stress and HCC. The association between resilience and HCC was primarily found in women who had not completed secondary level of education, suggesting resilience may have a greater protective effect on long-term HPA-axis function in women with lower levels of education. Our results underscore the importance of taking a broader perspective in stress research, which is largely pathology driven. Including assessments to tap into constructs related to protective factors, in addition to assessments of risk/psychopathology, can provide additional insights into the links between stressors, psychological responses and biological processes. Akin to biomedical research overall, there is a lack of ethnic, geographic and cultural diversity in research utilising HCC. Further research utilising HCC in more diverse settings and populations will improve our understanding of hair cortisol as a biomarker of stress, resilience and HPA-axis function.

5 Supplementary materials

Supplementary Table 1 Types of hair products used according to indication

Hair product type	Total	Hair product indication	
		Styling	Treatment
	Number (%)	Number (%)	Number (%)
Total	164	42	72
Product types			
Gel	16 (9.8)	16 (36.4)	6 (8.3)
Mousse	2 (1.2)	2 (4.5)	0 (0.0)
Spray	10 (6.1)	4 (9.5)	6 (8.3)
Cream	33 (20.1)	6 (13.6)	33 (45.8)
Serum	19 (11.6)	17 (38.6)	2 (2.8)
Grease	3 (1.8)	2 (4.5)	2 (2.8)
Oil	26 (15.9)	1 (2.3)	26 (36.1)
Silicone	8 (4.9)	5 (11.4)	4 (5.6)
Leave-in conditioner	4 (2.4)	0 (0.0)	4 (5.6)
Placenta	5 (3.0)	0 (0.0)	5 (6.9)

None of the individual product types were significantly associated with HCC
HCC, hair cortisol concentrations

6 References

Abell, J. G., Stalder, T., Ferrie, J. E., Shipley, M. J., Kirschbaum, C., Kivimäki, M., & Kumari, M. (2016). Assessing cortisol from hair samples in a large observational cohort : The Whitehall II study. *Psychoneuroendocrinology*, *73*, 148–156. https://doi.org/10.1016/j.psyneuen.2016.07.214

Bowes, L., & Jaffee, S. R. (2013). Biology, Genes, and Resilience. *Trauma, Violence, & Abuse*, *14*(3), 195–208. https://doi.org/10.1177/1524838013487807

Chrousos, G. P. (2009). Stress and disorders of the stress system. *Nature Reviews Endocrinology*, *5*(7), 374–381. https://doi.org/10.1038/nrendo.2009.106.

Cicchetti, D., & Rogosh, F. A. (2007). Personality, adrenal steroid hormones, and resilience in maltreated children: A multilevel perspective. *Development and Psychopathology*, *19*(3), 787–809. https://doi.org/10.1017/S0954579407000399

Clark, J. E., Osborne, J. W., Gallagher, P., & Watson, S. (2016). A simple method for optimising transformation of non-parametric data: an illustration by reference to cortisol assays. *Human Psychopharmacology: Clinical and Experimental*, *31*(4), 259–267.

https://doi.org/10.1002/hup.2528

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A Global Measure of Perceived Stress. *Journal of Health and Social Behavior*, *24*(4), 385–396. https://doi.org/10.1016/j.socscimed.2016.08.021

Connor, K. M., & Davidson, J. R. T. (2003). Development of a new resilience scale: the Connor-Davidson Resilience Scale (CD-RISC). *Depression and Anxiety*, *18*(2), 76–82. https://doi.org/10.1002/da.10113

Cooper, G. A., Kronstrand, R., & Kintz, P. (2012). Society of Hair Testing guidelines for drug testing in hair. *Forensic Science International*, *218*(1–3), 20–24. https://doi.org/10.1016/j.forsciint.2011.10.024

Dettenborn, L., Tietze, a., Kirschbaum, C., & Stalder, T. (2012). The assessment of cortisol in human hair: associations with sociodemographic variables and potential confounders. *Stress*, *15*(6), 578–588. https://doi.org/10.3109/10253890.2012.654479

Dockray, S., & Steptoe, A. (2010). Positive affect and psychobiological processes. *Neuroscience and Biobehavioral Reviews*, *35*(1), 69–75. https://doi.org/10.1016/j.neubiorev.2010.01.006

Faul, F., Erdfelder, E., Buchner, A., & Lang, A. G. (2009). Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behavior Research Methods*, *41*(4), 1149–1160. https://doi.org/10.3758/BRM.41.4.1149

Feller, S., Vigl, M., Bergmann, M. M., Boeing, H., Kirschbaum, C., & Stalder, T. (2014). Predictors of hair cortisol concentrations in older adults. *Psychoneuroendocrinology*, *39*(1), 132–140. https://doi.org/10.1016/j.psyneuen.2013.10.007

Gao, W., Stalder, T., Foley, P., Rauh, M., Deng, H., & Kirschbaum, C. (2013). Quantitative analysis of steroid hormones in human hair using a column-switching LC–APCI–MS/MS assay. *Journal of Chromatography B*, *928*, 1–8. https://doi.org/10.1016/j.jchromb.2013.03.008

Garcia-Leon, M. A., Peralta-Ramirez, M. I., Arco-Garcia, L., Romero-Gonzalez, B., Caparros-Gonzalez, R. A., Saez-Sanz, N., … Gonzalez-Perez, R. (2018). Hair cortisol concentrations in a

Spanish sample of healthy adults. *PLoS ONE*, *13*(9), 1–16. https://doi.org/10.1371/journal.pone.0204807

García-León, M. Á., Pérez-Mármol, J. M., Gonzalez-Pérez, R., García-Ríos, M. del C., & Peralta-Ramírez, M. I. (2019). Relationship between resilience and stress: Perceived stress, stressful life events, HPA axis response during a stressful task and hair cortisol. *Physiology and Behavior*, *202*(September 2018), 87–93. https://doi.org/10.1016/j.physbeh.2019.02.001

Grass, J., Miller, R., Carlitz, E. H., Patrovsky, F., Gao, W., Kirschbaum, C., & Stalder, T. (2016). In vitro influence of light radiation on hair steroid concentrations. *Psychoneuroendocrinology*, *73*, 109–116. https://doi.org/10.1016/j.psyneuen.2016.07.221

Gray, N. A., Dhana, A., Van Der Vyver, L., Van Wyk, J., Khumalo, N. P., & Stein, D. J. (2018). Determinants of hair cortisol concentration in children: A systematic review. *Psychoneuroendocrinology*, *87*(August 2017), 204–214. https://doi.org/10.1016/j.psyneuen.2017.10.022

Groer, M. W., Kane, B., Williams, S. N., & Duffy, A. (2015). Relationship of PTSD symptoms with combat exposure, stress, and inflammation in American soldiers. *Biological Research for Nursing*, *17*(3), 303–310. https://doi.org/10.1177/1099800414544949

Jackson, S. E., Kirschbaum, C., & Steptoe, A. (2017). Hair Cortisol and Adiposity in a Population-Based Sample of 2 , 527 Men and Women Aged 54 to 87 Years. *Obesity*, *25*(3), 539–544. https://doi.org/10.1002/oby.21733

Kalmakis, K. A., Meyer, J. S., Chiodo, L., & Leung, K. (2015). Adverse childhood experiences and chronic hypothalamic-pituitary-adrenal activity. *Stress*, *18*(4), 446–450. https://doi.org/10.3109/10253890.2015.1023791

Kuehl, L. K., Hinkelmann, K., Muhtz, C., Dettenborn, L., Wingenfeld, K., Spitzer, C., … Otte, C. (2015). Hair cortisol and cortisol awakening response are associated with criteria of the metabolic syndrome in opposite directions. *Psychoneuroendocrinology*, *51*, 365–370.

https://doi.org/10.1016/j.psyneuen.2014.09.012

Lee, E. H. (2012). Review of the psychometric evidence of the perceived stress scale. *Asian Nursing Research*, *6*(4), 121–127. https://doi.org/10.1016/j.anr.2012.08.004

Luthar, S. S., Cicchetti, D., & Becker, B. (2000). The construct of resilience: A critical evaluation and guidelines for future work. *Child Development*, *71*(3), 543–562. https://doi.org/10.1111/1467-8624.00164

Mewes, R., Reich, H., Skoluda, N., Seele, F., & Nater, U. M. M. (2017). Elevated hair cortisol concentrations in recently fled asylum seekers in comparison to permanently settled immigrants and non-immigrants. *Translational Psychiatry*, *7*(3), e1051. https://doi.org/10.1038/tp.2017.14

Milam, J., Slaughter, R., Verma, G., & McConnell, R. (2014). Hair cortisol, perceived stress and dispositional optimism: A pilot study among adolescents. *Journal of Traumatic Stress Disorders & Treatment*, *3*(3), 1000126. https://doi.org/10.4172/2324-8947.1000126

Muehlhan, M., Höcker, A., Miller, R., Trautmann, S., Wiedemann, K., Lotzin, A., … Schäfer, I. (2018). HPA axis stress reactivity and hair cortisol concentrations in recently detoxified alcoholics and healthy controls with and without childhood maltreatment. *Addiction Biology*, (August), 1–8. https://doi.org/10.1111/adb.12681

Orta, O. R., Tworoger, S. S., Terry, K. L., Coull, B. A., Gelaye, B., Kirschbaum, C., … Williams, M. A. (2018). Stress and hair cortisol concentrations from preconception to the third trimester. *Stress (Amsterdam, Netherlands)*, *0*(0), 1–10. https://doi.org/10.1080/10253890.2018.1504917

Osborne, J. W. (2010). Improving your data transformations : Applying the Box-Cox transformation. *Pratical Assessment, Research & Evaluation*, *15*(12), 1–9.

Pacella, M. L., Hruska, B., Steudte-Schmiedgen, S., George, R. L., & Delahanty, D. L. (2017). The utility of hair cortisol concentrations in the prediction of PTSD symptoms following traumatic physical injury. *Social Science & Medicine*, *175*(3), 228–234. https://doi.org/10.1016/j.socscimed.2016.12.046

Raul, J.-S. S., Cirimele, V., Ludes, B., & Kintz, P. (2004). Detection of physiological concentrations of cortisol and cortisone in human hair. *Clinical Biochemistry*, *37*(12), 1105–1111. https://doi.org/10.1016/j.clinbiochem.2004.02.010

Ruiz-Robledillo, N., De Andrés-García, S., Pérez-Blasco, J., González-Bono, E., & Moya-Albiol, L. (2014). Highly resilient coping entails better perceived health, high social support and low morning cortisol levels in parents of children with autism spectrum disorder. *Research in Developmental Disabilities*, *35*(3), 686–695. https://doi.org/10.1016/j.ridd.2013.12.007

Russell, E., Kirschbaum, C., Laudenslager, M. L., Stalder, T., Rijke, Y. De, van Rossum, E. F. C., … Koren, G. (2015). Toward Standardization of Hair Cortisol Measurement : Results of the First International Interlaboratory Round Robin. *Therapeutic Drug Monitoring*, *37*(1), 71–75. https://doi.org/10.1097/FTD.0000000000000148

Schreier, H. M. C., Bosquet Enlow, M., Ritz, T., Coull, B. A., Gennings, C., Wright, R. O., & Wright, R. J. (2016). Lifetime exposure to traumatic and other stressful life events and hair cortisol in a multi-racial/ethnic sample of pregnant women. *Stress (Amsterdam, Netherlands)*, *19*(1), 45–52. https://doi.org/10.3109/10253890.2015.1117447

Serwinski, B., Salavecz, G., Kirschbaum, C., & Steptoe, A. (2016). Associations between hair cortisol concentration, income, income dynamics and status incongruity in healthy middle-aged women. *Psychoneuroendocrinology*, *67*, 182–188. https://doi.org/https://doi.org/10.1016/j.psyneuen.2016.02.008

Sheehan, D. V, Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., … Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry*, *59*(Suppl 20), 22–33. Retrieved from https://psycnet.apa.org/record/1998-03251-004

Simmons, J. G., Badcock, P. B., Whittle, S. L., Byrne, M. L., Mundy, L., Patton, G. C., … Allen, N. B. (2016). The lifetime experience of traumatic events is associated with hair cortisol concentrations in community-based children. *Psychoneuroendocrinology*, *63*, 276–281.

https://doi.org/10.1016/j.psyneuen.2015.10.004

Stalder, T., & Kirschbaum, C. (2012). Analysis of cortisol in hair - State of the art and future directions. *Brain, Behavior, and Immunity*, *26*(7), 1019–1029. https://doi.org/10.1016/j.bbi.2012.02.002

Stalder, T., Kirschbaum, C., Alexander, N., Bornstein, S. R., Gao, W., Miller, R., … Fischer, J. E. (2013). Cortisol in hair and the metabolic syndrome. *The Journal of Clinical Endocrinology & Metabolism*, *98*(6), 2573–2580. https://doi.org/10.1210/jc.2013-1056

Stalder, T., Kirschbaum, C., Heinze, K., Steudte, S., Foley, P., Tietze, A., & Dettenborn, L. (2010). Use of hair cortisol analysis to detect hypercortisolism during active drinking phases in alcohol-dependent individuals. *Biological Psychology*, *85*(3), 357–360. https://doi.org/10.1016/j.biopsycho.2010.08.005

Stalder, T., Steudte-Schmiedgen, S., Alexander, N., Klucken, T., Vater, A., Wichmann, S., … Miller, R. (2017). Stress-related and basic determinants of hair cortisol in humans: A meta-analysis. *Psychoneuroendocrinology*, *77*, 261–274. https://doi.org/10.1016/j.psyneuen.2016.12.017

Statistics South Africa. (2013). *Gender statistics South Africa 2011*. https://doi.org/EB.AIR/WG.5/2001/7

Staufenbiel, S. M., Penninx, B. W. J. H., de Rijke, Y. B., van den Akker, E. L. T., & van Rossum, E. F. C. (2015). Determinants of hair cortisol and hair cortisone concentrations in adults. *Psychoneuroendocrinology*, *60*, 182–194. https://doi.org/10.1016/j.psyneuen.2015.06.011

Staufenbiel, S. M., Penninx, B. W. J. H., Spijker, A. T., Elzinga, B. M., & van Rossum, E. F. C. (2013). Hair cortisol, stress exposure, and mental health in humans: A systematic review. *Psychoneuroendocrinology*, *38*(8), 1220–1235. https://doi.org/10.1016/j.psyneuen.2012.11.015

Steptoe, A., Dockray, S., & Wardle, J. (2009). Positive Affect and Psychobiological Processes Relevant to Health. *Journal of Personality*, *77*(6), 1747–1776. https://doi.org/10.1111/j.1467-6494.2009.00599.x

Steudte-Schmiedgen, S., Wichmann, S., Stalder, T., Hilbert, K., Muehlhan, M., Lueken, U., & Beesdo-Baum, K. (2017). Hair cortisol concentrations and cortisol stress reactivity in generalized anxiety

disorder, major depression and their comorbidity. *Journal of Psychiatric Research*, *84*(2017), 184–190. https://doi.org/10.1016/j.jpsychires.2016.09.024

Ullmann, E., Barthel, A., Petrowski, K., Stalder, T., Kirschbaum, C., & Bornstein, S. R. (2016). Pilot study of adrenal steroid hormones in hair as an indicator of chronic mental and physical stress. *Scientific Reports*, *6*(May), 1–7. https://doi.org/10.1038/srep25842

Ursache, A., Merz, E., Melvin, S., Meyer, J., & Noble, K. G. (2017). Socioeconomic status, hair cortisol and internalizing symptoms in parents and children. *Psychoneuroendocrinology*, *78*, 142–150. https://doi.org/https://doi.org/10.1016/j.psyneuen.2017.01.020

Walker, F. R., Pfingst, K., Carnevali, L., Sgoifo, A., & Nalivaiko, E. (2017). In the search for integrative biomarker of resilience to psychological stress. *Neuroscience and Biobehavioral Reviews*, *74*, 310–320. https://doi.org/10.1016/j.neubiorev.2016.05.003

Wennig, R. (2000). Potential problems with the interpretation of hair analysis results. *Forensic Science International*, *107*(1), 5–12. https://doi.org/10.1016/S0379-0738(99)00146-2

Wester, V. L., Noppe, G., Savas, M., van den Akker, E. L. T., de Rijke, Y. B., & van Rossum, E. F. C. (2017). Hair analysis reveals subtle HPA axis suppression associated with use of local corticosteroids : The Lifelines cohort study. *Psychoneuroendocrinology*, *80*, 1–6. https://doi.org/10.1016/j.psyneuen.2017.02.024

Wester, V. L., van der Wulp, N. R. P., Koper, J. W., de Rijke, Y. B., & van Rossum, E. F. C. (2016). Hair cortisol and cortisone are decreased by natural sunlight. *Psychoneuroendocrinology*, *72*, 94–96. https://doi.org/10.1016/j.psyneuen.2016.06.016

Wikenius, E., Moe, V., Kjellevold, M., Smith, L., Lyle, R., Waagbø, R., … Myhre, A. M. (2016). The association between hair cortisol and self-reported symptoms of depression in pregnant women. *PLoS ONE*, *11*(9), 1–10. https://doi.org/10.1371/journal.pone.0161804

Windle, G., Bennett, K. M., & Noyes, J. (2011). A methodological review of resilience measurement scales. *Health and Quality of Life Outcomes*, *9*(1), 1. https://doi.org/10.1186/1477-7525-9-8

World Health Organization. (2005). *WHO STEPS surveillance manual: the WHO STEPwise approach to chronic disease risk factor surveillance*. Geneva: World Health Organization (WHO).

World Medical Association. (2013). World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA - Journal of the American Medical Association*, *310*(20), 2191–2194. https://doi.org/10.1001/jama.2013.281053

Wosu, A. C., Valdimarsdóttir, U., Shields, A. E., Williams, D. R., & Williams, M. A. (2013). Correlates of cortisol in human hair: Implications for epidemiologic studies on health effects of chronic stress. *Annals of Epidemiology*, *23*(12), 797–811. https://doi.org/10.1016/j.annepidem.2013.09.006

CHAPTER 7

The association between cognitive functioning and hair cortisol levels in South African mixed ancestry females

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The association between cognitive functioning and hair cortisol levels in South African mixed ancestry females

Abstract

Background: Glucocorticoids can influence cognitive function, particularly declarative memory and executive functions. Hair cortisol concentrations (HCC) reflect longer-term hypothalamic pituitary adrenal (HPA) axis function and can thus provide insight as to whether chronically dysregulated cortisol is associated with cognitive impairments.

Objectives: Our objectives were to investigate whether cognitive function in domains sensitive to glucocorticoids (declarative memory, executive functions) were associated with higher HCC as compared to other cognitive domains and global cognitive functioning in a sample of 153 mixed ancestry females, aged between 18 and 79 years.

Methods: Our sample comprised 153 females (mean age 46.1 years, *SD* = 15.1) who were control participants in a cross sectional case-control study (SHARED ROOTS), conducted in Cape Town, South Africa from May 2014 until June 2017. We examined whether performance on neurocognitive tests were associated with HCC in both unadjusted and adjusted linear regression models.

Results: Verbal working memory (digit span backwards) demonstrated a significant inverse association with HCC in both unadjusted ($p = 0.010$) and adjusted ($p = 0.043$) analyses. Performance on the other domains assessed (immediate and delayed memory, visuospatial, language, attention, and executive function) as well as global cognitive function were not associated with HCC in unadjusted and adjusted analyses.

Conclusions: Poorer performance on tests of working memory were associated with higher HCC, suggesting stress related effects may impair working memory. We thus demonstrated cortisol related effects in one of the cognitive domains frequently shown to be sensitive to the effects of glucocorticoids, but not for the other domains known to be influenced by glucocorticoids, such as immediate and delayed memory.

Keywords: Hair cortisol concentrations; working memory, declarative memory; cognitive function

1 Introduction

Glucocorticoids play a central role in learning and memory, especially pertaining to adaptations following exposure to stressors (de Kloet, Oitzl, & Joëls, 1999; Lupien, Maheu, Tu, Fiocco, & Schramek, 2007). Cortisol influences these processes by binding to glucocorticoid (GR) and mineralocorticoid (MR) receptors in the brain. MR are concentrated in the hippocampus, whereas GR are more widely distributed throughout the brain, with highest density demonstrated in the prefrontal cortex (de Kloet et al., 1999; Lupien et al., 2007; Lupien, McEwen, Gunnar, & Heim, 2009). Glucocorticoids can influence the structure and function of the hippocampus and prefrontal cortex through both genomic (gene-expression) and non-genomic (rapid-signalling) mechanisms (McEwen, Nasca, & Gray, 2016). MR receptors have a high affinity for cortisol and are usually occupied under basal conditions, whereas GR receptors have a low affinity and are occupied when cortisol levels increase, such as during stress (de Kloet et al., 1999; Lupien et al., 2007). GR and MR appear to play different roles in learning and memory processes and these are influenced by the comparative activation of GR and MR (de Kloet et al., 1999; Lupien et al., 2007). Mildly elevated glucocorticoids with associated increased GR activation enhance long-term potentiation (LTP), whereas when cortisol levels are too low, or too high LTP is decreased (de Kloet et al., 1999; Lupien et al., 2007). A dysregulated hypothalamic pituitary adrenal (HPA) axis can thus impair cognitive processes, particularly those related to the hippocampus (short and long-term memory) and the prefrontal cortex (executive functions and working memory) (Lupien et al., 2007; McEwen et al., 2016).

Both exogenous administration, and endogenous increases in glucocorticoids following stressful exposures, can influence memory and executive functions (Het, Ramlow, & Wolf, 2005; Sauro, Jorgensen, & Pedlow, 2003; Shields, Bonner, & Moons, 2015). The acute effects of cortisol are related to both improved and worsened cognitive function which appear to be time-dependent. For instance, glucocorticoids administered prior to retrieval impaired recall, but demonstrated mixed effects when administered prior to learning, impairing recall in the morning and improving recall in the afternoon (Het et al., 2005). Similarly exogenously administered glucocorticoids initially impaired working memory performance but demonstrated a delayed effect improving working memory performance, while the

converse was demonstrated for tests of inhibition (Shields et al., 2015). Meta-analyses have also demonstrated that acute stress exposure was associated with both increased glucocorticoid levels and poorer memory performance in animal and human studies, and in humans cortisol levels were correlated with declarative memory performance (Sauro et al., 2003). Although there are some inconsistencies, studies have also demonstrated that higher basal cortisol levels are associated with poorer performance on cognitive tests, particularly tests related to memory and executive functions (Feeney, O'Halloran, & Kenny, 2018; Franz et al., 2011; Li et al., 2006; Lupien et al., 2009; McLennan, Ihle, Steudte-Schmiedgen, Kirschbaum, & Kliegel, 2016; Pulopulos et al., 2014).

