HORMONES IN HAIR AS POSSIBLE PREDICTIVE BIOMARKERS OF POSTTRAUMATIC STRESS SYMPTOMS IN WOMEN WHO HAVE BEEN RAPED

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 part submitted it for obtaining any qualification.

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Summary

There is a gap in the literature with regard to researching long-term secretion of cortisol, as well as other hormones (cortisone, testosterone, progesterone, and dehydroepiandrosterone [DHEA]) in hair samples of women with posttraumatic stress symptomatology (PTSS) and who have been victims of rape. Cortisol and other steroid hormones measured in hair over a longer time window may be predictive biomarkers of PTSS.

This longitudinal study, based in Kwa-Zulu Natal (KZN), compared hormone concentrations between groups (rape-exposed [RE] and controls) over time. The first time point was at the baseline visit (samples and data collected within 20 days post rape), which provided an approximate three-month window of hormone concentrations, preceding the rape trauma. The second sampling was at three months post rape and this covered the window between the baseline assessment and three months post rape. The last time point was at six months post rape, providing concentrations in the window between three- and six months post rape.

Furthermore, the present study sought to examine differences in PTSS between and within groups at different time-points (baseline, three months, and six months post rape). The third aim of the present study was to conduct an analysis of temporal correlations between PTSS and hormones, as measured at baseline, three months, and six months post rape. Lastly, the study sought to establish whether pre-trauma hormone concentrations were predictive of the development of PTSS at baseline, three months and six months post rape.

There were no significant differences between groups at different time-points (baseline, three months, and six months) with regard to hair hormone concentrations. There were significant differences in PTSS between groups, and several, but weak, significant correlations were found between hormone concentrations and PTSS, as well as PTSD symptom clusters (re-experiencing/intrusion symptoms, avoidance/numbing symptoms, hyperarousal, as measured by the Davidson Trauma Scale [DTS]). Pre-trauma cortisol concentrations were significantly correlated with baseline (within 20 days post rape) total PTSD symptoms, re-experiencing/intrusion symptoms, avoidance/numbing symptoms, hyperarousal. Cortisone concentrations, as measured at six months (i.e. from three to six months post rape) significantly correlated with avoidance/numbing symptoms at three months post rape. A significant, but weak,

negative correlation was found between dehydroepiandrosterone (DHEA) concentrations as measured at three months (i.e. from baseline to three months post rape) and re-experiencing/intrusions at three months post rape. A significant, but weak, positive correlation was found between DHEA as measured at six months (i.e. from three to six months post rape) and total PTSS, as well as re-experiencing/intrusion symptoms at three months post rape.

Hormone concentrations were not predictive of the development of PTSS. Within 20 days post rape, three significant predictors of PTSS were identified. The strongest predictor of PTSS was depression, followed by previous trauma (trauma load / cumulative trauma), and perceived stress. At three-month follow-up, the strongest predictor of PTSS was trauma load, followed by depression. At six-month follow-up, no significant predictors of PTSS were identified.

This is the first study to examine hair cortisol and other hair hormone concentrations in female rape victims with PTSS compared to controls.

Opsomming

Daar is ‘n gaping in die literatuur ten opsigte van navorsing wat op die langtermyn sekresie van kortisol en ander hormone (kortisoon, testosteroon, progesteroon, en dehidro-epiandrosteroon [DHEA]) in haarmonsters van vrouens met posttraumatiese stres simptome (PTSS) en wie slagoffers van verkragting was, fokus. Kortisol en ander steroïde hormone wat oor ‘n langer tydperk in hare gemeet is, kan voorspellende biomerkers van PTSS wees.

Hierdie longitudinale studie, gebaseer in KwaZulu Natal (KZN), het hormoonkonsenstrasies oor ‘n tydperk tussen groepe (blootgestel aan verkragting [RE] en ‘n kontrole groep) vergelyk. Die eerste tydpunt was die beginpunt (monsters en data binne 20 dae na die verkragting ingesamel), wat 'n benaderde raamwerk van hormoonkonsenstrasies van drie maande voor die trauma van verkragting voorsien het. Die tweede steekproefneming was drie maande na verkragting en dit het die venster tussen die beginpunt en drie maande na verkragting gegee. Die laaste tydpunt was ses maande na verkragting, wat konsentrasies in die venster tussen drie en ses maande na verkragting gebied het.