The chronic effects secondary to a dysregulated HPA-axis are, however, likely to be different to the acute effects of cortisol and may be related to immune system dysfunction, and altered brain structure and function (Shields et al., 2015). Elevated glucocorticoids are associated with hippocampal atrophy and structural changes in the prefrontal cortex and amygdala (Lupien et al., 1998, 2009). The traditional samples utilised to measure basal cortisol levels (e.g. saliva, blood and urine) are better suited to determine acute cortisol levels (Stalder & Kirschbaum, 2012). In recent years hair cortisol concentrations (HCC) have been established as a useful marker of longer-term HPA axis function (Stalder et al., 2017). Studies are increasingly investigating the association between HCC and neurocognitive function in both clinical (Aas et al., 2019; Ben Assayag et al., 2017; Downey et al., 2015; Ogawa, Lee, Yamaguchi, Shibata, & Goto, 2017; Pereira et al., 2019) and community samples (Feeney et al., 2018; McLennan et al., 2016; Pulopulos et al., 2014). Most of the studies have, however, been conducted in developed regions and there is a lack of representation of studies examining associations between neuroendocrine markers of stress and cognitive performance from low and middle-income countries (James, Grace, Pan, Combrinck, & Thomas, 2019). Furthermore, as psychosocial stress exposure and HPA-axis function, including HCC, can vary according to ethnicity it is important to investigate for associations between HPA-axis function and cognitive performance in more diverse populations and settings (Abell et al., 2016; Schreier et al., 2016; Wosu, Valdimarsdóttir, Shields, Williams, & Williams, 2013). Our aim was to investigate, in a South African mixed ancestry sample, whether HCC were associated with cognitive domains known to be sensitive to glucocorticoids

(declarative memory, executive functioning and working memory) as compared to other cognitive domains and overall cognitive functioning.

2 Methods

2.1 Study design

Here we investigate one of the original aims of a neuroendocrine ancillary study in the control sample included in a cross-sectional matched case-control study (SHARED ROOTS [SR]). The controls were group matched based on age and gender to three neuropsychiatric disease (NPD) cohorts (posttraumatic stress disorder (PTSD), schizophrenia and Parkinson's disease). We have previously reported on the determinants of HCC in the current sample and for detailed methods consult Chapter 6.

Participants were assessed over 3 years from May 2014 until June 2017 in Cape Town, South Africa. This study was approved by the Health Research Ethics Committee at Stellenbosch University (HREC N13/08/115) and conducted according to ethical guidelines and principles of the seventh revision of the Declaration of Helsinki (World Medical Association, 2013).

2.2 Participants

Participants were mixed ancestry (coloured) adults, aged 18 years and older, who provided hair samples for neuroendocrine analyses. Further exclusions applied to this sample were (i) any current psychiatric disorder as determined by history, diagnostic interview or current medication use for a psychiatric condition, (ii) significant medical morbidity (such as cancer, auto-immune disorders or chronic infections), (iii) systemic or scalp steroid medication use, (iv) current pregnancy or pregnancy within the prior three months. We also excluded participants with any factors potentially influencing cognitive functioning, such a previous cerebrovascular incidents (CVAs, n = 4) and a history of a head injury with loss of consciousness (n = 7).

2.3 Procedures

Participants attended two to three study visits, each lasting around 3 hours. At the first visit, diagnostic, clinical and participant-administered measures were completed. The second visit occurred within 24-96

hours of the first visit and entailed neurocognitive testing, physical procedures and administration of additional lifestyle measures. Neurocognitive testing was performed by research and clinical psychologists.

2.3.1 Hair analysis

The proximal 3cm of hair samples cut from the posterior vertex scalp were analysed with liquid chromatography-tandem mass spectrometry (LC-MS/MS) at the TU Dresden laboratory (Prof Clemens Kirschbaum).

2.4 Measures

2.4.1 Neurocognitive assessments

2.4.1.1 The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) (Randolph, 1998)

The RBANS is a brief neurocognitive battery, with an administration time of 30 minutes and includes the following domains and tests: (i) immediate memory (list learning and story memory); (ii) visuospatial/constructional (figure copy and line orientation); (iii) language (picture naming and semantic fluency); (iv) attention (digit span and coding); (v) and delayed memory (list recall, list recognition, story memory, and figure recall). A total score, reflecting overall cognitive performance is derived by summing the scores for the various domains.

2.4.1.2 The Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999)

The two subtest version of the WASI was used to estimate intellectual functioning. This is a short (administration 15 minutes) reliable measure of intelligence and scores on vocabulary (verbal intelligence) and matrix reasoning (non-verbal intelligence) are combined to provide an impression of global intellectual ability.

2.4.1.3 Executive function and working memory tests

Additional tests of executive function were administered as the RBANS does not include executive function tests. These tests were only administered in the controls included in the PTSD and Parkinson's disease cohorts as the participants in the schizophrenia cohort completed a battery

specifically designed to assess cognition in individuals diagnosed with schizophrenia (Nuechterlein & Green, 2002). The following tests of executive function were included: The Ruff Figural Fluency Test (RFFT) (Ruff, 1996) to assess executive functioning and non-verbal fluency; (ii) The Stroop Color and Word Test (Golden & Freshwater, 1978) to assess selective attention, cognitive flexibility and resistance to interference from outside stimuli; Spatial span and digit span from the Wechsler memory scale (WMS-III) (Wechsler, 1997) to assess visuospatial (non-verbal) and verbal working memory respectively.

Higher scores on all the tests and domains indicate better performance, excluding only the error ratio from the RFFT.

Additional measures included demographic, medical history and hair questionnaires described in Chapter 6. Variables included in analyses were age, years of education completed, body mass index (BMI, kg/m2) and the determinants of HCC in this sample (see Chapter 6): (i) whether women were currently breastfeeding; (ii) whether any additional hair products had been used in the prior three months (such as hair styling and care products e.g. gels, oils and creams, excluding products used during hair washing); (iii) the average hours per day that hair was exposed to sunlight (less than one hour, 1-2 hours, more than 2 hours) and the duration of hair sample storage (less than 1 year, 1-2 years, 2-3.5 years).

2.5 Statistical analysis

We decided to limit our analyses to females only due to the small number of males (n = 3) with HCC available. Descriptive data, numbers and percentages for categorical variable and means and standard deviations for continuous variables are reported. To assess whether performance on neurocognitive domains were associated with HCC neurocognitive scores raw scores were z-transformed and were regressed on reciprocal square root transformed HCC in unadjusted and adjusted linear regression models. We adjusted for factors known to be associated with cognitive performance (age, years of education, and BMI) and covariates of HCC (additional hair products used, average hours in the sun, duration of storage and breastfeeding). Data were analysed with SPSS for windows, version 25.0, all tests were 2-tailed and the level of significance was set at .05.

3 Results

3.1 Participants

Descriptive details pertaining to the sample are available in Table 1. In brief, the mean age of participants was 46.1 years (*SD* 15.1), ranging from 18 to 79 years and the mean years of education completed were 10.4 (*SD* 2.5). The average raw scores for the neurocognitive domains evaluated are presented in Table 2.

Table 1 Descriptive features of the sample

Variables	Number (%)
Total sample	153 (100)
Socio-demographic	
Age	46.1 (15.1)[a]
Years of education completed	10.2 (2.5)[a]
Secondary education complete	
No	101 (66.0)
Yes	52 (34.0)
Employed	
No	97 (63.4)
Yes	56 (36.6)
Hair related	
Natural hair colour black	
No	84 (54.9)
Yes	69 (45.1)
Hair chemically treated	
No	46 (30.1)
Yes	107 (69.9)
Frequency of hair washing	
≥ 2 times a week	41 (26.8)
1 time a week	76 (49.7)
< 1 time a week	36 (23.5)
Add on hair products	
No	77 (50.3)
Yes	76 (49.7)
Average hours in sun[d]	
< 1 hour	71 (46.4)
1-2 hours	42 (27.5)
> 2 hours	37 (24.2)
Season sampled	
Summer	32 (20.9)
Autumn	41 (26.8)
Winter	45 (29.4)
Spring	35 (22.9)
Duration of storage	
Less than 1 year	28 (18.3)
1 - 2 years	51 (33.3)
2 – 3.5 years	74 (48.4)
Clinical	
Hormonal contraceptive	
No	124 (81.0)
Yes	29 (19.0)

Currently breastfeeding	
No	147 (96.1)
Yes	6 (3.9)
Topical steroid use	
No	146 (95.4)
Yes	7 (4.6)
Body mass index (BMI)	31.0 (8.0)[b]
Medical conditions	
No	74 (48.4)
Yes	79 (51.6)
Behavioural	
Tobacco use	
No	103 (67.3)
Yes	50 (32.7)
Alcohol use	
No	83 (54.2)
Yes	70 (45.8)
Level of physical activity	
High	15 (9.8)
Moderate	52 (34.0)
Low	86 (56.2)
Hair cortisol concentrations	1.2 (0.3)[a]

[a] Mean and standard deviation
[b] Three responses were missing for this item, n = 150

3.2 Neurocognitive function and hair cortisol concentrations (HCC) (Table 2)

3.2.1 RBANS

RBANS total and subscale scores (immediate memory, visuospatial/constructional, language, attention and delayed memory) were not significantly associated with HCC in unadjusted or adjusted analyses.

3.2.2 WASI

WASI verbal intelligence scores were inversely associated with HCC, demonstrating a trend towards significance in unadjusted ($p = 0.086$), but not adjusted ($p = 0.134$), analyses. WASI global and non-verbal intelligence scores were not significantly associated with HCC.

3.2.3 Executive function and working memory tests

Verbal working memory performance, as assessed with the digit span backwards, was significantly inversely associated with HCC in both unadjusted ($p = 0.010$) and adjusted ($p = 0.043$) analyses. The other tests of executive functioning were not associated with HCC in both unadjusted and adjusted analyses.

Table 2 Regression analyses of neurocognitive test scores on hair cortisol concentrations (HCC)

Variables	Raw score M(SD)	Simple linear regression ß (95% CI)	p-value	Multiple linear regression[a] ß (95% CI)	p-value
Total sample	n = 153				
The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)					
Total score	198.0 (28.8)	-0.02 (-0.08; 0.03)	0.365	-0.03 (-0.09; 0.03)	0.352
Immediate memory	44.6 (7.7)	-0.03 (-0.08; 0.03)	0.344	-0.04 (-0.10; 0.01)	0.126
Visuospatial/Constructional	31.8 (4.6)	-0.04 (-0.09; 0.01)	0.149	-0.01 (-0.06; 0.04)	0.705
Language	27.6 (4.7)	-0.03 (-0.08; 0.02)	0.267	-0.02 (-0.07; 0.03)	0.379
Attention	46.2 (12.6)	-0.00 (-0.05; 0.05)	0.960	-0.00 (-0.06; 0.06)	0.982
Delayed memory	47.8 (7.1)	-0.02 (-0.08; 0.03)	0.381	-0.02 (-0.08; 0.04)	0.446
The Wechsler Abbreviated Scale of Intelligence (WASI)[b]					
Total score	65.7 (15.1)	-0.03 (-0.08; 0.03)	0.321	-0.02 (-0.08; 0.03)	0.407
Vocabulary	49.5 (9.9)	-0.05 (-0.10; 0.01)	0.086	-0.04 (-0.10; 0.01)	0.134
Matrix reasoning	16.3 (7.7)	0.01 (-0.04; 0.06)	0.822	0.01 (-0.05; 0.06)	0.762
Ruff Figural Fluency Test[c]					
Error ratio	0.16 (0.2)	0.03 (-0.02; 0.08)	0.208	-0.00 (-0.05; 0.05)	0.929
The Stroop Color and Word Test[d]					
Interference	-1.9 (8.2)	0.02 (-0.03; 0.07)	0.492	0.02 (-0.03; 0.07)	0.386
Wechsler memory scale[e]					
Spatial span backwards	6.0 (2.2)	-0.03 (-0.08; 0.03)	0.358	0.01 (-0.05; 0.07)	0.702
Digit span backwards	5.0 (2.1)	-0.07 (-0.12; -0.02)	0.010*	-0.05 (-0.10; -0.00)	0.043*

[a] Multivariate adjusted model (adjusted for age, education, BMI, additional hair products, average hours in the sun, duration of storage, breastfeeding), n = 150; three missing responses on the item 'average hours per day that hair was exposed to sunlight'
[b] Missing for a single participant, n = 152
[c] Not administered in the controls included in the schizophrenia cohort, not completed in two other participants due to visual difficulties, n = 137
[d] Not administered in the controls included in the schizophrenia cohort, not completed in three other participants due to visual difficulties, n = 136
[e] Not administered in the controls included in the schizophrenia cohort, n = 139
* p-value < 0.05
BMI, body-mass index; HCC, hair cortisol concentrations; RBANS, The Repeatable Battery for the Assessment of Neuropsychological Status; RFFT, Ruff Figural Fluency Test; Stroop, the Stroop Color and Word Test WASI, the Wechsler Abbreviated Scale of Intelligence (WASI); WMS, Wechsler memory scale

4 Discussion

We aimed to investigate for stress related effects, as reflected by HCC, on cognitive performance in a sample of South African mixed ancestry females. We demonstrated that performance on one of the domains known to be associated with stress and elevated cortisol levels, namely working memory, was inversely associated with HCC. No other cognitive domains, as well as global cognitive functioning, were associated with HCC, including the other domains sensitive to glucocorticoids (declarative memory and executive function).

Other studies of HCC in non-clinical samples have demonstrated mixed results. A study in nurses (aged 21 - 62) found that HCC were not associated with performance on any of the cognitive domains assessed (McLennan et al., 2016). Whereas a study in healthy older adults (aged 56 – 77) found that higher HCC were associated with better cognitive performance (including working memory, learning and short- and long-term verbal memory) (Pulopulos et al., 2014). They also demonstrated that higher diurnal salivary cortisol levels were associated with poorer cognitive performance, thus suggesting diurnal salivary cortisol and hair cortisol reflect different aspects of HPA-axis function (Pulopulos et al., 2014). Conversely, in a large community cohort of older adults (aged 54 – 94 years) HCC were inversely associated with cognitive performance, including immediate and delayed recall (Feeney et al., 2018). Possible factors that may have influenced these differential results are that the two studies demonstrating significant results were limited to older individuals and the participants in Pulopulos et al. were older adults participating in a university study program and thus likely represented high functioning older individuals. Two randomised controlled trials (RCTs) evaluating psychosocial and physical interventions, one in adolescents (Panter-Brick, Wiley, Sancilio, Dajani, & Hadfield, 2019) and one in older adults (Jansen, Dahmen-Zimmer, Kudielka, & Schulz, 2017), also found that HCC were not associated with change in cognitive functioning over time.

The association between HCC and cognitive function may be more evident in individuals with increased vulnerability for cognitive impairment, such as those with existing brain disorders. For instance, a study in patients with schizophrenia, bipolar disorder and controls found that HCC was

inversely associated with working memory in the patient groups, but not in the control group (Aas et al., 2019). Similarly a study in children found that HCC were negatively correlated with spatial working memory performance in children with autism spectrum disorder (ASD), but not in typically developing children (Ogawa et al., 2017). Additionally, higher HCC in samples obtained directly following a stroke were associated with poorer cognitive performance post--stroke and at 24-months follow-up (Ben Assayag et al., 2017). In patients with multiple sclerosis (MS), however, HCC were not associated with executive dysfunction and processing speed (Pereira et al., 2019). A study in 3.4-methylenedioxymethamphetamine (MDMA) or 'Ecstasy' users and controls also demonstrated no correlations between memory function (immediate recall, delayed recall) and subjective memory problems and HCC (Downey et al., 2015).

Most studies demonstrate cognitive impairments in relation to elevated cortisol in cognitive domains related to brain regions with high MR and GR receptor densities, namely the hippocampus (declarative memory) and prefrontal cortex (working memory and executive functions). In our sample we only demonstrated significant associations for working memory (digit span backward) and not declarative memory and other executive function domains. Working memory appears to be more sensitive to the effects of glucocorticoids (Lupien et al., 2007) and working memory impairments have been demonstrated to be related to a GR mediated increase in dopamine release in the prefrontal cortex (Butts, Weinberg, Young, & Phillips, 2011). Working memory impairments have been demonstrated following glucocorticoid administration (Lupien, Gillin, & Hauger, 1999; Shields et al., 2015; Young, Sahakian, Robbins, & Cowen, 1999) and studies have also demonstrated that increased cortisol levels following psychosocial stressor exposures were associated with working memory impairments (Oei, Everaerd, Elzinga, Van Well, & Bermond, 2006; Taverniers, Van Ruysseveldt, Smeets, & Von Grumbkow, 2010). Contrary to our results, another HCC study in older adults demonstrated that higher HCC were associated with better performance on the digit span backward test (Pulopulos et al., 2014). Other studies utilising HCC have not demonstrated associations between HCC and working memory in non-clinical and control samples (Aas et al., 2019; Jansen et al., 2017; McLennan et al., 2016),

although inverse associations have been demonstrated in clinical samples (Aas et al., 2019; Ogawa et al., 2017).

Contrary to our results two studies have demonstrated significant inverse associations between HCC and short- and long-term memory (Ben Assayag et al., 2017; Feeney et al., 2018) although another demonstrated a positive association (Pulopulos et al., 2014). The relationship between cortisol levels and declarative memory appear to follow an inverse u-shaped curve and thus only cortisol levels below or exceeding a certain threshold may contribute to memory dysfunction (Lupien et al., 2007). For instance, a study administering low and high dose cortisol for four days, representing mild and more significant stress, found that high dose, but not low dose, cortisol resulted in impairments in verbal memory (Newcomer et al., 1999). It is possible that cortisol levels were not above the threshold associated with memory impairment for the majority of participants, possibly due to the inclusion of younger individuals and the exclusion of individuals with significant medical and psychiatric disorders. The lack of association between HCC and declarative memory could potentially also be ascribed to other factors related to stress influencing memory performance. A meta-analyses found that although stress led to both increased cortisol levels and memory impairments, that cortisol levels did not moderate the memory impairments (Shields, Sazma, McCullough, & Yonelinas, 2017). Stress therefore likely influences memory function through a combination of pathways in addition to cortisol, such as through effects of the sympathetic nervous system, other hormones and the immune system (Shields et al., 2017).

4.1 Strengths and limitations

Our study is cross-sectional and we cannot comment on any cause-effect relationships. Indeed, pre-morbid cognitive function may also be a vulnerability factor for HPA-axis dysfunction. In one longitudinal study cognitive performance at age 20 years was inversely associated with cortisol levels at age 55 years (Franz et al., 2011). The hippocampus regulates HPA-axis activity and thus hippocampal dysfunction may also contribute to HPA-axis dysregulation (Lupien et al., 2009). Smaller hippocampal volume has also been associated with increased risk for developing psychiatric disorders and cognitive

impairments and may represent greater vulnerability to environmental exposures (Lupien et al., 2009). Longitudinal studies utilising HCC are required to explicate these pathways.

Due to the small number of males with hair long enough to be sampled our sample was limited to a single sex and ethnicity limiting the generalisability of our findings. For instance, meta-analyses have revealed that stress related increases in cortisol levels are more pronounced in males (Shields et al., 2017) and thus sex may influence the association between HCC and cognitive performance. One of the benefits of our sample being limited to a single ethnicity and gender is that lifetime stress, and associated HPA-axis function, may vary according to gender and ethnicity (Abell et al., 2016; Schreier et al., 2016; Wosu et al., 2013), particularly in South Africa, a country with both a history of and ongoing racial and gender inequality (James et al., 2019; Mayosi et al., 2012), and that these factors may be more equivalent within our sample. Our sample is also unique in representing a geographic and cultural setting where there are notable psychosocial stressors, including high rates of trauma exposure, socio-economic strain and historical racially based trauma with enduring racial inequity. Indeed, only one other study has been conducted examining the association between cortisol levels and cognitive function in South Africa. Similar to our study they found that in older adults saliva cortisol levels were not significantly associated with overall cognitive and memory performance, in both the controls and patients with Alzheimer's disease (James et al., 2019). It also important to note that our control sample was recruited using purposive sampling approaches and does not represent a random population sample. Self-selection bias, such as employment status influencing the availability to participate in research, means that, for instance, our sample may be over-represented by individuals with specific stressors and factors influencing cognitive performance (such as unemployment). Notwithstanding, our sample appeared to be representative of South African mixed-ancestry women in terms of education and employment status, with the percentage that had completed secondary education (34.0%) roughly equivalent to the national average (30.0%), and if we excluded individuals aged 65 years and older the percentage who were employed (40.6%) was also roughly equivalent to the national average (42.3%) (Statistics South Africa, 2013). Considering the high stress burden in South Africa and the potential adverse effects on cognition further studies are required in our setting

including large community cohorts with broader ethnic, gender and cultural representation. Studies in clinical samples are also warranted as there appear to be greater associations between HCC and cognitive performance in individuals with known brain disorders (Aas et al., 2019; Ben Assayag et al., 2017; Ogawa et al., 2017). Further strengths of our study included that HCC were determined with LC-MS/MS, the gold standard method for HCC determination (Russell et al., 2015). Our cognitive battery included various domains thus ensuring we examined for associations between HCC and the major cognitive domains. Though the additional tests of executive function were not performed in the schizophrenia cohort, as they were administered a different cognitive battery specific to schizophrenia, and thus those analyses were conducted in a smaller sample.

4.2 Conclusions

In a sample of mixed ancestry females, poorer verbal working memory performance was associated with higher stress, as reflected by HCC. Performance on other domains know to be impaired by stress and elevated cortisol levels, including other tests of executive function and short- and long- term memory, were not associated with HCC. Studies combining HCC, acute basal and stress induced evaluations of cortisol may help to further elucidate the intricate pathways by which glucocorticoids influence cognitive function. Incorporating neuro-imaging alongside neurocognitive testing may also assist in determining whether HCC are associated with structural and functional changes in brain regions sensitive to glucocorticoids, such as the hippocampus and prefrontal cortex, and whether these align with performance on neurocognitive domains. Future studies in geographically, culturally and ethnically diverse populations are also required to better delineate how stress, HPA-axis dysfunction and cognitive performance are linked across different settings.