Verder het die huidige studie probeer om die verskille in PTSS tussen en binne groepe op verskillende tydpunte (basislyn, drie maande en ses maande na verkragting) te ondersoek. Die derde doel van die huidige studie was om 'n ontleding te doen van temporele korrelasies tussen PTSS en hormone, gemeet by die basislyn, drie maande en ses maande na verkragting. Laastens het die studie probeer vasstel of pre-trauma hormoonkonsenstrasies voorspelbaar was vir die ontwikkeling van PTSS by die beginpunt, drie maande en ses maande na verkragting.

Daar was geen beduidende verskille tussen groepe by verskillende tydpunte (basislyn, drie maande en ses maande) met betrekking tot hormoonkonsentrasies nie. Daar was beduidende verskille in PTSS tussen groepe, en verskeie, maar swak, beduidende korrelasies is gevind tussen hormoonkonsenstrasies en PTSS, sowel as PTSD-simptoom subgroepe (simptome wat herhaaldelik ervaar word / indringend is, simptome van vermyding / verdowing, hiper-opwekking, soos gemeet deur die Davidson Trauma Skaal [DTS]). Pre-trauma kortisolkonsentrasies was beduidend gekorreleerd met basislyn (binne 20 dae na verkragting) totale PTSV-simptome, simptome wat herhaaldelik ervaar word / indringend is, simptome van vermyding / verdowing, hiper-opwekking. Kortisoonkonsentrasies, gemeet op ses maande (d.w.s. drie tot ses maande na

verkragting), het aansienlik gekorreleer met vermyding / verdowingsimptome op drie maande na verkragting. 'n Beduidende, maar swak, negatiewe korrelasie is tussen DHEA-konsentrasies soos gemeet op drie maande (d.w.s. hormoonvlakke van die basislyn tot drie maande na verkragting) en die herervaring / indringing op drie maande na verkragting gevind. ‘n Beduidende, maar swak, positiewe korrelasie tussen DHEA soos gemeet op ses maande (d.w.s. hormoonvlakke van drie tot ses maande na verkragting) en totale PTSS, sowel as simptome van herervaring / indringing op drie maande na verkragting, is gevind.

Hormoonkonsenstrasies was nie 'n voorspelling van die ontwikkeling van PTSS nie. By die basismeting (binne 20 dae na verkragting) is drie beduidende voorspellers van PTSS egter geïdentifiseer. Die sterkste voorspeller van PTSS was depressie, gevolg deur vorige trauma (traumavrag / kumulatiewe trauma), en waargenome spanning. By die opvolging van drie maande is twee beduidende voorspellers van PTSS geïdentifiseer: Die sterkste voorspeller van PTSS was traumavrag, gevolg deur depressie. Na ses maande opvolg is geen beduidende voorspellers van PTSS geïdentifiseer nie.

Dit is die eerste studie wat ondersoek ingestel het na haarkortisol en ander konsentrasies van haarhormone by vroulike verkragtingslagoffers met PTSS in vergelyking met ‘n kontrole groep.