5 References

Aas, M., Pizzagalli, D. A., Laskemoen, J. F., Reponen, E. J., Ueland, T., Melle, I., … Andreassen, O. A. (2019). Elevated hair cortisol is associated with childhood maltreatment and cognitive impairment in schizophrenia and in bipolar disorders. *Schizophrenia Research*, (xxxx), 1–7. https://doi.org/10.1016/j.schres.2019.01.011

Abell, J. G., Stalder, T., Ferrie, J. E., Shipley, M. J., Kirschbaum, C., Kivimäki, M., & Kumari, M. (2016). Assessing cortisol from hair samples in a large observational cohort : The Whitehall II study. *Psychoneuroendocrinology*, *73*, 148–156. https://doi.org/10.1016/j.psyneuen.2016.07.214

Ben Assayag, E., Tene, O., Korczyn, A. D., Shopin, L., Auriel, E., Molad, J., … Stalder, T. (2017). High hair cortisol concentrations predict worse cognitive outcome after stroke: Results from the TABASCO prospective cohort study. *Psychoneuroendocrinology*, *82*(November 2016), 133–139. https://doi.org/10.1016/j.psyneuen.2017.05.013

Butts, K. A., Weinberg, J., Young, A. H., & Phillips, A. G. (2011). Glucocorticoid receptors in the prefrontal cortex regulate stress-evoked dopamine efflux and aspects of executive function. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(45), 18459–18464. https://doi.org/10.1073/pnas.1111746108

de Kloet, E. R., Oitzl, M. S., & Joëls, M. (1999). Stress and cognition: are corticosteroids good or bad guys? *Trends in Neuroscience*, *22*(10), 422–426. https://doi.org/https://doi.org/10.1016/S0166-2236(99)01438-1

Downey, L. A., Sands, H., Jones, L., Clow, A., Evans, P., Stalder, T., & Parrott, A. C. (2015). Reduced memory skills and increased hair cortisol levels in recent Ecstasy/MDMA users: Significant but independent neurocognitive and neurohormonal deficits. *Human Psychopharmacology*, *30*(3), 199–207. https://doi.org/10.1002/hup.2474

Feeney, J. C., O'Halloran, A. M., & Kenny, R. A. (2018). The Association Between Hair Cortisol, Hair Cortisone, and Cognitive Function in a Population-Based Cohort of Older Adults: Results From The Irish Longitudinal. *The Journals of Gerontology: Series A*. https://doi.org/https://doi.org/10.1093/gerona/gly258

Franz, C. E., O'Brien, R. C., Hauger, R. L., Mendoza, S. P., Panizzon, M. S., Prom-Wormley, E., … Kremen, W. S. (2011). Cross-sectional and 35-year longitudinal assessment of salivary cortisol and cognitive functioning: The Vietnam Era Twin Study of Aging. *Psychoneuroendocrinology*, *36*(7), 1040–1052. https://doi.org/10.1016/j.psyneuen.2011.01.002

Golden, C. J., & Freshwater, S. M. (1978). *Stroop color and word test*. Chicago, IL: Stoelting.

Het, S., Ramlow, G., & Wolf, O. T. (2005). A meta-analytic review of the effects of acute cortisol administration on human memory. *Psychoneuroendocrinology*, *30*(8), 771–784. https://doi.org/10.1016/j.psyneuen.2005.03.005

James, K. A., Grace, L. K., Pan, C. Y., Combrinck, M. I., & Thomas, K. G. F. (2019). Psychosocial stress associated with memory performance in older South African adults. *Aging, Neuropsychology, and Cognition*, *00*(00), 1–14. https://doi.org/10.1080/13825585.2019.1645809

Jansen, P., Dahmen-Zimmer, K., Kudielka, B. M., & Schulz, A. (2017). Effects of Karate Training Versus Mindfulness Training on Emotional Well-Being and Cognitive Performance in Later Life. *Research on Aging*, *39*(10), 1118–1144. https://doi.org/10.1177/0164027516669987

Li, G., Cherrier, M. M., Tsuang, D. W., Petrie, E. C., Colasurdo, E. A., Craft, S., … Wilkinson, C. W. (2006). Salivary cortisol and memory function in human aging. *Neurobiology of Aging*, *27*(11), 1705–1714. https://doi.org/10.1016/j.neurobiolaging.2005.09.031

Lupien, S. J., De Leon, M., De Santi, S., Convit, A., Tarshish, C., Nair, N. P. V., … Meaney, M. J. (1998). Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nature Neuroscience*, *1*(1), 69–73. https://doi.org/10.1038/271

Lupien, S. J., Gillin, C. J., & Hauger, R. L. (1999). Working memory is more sensitive than declarative memory to the acute effects of corticosteroids: A dose-response study in humans. *Behavioral Neuroscience*, *113*(3), 420–430. https://doi.org/10.1037/0735-7044.113.3.420

Lupien, S. J., Maheu, F., Tu, M., Fiocco, A., & Schramek, T. E. (2007). The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. *Brain and Cognition*, *65*(3), 209–237. https://doi.org/10.1016/j.bandc.2007.02.007

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, *10*(6), 434–445. https://doi.org/10.1038/nrn2639

Mayosi, B. M., Lawn, J. E., Van Niekerk, A., Bradshaw, D., Abdool Karim, S. S., & Coovadia, H. M. (2012). Health in South Africa: Changes and challenges since 2009. *The Lancet*, *380*(9858), 2029–2043. https://doi.org/10.1016/S0140-6736(12)61814-5

McEwen, B. S., Nasca, C., & Gray, J. D. (2016). Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex. *Neuropsychopharmacology*, *41*(1), 3–23. https://doi.org/10.1038/npp.2015.171

McLennan, S. N., Ihle, A., Steudte-Schmiedgen, S., Kirschbaum, C., & Kliegel, M. (2016). Hair cortisol and cognitive performance in working age adults. *Psychoneuroendocrinology*, *67*, 100–103. https://doi.org/10.1016/j.psyneuen.2016.01.029

Newcomer, J. W., Selke, G., Melson, A. K., Hershey, T., Craft, S., Richards, K., & Alderson, A. L. (1999). Decreased memory performance in healthy humans induced by stress-level cortisol treatment. *Archives of General Psychiatry*, *56*(6), 527–533. https://doi.org/10.1001/archpsyc.56.6.527

Nuechterlein, K., & Green, M. (2002). *Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Consensus Cognitive Battery (MCCB)*. Los Angeles, CA: Harcourt.

Oei, N. Y. L., Everaerd, W. T. A. M., Elzinga, B. M., Van Well, S., & Bermond, B. (2006). Psychosocial stress impairs working memory at high loads: An association with cortisol levels and memory retrieval. *Stress*, *9*(3), 133–141. https://doi.org/10.1080/10253890600965773

Ogawa, S., Lee, Y. A., Yamaguchi, Y., Shibata, Y., & Goto, Y. (2017). Associations of acute and chronic stress hormones with cognitive functions in autism spectrum disorder. *Neuroscience*, *343*, 229–239. https://doi.org/10.1016/j.neuroscience.2016.12.003

Panter-Brick, C., Wiley, K., Sancilio, A., Dajani, R., & Hadfield, K. (2019). C-reactive protein, Epstein-Barr virus, and cortisol trajectories in refugee and non-refugee youth: Links with stress, mental health, and cognitive function during a randomized controlled trial. *Brain, Behavior, and Immunity*, (July 2018). https://doi.org/10.1016/j.bbi.2019.02.015

Pereira, G. M., Becker, J., Soares, N. M., de Azeredo, L. A., Grassi- Oliveira, R., Rysdyk, A., & de Almeida, R. M. M. (2019). Hair cortisol concentration, cognitive, behavioral, and motor impairment in multiple sclerosis. *Journal of Neural Transmission*, (0123456789). https://doi.org/10.1007/s00702-019-02040-w

Pulopulos, M. M., Hidalgo, V., Almela, M., Puig-Perez, S., Villada, C., & Salvador, A. (2014). Hair cortisol and cognitive performance in healthy older people. *Psychoneuroendocrinology*, *44*, 100–111. https://doi.org/10.1016/j.psyneuen.2014.03.002

Randolph, C. (1998). RBANS manual: Repeatable battery for the assessment of neuropsychological status. *San Antonio, TX: The Psychological Corporation*.

Ruff, R. M. (1996). *RFFT: Ruff figural fluency test: Professional manual*. Lutz, FL: Psychological Assessment Resources.

Russell, E., Kirschbaum, C., Laudenslager, M. L., Stalder, T., Rijke, Y. De, van Rossum, E. F. C., … Koren, G. (2015). Toward Standardization of Hair Cortisol Measurement : Results of the First International Interlaboratory Round Robin. *Therapeutic Drug Monitoring*, *37*(1), 71–75. https://doi.org/10.1097/FTD.0000000000000148

Sauro, M. D., Jorgensen, R. S., & Pedlow, C. T. (2003). Stress, Glucocorticoids, and Memory: A Meta-analytic Review. *Stress*, *6*(4), 235–245. https://doi.org/10.1080/10253890310001616482

Schreier, H. M. C., Bosquet Enlow, M., Ritz, T., Coull, B. A., Gennings, C., Wright, R. O., & Wright, R. J. (2016). Lifetime exposure to traumatic and other stressful life events and hair cortisol in a multi-racial/ethnic sample of pregnant women. *Stress (Amsterdam, Netherlands)*, *19*(1), 45–52. https://doi.org/10.3109/10253890.2015.1117447

Shields, G. S., Bonner, J. C., & Moons, W. G. (2015). Does cortisol influence core executive functions? A meta-analysis of acute cortisol administration effects on working memory, inhibition, and set-shifting. *Psychoneuroendocrinology*, *58*, 91–103. https://doi.org/https://doi.org/10.1016/j.psyneuen.2015.04.017

Shields, G. S., Sazma, M. A., McCullough, A. M., & Yonelinas, A. P. (2017). The effects of acute stress on episodic memory: A meta-analysis and integrative review. *Psychological Bulletin*, *143*(6), 636–675. https://doi.org/10.1037/bul0000100

Stalder, T., & Kirschbaum, C. (2012). Analysis of cortisol in hair - State of the art and future directions. *Brain, Behavior, and Immunity*, *26*(7), 1019–1029. https://doi.org/10.1016/j.bbi.2012.02.002

Stalder, T., Steudte-Schmiedgen, S., Alexander, N., Klucken, T., Vater, A., Wichmann, S., … Miller, R. (2017). Stress-related and basic determinants of hair cortisol in humans: A meta-analysis. *Psychoneuroendocrinology*, *77*, 261–274. https://doi.org/10.1016/j.psyneuen.2016.12.017

Taverniers, J., Van Ruysseveldt, J., Smeets, T., & Von Grumbkow, J. (2010). High-intensity stress elicits robust cortisol increases, and impairs working memory and visuo-spatial declarative memory in Special forces candidates: A field experiment. *Stress*, *13*(4), 323–333. https://doi.org/10.3109/10253891003642394

Wechsler, D. (1997). *Wechsler memory scale (WMS-III)*. New York, NY: Psychological Corporation.

Wechsler, D. (1999). *Manual for the wechsler abbreviated scale of intelligence (WASI)*. New York, NY: The Psychological Corporation: Harcourt Brace & Company.

Wosu, A. C., Valdimarsdóttir, U., Shields, A. E., Williams, D. R., & Williams, M. A. (2013). Correlates of cortisol in human hair: Implications for epidemiologic studies on health effects of chronic stress. *Annals of Epidemiology*, *23*(12), 797–811. https://doi.org/10.1016/j.annepidem.2013.09.006

Young, A. H., Sahakian, B. J., Robbins, T. W., & Cowen, P. J. (1999). The effects of chronic administration of hydrocortisone on cognitive function in normal male volunteers. *Psychopharmacology*, *145*(3), 260–266. https://doi.org/10.1007/s002130051057

CHAPTER 8

Conclusion

Conclusion

The key findings and scientific contributions of this study are summarised and then elaborated for each chapter. Suggestions and recommendations for future research are then outlined, including further research planned in this sample.

1 Summary of contributions to existing knowledge

Our study demonstrated that HPA-axis function was altered in the three neuropsychiatric disorders (NPDs), with higher hair cortisol concentrations (HCC) in posttraumatic stress disorder (PTSD) patients than trauma exposed controls (TEC), lower HCC in patients with schizophrenia than controls and higher hair cortisone levels, but not HCC, in Parkinson's disease patients than controls. These findings align with our hypotheses, excluding the schizophrenia cohort where we expected to find higher HCC in patients than controls. Furthermore, HCC were associated with severity of PTSD and hair cortisone with non-motor symptoms related to depression and anxiety in Parkinson's disease patients. Thus our results suggest that stress, as reflected by long-term HPA-axis dysfunction, is associated with psychopathology. These results underscore the need to address stress occurring in NPDs and consideration of interventions are required to improve resilience and stress coping ability, especially as greater resilience was also associated with lower HCC in the control sample.

We found that metabolic syndrome (MetS) was not associated with HCC in the three NPD cohorts, excluding only significant positive associations demonstrated in the longitudinal investigation conducted in the schizophrenia cohort. We also found that in our combined analysis that there was a trend toward significance demonstrated for higher HCC in those with MetS when we excluded PTSD patients, the only group where HCC were lower in those with MetS. The findings did not completely align with our hypothesis as we expected to find higher HCC in NPD patients with MetS than in patients without MetS. The lack of significant findings related to MetS may be due to the small samples and lack of statistical power to detect associations in the individual cohorts.

Our exploratory analyses investigating associations between the individual cardiovascular disease (CVD) risk factors and HCC demonstrated persistent associations between high density lipoprotein cholesterol (HDL-C) and HPA-axis function, with low HDL-C associated with elevated glucocorticoids across the different cohorts. Furthermore, waist-to-hip ratio demonstrated a positive association with hair cortisone in the Parkinson's disease cohort and an inverse association with HCC in patients with PTSD. Our results thus suggest that these CVD risk factors may be associated with HPA-axis dysfunction in NPDs and warrant closer investigation in studies of HCC in these NPDs.

Very few studies have directly investigated links between hypothalamic pituitary adrenal (HPA) axis, NPDs and cardiovascular disease (CVD) risk and our study is the first study to utilise a measure of longer-term HPA axis dysfunction to address this. Considering the high burden of CVD in NPDs, our study provides a step towards better understanding of the role played by chronic stress, as reflected by long-term HPA axis dysfunction, in the co-occurrence of CVD in NPDs. Our results suggest that the pathways whereby HPA-axis dysfunction and MetS in NPDs are linked may be complex and vary according to NPD.

Regarding our secondary aims, contrary to what we hypothesized we demonstrated that HCC were lower in patients with first episode psychosis (FEP) than controls at baseline and increased following a course of treatment with a flupenthixol decanoate, demonstrating a trend towards significance. Our results suggest that in a subset of FEP patients that acute psychosis may be associated with a blunted HPA-axis with lower HCC and that this may be normalised with antipsychotic treatment. In our control sample we found some analogous associations between HCC and basic determinants to what has been found before (lower HCC with higher level of education and increased duration of storage) as well as some dissimilarities (inverse associations between HCC and age). Our results provide insight as to the main determinants of HCC in South African mixed ancestry females thus informing future research in representative populations. We also identified novel associations with HCC (duration of sun exposure, hair product use and breastfeeding in women) that warrant further investigation. In alignment with our hypothesis we also demonstrated that HCC were inversely associated with resilience, although HCC were not significantly associated with self-perceived stress. We thus emphasise the

importance of investigating the biological pathways underpinning resilience and taking a broader perspective in stress research. In partial alignment with our hypothesis we also found that poorer working memory performance, but not semantic memory and other executive functions, was associated with higher HCC. Our results thus suggest that chronic stress, as reflected by long-term cortisol levels, may be associated with working memory impairments.

This study is one of the first studies to utilise HCC as a marker of longer-term HPA axis function in a South African sample. Our study thus provides insights into the role of chronic stress and associated HPA-axis dysfunction in clinical conditions of relevance to South Africa and contributes to broader geographic, cultural and ethnic representation in hair cortisol research. Limitations identified in this study, such as those pertaining to hair length, also suggest ways in which hair neuroendocrine studies need to be adapted to allow for greater gender and ethnic representation across different cultures and settings. Our study is also notable in that we investigated the role of HPA-axis dysfunction across three different NPDs and thus were able to identify possible shared and unique pathways across NPDs.

A significant limitation to this study is that the cross-sectional design does not allow for the establishment of cause-effect relationships. This study addresses the question of whether HPA-axis dysfunction, as measured by HCC, is associated with the diagnosis of the three NPDs and furthermore the comorbidity of MetS within these NPDs. This study does not address the pathways through which HPA-axis dysfunction, NPDs and MetS are linked, but only investigates for possible associations. It thus remains unclear exactly how the NPDs, MetS and a dysregulated HPA axis, as measured by HCC, relate to each other. Each of these factors could theoretically increase the risk for the developing the others and it is very likely that these factors inter-relate and influence each other. For instance, HPA-axis dysfunction occurring within the context of NPDs could contribute to metabolic derangement and increase the risk for MetS, but conversely MetS could contribute to HPA-axis dysfunction observed in NPDs. Alternatively MetS and HPA-axis dysfunction, by influencing brain function, could increase the risk of developing NPDs. There may also be other shared pathways contributing to the co-occurrence of these factors. Longitudinal studies will be required to better delineate how these factors are related to and influence each other over time.

A further limitation is the way the groups have been categorised, both in terms of NPD diagnoses, and MetS status, and that these rely on existing conceptualisations of the disorders and do not take a more trans-diagnostic or dimensional approach. As the classification of NPDs and MetS are a component of the SHARED ROOTS study design they need to be considered in analyses employed, however alternative statistical modelling approaches can be utilised to further examine the how the various factors interact and relate to each other. For instance, different methods of determining cardiovascular risk can be employed, such as using alternative risk stratification algorithms, such as the Framingham, SCORE, and CUORE risk scores (Castelli, Anderson, Wilson, & Levy, 1992; Conroy et al., 2003; D'Agostino et al., 2008; Gaziano et al., 2013; Giampaoli et al., 2007; Joseph et al., 2018) or using modelling techniques, such as latent class analysis (LCA) or latent profile analysis (LPA) to identify subtypes of cardiovascular risk groups (Hajian-Tilaki, 2018; Khodarahmi, Asghari-Jafarabadi, & Farhangi, 2019; Roman-Urrestarazu et al., 2016). LCA, or other modelling techniques, can also be employed to identify subtypes within and across the NPDs (Rahman et al., 2018; Starkstein et al., 2011; Tsai & Rosenheck, 2013; Zahodne, Marsiske, & Bowers, 2013). The association between these subtypes of NPDs, CVD risk and HCC can then be examined utilising structural equation modelling (SEM) or machine learning based approaches (Adams & Boscarino, 2011; Galatzer-Levy, Ma, Statnikov, Yehuda, & Shalev, 2017; M. W. Miller, Wolf, Martin, Kaloupek, & Keane, 2008).

1.1 Chapter 2 - Hair cortisol levels in posttraumatic stress disorder and metabolic syndrome: A case-control study in South African mixed ancestry females

We demonstrated higher HCC in patients with PTSD as compared to TEC. Although this association is the converse of what has generally been found (Meewisse, Reitsma, de Vries, Gersons, & Olff, 2007; Morris, Compas, & Garber, 2012; Schumacher et al., 2019), the context of ongoing stress and trauma in which our study was conducted may explain the contrasting findings (G. E. Miller, Chen, & Zhou, 2007; Steudte-Schmiedgen, Kirschbaum, Alexander, & Stalder, 2016). Moreover, we demonstrated a dose-response association between PTSD severity and HCC with the clinical phenotype (PTSD), rather than trauma load, significantly associated with HCC. Furthermore, self-perceived stress was also associated with HCC providing further support that it is the psychological stress experienced by PTSD

sufferers that is associated with increased cortisol output. We did not find an association between MetS and HCC, although HCC were significantly higher in individuals with low HDL-C. We also found that in PTSD patients there was an inverse association between waist-to-hip ratio and HCC, which is counter to what is generally found (Stalder et al., 2017). Our study is one of the largest investigations of the association between PTSD and HCC to date and represents a different context than the studies that have been conducted so far. It is furthermore, one of the few studies (Blessing et al., 2017) that have directly examined the association between PTSD, CVD risk and cortisol levels and the first to use HCC to address this aim.

1.2 Chapter 3 - Hair glucocorticoid levels in females with Parkinson's disease

We found that hair cortisone, but not hair cortisol, levels were significantly higher in patients with Parkinson's disease than in controls. Furthermore, hair cortisone was positively associated with non-motor symptoms of Parkinson's disease related to depression, anhedonia and anxiety and with waist-to-hip ratio. Similar to other studies (Davison, Singh, & McFarlane, 2019; Stalder et al., 2013) we demonstrated stronger associations and less variability in hair cortisone than hair cortisol, supporting the utility of hair cortisone levels as an additional biomarker of HPA-axis function. This study is the first to report on hair glucocorticoid levels in Parkinson's disease and provides further evidence that patients with Parkinson's disease exhibit a dysregulated HPA-axis and that stress and symptoms of depression and anxiety may play a role. It is also the first study to directly examine the association between Parkinson's disease, CVD risk and cortisol levels.

1.3 Chapter 4 – Hair cortisol levels in schizophrenia and metabolic syndrome

We demonstrated that HCC were significantly lower in patients with schizophrenia than controls, and in particular in first-episode psychosis (FEP) patients than controls. These findings are contradictory to the majority of studies that have examined basal cortisol levels in schizophrenia (Borges, Gayer-Anderson, & Mondelli, 2013; Bradley & Dinan, 2010; Gajsak, Gelemanovic, Kuzman, & Puljak, 2017; Girshkin, Matheson, Shepherd, & Green, 2014; Hubbard & Miller, 2019). Findings of studies investigating HCC in schizophrenia have been mixed, with most demonstrating no association between schizophrenia and HCC (Aas et al., 2019; Andrade et al., 2016; Streit et al., 2016; Touskova et al., 2018) while other

studies, particularly in FEP samples, have demonstrated lower cortisol levels in patients than controls (Bradley & Dinan, 2010; Mondelli et al., 2015; Seitz et al., 2019). We also found that HCC increased following treatment with a depot antipsychotic, demonstrating a trend toward significance, and in exploratory analysis that HCC were lower in non-remitted patients than in controls and in putatively remitted patients, suggesting that lower HCC are associated with symptomatic status. In the context of a large body of literature demonstrating higher basal cortisol levels and increased stress in patients with schizophrenia and those at increased risk of developing schizophrenia we postulate that in a subset of patients lower long-term cortisol levels may reflect an overburdened HPA-axis that becomes decompensated during periods of active psychosis, particularly during the first episode of illness. This theory is also partly supported by the inverse association demonstrated between self-perceived stress scores and HCC in this cohort. These findings can at best be considered as preliminary and in view of the very small sample will require replication in larger samples. Similar to the other samples we demonstrated that at baseline MetS and CVD risk factors were not associated with HCC, although HCC increased from baseline to month-12 in those with MetS and MetS and low HDL-C were associated with higher HCC at month-12. This is one of the few studies (Manzanares et al., 2014; Vuksan-Cúsa, Săgud, Mihaljevic-Peleš, Jaksĭć, & Jakovljević, 2014) that have examined cortisol levels in relation to CVD risk in schizophrenia and the first utilising HCC. Although the schizophrenia sample was very small, there were some notable findings and these require replication in larger samples.