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List of Abbreviations

ACE: Adverse Childhood Experiences

ACTH: Adrenocorticotropic Hormone

AI: Adrenal Insufficiency

AIDS: Acquired Immunodeficiency Syndrome

ANOVA: Analysis of Variance

APA: American Psychiatric Association

AUDIT: Alcohol Use Disorders Identification Test

AUDIT-C: Alcohol Use Disorders Identification Test - Consumption

BD: Bipolar Disorder

BMI: Body Mass Index

CAPS: Clinician Administered Posttraumatic Stress Disorder Scale

CAR: Cortisol Awakening Response

CES-D: Center for Epidemiologic Studies Depression Scale

CFS: Chronic Fatigue Syndrome

CRH: Corticotrophin Releasing Hormone

CR-PTSD: Combat Related Posttraumatic Stress Disorder

CS: Cushing syndrome

CSA: Childhood Sexual Abuse

CTQ: Childhood Trauma Questionnaire

CTQ-SF Childhood Trauma Questionnaire – Short Form

CVD: Cardiovascular Disease

DAST: Drug Abuse Screening Test

DEX: Dexamethasone

DHEA: Dehydroepiandrosterone

DHEA-S: Dehydroepiandrosterone-sulphate

DSM: Diagnostic and Statistical Manual of Mental Disorders

DTS: Davidson Trauma Scale

DUDIT: Drug Use Disorders Identification Test

FSH: Follicle-Stimulating Hormone

GABA-R: Gamma-Aminobutyric Acid Receptor

GEE: Generalised Estimating Equation

GAD: Generalised Anxiety Disorder

GnRH: Gonadotropin Releasing Hormone

HCC: Hair Cortisol Concentration

HIV: Human Immunodeficiency Virus

HPA: Hypothalamic Pituitary Adrenal Axis

HREC: Health Research Ethics Committee

IC: Informed Consent

IES: Impact of Event Scale

IPV-PTSD: Intimate Partner Violence related Posttraumatic Stress Disorder

KZN: Kwa-Zulu Natal

LEC: Life Events Checklist

LH: Luteinizing Hormone

LSD: Fisher's Least Significant Difference

MDD: Major Depressive Disorder

MINI: The Mini International Neuropsychiatric Interview

MPSS: Multidimensional Scale of Perceived Social Support

MRC: Medical Research Council

NPA: National Prosecuting Authority

OCD: Obsessive-Compulsive Disorder

PI: Principal Investigator

PDA: Personal Digital Assistant

PSS: Perceived Stress Scale

PTSD: Posttraumatic Stress Disorder

PTSS: Posttraumatic Stress Symptomatology

RE: Rape-Exposed

RICE: Rape Intervention Cohort Evaluation

SCID: Structured Clinical Interview for DSM-III-R

SA MRC REC: South African Medical Research Council's Ethics Committee

SAPS: South African Police Services

SASH: South African Stress and Health

SD: Standard Deviation

SU: Stellenbosch University

TCC: Thuthuzela Care Centre

USAID: United States Agency for International Development

WHO: World Health Organisation

WMH: World Mental Health

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1. INTRODUCTION

1.1 Introduction

The introduction chapter will provide definitions of sexual violence and rape, followed by global prevalence of rape, statistics and prevalence of rape in South Africa, as well as under-reporting of rape to police. The link between rape and psychopathology, including posttraumatic stress disorder (PTSD) is demonstrated. This section is followed by the diagnostic criteria of PTSD and a brief section of global prevalence of PTSD, as well as in South Africa. Risk factors for the development of PTSD and/or symptoms of PTSD (PTSS) are also discussed. This is followed by a section on the novelty, rationale and aims of the present study. Furthermore, the hypotheses for the present study, as well as an overview of the chapters that are presented within the present dissertation are given.

1.2 Literature and statistics of rape and PTSD

1.2.1 Sexual violence

Sexual violence is defined by the World Health Organisation (WHO, 2011) as "Any sexual act, attempt to obtain a sexual act, unwanted sexual comments or advances, or acts to traffic or otherwise directed against a person's sexuality using coercion, by any person regardless of their relationship to the victim, in any setting, including but not limited to home and work."

1.2.2 Rape

Rape is a form of sexual violence (WHO, 2011) and is broadly defined in South Africa (SA) by the Criminal Law (Sexual Offences and Related Matters) Amendment Act 21 of 2007 as "any person ('A') who unlawfully and intentionally commits an act of sexual penetration with a complainant ('B'), without the consent of B, is guilty of the offence of rape". The definition of rape differs between countries and is defined by each country's national law. The definition that has been used by the "The impact of rape in women on Human Immunodeficiency Virus (HIV) acquisition and retention and linkages to care: a longitudinal study" (Rape Intervention Cohort Evaluation – RICE, *see RICE protocol article: Abrahams et al., 2017*), of which the present study was a part of, is "an act of coerced vaginal or anal penetration by a male perpetrator who may be a husband, boyfriend (a partner) or stranger, acquaintance, or man of any other relationship to the victim (non-partner)". The present study only focused on the rape of females (aged 18-40 years).

1.2.2.1 Global prevalence of rape

Global prevalence studies suggest that lifetime exposure to rape and other forms of sexual violence is relatively high in most countries (De Vries et al., 2013; WHO, 2013). Globally, one in fifteen women has been raped by a non-partner male (Abrahams et al., 2014). The Federation of students in Canada suggests that one woman is sexually abused every one minute (Chivers-Wilson, 2006). African studies found that more than half of women from Malawi and 20% from Nigeria expressed that their first sexual intercourse was against their will (Manzini, 2001). Forced first sexual intercourse is often used by studies to measure rape. A systematic review by WHO (2013), that investigated non-partner sexual violence and intimate partner violence, found that, globally, one in three women will experience physical and/or sexual violence by a partner or sexual violence by a non-partner. This report was the first to show combined global and regional prevalence rates of non-partner sexual violence and intimate partner violence. WHO (2013) found that globally, 35% of women have experienced either physical and/or sexual non-partner sexual violence or intimate partner violence. Higher rates of health difficulties have been reported by women who have been sexually or physically abused. WHO (2013) points out that violence against women is a worldwide public health problem that needs to be addressed. However, sexual violence can be difficult to measure and most studies measure the broader experience of sexual violence opposed to rape (as defined by the law).

1.2.2.2 Rape statistics and prevalence in SA

In South Africa, 49 660 sexual offence cases have been reported to the police between March 2016 to April 2017 (SAPS, 2018) of which 39 828 were rape, followed by 6271 sexual assault cases. Since April 2017 to March 2018, slightly higher rates were reported to the South African police: 50 108 sexual offence cases, of which 40 035 were rape and 6786 were sexual assault (SAPS, 2018). According to Statistics South Africa (2019), 52 420 rape cases have been reported during the 2018/2019 financial year. In KZN, 8484 cases of sexual offence were reported between March 2016 to April 2017, of which 7032 were rape and 1039 sexual assaults (SAPS, 2018). Slightly higher rates were reported from April 2017 to March 2018: 8759 sexual offence, with 7243 rape and 1148 sexual assault cases (SAPS, 2018). More specifically, the police stations with the highest rape reported since April 2017 to March 2018, is Inandi (278 reported rape cases) and Umlazi (252 reported rape cases) stations, both based in KZN (SAPS, 2018).

In South Africa, according to the SASH study, it was found that over 33% of the population is exposed to a type of violence in their life (Kaminer, Grimsrud, Myer, Stein, and Williams, 2008), specifically a prevalence rate of any lifetime traumatic event has been documented to be 73.8% (Atwoli et al., 2013). A limitation of the study by Kaminer et. al. (2008), is that the findings were self-reported and that the violence that were assessed, such as rape, may be underreported by participants. A limitation of the Atwoli et al. (2013) population study, is that they relied on the retrospective reporting by participants and therefore the traumatic events may also have been underreported.

Rape is a commonly reported criminal offence in South Africa (SAPS, 2018) and can lead to severe physical, emotional and psychological harm (Jewkes, Sikweyiya, Morrell, & Dunkle, 2011; Jina & Thomas 2013). The study by Jewkes et al. (2011) was cross-sectional and therefore does not document the long-term effects in the aftermath of rape. A prevalence rate of 7.6% for sexual violence, 1.6% for sexual assault and 2.1% for rape has been documented in the South African Stress and Health (SASH) study (Atwoli et al., 2013). Jewkes et al. (2011) reported that more than one in five men in South Africa reported raping a woman. In this case, the victim was not the perpetrator's partner (i.e. a stranger, family member or acquaintance) (Jewkes et al., 2011). Jewkes et al. (2011) also reported that one in seven men reported raping a current or former partner. In a study by Jewkes, Sikweyiya, Morrell, and Dunkle. (2009), in KwaZulu-Natal (KZN) and the Eastern Cape specifically, of the 222 men interviewed, 27,6% have raped a female, and attempted rape was reported by 16,8%. The study by Jewkes at al. (2009) was not a prospective study, and another limitation is that the age of the sample was not a reflection of that in the general population, most men were under 30 years of age.