1.4 Chapter 5 - Hair cortisol as a neuroendocrine biomarker to evaluate the impact of chronic stress on the interaction between neuropsychiatric disorders and metabolic syndrome in females

Our pooled analysis provided some additional insights. Firstly our analyses showed that HCC, as well as various other factors, differed significantly between the three cohorts overall, suggesting that within cohort case-control comparisons provided better estimates of the patterns of HPA-axis dysfunction in the NPDs than cross-cohort comparisons. Thus although HCC largely did not differ significantly between the three NPDs different patterns emerged in the individual cohorts as discussed above. The results obtained from the combined analyses also aligned with those demonstrated in individual

cohorts, providing some support for the veracity of results. The opposite direction of association between cases and controls and HCC in the PTSD and schizophrenia cohorts were also reflected in the association patterns between self-perceived stress and HCC in these cohorts, suggesting that psychological stress is associated with increased cortisol output in PTSD and a blunted HPA-axis in schizophrenia. MetS was not associated with HCC in the overall sample. When PTSD patients, the only group demonstrating an opposite direction, were excluded from the analysis there was a trend toward statistical significance demonstrated for higher HCC in individuals with MetS. Our results highlight that although there may be limitations to cross-disorder comparisons that including different disorders within the same study allow for a clearer delineation of unique versus shared pathways.

1.5 Chapter 6 - Hair cortisol as a biomarker of stress and resilience in South African mixed ancestry females

Our study is one of the first to examine the basic determinants of HCC in a sample of non-clinical controls representing a different geographic, ethnic and cultural setting than in most studies that have been conducted to date. Furthermore, our study is also one of the first to examine for associations between HCC and a measure of resilience alongside a measure of self-perceived stress. We demonstrated a number of expected associations between HCC and basic determinants, as well as some novel associations. Specifically, HCC declined with age, which is the converse to what has mostly been demonstrated (Stalder et al., 2017; Wosu, Valdimarsdóttir, Shields, Williams, & Williams, 2013). Completion of secondary education was associated with lower HCC, which aligns with existing studies (Orta et al., 2018; Wosu et al., 2013). Our study is also one of the first studies to demonstrate that sun exposure has an effect on HCC under naturalistic conditions. We also demonstrated significant associations between HCC and other factors that are not frequently reported on in studies, such as hair product use, duration of storage and breastfeeding in women. We demonstrated a significant inverse association between HCC and resilience scores, alongside a non-significant positive association between self-perceived stress and HCC. As such, our results underscore the importance of taking a broader perspective in stress research, which is largely pathology driven. Our study also

highlights important limitations when hair sampling is used in different contexts and that methods need to be adapted to allow for proportionate inclusion of participants of different sexes and ethnic groups.

1.6 Chapter 7 - The association between cognitive functioning and hair cortisol levels in South African mixed ancestry females

We demonstrated that overall cognitive performance, as well as performance on the majority of cognitive domains were not associated with HCC. With respect to the cognitive domains (declarative memory, executive functions and working memory) that are sensitive to the effects of stress and cortisol, only verbal working memory was inversely associated with HCC. Our results align with other research suggesting working memory may be more sensitive to the effects of glucocorticoids (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007). Our study is one of the few to examine for associations between cognitive performance and cortisol levels in South Africa (James, Grace, Pan, Combrinck, & Thomas, 2019) and represents a different ethnic and cultural context than the majority of studies thus far.

2 Future research directions

2.1 Further studies planned in this sample

There are various research questions that remain to be addressed in the SR sample and some of the investigations planned are discussed here.

To further examine the pathways connecting stress, NPDs, MetS and HPA-axis dysfunction we plan to use SEM based modelling techniques. An example of a theoretical model that can be tested with SEM is illustrated below (Figure 1). Stress experienced by individuals can contribute to the development of NPDs, increased CVD risk and HPA-axis dysfunction. Thus we postulate that there is a latent variable ‘stress’ that is increasing the risk of developing brain, metabolic and HPA-axis dysfunction. We can utilise observed variables, such as childhood maltreatment, trauma exposures and socio-economic stressors to model the underlying latent variable ‘stress’. We can also utilise observed variables related to NPDs, CVD risk and HPA-axis function to model, or provide subtypes, of NPDs, CVD risk and HPA-axis dysfunction. We can then examine the pathways by which stress is associated with these three

latent variables and how they inter-relate. The role of other potentially mediating/moderating factors, such as age, gender, social support, resilience and lifestyle or behavioural factors can also be tested.

We plan to evaluate whether HCC are associated with structural and functional neuroimaging features and how this relates to phenotypes and clinical features. We also aim to investigate the interaction between HCC and inflammatory cytokines in the NPDs, and its role in comorbid CVD risk. We further plan to investigate whether HCC are associated with genomic, transcriptomic and epigenetic factors, particularly those related to cortisol [e.g. genes related to FK506 binding protein 51 (FKBP5), corticotrophin releasing hormone (CRH) receptors (CRHR1, CRHR2), corticotropin receptors (or melanocortin receptors, MC1R-MC5R), glucocorticoid receptors (NR3C1), and mineralocorticoid receptors (NR3C2)]. We demonstrated a dissociation between HCC and hair cortisone levels in relation to Parkinson's disease and CVD risk factors. Further studies are planned combining different hair neuroendocrine levels (cortisone, testosterone, progesterone, and dehydroepiandrosterone [DHEA]) with HCC and examining their interaction in relation to clinical factors and other variables of interest. Studies have demonstrated that the association between HCC and cognitive performance differs between healthy controls and individuals with NPDs (Aas et al., 2019; Ogawa, Lee, Yamaguchi, Shibata, & Goto, 2017); we thus plan to investigate the association between HCC and cognitive performance in NPD patients compared to the controls. We also plan to investigate how factors associated with stress, such as trauma exposure, perceived stress and resilience influence the associations between HCC and cognitive performance. In our PTSD sample we want to perform more in-depth investigations of the association between trauma type and differential childhood maltreatment exposures and altered HCC observed in this sample. LCA, or alternative modelling techniques will also be employed to identify polytrauma typologies (Contractor, Brown, & Weiss, 2018; Sullivan, Contractor, Gerber, & Neumann, 2017). We also aim to investigate whether haircare practices are directly associated with clinical measures, as explained below. SR was designed to utilise a multi-omics approach combining different techniques to investigate the comorbidity of NPDs and MetS. The final aim will be to incorporate HCC along with these other measures to build a mechanistic model of how the HPA-axis links with other factors, such as genetic, gene transcription, and epigenetic factors,

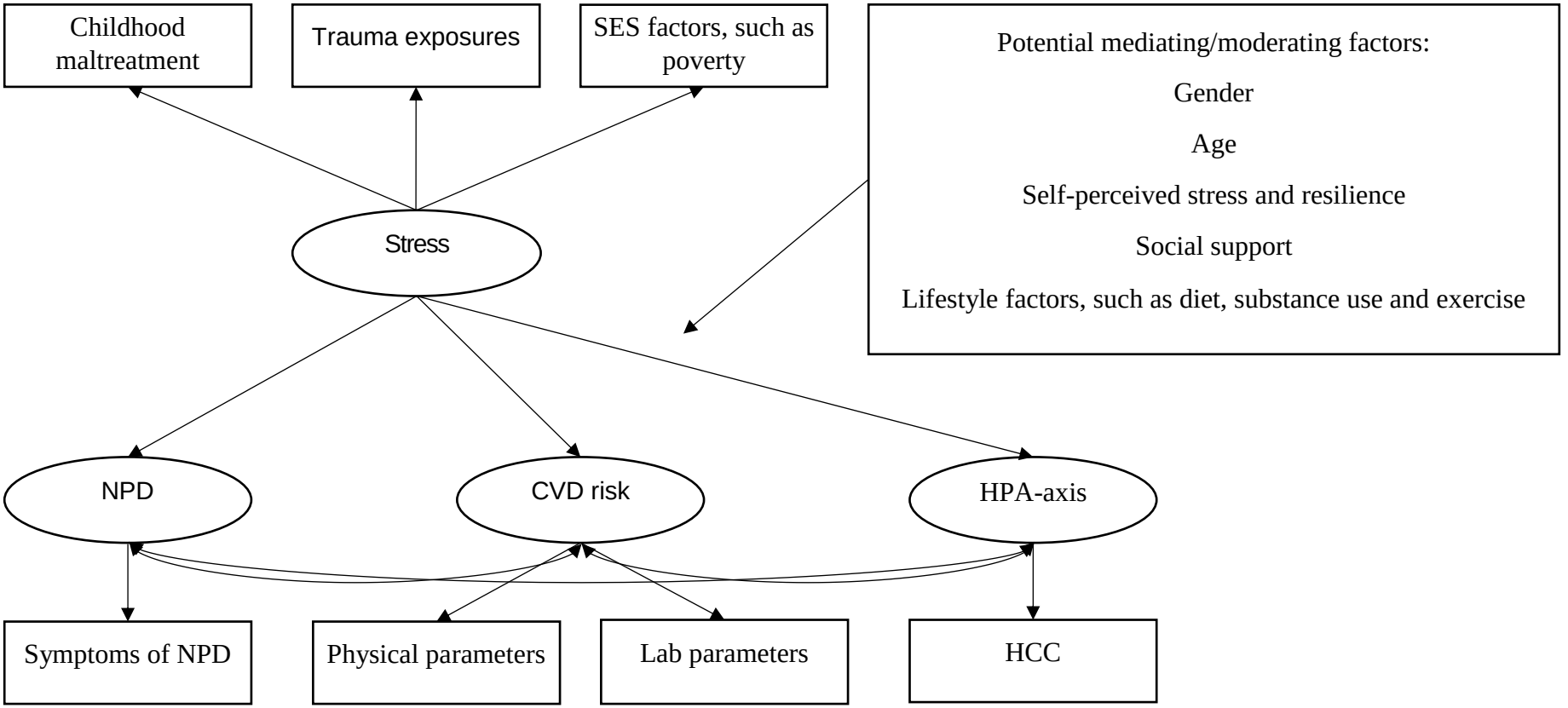

Figure 1 A theoretical model of how stress may be related to NPDs, CVD risk and HPA-axis dysfunction

A theoretical model of how stress may be related to NPDs, CVD risk and HPA-axis dysfunction that can be evaluated utilising structural equation modelling approaches. The model illustrates how different observed variables, such as childhood maltreatment, traumatic exposures and socio-economic stressors can be modelled to represent the latent variable 'stress'. In turn the observed variables representing NPDs, CVD risk and HPA-axis function can be modelled to represent each of those latent variables. The model can then examine how stress relates to NPDs, CVD risk and HPA-axis function and how these factors inter-relate. The role of other potentially mediating or moderating variables can also be tested.

structural and functional neuro-imaging, the microbiome and inflammatory cytokines in contributing to increased CVD risk in NPDs.

2.2 Optimising and standardising hair sampling procedures to ensure broader inclusion of participants

There are certain limitations to the study and to using HCC that can inform future research. Although there are various benefits to utilising hair samples there are also notable limitations and some of these became evident in this study. The limitation with the largest influence was the requirement that hair samples had to be 3cm or longer resulting in exclusion of the majority of males as well as many females. Other studies have similarly demonstrated that males and certain ethnic groups are disproportionately excluded (Kalmakis, Meyer, Chiodo, & Leung, 2015; Pacella, Hruska, Steudte-Schmiedgen, George, & Delahanty, 2017; Simmons et al., 2016). Investigators have employed different methods to circumvent the limitation of short hair length in males, such as using shorter samples of hair from the posterior vertex scalp, having hairdressers performing sampling whilst simultaneously providing complimentary haircuts, and shaving the forearm in military samples (Dajani, Hadfield, van Uum, Greff, & Panter-Brick, 2018; Groer, Kane, Williams, & Duffy, 2015; Mewes, Reich, Skoluda, Seele, & Nater, 2017). The length of hair samples have further implications, for instance, as hair length used across studies is not uniform this limits comparability across studies (Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, 2013). Furthermore, the period of time reflected by hair samples (e.g. 1cm representing one month window) needs to align better with the period of time specified in clinical measures (e.g. symptoms in the last month). In sum, methods used for hair sampling need to be standardised as well as adapted to allow for greater representation of different sexes and ethnicities. Utilising 1cm hair samples from the posterior vertex scalp may be the best strategy at present although adaptions should be made depending on the context in which the study is conducted.

2.3 Identifying and controlling for potentially confounding factors in hair cortisol studies

In our control sample we identified specific determinants (e.g. age, level of education, hair product use, duration of sun exposure, duration of storage, batch analysed, and breastfeeding) that can influence

HCC. Some of these factors, such as hair product use, duration of sun exposure and breastfeeding, have not previously been associated with HCC. These findings need to be replicated in other settings, particularly as they are not routinely assessed for or reported on in hair cortisol studies. We also identified an inverse association with age which is counter to what is generally found (Stalder et al., 2017; Wosu et al., 2013). Furthermore, we identified that study design related factors, such as the duration of sample storage and the batch analysed were associated with HCC and very few studies report on these factors. Our results highlight that though HCC can offer great insights regarding longer-term HPA-axis dysfunction that investigators need to take cognisance of potential confounders. These factors need to be considered in study design and conduct and in data analysis. Our results also highlight that determinants of hair cortisol may vary according to study population and setting and that future studies identifying the determinants of HCC in diverse settings and populations are required to provide greater representation as well as expand current understanding of how HCC are influenced by different factors.

2.4 Optimising and utilising haircare data

Hair care practices may vary according to culture and ethnicity and the influence of these factors on HCC need to be examined across different contexts and in more heterogeneous samples that are more representative of clinical and non-clinical populations. Although our study is a step in this direction, there is a need for greater population representivity in HCC research, and in health research in general.

Additional work also needs to go into building a scientific database of haircare products. These products are largely commercially defined and thus names and branding may not fully capture the contents of these products. Developing a database of haircare products and core ingredients may assist in standardising and better identifying hair care practices and products that influence HCC and other hormones in hair.

There is a general assumption that haircare practices directly influence cortisol levels in the hair samples. Another possibility is that haircare practices may reflect behavioural patterns that are linked to stress and thereby influence HCC. For instance, differences in additional hair product use and chemical

treatment of hair were observed across some of the groups in this study. Studies evaluating whether haircare practices are directly associated with psychosocial measures and clinical outcomes can assist in better delineating how these factors influence HCC. Furthermore, poor self-care practices are a diagnostic feature of various psychiatric disorders, but this is rarely quantified. Given that data pertaining to haircare practices are already being collected in hair neuroendocrine studies, these data can be utilised to investigate whether haircare practices provide a quantifiable proxy measure of self-care and its relationship to psychopathology and well-being.

2.5 Studies seizing on the benefits of hair sampling

Due to the simplicity of hair sampling, hair neuroendocrine levels have also increasingly been investigated in animal research. HCC may be an ideal measure of long-term HPA-axis function to utilise in translational research. For instance, HCC have already been used in animal models of depression and Parkinson's disease (Chu et al., 2014; Franke et al., 2016; Qin et al., 2019) and can assist in further elucidating the role of stress in various contexts and conditions.

Another area where hair neuroendocrine research may be beneficial is in post-mortem research. In post-mortem studies hair neuroendocrine levels can be correlated with specific brain pathophysiologic abnormalities observed. This may also be of particular use in research pertaining to suicide. As HCC reflect systemic cortisol levels for the period leading up to suicide these can provide insight as to whether stress and dysregulated HPA-axis function are present in the weeks prior to suicide completion and may thus help identify pathways contributing to suicide and thereby possibly inform preventive strategies.

2.6 Collaborations and studies with increased power

Studies with larger sample sizes that provide greater statistical power to detect case-control differences are needed to further investigate the questions addressed in this study, but also in HCC research overall. For instance, the effects related to MetS appeared to be smaller in magnitude than those pertaining to NPDs, and thus to adequately investigate the association between HPA-axis, NPDs and CVD risk, larger samples of patients both with NPDs and MetS are required. One mechanism, discussed above, is to adapt hair sampling approaches to allow for inclusion of a greater percentage of

participants in hair neuroendocrine studies. Another approach to increase power will be to conduct multicentre collaborative studies or to form consortia that will *a priori* apply harmonized protocols to allow for pooling of samples and clinical data (Boksa, 2013; Pinto, Moulin, & Amaral, 2017). These collaborations can also increase reproducibility, and by including members from lower resource settings improve the sharing of resources, knowledge and skills, and aid in capacity building. These efforts will also benefit by including samples across varied NPDs and implementing trans-diagnostic or dimensional approaches (Cuthbert & Insel, 2013; Insel, 2014). Limitations of our study can inform the design of cross-diagnostic studies, such as standardisation of sample characteristics across disorders, e.g. limiting samples to similar ages, stage of illness and with respect to treatment histories and methodological protocols. Furthermore, investigations across disorders, and evaluation of comorbidity of NPDs and somatic conditions, can increase cross-specialty collaborations and knowledge exchange. Cross-disorder and trans-diagnostic approaches align better with models whereby the traditional boundaries between mental and somatic disorders decrease allowing for a more holistic and integrated approach.

2.7 Longitudinal studies

Longitudinal studies with repeated hair sampling are required to determine the course and causal associations between NPDs, MetS and the HPA-axis. It is unclear how these factors are linked together and longitudinal studies can provide insights regarding the underlying mechanisms and the sequence in which the factors influence each other. Prospective studies can also elucidate the pathway between incident trauma, PTSD symptomatology and HPA-axis function. One of the key benefits of hair sampling is that it reflects retrospective systemic cortisol levels (Steudte-Schmiedgen et al., 2016). Hair samples obtained directly following trauma exposure will thus reflect cortisol levels prior to traumatisation. By conducting prospective studies in recently trauma exposed samples this will provide insights into HPA-axis dysfunction as a predisposing risk factor for the development of PTSD versus a consequential factor of trauma exposure and/or PTSD. In particular, repeated sampling will assist in tracing the course of cortisol levels following trauma exposure in those who develop psychopathology versus individuals who do not.

2.8 Integrating biomarkers

In this study the focus has been on HCC and its relationship to various clinical outcomes. HCC reflects one method of determining HPA-axis functioning and there are various other methods available that are utilised to examine HPA-axis function, such as psychological and pharmacological stress tests and measuring other proteins and hormones related to the HPA-axis (Allen et al., 2017; de Kloet et al., 2006; Gunnar, 2010; J.Yeo, Babic, Hannoush, & Weiss, 2000; Zorn et al., 2017). There is a need for more studies that combine different methods of HPA-axis function determination that will allow for a better integration of how HCC relates to other aspects of HPA-axis functioning. For instance, some studies have incorporated the Trier Social Stress Test (TSST) and other measures of HPA-axis function in hair cortisol studies (Muehlhan et al., 2018; Steudte-Schmiedgen et al., 2015, 2017). Furthermore, within the context of stress related adaptations, the HPA-axis does not function in isolation, but is one component of multiple integrated biological systems, including primary mediators of stress, such as stress hormones and inflammatory cytokines, and secondary outcomes, such a metabolic, cardiovascular and immune effects (Juster, McEwen, & Lupien, 2010). Biological markers of these different systems are frequently combined in studies to provide an allostatic load index, allowing for better prediction of risk and greater insight into the overall effects associated with stress related dysregulation (Juster et al., 2010). Studies evaluating how HCC interact with current allostatic load index approaches, as well as whether HCC can potentially be integrated into allostatic load algorithms, are needed.

Furthermore, similar to many other biomarkers, alterations in cortisol levels are non-specific and are observed across various disorders (Boksa, 2013; Pinto et al., 2017). To better distinguish specific disorders, multiple markers may need to be combined (Boksa, 2013). The HPA-axis regulates inflammatory and immune pathways and these are also altered in NPDs and in CVD and thus adding inflammatory markers to hair neuroendocrine studies can help determine how these factors intersect. There may also be shared genetic risk factors that underlie the co-occurrence of NPDs and CVD and that are also related to the HPA-axis. Genetic, transcriptomic and epigenetic studies incorporating HCC can provide greater insights. Linking hair neuroendocrine studies where DNA and RNA have been

collected to existing consortia may be a step towards addressing these aims. Incorporating HCC in functional and structural neuro-imaging studies can address the question as to whether dysregulated HPA-axis is associated with structural and functional changes in the brain regions sensitive to these effects, such as the hippocampus, amygdala, and prefrontal cortices. Other variables, such as markers of autonomic nervous system (ANS) function, oxidative stress, other cortisol related measures and a combination of different neuroendocrine hormones, can be included in HCC studies to allow for a more holistic investigation of the pathways involved in NPD-CVD co-occurrence (De Hert, Detraux, & Vancampfort, 2018; Vancampfort et al., 2015). Alternative statistical modelling approaches, such as SEM and network analyses, can be employed to better delineate the pathways through which HCC and these factors relate. Indeed, very few HCC studies have employed these types of modelling approaches (Gerber et al., 2013; Khoury, Bosquet Enlow, Plamondon, & Lyons-Ruth, 2019; Mustonen et al., 2019; Stalder et al., 2012) which can expand on and provide a more in-depth understanding of the underlying pathways involved.

2.9 Incorporating advances in technology

Advances in technology have opened up new avenues in the ways research is conducted as well as how data are collected and analysed. Personal devices, such as smartphones and wearables, have enhanced monitoring of physiology, behaviour and symptoms. Mobile technologies allow for real-time assessments of affect, cognitions and behaviours or 'Ecological Momentary Assessment (EMA)' (Reinertsen & Clifford, 2018). Furthermore, various activities reflecting locomotor and social behaviours can be tracked with smartphones and wearables can monitor features of ANS function (Reinertsen & Clifford, 2018). With advances in machine learning and artificial intelligence it is now possible to extract useful information from complex data and these technologies can thus assist with improved phenotyping of mental disorders (Insel, 2017). These technologies are already in use to measure movements and behaviour patterns in Parkinson's disease, schizophrenia and PTSD, although the studies in PTSD and schizophrenia are limited and there remains much scope for further investigations (Reinertsen & Clifford, 2018). Studies incorporating these advances in technology with hair neuroendocrine measures can build better models of how stress, the HPA-axis and complex

behaviours are linked. For instance, ANS monitoring, reflecting another central stress mediation pathway can be correlated with HCC reflecting the same time periods, thereby further demonstrating how these pathways are linked and how stress related adverse effects may be mediated through them.

These mobile technologies can also be used to better quantify stress and trauma exposure in contexts of ongoing stress and trauma. For instance, our sample had very complex trauma histories and the administered measures were limited in their ability to accurately capture the complexity and the severity of trauma exposure, particularly pertaining to ongoing trauma exposure. Many of our participants were from communities with high rates of community violence or had been exposed to intimate partner violence. They thus lived in settings of repeated traumatisation as well as ongoing exposure to trauma related cues. Within this context it is difficult to estimate the true extent of current stress and trauma and how this relates to the clinical picture. In-depth measures that better capture trauma histories and link these to biological markers, psychopathology and functioning are needed. This is a context where technology can be utilised to report real-time exposures as well as associated psychological measures. Conducting prospective studies in environments with high rates of ongoing trauma exposure, utilising mobile applications that allow for real-time reporting of trauma exposures or exposure to trauma related cues alongside EMA may allow us to better define trauma exposure as well as to how it correlates with environmental and clinical features. HCC and ANS system monitoring can also be incorporated into these studies to provide biological measures of stress alongside these in-depth trauma characterisations.

Beyond these advances mobile technology also allows for participation in research by completing measures online thus obviating the need for physical contact and travel to study sites. Some of the benefits pertaining to hair sampling, namely simplicity of sampling procedures, minimal storage and transport requirements, make it an ideal biological measure to incorporate into studies involving minimal or no clinical contact. Hair sampling procedures can easily be adapted to allow participants to obtain samples within their home environments and can be transported to researchers using existing postal facilities. Commercial laboratories offering substance and micronutrient testing already provide services whereby clients can obtain and send their own hair samples for analyses. One study in

children provided the parents who could not attend the study visits with instructions on how to collect hair samples from their children (Larsen, Fahrenkrug, Olsen, & Heitmann, 2016). Conducting research through these distance-based approaches decreases the costs and burden for both participants and researchers as participants can perform assessments at their own convenience and do not need to travel in to study sites and less research resources have to be expended on performing assessments. Hair sampling for neuroendocrine analyses is thus an ideal biological measure to incorporate into large population based studies utilising online or posted questionnaires. By utilising technological advances to improve the capacity to collect data and to provide mobile health (m-health) interventions to a greater number of people these technologies have the potential to affect global mental health (Insel, 2017).

2.10 Interventions

Delivery of adequate mental health services remains a major challenge globally (Patel et al., 2018). There is a need for mental health services to be integrated into other health care services and for special attention to be paid to the physical health of individuals with mental illness (Patel et al., 2018). Understanding the pathways contributing to increased CVD risk in NPDs can assist in informing interventions aimed at improving outcomes and reducing the burden of both CVD and NPDs. Considering the evidence positing that chronic stress plays a role in both NPDs and CVD, interventions aimed at reducing stress and increasing resilience can potentially address multiple negative sequelae. Studies are required to investigate whether interventions focused on alleviating stress, such as mindfulness and relaxation-based and exercise interventions, improve clinical outcomes pertaining to both NPDs and CVD and how such interventions can be incorporated into existing healthcare frameworks. For instance, South African patients with chronic medical conditions expressed a need for mental health services and agreed with the integration of these services within their chronic disease care, particularly counselling pertaining to stress coping strategies (Myers et al., 2018). Furthermore, HCC can be utilised as a marker of treatment response and there are already studies evaluating the efficacy of psychosocial interventions that have incorporated HCC to investigate how these relate to clinical outcomes (Dajani et al., 2018; Guo et al., 2018).

2.11 The role of HPA-axis function in resilience and positive adaptations

Very few studies have investigated whether cortisol levels are associated with resilience or stress-coping ability (Dockray & Steptoe, 2010; García-León, Pérez-Mármol, Gonzalez-Pérez, García-Ríos, & Peralta-Ramírez, 2019; Milam, Slaughter, Verma, & McConnell, 2014; Steptoe, Dockray, & Wardle, 2009; Ullmann et al., 2016) and less is known about the biological processes underpinning resilience as compared to stress. We demonstrated that greater resilience was associated with lower HCC in our control sample. This research needs to be extended into clinical samples. For instance, another outcome of trauma exposure is posttraumatic growth (PTG). Unlike resilience and stress coping that focus on adaptations that allow for return to or continued functioning despite adversity, PTG denotes an increase in psychological functioning following trauma or stress exposure (Schubert, Schmidt, & Rosner, 2016). Similar to resilience and other positive psychological constructs there is very limited research investigating the biological factors underpinning PTG (Schubert et al., 2016). There is a need for more research investigating how PTG, resilience and other protective factors interact with HPA-axis function that can help inform strategies aimed at improving positive outcomes.

2.12 Studies in particular disorders

2.12.1 Examining the role of stress in HIV-NPD comorbidity

South Africa has the highest rates of HIV globally, with an estimated 7.7 million people living with HIV in 2018 (UNAIDS, 2018). Due to decreased mortality rates and increased longevity the prevalence has continued to rise despite a decrease in new infections. As the nature of HIV is changing into a chronic medical condition the burden of illness has shifted to non-AIDS related conditions, such as neuropsychiatric disorders (NPDs), including HIV associated neurocognitive disorders (HAND) (van den Heuvel, Seedat, & Fennema-Notestine, 2016). Individuals with HIV frequently demonstrate higher rates of NPDs than observed in the general population, including common mental disorders, such as depression and anxiety, and neurocognitive impairments (Bernard, Dabis, & De Rekeneire, 2017; Chaponda et al., 2018; Freeman, Nkomo, Kafaar, & Kelly, 2008; Habib et al., 2013; Joska, Fincham, Stein, Paul, & Seedat, 2010; Joska et al., 2011; Kagee & Martin, 2010; Myer et al., 2008; Niu, Luo, Liu, Silenzio, & Xiao, 2016; Witten, Thomas, Westgarth-Taylor, & Joska, 2015).

In HIV different markers of stress, including psychosocial and biological measures, and specifically cortisol levels have been associated with lower CD4 cell counts, higher viral loads and rapider disease progression as well as poorer adherence to treatment (Weinstein & Li, 2016). Altered HPA-axis function may be one of the pathways contributing to NPD-HIV comorbidity and through which NPDs contribute to HIV disease progression (Arseniou, Arvaniti, & Samakouri, 2014; Weinstein & Li, 2016). The HPA-axis regulates immune and inflammatory pathways and this is one mechanism whereby a dysregulated HPA-axis can influence HIV related outcomes (Arseniou et al., 2014). For instance, stress and elevated cortisol levels can influence cytokine production by T lymphocytes contributing to CD4 lymphocyte destruction and viral replication (Leserman et al., 2000). Furthermore, one South African study demonstrated that neurocognitive impairment in HIV positive females was associated with single-nucleotide polymorphisms (SNPs) of two HPA axis genes, namely corticotrophin-releasing hormone receptor 1 (CRHR1) and corticotrophin-releasing hormone-binding protein (CRHBP) (Jacobs et al., 2018). A dysregulated HPA-axis may therefore play a role in the comorbidity of NPDs in HIV, as well as increased adverse effects associated with NPD-HIV comorbidity, such as HIV disease progression.

There are a limited number of studies that have examined HCC in individuals with HIV. A study in Chinese people living with HIV demonstrated that HCC were higher in those with higher stress levels and were correlated with anxiety (Qiao et al., 2017). Another study from the Netherlands found that though HCC were not associated with HIV status, that in those with HIV HCC were inversely associated with MetS, suggesting altered HPA-axis function is associated with multiple morbidity (Langerak et al., 2015). No studies utilising HCC have however been conducted in South Africa, the country with the highest prevalence of HIV. HCC as a marker of long-term HPA-axis function can provide insights into the role chronic stress plays in HIV-NPD comorbidity and HIV disease progression.

A better understanding of the role of stress and HPA-axis dysfunction in HIV-NPD comorbidity can help inform treatment strategies. For instance, stress management interventions have been associated with reductions in cortisol levels in HIV positive individuals (Antoni, 2003; Jones, Owens, Kumar, Cook, & Weiss, 2014). HCC and other markers of allostatic load can also be used as biological markers of

treatment response in intervention studies aimed at treating NPDs in HIV positive individuals. A randomised control trial (RCT) in China is utilising HCC as a stress-related outcome marker for an mobile-health intervention aimed at increasing physical activity in HIV positive individuals (Guo et al., 2018). More research regarding the role of stress, and HPA-axis dysfunction in HIV-NPD comorbidity is needed, especially in populations experiencing the highest burden of the disease, such as South Africa and insights gained need to be utilised in the formulation and implementation of treatment approaches, particularly those addressing stress reduction.

2.12.2 Investigating and addressing physician burnout

Burnout, a stress related syndrome that is directly linked to stress experienced within the context of work, is very prevalent among physicians, with some studies reporting rates as high as 50% (Dewa, Loong, Bonato, & Trojanowski, 2017; Rothenberger, 2017). Burnout is generally considered to involve three dimensions, emotional exhaustion, depersonalisation and low personal accomplishment (Maslach & Jackson, 1981). Burnout is linked to increased health problems, depression and suicidal ideation among physicians (Azam, Khan, & Alam, 2017; Kuhn & Flanagan, 2017) and can also have adverse impacts beyond the individual by affecting productivity and the quality of healthcare provided (Dewa, Loong, Bonato, Thanh, & Jacobs, 2014; Dewa et al., 2017). Risk factors include both individual features and organizational factors and although both individual and organisational interventions can improve burnout in physicians, the effects are significantly larger for organisational than individually focused interventions (Azam et al., 2017; Panagioti et al., 2017; West, Dyrbye, Erwin, & Shanafelt, 2016). Considering the significant adverse consequences to both physicians and healthcare structures further studies are required that investigate the risk factors for burnout and that evaluate the efficacy of interventions. HCC as a marker of chronic stress and associated neuroendocrine dysfunction can help identify risk factors for burnout and inform the development of strategies to address modifiable factors, and to design preventative approaches as well as interventions for physician burnout. The few studies that have been conducted utilising HCC have already provided evidence of HPA-axis dysregulation, with elevated HCC, in individuals with burnout (Penz et al., 2018; Wang, Dai, & Li, 2019). A Chinese study in hospital employees, including physicians, found that HCC were increased in burnout and that

these mediated the association between burnout and insomnia (Wang et al., 2019). Furthermore, a prospective burnout study also demonstrated that effort-reward imbalance contributed to a blunted HPA-axis with reduced HCC over time (Penz et al., 2019). HCC can also be incorporated into intervention studies to investigate whether successful interventions are associated with normalisation of the HPA-axis and how this relates to clinical outcomes. HCC studies demonstrating that burnout is a stress related condition can help set the agenda towards improving working conditions, individual well-being, as well as patient care.

2.13 Improving representation and resource allocation in health research and healthcare provision

Some of the results obtained in our study were contrary to what has generally been found e.g. lower HCC in patients with schizophrenia, higher HCC in patients with PTSD, inverse associations between HCC and age. Although we cannot distinguish to what extent the composition of our sample may have influenced these outcomes our results still highlight why research needs to be conducted in more diverse populations and settings. Our understanding of HPA-axis function in relation to most outcomes is largely driven by research conducted in developed regions, underscoring the need for more studies to be conducted in different geographic, ethnic and cultural contexts to both increase equity in healthcare research but to also broaden current understanding and thereby advance science and improve health and well-being for all.

There is also a particular need in South Africa to investigate how stress is linked to adverse health outcomes. Much of the poor health (and mental health) in South Africa can be ascribed to social determinants including poverty, unemployment, and socioeconomic-, racial- and gender- inequality (Lund, Breen, Flisher, & Kakuma, 2010; Lund et al., 2018; Mayosi et al., 2012). Chronic stress, and associated HPA-axis dysfunction, may be one of the routes through which these larger societal factors influence health outcomes. The mechanisms through which these social determinants influence health and mental health warrant further investigation and studies that both address these aims and investigate strategies aimed at alleviating their effects need to be prioritised.

Mental disorders are very prevalent and are a major cause of disability globally, as well as in South Africa (Herman et al., 2009; Suliman, Stein, Myer, Williams, & Seedat, 2010; Whiteford et al., 2013). Despite the high burden of NPDs, funding allocation for research is disproportionately low (Collins et al., 2011). Mental disorders frequently also remain undiagnosed and under-treated and appropriate mental health services are frequently lacking (Patel et al., 2018; Seedat et al., 2008; Suliman et al., 2010). Furthermore, despite higher rates of CVD and increased mortality risk, individuals with mental disorders are less likely to receive appropriate medical care (Laursen, Nordentoft, & Mortensen, 2014; Penninx & Lange, 2018; Walker, McGee, & Drus, 2015). Increased research efforts are required to better understand neuroendocrine and related biological pathways leading to mental disorders, as well as co-occurring somatic conditions, and to investigate preventive and treatment strategies to address these problems. Mental health needs to be prioritised in both research and healthcare provision and resource allocation ought to be more equitable.

3 References

Aas, M., Pizzagalli, D. A., Laskemoen, J. F., Reponen, E. J., Ueland, T., Melle, I., … Andreassen, O. A. (2019). Elevated hair cortisol is associated with childhood maltreatment and cognitive impairment in schizophrenia and in bipolar disorders. *Schizophrenia Research*, (xxxx), 1–7. https://doi.org/10.1016/j.schres.2019.01.011

Adams, R. E., & Boscarino, J. A. (2011). A structural equation model of perievent panic and posttraumatic stress disorder after a community disaster. *Journal of Traumatic Stress*, *24*(1), 61–69. https://doi.org/10.1002/jts.20603

Allen, A. P., Kennedy, P. J., Dockray, S., Cryan, J. F., Dinan, T. G., & Clarke, G. (2017). The Trier Social Stress Test: Principles and practice. *Neurobiology of Stress*, *6*, 113–126. https://doi.org/10.1016/j.ynstr.2016.11.001

Andrade, E. H., Rizzo, L. B., Noto, C., Ota, V. K., Gadelha, A., Daruy-Filho, L., … Brietzke, E. (2016). Hair cortisol in drug-naïve first-episode individuals with psychosis. *Revista Brasileira de*

Psiquiatria, *38*(1), 11–16. https://doi.org/10.1590/1516-4446-2014-1634

Antoni, M. H. (2003). Stress management effects on psychological, endocrinological, and immune functioning in men with HIV infection: Empirical support for a psychoneuroimmunological model. *Stress*, *6*(3), 173–188. https://doi.org/10.1080/1025389031000156727

Arseniou, S., Arvaniti, A., & Samakouri, M. (2014). HIV infection and depression. *Psychiatry and Clinical Neurosciences*, *68*(2), 96–109. https://doi.org/10.1111/pcn.12097

Azam, K., Khan, A., & Alam, M. T. (2017). Causes and adverse impact of physician burnout: A systematic review. *Journal of the College of Physicians and Surgeons Pakistan*, *27*(8), 495–501.

Bernard, C., Dabis, F., & De Rekeneire, N. (2017). Prevalence and factors associated with depression in people living with HIV in sub-Saharan Africa: A systematic review and meta-analysis. *PLoS ONE*, *12*(8), 1–22. https://doi.org/10.1371/journal.pone.0181960

Blessing, E. M., Reus, V., Mellon, S. H., Wolkowitz, O. M., Flory, J. D., Bierer, L., … Marmar, C. R. (2017). Biological predictors of insulin resistance associated with posttraumatic stress disorder in young military veterans. *Psychoneuroendocrinology*, *82*(April), 91–97. https://doi.org/10.1016/j.psyneuen.2017.04.016

Boksa, P. (2013). A way forward for research on biomarkers for psychiatric disorders. *Journal of Psychiatry and Neuroscience*, *38*(2), 75–77. https://doi.org/10.1503/jpn.130018

Borges, S., Gayer-Anderson, C., & Mondelli, V. (2013). A systematic review of the activity of the hypothalamic-pituitary-adrenal axis in first episode psychosis. *Psychoneuroendocrinology*, *38*(5), 603–611. https://doi.org/10.1016/j.psyneuen.2012.12.025

Bradley, A. J., & Dinan, T. G. (2010). A systematic review of hypothalamic-pituitary-adrenal axis function in schizophrenia: implications for mortality. *Journal of Psychopharmacology (Oxford, England)*, *24*(4 Suppl), 91–118. https://doi.org/10.1177/1359786810385491; 10.1177/1359786810385491

Castelli, W. P., Anderson, K., Wilson, P. W. F., & Levy, D. (1992). Lipids and risk of coronary heart

disease The Framingham Study. *Annals of Epidemiology*, *2*(1–2), 23–28. https://doi.org/10.1016/1047-2797(92)90033-M

Chaponda, M., Aldhouse, N., Kroes, M., Wild, L., Robinson, C., & Smith, A. (2018). Systematic review of the prevalence of psychiatric illness and sleep disturbance as co-morbidities of HIV infection in the UK. *International Journal of STD & AIDS*, *29*(7), 704–713. https://doi.org/10.1177/0956462417750708

Chu, X. X., Rizak, J. D., Yang, S. C., Wang, J. H., Ma, Y. Y., & Hu, X. T. (2014). A natural model of behavioral depression in postpartum adult female cynomolgus monkeys (Macaca fascicularis). *Zoological Research*, *35*(3), 174–181. https://doi.org/10.11813/j.issn.0254-5853.2014.3.174

Collins, P. Y., Joestl, S. S., March, D., Insel, T. R., Daar, A. S., & Scientific Advisory Board and the Executive Committee of the Grand Challenges in Global Mental Health. (2011). Grand challenges in global mental health: A consortium of researchers, advocates and clinicians announces here research priorities for improving the lives of people with mental illness around the world, and calls for urgent action and investment. *Nature*, *475*(7354), 27–30. https://doi.org/10.1038/475027a.Grand

Conroy, R. M., Pyörälä, K., Fitzgerald, A. P., Sans, S., Menotti, A., De Backer, G., … Graham, I. M. (2003). Estimation of ten-year risk of fatal cardiovascular disease in Europe: The SCORE project. *European Heart Journal*, *24*(11), 987–1003. https://doi.org/10.1016/S0195-668X(03)00114-3

Cuthbert, B. N., & Insel, T. R. (2013). Toward the future of psychiatric diagnosis: The seven pillars of RDoC. *BMC Medicine*, *11*(1). https://doi.org/10.1186/1741-7015-11-126

D'Agostino, R. B., Vasan, R. S., Pencina, M. J., Wolf, P. A., Cobain, M., Massaro, J. M., & Kannel, W. B. (2008). General cardiovascular risk profile for use in primary care: The Framingham heart study. *Circulation*, *117*(6), 743–753. https://doi.org/10.1161/CIRCULATIONAHA.107.699579

Dajani, R., Hadfield, K., van Uum, S., Greff, M., & Panter-Brick, C. (2018). Hair cortisol concentrations in war-affected adolescents: A prospective intervention trial. *Psychoneuroendocrinology*,

89(December 2017), 138–146. https://doi.org/10.1016/j.psyneuen.2017.12.012

Davison, B., Singh, G. R., & McFarlane, J. (2019). Hair cortisol and cortisone as markers of stress in indigenous and non-indigenous young adults. *Stress*, *22*(2), 210–220. https://doi.org/10.1080/10253890.2018.1543395

De Hert, M., Detraux, J., & Vancampfort, D. (2018). The intriguing relationship between coronary heart disease and mental disorders. *Dialogues in Clinical Neuroscience*, *20*(1), 31–40.

de Kloet, C. S., Vermetten, E., Geuze, E., Kavelaars, A., Heijnen, C. J., & Westenberg, H. G. M. (2006). Assessment of HPA-axis function in posttraumatic stress disorder: Pharmacological and non-pharmacological challenge tests, a review. *Journal of Psychiatric Research*, *40*(6), 550–567. https://doi.org/10.1016/j.jpsychires.2005.08.002

Dewa, C. S., Loong, D., Bonato, S., Thanh, N. X., & Jacobs, P. (2014). How does burnout affect physician productivity? A systematic literature review. *BMC Health Services Research*, *14*(1), 1–10. https://doi.org/10.1186/1472-6963-14-325

Dewa, C. S., Loong, D., Bonato, S., & Trojanowski, L. (2017). The relationship between physician burnout and quality of healthcare in terms of safety and acceptability: A systematic review. *BMJ Open*, *7*(6). https://doi.org/10.1136/bmjopen-2016-015141

Dockray, S., & Steptoe, A. (2010). Positive affect and psychobiological processes. *Neuroscience and Biobehavioral Reviews*, *35*(1), 69–75. https://doi.org/10.1016/j.neubiorev.2010.01.006

Franke, S. K., Van Kesteren, R. E., Hofman, S., Wubben, J. A. M., Smit, A. B., & Philippens, I. H. C. H. M. (2016). Individual and familial susceptibility to MPTP in a common marmoset model for Parkinson's disease. *Neurodegenerative Diseases*, *16*(5–6), 293–303. https://doi.org/10.1159/000442574

Freeman, M., Nkomo, N., Kafaar, Z., & Kelly, K. (2008). Mental disorder in people living with HIV/AIDS in South Africa. *South African Journal of Psychology*, *38*(3), 489–500. https://doi.org/10.1177/008124630803800304

Gajsak, L. R., Gelemanovic, A., Kuzman, M. R., & Puljak, L. (2017). Impact of stress response in development of first-episode psychosis in schizophrenia: An overview of systematic reviews. *Psychiatria Danubina*, *29*(1), 14–23.

Galatzer-Levy, I. R., Ma, S., Statnikov, A., Yehuda, R., & Shalev, A. Y. (2017). Utilization of machine learning for prediction of post-traumatic stress: A re-examination of cortisol in the prediction and pathways to non-remitting PTSD. *Translational Psychiatry*, *7*(3), 1–8. https://doi.org/10.1038/tp.2017.38

García-León, M. Á., Pérez-Mármol, J. M., Gonzalez-Pérez, R., García-Ríos, M. del C., & Peralta-Ramírez, M. I. (2019). Relationship between resilience and stress: Perceived stress, stressful life events, HPA axis response during a stressful task and hair cortisol. *Physiology and Behavior*, *202*(September 2018), 87–93. https://doi.org/10.1016/j.physbeh.2019.02.001

Gaziano, T. A., Pandya, A., Steyn, K., Levitt, N., Mollentze, W., Joubert, G., … Laubscher, R. (2013). Comparative assessment of absolute cardiovascular disease risk characterization from non-laboratory-based risk assessment in South African populations. *BMC Medicine*, *11*(1). https://doi.org/10.1186/1741-7015-11-170

Gerber, M., Kalak, N., Elliot, C., Holsboer-Trachsler, E., Pühse, U., & Brand, S. (2013). Both hair cortisol levels and perceived stress predict increased symptoms of depression: An exploratory study in young adults. *Neuropsychobiology*, *68*(2), 100–109. https://doi.org/10.1159/000351735

Giampaoli, S., Palmieri, L., Donfrancesco, C., Panico, S., Pilotto, L., Ferrario, M., … Mattiello, A. (2007). Cardiovascular risk assessment in Italy: the CUORE Project risk score and risk chart. *Italian Journal of Public Health*, *4*(2). https://doi.org/10.2427/5885

Girshkin, L., Matheson, S. L., Shepherd, A. M., & Green, M. J. (2014). Morning cortisol levels in schizophrenia and bipolar disorder: A meta-analysis. *Psychoneuroendocrinology*, *49*(1), 187–206. https://doi.org/10.1016/j.psyneuen.2014.07.013

Groer, M. W., Kane, B., Williams, S. N., & Duffy, A. (2015). Relationship of PTSD symptoms with

combat exposure, stress, and inflammation in American soldiers. *Biological Research for Nursing*, *17*(3), 303–310. https://doi.org/10.1177/1099800414544949

Gunnar, M. R. (2010). Stressor paradigms in developmental studies. *Psychoneuroendocrinology*, *34*(7), 953–967. https://doi.org/10.1016/j.psyneuen.2009.02.010.Stressor

Guo, Y., Hong, Y. A., Qiao, J., Xu, Z., Zhang, H., Zeng, C., … Yang, C. (2018). Run4Love, a mHealth (WeChat-based) intervention to improve mental health of people living with HIV: A randomized controlled trial protocol. *BMC Public Health*, *18*(1), 1–10. https://doi.org/10.1186/s12889-018-5693-1

Habib, A. G., Yakasai, A. M., Owolabi, L. F., Ibrahim, A., Habib, Z. G., Gudaji, M., … Nashabaru, I. (2013). Neurocognitive impairment in HIV-1-infected adults in Sub-Saharan Africa: A systematic review and meta-analysis. *International Journal of Infectious Diseases*, *17*(10), e820–e831. https://doi.org/10.1016/j.ijid.2013.06.011

Hajian-Tilaki, K. (2018). Comparison of competitive models of metabolic syndrome using structural equation modeling: A confirmatory factor analysis. *Diabetes and Metabolism Journal*, *42*(5), 433–441. https://doi.org/10.4093/dmj.2018.0010

Herman, A. A., Stein, D. J., Seedat, S., Heeringa, S. G., Moomal, H., & Williams, D. R. (2009). The South African Stress and Health (SASH) study: 12-month and lifetime prevalence of common mental disorders. *South African Medical Journal*, *99*(5), 339–344.

Hubbard, D. B., & Miller, B. J. (2019). Meta-analysis of blood cortisol levels in individuals with first-episode psychosis. *Psychoneuroendocrinology*, *104*(September 2018), 269–275. https://doi.org/10.1016/j.psyneuen.2019.03.014

Insel, T. R. (2014). The NIMH research domain criteria (RDoC) project: precision medicine for psychiatry. *American Journal of Psychiatry*, *171*(4), 395–397. https://doi.org/https://doi.org/10.1176/appi.ajp.2014.14020138

Insel, T. R. (2017). Digital phenotyping: Technology for a new science of behavior. *JAMA - Journal of*

the American Medical Association, *318*(13), 1215–1216. https://doi.org/10.1001/jama.2017.11295

J.Yeo, K.-T., Babic, N., Hannoush, Z. C., & Weiss, R. E. (2000). *Endocrine Testing Protocols: Hypothalamic Pituitary Adrenal Axis*. (K. Feingold, B. Anawalt, A. Boyce, G. Chrousos, K. Dungan, A. Grossman, … D. Wilson, Eds.), *Endotext*. South Dartmouth (MA): MDText.com, Inc. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/25905177

Jacobs, S., Moxley, K., Womersley, J. S., Spies, G., Hemmings, S. M. J., & Seedat, S. (2018). HPA-axis genes as potential risk variants for neurocognitive decline in trauma-exposed, HIV-positive females. *Neuropsychiatric Disease and Treatment*, *14*, 2497–2504. https://doi.org/10.2147/NDT.S166992

James, K. A., Grace, L. K., Pan, C. Y., Combrinck, M. I., & Thomas, K. G. F. (2019). Psychosocial stress associated with memory performance in older South African adults. *Aging, Neuropsychology, and Cognition*, *00*(00), 1–14. https://doi.org/10.1080/13825585.2019.1645809

Jones, D., Owens, M., Kumar, M., Cook, R., & Weiss, S. M. (2014). The Effect of Relaxation Interventions on Cortisol Levels in HIV-Seropositive Women. *Journal of the International Association of Providers of AIDS Care (JIAPAC)*, *13*(4), 318–323. https://doi.org/10.1177/2325957413488186

Joseph, P., Yusuf, S., Lee, S. F., Ibrahim, Q., Teo, K., Rangarajan, S., … Anand, S. S. (2018). Prognostic validation of a non-laboratory and a laboratory based cardiovascular disease risk score in multiple regions of the world. *Heart*, *104*(7), 581–587. https://doi.org/10.1136/heartjnl-2017-311609

Joska, J. A., Fincham, D. S., Stein, D. J., Paul, R. H., & Seedat, S. (2010). Clinical correlates of HIV-associated neurocognitive disorders in South Africa. *AIDS and Behavior*, *14*(2), 371–378. https://doi.org/10.1007/s10461-009-9538-x

Joska, J. A., Westgarth-Taylor, J., Myer, L., Hoare, J., Thomas, K. G. F., Combrinck, M., … Flisher, A.

J. (2011). Characterization of HIV-Associated Neurocognitive Disorders among individuals starting antiretroviral therapy in South Africa. *AIDS and Behavior*, *15*(6), 1197–1203. https://doi.org/10.1007/s10461-010-9744-6

Juster, R. P., McEwen, B. S., & Lupien, S. J. (2010). Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neuroscience and Biobehavioral Reviews*, *35*(1), 2–16. https://doi.org/10.1016/j.neubiorev.2009.10.002

Kagee, A., & Martin, L. (2010). Symptoms of depression and anxiety among a sample of South African patients living with HIV. *AIDS Care - Psychological and Socio-Medical Aspects of AIDS/HIV*, *22*(2), 159–165. https://doi.org/10.1080/09540120903111445

Kalmakis, K. A., Meyer, J. S., Chiodo, L., & Leung, K. (2015). Adverse childhood experiences and chronic hypothalamic-pituitary-adrenal activity. *Stress*, *18*(4), 446–450. https://doi.org/10.3109/10253890.2015.1023791

Khodarahmi, M., Asghari-Jafarabadi, M., & Farhangi, M. A. (2019). A structural equation modeling approach for the association of a healthy eating index with metabolic syndrome and cardio-metabolic risk factors among obese individuals. *PLoS ONE*, *14*(7), 1–20. https://doi.org/10.1371/journal.pone.0219193

Khoury, J. E., Bosquet Enlow, M., Plamondon, A., & Lyons-Ruth, K. (2019). The association between adversity and hair cortisol levels in humans: A meta-analysis. *Psychoneuroendocrinology*, *103*(January), 104–117. https://doi.org/10.1016/j.psyneuen.2019.01.009

Kuhn, C. M., & Flanagan, E. M. (2017). Self-care as a professional imperative: physician burnout, depression, and suicide. *Canadian Journal of Anesthesia*, *64*(2), 158–168. https://doi.org/10.1007/s12630-016-0781-0

Langerak, T., van den Dries, L. W. J., Wester, V. L., Staufenbiel, S. M., Manenschijn, L., van Rossum, E. F. C., & van Gorp, E. C. M. (2015). The relation between long-term cortisol levels and the metabolic syndrome in HIV-infected patients. *Clinical Endocrinology*, *83*(2), 167–172.

https://doi.org/10.1111/cen.12790

Larsen, S. C., Fahrenkrug, J., Olsen, N. J., & Heitmann, B. L. (2016). Association between Hair Cortisol Concentration and Adiposity Measures among Children and Parents from the " Healthy Start " Study, 1–11. https://doi.org/10.1371/journal.pone.0163639

Laursen, T. M., Nordentoft, M., & Mortensen, P. B. (2014). Excess Early Mortality in Schizophrenia. *Annual Review of Clinical Psychology*, *10*(1), 425–448. https://doi.org/10.1146/annurev-clinpsy-032813-153657

Leserman, J., Petitto, J. M., Golden, R. N., Gaynes, B. N., Gu, H., Perkins, D. O., … Evans, D. L. (2000). Impact of stressful life events, depression, social support, coping, and cortisol on progression to aids. *American Journal of Psychiatry*, *157*(8), 1221–1228. https://doi.org/10.1176/appi.ajp.157.8.1221

Lund, C., Breen, A., Flisher, A., & Kakuma, R. (2010). Poverty and common mental disorders in low and middle income countries: a systematic review. *Social Science &*. Retrieved from http://www.sciencedirect.com/science/article/pii/S0277953610003576

Lund, C., Brooke-Sumner, C., Baingana, F., Baron, E. C., Breuer, E., Chandra, P., … Saxena, S. (2018). Social determinants of mental disorders and the Sustainable Development Goals: a systematic review of reviews. *The Lancet Psychiatry*, *5*(4), 357–369. https://doi.org/10.1016/S2215-0366(18)30060-9

Lupien, S. J., Maheu, F., Tu, M., Fiocco, A., & Schramek, T. E. (2007). The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. *Brain and Cognition*, *65*(3), 209–237. https://doi.org/10.1016/j.bandc.2007.02.007

Manzanares, N., Monseny, R., Ortega, L., Montalvo, I., Franch, J., Gutiérrez-Zotes, A., … Labad, J. (2014). Unhealthy lifestyle in early psychoses: The role of life stress and the hypothalamic-pituitary-adrenal axis. *Psychoneuroendocrinology*, *39*(1), 1–10. https://doi.org/10.1016/j.psyneuen.2013.09.023

Maslach, C., & Jackson, S. E. (1981). The measurement of experienced burnout. *Journal of Organizational Behavior*, *2*(2), 99–113. https://doi.org/10.1002/job.4030020205

Mayosi, B. M., Lawn, J. E., Van Niekerk, A., Bradshaw, D., Abdool Karim, S. S., & Coovadia, H. M. (2012). Health in South Africa: Changes and challenges since 2009. *The Lancet*, *380*(9858), 2029–2043. https://doi.org/10.1016/S0140-6736(12)61814-5

Meewisse, M. L., Reitsma, J. B., de Vries, G. J., Gersons, B. P., & Olff, M. (2007). Cortisol and post-traumatic stress disorder in adults: systematic review and meta-analysis. *The British Journal of Psychiatry : The Journal of Mental Science*, *191*, 387–392. https://doi.org/191/5/387 [pii]

Mewes, R., Reich, H., Skoluda, N., Seele, F., & Nater, U. M. M. (2017). Elevated hair cortisol concentrations in recently fled asylum seekers in comparison to permanently settled immigrants and non-immigrants. *Translational Psychiatry*, *7*(3), e1051. https://doi.org/10.1038/tp.2017.14

Milam, J., Slaughter, R., Verma, G., & McConnell, R. (2014). Hair cortisol, perceived stress and dispositional optimism: A pilot study among adolescents. *Journal of Traumatic Stress Disorders & Treatment*, *3*(3), 1000126. https://doi.org/10.4172/2324-8947.1000126

Miller, G. E., Chen, E., & Zhou, E. S. (2007). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychological Bulletin*, *133*(1), 25–45. https://doi.org/10.1037/0033-2909.133.1.25

Miller, M. W., Wolf, E. J., Martin, E., Kaloupek, D. G., & Keane, T. M. (2008). Structural equation modeling of associations among combat exposure, PTSD symptom factors, and Global Assessment of Functioning. *Journal of Rehabilitation Research and Development*, *45*(3), 359–370. https://doi.org/10.1682/JRRD.2007.06.0085

Mondelli, V., Ciufolini, S., Murri, M. B., Bonaccorso, S., Di Forti, M., Giordano, A., … Dazzan, P. (2015). Cortisol and inflammatory biomarkers predict poor treatment response in first episode psychosis. *Schizophrenia Bulletin*, *41*(5), 1162–1170. https://doi.org/10.1093/schbul/sbv028

Morris, M. C., Compas, B. E. B. E., & Garber, J. (2012). Relations among posttraumatic stress

disorder, comorbid major depression, and HPA function: a systematic review and meta-analysis. *Clinical Psychology Review*, *32*(4), 301–315. https://doi.org/10.1016/j.cpr.2012.02.002.Relations

Muehlhan, M., Höcker, A., Miller, R., Trautmann, S., Wiedemann, K., Lotzin, A., … Schäfer, I. (2018). HPA axis stress reactivity and hair cortisol concentrations in recently detoxified alcoholics and healthy controls with and without childhood maltreatment. *Addiction Biology*, (August), 1–8. https://doi.org/10.1111/adb.12681

Mustonen, P., Karlsson, L., Kataja, E. L., Scheinin, N. M., Kortesluoma, S., Coimbra, B., … Karlsson, H. (2019). Maternal prenatal hair cortisol is associated with prenatal depressive symptom trajectories. *Psychoneuroendocrinology*, *109*(July), 104383. https://doi.org/10.1016/j.psyneuen.2019.104383

Myer, L., Smit, J., Roux, L. Le, Parker, S., Stein, D. J., & Seedat, S. (2008). Common mental disorders among HIV-infected individuals in South Africa: Prevalence, predictors, and validation of brief psychiatric rating scales. *AIDS Patient Care and STDs*, *22*(2), 147–158. https://doi.org/10.1089/apc.2007.0102

Myers, B., Joska, J. A., Lund, C., Levitt, N. S., Butler, C. C., Naledi, T., … Sorsdahl, K. (2018). Patient preferences for the integration of mental health counseling and chronic disease care in South Africa. *Patient Preference and Adherence*, *12*, 1797–1803. https://doi.org/10.2147/PPA.S176356

Niu, L., Luo, D., Liu, Y., Silenzio, V. M. B., & Xiao, S. (2016). The Mental Health of People Living with HIV in China, 1998-2014: A Systematic Review. *PloS One*, *11*(4), e0153489. https://doi.org/10.1371/journal.pone.0153489

Orta, O. R., Tworoger, S. S., Terry, K. L., Coull, B. A., Gelaye, B., Kirschbaum, C., … Williams, M. A. (2018). Stress and hair cortisol concentrations from preconception to the third trimester. *Stress (Amsterdam, Netherlands)*, *0*(0), 1–10. https://doi.org/10.1080/10253890.2018.1504917

Pacella, M. L., Hruska, B., Steudte-Schmiedgen, S., George, R. L., & Delahanty, D. L. (2017). The utility of hair cortisol concentrations in the prediction of PTSD symptoms following traumatic

physical injury. *Social Science & Medicine*, *175*(3), 228–234. https://doi.org/10.1016/j.socscimed.2016.12.046

Panagioti, M., Panagopoulou, E., Bower, P., Lewith, G., Kontopantelis, E., Chew-Graham, C., … Esmail, A. (2017). Controlled interventions to reduce burnout in physicians: a systematic review and meta-analysis. *JAMA Internal Medicine*, *177*(2), 195–205. https://doi.org/10.1001/jamainternmed.2016.7674

Patel, V., Saxena, S., Lund, C., Thornicroft, G., Baingana, F., Bolton, P., … UnÜtzer, Jü. (2018). The Lancet Commission on global mental health and sustainable development. *The Lancet*, *392*(10157), 1553–1598. https://doi.org/10.1016/S0140-6736(18)31612-X

Penninx, B. W. J. H., & Lange, S. M. M. (2018). Metabolic syndrome in psychiatric patients: Overview, mechanisms, and implications. *Dialogues in Clinical Neuroscience*, *20*(1), 63–73.

Penz, M., Siegrist, J., Wekenborg, M. K., Rothe, N., Walther, A., & Kirschbaum, C. (2019). Effort-reward imbalance at work is associated with hair cortisol concentrations: Prospective evidence from the Dresden Burnout Study. *Psychoneuroendocrinology*, *109*(March), 104399. https://doi.org/10.1016/j.psyneuen.2019.104399

Penz, M., Stalder, T., Miller, R., Ludwig, V. M., Kanthak, M. K., & Kirschbaum, C. (2018). Hair cortisol as a biological marker for burnout symptomatology. *Psychoneuroendocrinology*, *87*(July 2017), 218–221. https://doi.org/10.1016/j.psyneuen.2017.07.485

Pinto, J. V., Moulin, T. C., & Amaral, O. B. (2017). On the transdiagnostic nature of peripheral biomarkers in major psychiatric disorders: A systematic review. *Neuroscience and Biobehavioral Reviews*, *83*(21), 97–108. https://doi.org/10.1016/j.neubiorev.2017.10.001

Qiao, S., Li, X., Zilioli, S., Chen, Z., Deng, H., Pan, J., & Guo, W. (2017). Hair Measurements of Cortisol, DHEA, and DHEA to Cortisol Ratio as Biomarkers of Chronic Stress among People Living with HIV in China: Known-Group Validation. *PLoS One*, *12*(1). https://doi.org/http://dx.doi.org/10.1371/journal.pone.0169827

Qin, D., Li, Z., Li, Z., Wang, L., Hu, Z., Lü, L., … Hu, X. (2019). Chronic Glucocorticoid Exposure Induces Depression-Like Phenotype in Rhesus Macaque (Macaca Mulatta). *Frontiers in Neuroscience*, *13*(March), 1–9. https://doi.org/10.3389/fnins.2019.00188

Rahman, A. F., Manatunga, A., Guo, Y., Peng, L., Warnock, M., Ressler, K. J., & Jovanovic, T. (2018). A latent class analysis of PTSD symptoms among inner city primary care patients. *Journal of Psychiatric Research*, *98*, 1–8. https://doi.org/10.1016/j.jpsychires.2017.11.015

Reinertsen, E., & Clifford, G. D. (2018). A review of physiological and behavioral monitoring with digital sensors for neuropsychiatric illnesses. *Physiological Measurement*, *39*(5). https://doi.org/https://doi.org/10.1088/1361-6579/aabf64

Roman-Urrestarazu, A., Ali, F. M. H., Reka, H., Renwick, M. J., Roman, G. D., & Mossialos, E. (2016). Structural equation model for estimating risk factors in type 2 diabetes mellitus in a Middle Eastern setting: Evidence from the STEPS Qatar. *BMJ Open Diabetes Research and Care*, *4*(1). https://doi.org/10.1136/bmjdrc-2016-000231

Rothenberger, D. A. (2017). Physician Burnout and Well-Being: A Systematic Review and Framework for Action. *Diseases of the Colon and Rectum*, *60*(6), 567–576. https://doi.org/10.1097/DCR.0000000000000844

Schubert, C. F., Schmidt, U., & Rosner, R. (2016). Posttraumatic Growth in Populations with Posttraumatic Stress Disorder—A Systematic Review on Growth-Related Psychological Constructs and Biological Variables. *Clinical Psychology and Psychotherapy*, *23*(6), 469–486. https://doi.org/10.1002/cpp.1985

Schumacher, S., Niemeyer, H., Engel, S., Cwik, J. C., Laufer, S., Klusmann, H., & Knaevelsrud, C. (2019). HPA axis regulation in posttraumatic stress disorder: A meta-analysis focusing on potential moderators. *Neuroscience and Biobehavioral Reviews*, *100*(June 2018), 35–57. https://doi.org/10.1016/j.neubiorev.2019.02.005

Seedat, S., Stein, D. J., Herman, A., Kessler, R., Sonnega, J., Heeringa, S., … Williams, D. (2008).

Twelve-month treatment of psychiatric disorders in the South African Stress and Health Study (World Mental Health Survey Initiative). *Social Psychiatry and Psychiatric Epidemiology*, *43*(11), 889–897. https://doi.org/10.1007/s00127-008-0399-9

Seitz, R., Vracotas, N., Bechard-Evans, L., King, S., Abadi, S., Joober, R., … Pruessner, M. (2019). The Trier Social Stress Test in first episode psychosis patients: Impact of perceived stress, protective factors and childhood trauma. *Psychoneuroendocrinology*, *105*(July 2018), 155–163. https://doi.org/10.1016/j.psyneuen.2019.01.010

Simmons, J. G., Badcock, P. B., Whittle, S. L., Byrne, M. L., Mundy, L., Patton, G. C., … Allen, N. B. (2016). The lifetime experience of traumatic events is associated with hair cortisol concentrations in community-based children. *Psychoneuroendocrinology*, *63*, 276–281. https://doi.org/10.1016/j.psyneuen.2015.10.004

Stalder, T., Kirschbaum, C., Alexander, N., Bornstein, S. R., Gao, W., Miller, R., … Fischer, J. E. (2013). Cortisol in hair and the metabolic syndrome. *The Journal of Clinical Endocrinology & Metabolism*, *98*(6), 2573–2580. https://doi.org/10.1210/jc.2013-1056

Stalder, T., Steudte-Schmiedgen, S., Alexander, N., Klucken, T., Vater, A., Wichmann, S., … Miller, R. (2017). Stress-related and basic determinants of hair cortisol in humans: A meta-analysis. *Psychoneuroendocrinology*, *77*, 261–274. https://doi.org/10.1016/j.psyneuen.2016.12.017

Stalder, T., Steudte, S., Miller, R., Skoluda, N., Dettenborn, L., & Kirschbaum, C. (2012). Intraindividual stability of hair cortisol concentrations. *Psychoneuroendocrinology*, *37*(5), 602–610. https://doi.org/https://doi.org/10.1016/j.psyneuen.2011.08.007

Starkstein, S., Dragovic, M., Jorge, R., Brockman, S., Merello, M., Robinson, R. G., … Wilson, M. (2011). Diagnostic criteria for depression in Parkinson's disease: A study of symptom patterns using latent class analysis. *Movement Disorders*, *26*(12), 2239–2245. https://doi.org/10.1002/mds.23836

Staufenbiel, S. M., Penninx, B. W. J. H., Spijker, A. T., Elzinga, B. M., & van Rossum, E. F. C. (2013).

Hair cortisol, stress exposure, and mental health in humans: A systematic review. *Psychoneuroendocrinology*, *38*(8), 1220–1235. https://doi.org/10.1016/j.psyneuen.2012.11.015

Steptoe, A., Dockray, S., & Wardle, J. (2009). Positive Affect and Psychobiological Processes Relevant to Health. *Journal of Personality*, *77*(6), 1747–1776. https://doi.org/10.1111/j.1467-6494.2009.00599.x

Steudte-Schmiedgen, S., Kirschbaum, C., Alexander, N., & Stalder, T. (2016). An integrative model linking traumatization, cortisol dysregulation and posttraumatic stress disorder: Insight from recent hair cortisol findings. *Neuroscience and Biobehavioral Reviews*, *69*, 124–135. https://doi.org/10.1016/j.neubiorev.2016.07.015

Steudte-Schmiedgen, S., Stalder, T., Schonfeld, S., Wittchen, H.-U. H.-U., Trautmann, S., Alexander, N., … Kirschbaum, C. (2015). Hair cortisol concentrations and cortisol stress reactivity predict PTSD symptom increase after trauma exposure during military deployment. *Psychoneuroendocrinology*, *59*, 123–133. https://doi.org/10.1016/j.psyneuen.2015.05.007

Steudte-Schmiedgen, S., Wichmann, S., Stalder, T., Hilbert, K., Muehlhan, M., Lueken, U., & Beesdo-Baum, K. (2017). Hair cortisol concentrations and cortisol stress reactivity in generalized anxiety disorder, major depression and their comorbidity. *Journal of Psychiatric Research*, *84*(2017), 184–190. https://doi.org/10.1016/j.jpsychires.2016.09.024

Streit, F., Memic, A., Hasandedić, L., Rietschel, L., Frank, J., Lang, M., … Rietschel, M. (2016). Perceived stress and hair cortisol: Differences in bipolar disorder and schizophrenia. *Psychoneuroendocrinology*, *69*, 26–34. https://doi.org/10.1016/j.psyneuen.2016.03.010

Suliman, S., Stein, D. J., Myer, L., Williams, D. R., & Seedat, S. (2010). Disability and treatment of psychiatric and physical disorders in South Africa. *Journal of Nervous and Mental Disease*, *198*(1), 8–15. https://doi.org/10.1097/NMD.0b013e3181c81708

Touskova, T. P., Bob, P., Pec, O., Mishara, A., Vanickova, Z., Raboch, J., & Lysaker, P. (2018). Insight and cortisol responses in women with first episode psychosis. *Schizophrenia Research*, *201*,

428–429. https://doi.org/10.1016/j.schres.2018.06.002

Tsai, J., & Rosenheck, R. A. (2013). Psychiatric comorbidity among adults with schizophrenia: A latent class analysis. *Psychiatry Research*, *210*(1), 16–20. https://doi.org/10.1016/j.psychres.2013.05.013

Ullmann, E., Barthel, A., Petrowski, K., Stalder, T., Kirschbaum, C., & Bornstein, S. R. (2016). Pilot study of adrenal steroid hormones in hair as an indicator of chronic mental and physical stress. *Scientific Reports*, *6*(May), 1–7. https://doi.org/10.1038/srep25842

UNAIDS. (2018). AIDSinfo Country factsheets South Africa 2018. Retrieved from https://www.unaids.org/en/regionscountries/countries/southafrica

van den Heuvel, L., Seedat, S., & Fennema-Notestine, C. (2016). NeuroAIDS in Resource-Poor Settings, Assessment, and Treatment of. In S. M. Hope T.J., Richman D.D. (Ed.), *Encyclopedia of AIDS*. New York, NY: Springer. https://doi.org/https://doi.org/10.1007/978-1-4939-7101-5_295

Vancampfort, D., Stubbs, B., Mitchell, A. J., De Hert, M., Wampers, M., Ward, P. B., … Correll, C. U. (2015). Risk of metabolic syndrome and its components in people with schizophrenia and related psychotic disorders, bipolar disorder and major depressive disorder: A systematic review and meta-analysis. *World Psychiatry*, *14*(3), 339–347. https://doi.org/10.1002/wps.20252

Vuksan-Cúsa, B., Săgud, M., Mihaljevic-Peleš, A., Jaksǐć, N., & Jakovljević, M. (2014). Metabolic syndrome and cortisol/dheas ratio in patients with bipolar disorder and schizophrenia. *Psychiatria Danubina*, *26*(2), 187–189.

Walker, E. R., McGee, R. E., & Drus, B. G. (2015). Mortality in Mental Disorders and Global Disease Burden Implications: A Systematic Review and Meta-analysis. *JAMA Psychiatry*, *72*(4), 334–341. https://doi.org/10.1001/jamapsychiatry.2014.2502

Wang, C., Dai, J., & Li, J. (2019). Mediating effects of hair cortisol on the mutual association of job burnout and insomnia: A retrospective exploratory study. *Journal of Psychiatric Research*, *117*(February), 62–67. https://doi.org/10.1016/j.jpsychires.2019.07.001

Weinstein, T. L., & Li, X. (2016). The relationship between stress and clinical outcomes for persons living with HIV/AIDS: A systematic review of the global literature. *AIDS Care - Psychological and Socio-Medical Aspects of AIDS/HIV*, *28*(2), 160–169. https://doi.org/10.1080/09540121.2015.1090532

West, C. P., Dyrbye, L. N., Erwin, P. J., & Shanafelt, T. D. (2016). Interventions to prevent and reduce physician burnout: a systematic review and meta-analysis. *The Lancet*, *388*(10057), 2272–2281. https://doi.org/10.1016/S0140-6736(16)31279-X

Whiteford, H. A., Degenhardt, L., Rehm, J., Baxter, A. J., Ferrari, A. J., Erskine, H. E., … Johns, N. (2013). Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *The Lancet*, *382*(9904), 1575–1586. https://doi.org/https://doi.org/10.1016/S0140-6736(13)61611-6

Witten, J. A., Thomas, K. G. F., Westgarth-Taylor, J., & Joska, J. A. (2015). Executive Dyscontrol of Learning and Memory: Findings from a Clade C HIV-positive South African Sample. *Clinical Neuropsychologist*, *29*(7), 956–984. https://doi.org/10.1080/13854046.2015.1108455

Wosu, A. C., Valdimarsdóttir, U., Shields, A. E., Williams, D. R., & Williams, M. A. (2013). Correlates of cortisol in human hair: Implications for epidemiologic studies on health effects of chronic stress. *Annals of Epidemiology*, *23*(12), 797–811. https://doi.org/10.1016/j.annepidem.2013.09.006

Zahodne, L. B., Marsiske, M., & Bowers, D. (2013). A latent class analysis of psychological disturbance in Parkinson's disease. *International Journal of Geriatric Psychiatry*, *28*(10), 1054–1060. https://doi.org/10.1002/gps.3927

Zorn, J. V., Schür, R. R., Boks, M. P., Kahn, R. S., Joëls, M., & Vinkers, C. H. (2017). Cortisol stress reactivity across psychiatric disorders: A systematic review and meta-analysis. *Psychoneuroendocrinology*, *77*, 25–36. https://doi.org/10.1016/j.psyneuen.2016.11.036

APPENDIX A

Cortisol levels in different tissue samples in posttraumatic stress disorder patients versus controls: A systematic review and meta-analysis protocol

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Cortisol levels in different tissue samples in posttraumatic stress disorder patients versus controls: a systematic review and meta-analysis protocol

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Abstract

Background: Posttraumatic stress disorder (PTSD) is a disorder that develops following exposure to severely stressful events. Altered cortisol secretion has been reported in PTSD; however, results have been inconsistent. Previous meta-analyses of cortisol levels in PTSD have combined results of studies that have used different tissue samples (blood, saliva, urine) for cortisol measurement and have not included newer methods of determining cortisol levels (e.g. hair samples). In this systematic review, we will synthesise evidence from studies evaluating basal cortisol levels in PTSD patients versus controls and stratify studies according to tissue type used for cortisol measurement. We will also determine whether results from different tissue types can be pooled and if any specific tissue samples have better utility in research studies on PTSD.

Methods: We will perform a systematic review of the scientific literature including all studies that have evaluated basal or baseline cortisol levels in adults with current PTSD versus controls, with and without trauma exposure. Independent reviewers will conduct searches in electronic databases (Medline, CINAHL, PTSDpubs, Web of Science, Scopus, ProQuest Dissertations & Theses A&I, ClinicalTrials.gov, and ICTRP), and additional studies will be obtained by searching the reference lists of articles. Two reviewers (LLvdH and SW) will independently conduct standardised screening, eligibility assessments, data extraction, and quality assessments before qualitative and, if appropriate, quantitative (meta-analysis and meta-regression) synthesis. Disagreements that arise at any stage will be resolved by a third reviewer (ShS).

Discussion: In line with previous reviews, we expect that cortisol levels will be lower in PTSD patients than in controls, but that patterns may vary somewhat according to the tissue sample in which cortisol is measured. This systematic review will assist in developing a better understanding of the acute and chronic patterns of basal cortisol secretion in PTSD and will inform future research.

Systematic review registration: PROSPERO CRD42018091874

Keywords: Posttraumatic stress disorder (PTSD), Trauma, Cortisol

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Background

Posttraumatic stress disorder (PTSD) develops following exposure to an extreme stressor(s) or traumatic event(s), such as being confronted with actual or threatened death, serious injury, or sexual violence [1]. Symptoms, causing significant distress or impairment of functioning, persist for at least a month and involve repeated re-experiencing of the traumatic event, avoidance of trauma-related cues, negative changes in thinking and mood, and increased arousal. In the World Health Organization (WHO) World Mental Health (WMH) surveys, the 12-month prevalence of PTSD in the total sample was 1.1% [2] and the estimated lifetime prevalence of PTSD was 2.9% [3]. Of those with PTSD, 42% reported severe role impairment in at least one domain (work, social life, close relationships, or home maintenance) [2] and PTSD was one of the three most disabling disorders [4]. PTSD and other trauma- and stressor-related disorders are distinguished from other psychiatric disorders in their requirement of exposure to stressful events to make a diagnosis [1]. For PTSD, the stressful event(s) must be of a severe or life-threatening nature; PTSD is, therefore, unique in that severe stress plays a central aetiological role in the development of a set of characteristic and persisting symptoms.

The glucocorticoid, cortisol, is generally viewed as the body's chief stress hormone. Cortisol is synthesised and released from the adrenal cortex which is a component of the neuro-endocrine hypothalamic–pituitary–adrenal (HPA) axis. Cortisol influences processes such as metabolism, immune function, digestion, and behaviour [5]. Cortisol has a baseline diurnal secretion pattern but is also released following exposure to a stressor. It has thus emerged as an objective biological marker of the stress response [5–7]. A negative feedback loop in the HPA axis regulates cortisol secretion and allows for the maintenance of homeostasis. Prolonged or severe stress can lead to dysfunction of the HPA axis with resultant dysregulation of cortisol secretion and associated adverse health outcomes [8, 9]. Cortisol levels can be measured in various tissue samples reflecting cortisol secretion over different time periods. Traditional sampling methods, such as blood (serum and plasma), saliva, and urine are useful for assessing acute cortisol secretion (less than 24 h); however, newer approaches utilising hair and nail samples can provide retrospective and chronic patterns (weeks to months) of cortisol secretion [10–12].

The nature of PTSD as a disorder involving a chronic maladaptive behavioural response to an extreme stressor(s) suggests that it likely has a relationship with a dysregulated endocrine-mediated stress response. Indeed, HPA axis dysregulation has been reported in PTSD; however, results have been inconsistent. A previous systematic review and meta-analysis of basal cortisol levels in PTSD and controls reported no difference in cortisol levels between PTSD patients and controls [13]. The authors pooled results from different tissue samples for their primary analysis and documented significant heterogeneity. In subgroup analysis, they found that plasma and serum cortisol levels were significantly lower in PTSD patients versus trauma unexposed controls (TUC), suggesting that cortisol findings may vary according to tissue type sampled. Furthermore, a recent meta-analysis that only included salivary cortisol levels reported lower cortisol levels in PTSD patients than in controls [14]. Trauma exposure in controls may be another confounding factor, as another meta-analysis that examined the association between trauma exposure in adulthood and cortisol levels found no difference in basal cortisol levels between PTSD patients and trauma-exposed controls (TEC) [15]. Contrary to these findings, a meta-analysis that separately evaluated PTSD patients and PTSD patients with comorbid depression reported lower daily cortisol output for both groups compared to TUC [16]. Inconsistent findings in meta-analyses could also be ascribed to variations in tissue sample type in the included studies and methods used in aggregating results in meta-analysis. Systematic reviews evaluating cortisol levels in PTSD have also not included newer tissue sampling methods, such as hair and nail cortisol measurements. A recent meta-analysis of hair cortisol studies reported lower hair cortisol levels in patients with anxiety disorders (PTSD and generalised anxiety disorder [GAD]) [17], suggesting that adding newer tissue sampling methods could enhance the understanding of HPA axis function in PTSD.

In this systematic review, we aim to bring together all studies that have evaluated basal cortisol levels in PTSD patients versus controls according to the type of tissue sampled. By analysing tissue type, we seek to develop a better understanding of measurement of acute and chronic patterns of basal cortisol secretion in PTSD patients versus controls. We will also seek to establish whether data from different tissue types can be combined and whether sampling a specific tissue for cortisol measurement has greater utility in PTSD studies.

Objectives

Our objectives are as follows:

1. Our primary objective is to evaluate whether PTSD is associated with altered basal cortisol levels by synthesising the available evidence from primary studies. A previous study that addressed this aim was published more than 10 years ago [13]. Our study involves important differences in terms of methodological approach. In both qualitative and quantitative synthesis (meta-analyses), we will group studies according to the tissue type sampled.

See 'Additional file 1: Table summarising published systematic reviews examining basal cortisol levels in posttraumatic stress disorder' for a summary of the results of existing systematic reviews and explanation of main differences as compared to this protocol.

2. To evaluate whether pooling results from different tissue samples is meaningful as this may inform future approaches to sampling cortisol.
3. To determine the factors that influence the relationship between basal cortisol levels and PTSD by performing meta-regression where feasible.
4. To perform a critical evaluation of the available literature with a view to identifying areas that require further research.

See Table 1 for research question in PICOTS format.

Methods

This protocol was designed in accordance with the guidelines set forth by The Cochrane Collaboration [18] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [19] Statement. The systematic review protocol has been registered with the PROSPERO International prospective register of systematic reviews database (PROSPERO registration number: CRD42018091874) [20]. A PROSPERO search identified two other systematic reviews registered evaluating cortisol levels in PTSD. The first aims to evaluate broader HPA axis function in PTSD, including factors such as dehydroepiandrosterone (DHEA) levels and changes due to psychotherapeutic treatment [21], and the second is focused on evaluating 24-h urinary cortisol levels in PTSD patients and was registered more recently than our systematic review protocol [22]. Two reviewers (LLvdH and SW) will independently conduct standardised screening, eligibility assessments, data extraction, and quality assessments prior to qualitative and quantitative (meta-analyses and meta-regression) synthesis.

Search strategy

Two independent reviewers (LLvdH and SW) will perform searches in electronic databases (PubMed/MEDLINE, Cumulative Index to Nursing and Allied Health Literature (CINAHL), PTSDpubs, Web of Science, Scopus, and ProQuest Dissertations & Theses A&I) and trial registries (ClinicalTrials.gov and International Clinical Trials Registry Platform [ICTRP]) for published and unpublished studies. Additional studies will be identified by searching the reference lists of relevant reviews and included studies. No limits will be placed on publication date or language; however, articles will only be translated into English and/or authors contacted for information if a study is likely to meet our inclusion criteria conditional upon the title and abstract being available in English. Search terms based on 'PTSD' and 'cortisol' and applicable synonyms and controlled vocabulary (MeSH terms) will be used where available. The primary search terms will first be formulated in MEDLINE (PubMed) and will then be translated to the other databases. In the databases, all fields will be searched, excluding SCOPUS where title, abstract, and keywords will be searched. We will not place any limitations, such as age group and study design, on searches, but will manually exclude studies according to inclusion and exclusion criteria. We obtained independent peer review from an information specialist who utilised the PRESS methodology [23] and made changes to our search strategy and terms according to their recommendations. The full search terms for each of the databases are included in 'Additional file 2: Search terms'. Searches in databases will be rerun just prior to analysis to identify any new studies qualifying

Table 1 Research question in PICOTS format

PICOTS	Inclusion and exclusion criteria
Patients or populations	Adults aged 18 years or older
	Patients with current PTSD based on DSM/ICD criteria
Exposures	Trauma exposure fulfilling DSM/ICD criteria occurring at least a month prior to assessment in PTSD patients and TEC
Comparison group(s)	PTSD patients versus all controls
	Subgroup analysis:
	PTSD patients versus TEC
	PTSD patients versus TUC
Outcomes	Basal or baseline cortisol levels (mean levels and standard deviation) measured in different tissue types
Timing	At least 1 month since trauma exposure in PTSD patients and TEC
Setting	Any setting (inpatient, outpatient, community settings)
Study design	Any study design where cortisol levels are compared in patients versus controls (e.g. cross-sectional, case-control and cohort)

DSM, Diagnostic and Statistical Manual of Mental Disorders; *ICD, International Statistical Classification of Diseases and Related Health Problems*; PICOTS, Population, Intervention, Comparison, Outcomes, Timing, Setting; *PTSD*, posttraumatic stress disorder; *TEC*, trauma-exposed controls; *TUC*, trauma-unexposed controls

for inclusion. Searches will be saved and managed utilising the reference manager 'Mendeley' where duplicates will be identified and removed. Results from published and unpublished or 'grey' literature will be included, provided studies fulfil the inclusion and exclusion criteria and sufficient information is available to assess study quality. Search results will be presented in a PRISMA flowchart according to PRISMA guidelines (Additional file 3: Figure S1 PRISMA flow diagram).

Selection criteria

Study design

Any study design that compares cortisol levels between PTSD patients and controls will be included. The most common designs will be cross-sectional and case–control studies, but cohort and other study designs will also be included, as long as the studies address the main question of this review and fulfil the inclusion/exclusion criteria. If multiple papers related to a single study have been published, the data from the study results will either be combined or the article with the largest sample size will be included.

Participants

We will include studies with adults aged 18 years and older that compare cortisol levels between PTSD patients and controls. We will only include patients with current, and not lifetime, PTSD according to DSM/ICD criteria. Controls, with and without, trauma exposure and without a history of prior PTSD will be included. For each tissue type, we will compare cortisol levels in PTSD patients versus all controls (AC). We will also perform subgroup analyses comparing PTSD patients versus TEC and versus TUC. In both patients and TEC, trauma exposure should fulfil DSM/ICD criteria of trauma exposure and should have occurred at least a month prior to assessment. We will include studies where trauma exposure is not clearly defined in our AC group, but not in the subgroup analysis. Including a group of AC, alongside trauma-specified controls, will increase the number of qualifying studies and subgroup analysis will help elucidate the importance of accounting for trauma exposure in controls. We will include studies with medical or psychiatric comorbidity provided the details of comorbidity are well described and comorbidity is not expected to have a substantial impact on outcomes. If possible, psychiatric comorbidity will be included as a factor in meta-regression.

Outcomes

To be included in the review, sufficient data to compute effect sizes (mean cortisol levels and standard deviations) must be included in the articles or be made available from the authors on request. Baseline or basal cortisol measurement in any tissue sample type (plasma, serum, whole blood, saliva, urine, hair, nails, and any others) will be included. Different time periods of sampling will be included, such as morning, evening, 24-h output, and different lengths of hair/nail samples. Mean cortisol levels from repeated cortisol measures (e.g. over 24 h) will be included as daily cortisol output. We will exclude studies evaluating cortisol levels in response to psychological or pharmacological stress tests. We will only include studies where the methods to obtain samples (e.g. time of day for acute measures, area on body hair samples obtained from) and to determine cortisol levels (e.g. storage conditions and assay methods used) are clearly described. We will highlight and report separately on studies that utilised more than one tissue sample to determine cortisol levels as these studies may assist in directly evaluating the utility of specific sampling methods in PTSD studies. In our meta-analysis, we will include each study only once and we will prioritise the results according to the largest sample size, the sample with the most complete data, and the sample with the newer tissue sample type (e.g. hair or nails), as the more established sampling approaches have been evaluated in prior meta-analyses.

Study selection

We will first screen the titles and abstracts of articles and exclude articles based on inclusion/exclusion criteria. We will then screen the full text of the remaining articles and further sort the articles based on inclusion/exclusion criteria. We will utilise an eligibility form to capture and note reasons for including or excluding articles at this stage (Additional file 4: Eligibility form). The eligibility form will be piloted on a subset of studies, and if necessary, modifications will be made. Titles, abstracts, and full texts will be independently reviewed by reviewers 1 and 2 (LLvdH and SW). Any disagreements will be discussed, and if not resolved, eligibility will be determined with the assistance of a third reviewer (ShS). For each stage of review, we will calculate inter-rater agreement utilising the kappa statistic.

Corresponding authors of manuscripts will be contacted via email in the following cases: to obtain a copy of the manuscript if one cannot be obtained; if a manuscript in a foreign language appears eligible for inclusion, the authors will be contacted to enquire whether the details of the study including the results are available in English; if additional information is required to determine study eligibility; to ask for clarification of methods and specific results if these were not included in the manuscript; authors on trial databases will be contacted to enquire whether study details and results are available. If no response is received within 2 weeks, a follow-up email will be sent. If no response is received

within another 2 weeks, non-response will be noted and the study excluded. Studies may still be included if authors respond prior to final data analysis.

Data extraction

Data will be abstracted by two independent reviewers (LLvdH and SW). Data will be entered and managed utilising the Research Electronic Data Capture (REDCap) database application [24]. REDCap is a system for structured, clinical study data capture and is designed to comply with HIPAA regulations. Access control will be password-protected and role-based. The data extraction form will be piloted prior to formal data abstraction, and if necessary, modifications will be made (Additional file 5: Data extraction form). Data extracted will include study characteristics such as study design, setting, and sample size; basic descriptive data (e.g. age, gender, ethnicity) of patients and controls; trauma-related data such as trauma type, trauma load, and duration since trauma; PTSD-related data such as method to determine diagnosis, PTSD severity, duration of illness; physical data such as BMI and blood pressure and any medical or psychiatric comorbidity; cortisol-related data such as tissue type, date and time of sampling, method used to sample and analyse cortisol, mean cortisol levels and standard deviations, and the unit of measurement for patients and controls. If cortisol is measured at multiple time points, we will extract data for each time point and the mean total cortisol over a set period (e.g. 24 h) if available.

Quality (risk of bias) assessment

Two reviewers (LLvdH and SW) will perform independent quality or risk of bias (ROB) assessments, and any disagreements will be resolved by a third reviewer (ShS). Inter-rater reliability will be determined (kappa statistic). We will perform ROB assessments with a modified version of the Newcastle–Ottawa scale (NOS) [25] adapted for use in observational studies [26] (Additional file 6: Modified Newcastle Ottawa Scale). The scale assesses for four types of bias (selection bias, performance bias, detection bias, and information bias) with seven questions rated from 0 to 3 with higher scores reflecting lower ROB. In addition, we will pilot a tool for ROB assessment designed for specific use in our review (Additional file 7: Risk of bias (ROB) assessment), based on the guidelines provided by the Agency for Healthcare Research and Quality (AHRQ) [27]. We utilised the approach and applicable components (blinding of outcome, incomplete outcome data, selective reporting) from the Cochrane Risk Of Bias Tool [28] as well as modified components from the NOS. The ROB assessment contains 11 items assessing for selection bias, performance bias, detection bias, attrition bias, reporting bias, and funding or conflict of interest bias. Each item will be rated as 'low risk of bias', 'high risk of bias', or 'unclear risk of bias', and the results for each study will be presented graphically (Additional file 8: Example risk of bias (ROB) figure). The ROB assessment tool will be piloted on a subset of studies, and if required, revisions will be made. We will report on and compare ROB bias assessments obtained with both tools as the modified NOS will allow for comparability with existing studies, whereas the ROB assessment tool designed for our study utilises examples and explanations specific to PTSD and cortisol studies, such as specific methods to determine TE and patient status and includes assessments for additional types of bias not assessed with the modified NOS. We will utilise the Grading of Recommendations, Assessment and Evaluation (GRADE) approach to report the quality of evidence and strength of recommendations [29]. The influence of bias on quantitative outcomes will be assessed by performing sensitivity analysis.

Data synthesis

Qualitative synthesis Individual studies will be summarised utilising evidence tables. At a minimum, we will include the authors, year of publication, setting, study design, sample sizes, age, sex, ethnicity, trauma type, time since trauma, trauma and PTSD measures, PTSD severity, PTSD duration, comorbidity, medication use, time or time period of cortisol assessment, cortisol levels, measurement of cortisol levels, and confounders (see 'Additional file 9: List of potential moderators' for a full list of moderators that will be included). Data will be organised according to patient and control groups for each study. We will perform a qualitative evaluation of heterogeneity by examining factors such as study design, trauma exposure, settings, populations, and outcome measurements.

Quantitative synthesis Where appropriate, we will perform a meta-analysis for each tissue type sampled. Meta-analysis will only be performed where there are at least two comparable studies with sufficient data available. In articles where results are not reported as means and standard deviations, study authors will be contacted to obtain these summary statistics. If these summary statistics cannot be obtained, we will transform available summary statistics (e.g. median and interquartile range) utilising available formulas [30]. We will compare standardised mean difference (SMD) in cortisol levels between PTSD cases and all controls, and if possible, we will perform a subgroup analysis based on trauma exposure status of controls (TEC and TUC). We will utilise the SMD to allow for pooling of data from studies utilising different methods to determine cortisol (e.g. ELISA versus LC-MS and different tissue samples). Hedge's g will be used for studies with smaller sample

sizes. As we expect there to be heterogeneity in study design, we will utilise a random-effects model (DerSimonian and Laird) [31]. Results will be graphically represented utilising forest plots. Heterogeneity will be assessed utilising the Cochrane's Q (chi-squared test) and I^2 statistics and by visually inspecting the forest plots. If significant heterogeneity exists, we will evaluate whether any specific studies significantly influenced the results by excluding each individual study and examining its impact on the pooled SMD and between-study heterogeneity. If there are a sufficient number of studies per tissue type (e.g. ten), we will perform meta-regression, in addition to the subgroup analysis according to trauma exposure status of controls. Potential moderators that will be included in the meta-regression will be year of publication, age, sex, trauma type, time since index trauma, developmental stage of trauma exposure, PTSD severity, psychiatric comorbidity, time period of sampling (e.g. time of day for acute measures and length of hair sample representing retrospective window for hair sampling), and method used to determine cortisol level (ELISA or LC-MS). Moderators will first be entered individually and those with a significance level of 0.1 will be entered in a multivariate meta-regression. The meta-regression will be conducted using a restricted maximum likelihood (REML) model. To address the issue of statistically dependent effect sizes that may arise from multiple methods of cortisol assessment within a study or from either repeated measures within a study that may have a longitudinal design, robust variance estimation procedures in meta-regression will be used. We will evaluate for effect of studies at high ROB and for the effect of study design type by entering the total score and domain sub-scores obtained on the modified NOS and study design type into separate regression models. Further sensitivity analyses will be performed to assess the influence of studies assessed as having a high ROB, by excluding studies with a high ROB and evaluating the impact on main outcomes. We will assess for small study effects with funnel plots (for analyses with 10 or more studies), and statistical tests for asymmetry (Egger test) will be performed where appropriate. If asymmetry is present, we will perform the trim-and-fill procedure [32]. Data will be analysed with STATA IC version 15.

We will present the results for each tissue type sampled in a summary of findings table. As a final step, we will pool the results of studies utilising different tissue types together utilising a cumulative approach to demonstrate trends according to tissue type sampled. We anticipate that we may have to make certain modifications to our meta-analysis approach based on the data collected (e.g. certain important variations may only be evident once the data have been collated). We will stipulate the rationale for any modifications and clearly specify post-hoc analyses.

Dissemination of results

The manuscript will be submitted for publication to an appropriate peer-reviewed journal, with preference for an open-access journal to enhance both accessibility and visibility. The results will also be presented at relevant conferences and meetings.

Discussion

In line with previous reviews, we expect that cortisol levels will generally be lower in PTSD patients than in controls, with larger difference observed when compared to TUC than TEC. We expect that patterns (i.e. the directionality of cortisol levels in PTSD cases compared with controls) will be similar across tissue type but case–control differences may not be statistically significant for all tissue types sampled. Some of the variability may be the result of the time window of cortisol measurement, as reflected by the tissue type sampled. Systematically identifying this variability may assist in better delineating acute and chronic patterns of basal cortisol secretion in PTSD. We will utilise outcomes of this review to identify aspects requiring additional investigation as well as provide suggestions as to which tissue sample types may have better utility for PTSD studies.

Additional files

Additional file 1: Table summarising published systematic reviews examining basal cortisol levels in posttraumatic stress disorder. (DOCX 17 kb)

Additional file 2: Search terms. (DOCX 14 kb)

Additional file 3: Figure S1. PRISMA flow diagram. (DOC 29 kb)

Additional file 4: Eligibility form. (PDF 47 kb)

Additional file 5: Data extraction form. (PDF 92 kb)

Additional file 6: Modified Newcastle Ottawa Scale. (PDF 40 kb)

Additional file 7: Risk of bias (ROB) assessment. (PDF 50 kb)

Additional file 8: Example risk of bias (ROB) figure. (DOCX 13 kb)

Additional file 9: List of potential moderators. (DOCX 15 kb)

Additional file 10: PRISMA-P Checklist. (DOCX 30 kb)

Abbreviations

AC: All controls; AHRQ: Agency for Healthcare Research and Quality; BMI: Body mass index; DSM: *Diagnostic and Statistical Manual of Mental Disorders*; ELISA: Enzyme-linked immunosorbent assay; GAD: Generalised anxiety disorder; HIPAA: Health Insurance Portability and Accountability Act of 1996; HPA: Hypothalamic–pituitary–adrenal; ICD: *International Statistical Classification of Diseases and Related Health Problems*; LC-MS: Liquid chromatography–mass spectrometry; NOS: Newcastle–Ottawa scale; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PTSD: Posttraumatic stress disorder; REDCap: Research Electronic Data Capture; ROB: Risk of bias; TEC: Trauma-exposed controls; TUC: Trauma-unexposed controls; WHO: World Health Organization; WMH: World Mental Health

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Funders have played no role in the study design, data collection, analysis, and interpretation and in writing the manuscript.

Availability of data and materials

Not applicable at this stage. Once the systematic review and meta-analysis has been completed, data will be made available as supplementary files.

Authors' contributions

LLvdH designed the study and wrote the manuscript and is the guarantor of the review. All authors contributed to, read, and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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References

1. American Psychiatric Association [APA]. Diagnostic and statistical manual of mental disorders, fifth edition (DSM-5®). Arlington: American Psychiatric Publishing; 2013.
2. Karam EG, Friedman MJ, Hill ED, Kessler RC, McLaughlin KA, Petukhova M, et al. Cumulative traumas and risk thresholds: 12-month PTSD in the World Mental Health (WMH) surveys. Depress Anxiety. 2014;31:130–42.
3. Kessler RC, Ormel J, Petukhova M, McLaughlin KA, Green JG, Russo LJ, et al. Development of lifetime comorbidity in the World Health Organization world mental health surveys. Arch Gen Psychiatry. 2011;68:90–100.
4. Alonso J, Petukhova M, Vilagut G, Chatterji S, Heeringa S, Üstün TB, et al. Days out of role due to common physical and mental conditions: results from the WHO World Mental Health surveys. Mol Psychiatry. 2011;16:1234–46.
5. Chrousos GP. Stress and disorders of the stress system. Nat Rev Endocrinol. 2009;5:374–81.
6. Jones T, Moller MD. Implications of hypothalamic-pituitary-adrenal axis functioning in posttraumatic stress disorder. J Am Psychiatr Nurses Assoc. 2011;17:393–403. https://doi.org/10.1177/1078390311420564.
7. King SL, Hegadoren KM. Stress Hormones: How Do They Measure Up? Biol Res Nurs. 2002;4:92–103. https://doi.org/10.1177/1099800402238334.
8. McEwen BS. Central effects of stress hormones in health and disease: understanding the protective and damaging effects of stress and stress mediators. Eur J Pharmacol. 2008;583:174–85.
9. Mcewen BS, Gianaros PJ. Central role of the brain in stress and adaptation: links to socioeconomic status, health, and disease. Ann N Y Acad Sci. 2010; 1186:190–222.
10. Pereg D, Gow R, Mosseri M, Lishner M, Rieder M, Van Uum S, et al. Hair cortisol and the risk for acute myocardial infarction in adult men. Stress. 2011;14:73–81.
11. Higashi T, Yamagata K, Kato Y, Ogawa Y, Takano K, Nakaaze Y, et al. Methods for determination of fingernail steroids by LC/MS/MS and differences in their contents between right and left hands. Steroids. 2016; 109:60–5. https://doi.org/10.1016/j.steroids.2016.02.013.
12. Stalder T, Kirschbaum C. Analysis of cortisol in hair–state of the art and future directions. Brain Behav Immun. 2012;26:1019–29.
13. Meewisse ML, Reitsma JB, de Vries GJ, Gersons BP, Olff M. Cortisol and post-traumatic stress disorder in adults: systematic review and meta-analysis. Br J Psychiatry. 2007;191:387–92.
14. Pan X, Wang Z, Wu X, Wen SW, Liu A. Salivary cortisol in post-traumatic stress disorder: a systematic review and meta- analysis. BMC Psychiatry. 2018;18:324.
15. Klaassens ER, Giltay EJ, Cuijpers P, van Veen T, Zitman FG. Adulthood trauma and HPA-axis functioning in healthy subjects and PTSD patients: a meta-analysis. Psychoneuroendocrinology. 2012;37:317–31. https://doi.org/10.1016/j.psyneuen.2011.07.003.
16. Morris MC, Compas BEBE, Garber J. Relations among posttraumatic stress disorder, comorbid major depression, and HPA function: a systematic review and meta-analysis. Clin Psychol Rev. 2012;32:301–15. https://doi.org/10.1016/j.cpr.2012.02.002.Relations.
17. Stalder T, Steudte-Schmiedgen S, Alexander N, Klucken T, Vater A, Wichmann S, et al. Stress-related and basic determinants of hair cortisol in humans: a meta-analysis. Psychoneuroendocrinology. 2017;77:261–74. https://doi.org/10.1016/j.psyneuen.2016.12.017.
18. The Cochrane Collaboration. Cochrane handbook for systematic reviews of interventions version 51.0 [updated March 2011]. 2011.
19. Moher D, Liberati A, Tetzlaff J, Altman DG, Altman D, Antes G, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;151:264–9.
20. van den Heuvel L, Wright S, Suliman S, Stalder T, Kirschbaum C. Cortisol levels in different tissue samples in posttraumatic stress disorder patients versus controls: a systematic review and meta-analysis, PROSPERO 2018 CRD42018091874. International prospective register of systematic reviews; 2018. p. 1–5. https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=91874
21. Schumacher S, Niemeyer H, Knaevelsrud C. HPA-axis function in posttraumatic stress disorder: a meta-analysis, PROSPERO 2016 CRD42016041690. International prospective register of systematic reviews; 2016. p. 1–4. https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=41690
22. Pan X. Post-traumatic stress disorder and 24-h urinary cortisol: a systematic review and meta-analysis, PROSPERO 2018 CRD42018109958. International prospective register of systematic reviews; 2018. p. 1–4. https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=109958
23. McGowan J, Sampson M, Salzwedel DM, Cogo E, Foerster V, Lefebvre C. PRESS peer review of electronic search strategies: 2015 guideline statement. J Clin Epidemiol. 2016;75:40–6. https://doi.org/10.1016/j.jclinepi.2016.01.021.
24. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42:377–81.
25. Wells GA, Shea B, O'connell D, Peterson J, Welch V, Losos MTP. The Newcastle-Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses: Ottawa Hospital Research Institute; 2009. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed 1 Feb 2018
26. Bawor M, Dennis BB, Anglin R, Steiner M, Thabane L, Samaan Z. Sex differences in outcomes of methadone maintenance treatment for opioid addiction: a systematic review protocol. Syst Rev. 2014;3:1–7.

27. Viswanathan M, Ansari M, Berkman N, Chang S, Hartling L, McPheeters L, et al. Assessing the risk of bias of individual studies in systematic reviews of health care interventions. In: Methods guide for effectiveness and comparative effectiveness reviews. Rockville: Agency for Healthcare Research and Quality Methods; 2017. p. 1–33. PMID:22479713.
28. Higgins JPT, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343:1–9.
29. Balshem H, Helfand M, Schünemann HJ, Oxman AD, Kunz R, Brozek J, et al. GRADE guidelines: 3. Rating the quality of evidence. J Clin Epidemiol. 2011; 64:401–6.
30. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. BMC Med Res Methodol. 2014;14:1–13.
31. Dersimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7:177–88.
32. Duval S, Tweedie R. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. Biometrics. 2000; 56:455–63. https://doi.org/10.1111/j.0006-341X.2000.00455.x.

APPENDIX B: Associated publications

Suliman S, **van den Heuvel L**, Suryapranata A, Bisson JI, Seedat S. Publication and non-publication of clinical trials in PTSD: an overview. Res Integr Peer Rev. 2019;4:15. doi: 10.1186/s41073-019-0074-6. eCollection 2019. PubMed PMID: 31372244; PubMed Central PMCID: PMC6659272.

du Plessis S, Bossert M, Vink M, **van den Heuvel L**, Bardien S, Emsley R, Buckle C, Seedat S, Carr J. Reward processing dysfunction in ventral striatum and orbitofrontal cortex in Parkinson's disease. Parkinsonism Relat Disord. 2018 Mar;48:82-88. doi: 10.1016/j.parkreldis.2017.12.024. Epub 2017 Dec 24. PubMed PMID: 29307561.

Hemmings SMJ, Malan-Muller S, **van den Heuvel LL**, Demmitt BA, Stanislawski MA, Smith DG, Bohr AD, Stamper CE, Hyde ER, Morton JT, Marotz CA, Siebler PH, Braspenning M, Van Criekinge W, Hoisington AJ, Brenner LA, Postolache TT, McQueen MB, Krauter KS, Knight R, Seedat S, Lowry CA. The Microbiome in Posttraumatic Stress Disorder and Trauma-Exposed Controls: An Exploratory Study. Psychosomatic medicine. 2017; PubMed PMID: 28700459

Suliman S, Anthonissen L, Carr J, du Plessis S, Emsley R, Hemmings SM, Lochner C, McGregor N, **van den Heuvel L**, Seedat S. Posttraumatic Stress Disorder, Overweight, and Obesity: A Systematic Review and Meta-analysis. Harv Rev Psychiatry. 2016 Jul-Aug;24(4):271-93. doi: 10.1097/HRP.0000000000000106. Review. PubMed PMID: 27384397.

Malan-Müller S, Kilian S, **van den Heuvel LL**, Bardien S, Asmal L, Warnich L, Emsley RA, Hemmings SM, Seedat S. A systematic review of genetic variants associated with metabolic syndrome in patients with schizophrenia. Schizophr Res. 2016 Jan;170(1):1-17. doi: 10.1016/j.schres.2015.11.011. Epub 2015 Nov 25. Review. PubMed PMID: 26621002.

APPENDIX C1: Declaration of contribution to Chapter 2

With regard to Chapter 2 'Hair cortisol levels in posttraumatic stress disorder and metabolic syndrome: A case-control study in South African mixed ancestry females', pages 33-70, the nature and scope of my contribution were as follows:

Nature of contribution	**Extent of contribution (%)**
Data acquisition Statistical analysis Drafting and revision of manuscript	55%

The following co-authors have contributed to Chapter 2, pages 33-70:

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Date: 10/10/2019

Declaration by co-authors:

The undersigned hereby confirm that

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 2, pages 33-70,

2. no other authors contributed to Chapter 2, pages 33-70, besides those specified above, and

3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 2, pages 33-70, of this dissertation.

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APPENDIX C2: Declaration of contribution to Chapter 3

With regard to Chapter 3 'Hair glucocorticoid levels in in females with Parkinson's disease', pages 71-108, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Data acquisition Statistical analysis Drafting and revision of manuscript	50%

The following co-authors have contributed to Chapter 3, pages 71-108:

Name	e-mail address	Nature of contribution	Extent of contribution (%)
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Declaration by co-authors:

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1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 3, pages 71-108,

2. no other authors contributed to Chapter 3, pages 71-108, besides those specified above, and

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APPENDIX C3: Declaration of contribution to Chapter 4

With regard to Chapter 4 'Hair cortisol levels in schizophrenia and metabolic syndrome', pages 109-149, the nature and scope of my contribution were as follows:

Nature of contribution	**Extent of contribution (%)**
Statistical analysis Drafting and revision of manuscript	50%

The following co-authors have contributed to Chapter 4, pages 109-149:

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1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 4, pages 109-149,

2. no other authors contributed to Chapter 4, pages 109-149, besides those specified above, and

3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 4, pages 109-149, of this dissertation.

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APPENDIX C4: Declaration of contribution to Chapter 5

With regard to Chapter 5 'Hair cortisol as a neuroendocrine biomarker to evaluate the impact of chronic stress on the interaction between neuropsychiatric disorders and metabolic syndrome in females', pages 150-178, the nature and scope of my contribution were as follows:

Nature of contribution	**Extent of contribution (%)**
Data acquisition Statistical analysis Drafting and revision of manuscript	60%

The following co-authors have contributed to Chapter 5, pages 150-178:

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1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 5, pages 150-178,

2. no other authors contributed to Chapter 5, pages 150-178, besides those specified above, and

3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 5, pages 150-178, of this dissertation.

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APPENDIX C5: Declaration of contribution to Chapter 6

With regard to Chapter 6 'Hair cortisol as a biomarker of stress and resilience in South African mixed ancestry females', pages 179-212, the nature and scope of my contribution were as follows:

Nature of contribution	**Extent of contribution (%)**
Data acquisition Statistical analysis Drafting and revision of manuscript	50%

The following co-authors have contributed to Chapter 6, pages 179-212:

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1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 6, pages 179-212,

2. no other authors contributed to Chapter 6, pages 179-212, besides those specified above, and

3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 6, pages 179-212, of this dissertation.

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APPENDIX C6: Declaration of contribution to Chapter 7

With regard to Chapter 7 'The association between cognitive functioning and hair cortisol levels in South African mixed ancestry females', pages 213-233, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Data acquisition Statistical analysis Drafting and revision of manuscript	50%

The following co-authors have contributed to Chapter 7, pages 213-233:

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1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 7, pages 213-233,

2. no other authors contributed to Chapter 7, pages 213-233, besides those specified above, and

3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 7, pages 213-233, of this dissertation.

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APPENDIX C7: Declaration of contribution to Appendix A

With regard to Appendix A 'Cortisol levels in different tissue samples in posttraumatic stress disorder patients versus controls: A systematic review and meta-analysis protocol', pages 274-282, the nature and scope of my contribution were as follows:

Nature of contribution	**Extent of contribution (%)**
Drafting and revision of manuscript	60%

The following co-authors have contributed to Appendix A, pages 274-282:

Name	**e-mail address**	**Nature of contribution**	**Extent of contribution (%)**
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Declaration by co-authors:

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1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Appendix A, pages 274-282,

2. no other authors contributed to Appendix A, pages 274-282, besides those specified above, and

3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Appendix A, pages 274-282, of this dissertation.

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[Tobias Stalder] Declaration with signature in possession of candidate and supervisor	University of Siegen, Siegen, Germany	22/10/2019
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