KZN has high levels of poverty and has been identified by SAPS to be South Africa's murder capital. KZN has the largest population in South Africa (Statistics South Africa, 2019). Living in KZN under these conditions may further exacerbate symptoms of mental illness, such as posttraumatic stress post rape (Mbalo, Zhang, & Sam, 2017). However, the study by Mbalo et al. (2017) is only one study, the sample size was small, and no large comparative study is available. The present study was conducted in KZN.

Understanding the scope of the problem remains difficult and therefore more studies should be conducted in this regard.

1.2.2.3 Under-reporting to police

Globally, rape is under-reported to police (Kaminer et al., 2008; Schaeffer, 2000), as well as in South Africa (SAPS, 2013). In South Africa, only 1 in 25 women who have been raped are estimated to have ever reported it to the police (Machisa et al., 2011). There are no studies that present national statistics of under-reporting of rape to the police, but data that has been collected from surveys suggest very few (between 2.1% - 15.2%) women report being raped to the police. Therefore, the prevalence of rape is most likely to be much higher compared to what is reported from SA crime statistics (Jewkes & Abrahams, 2002; Machisa, Jewkes, Lowe-Morna, & Rama, 2011).

Furthermore, when survivors of rape experience negative social reactions when they disclose information to professionals (e.g. police), friends or family, they could experience more symptoms of PTSD and are less likely to disclose information in future (Ullman & Peter-Hagene, 2014). More research supports the notion that survivors of rape are likely to not disclose information, as opposed to experiencing more symptoms of PTSD post rape. The stigma associated with rape may also cause victims to not report the incident (Kennedy & Prock, 2016). Victims of rape may internalise this stigma, leaving them to blame themselves, feeling ashamed and thereby not seeking help (Kennedy & Prock, 2016).

1.2.3 Rape and psychopathology

Women who have been sexually abused are at higher risk for psychiatric disorders than those who have not been sexually abused (Campbell, 2002; Ellsberg et al., 2008). Sexual assault is thought to be associated with a broad spectrum of psychopathology and not only PTSD (Dworkin, Menon, Bystrynski, & Allen, 2017). The strongest link between sexual abuse and the following psychopathologies have been identified: depression, (Mbalo et al. (2017), suicide attempts (Chen et al., 2010; Gilbert et al., 2009) and PTSD (Mbalo et al. (2017).

1.2.3.1 Depression

According to the National Center for PTSD, depression is one of the effects that should be considered in the aftermath of sexual assault. In the literature, it is evident that participants with PTSD are frequently diagnosed with comorbid depression (Perkonigg, Kessler, Storz, & Wittchen, 2000). Research has found that women who experienced rape trauma in KZN are seven times more likely to experience depression symptoms compared to other provinces in South Africa (Mbalo et al., 2017). Participants for the present study were recruited in KZN.

1.2.3.2 Suicidality

In a study by Dworkin et al. (2017), sexual assault survivors were at an increased risk to develop suicidal behaviour (ideation and attempts) compared to other conditions. Sexual assault was associated with increased risk for suicidality (Dworkin et al., 2017). When controlling for other risk factors, these findings were in congruence with previous epidemiological studies of the association between sexual assault and suicidality (Ullman & Brecklin, 2002; Stein et al., 2010). Furthermore, Chen et al. (2010) have also identified a link between sexual abuse and suicide attempts. However, the strongest association was found between sexual assault and PTSD, although PTSD is not seen as the only psychopathology in the aftermath of sexual assault (Dworkin et al., 2017).

1.2.3.3 PTSD/PTSS

According to the WMH surveys, Kessler et al. (2017) reported that globally, intimate partner sexual violence, including rape and sexual assault, has been associated with the highest risk for PTSD (Kessler et al., 2017). Among survivors of sexual assault, PTSD prevalence has been reported to be 20.2% (Scott et al., 2018). The Australian National Survey suggested that the lifetime prevalence of PTSD in women who have been sexually abused is 50% (Creamer, Burgess, & McFarlane, 2001). According to the National Center for PTSD, in one study 94% of women who experienced sexual assault presented with symptoms of PTSD during the first two weeks post rape, 45% of women who experienced rape trauma met diagnostic criteria for PTSD. Sexual assault (including rape) was the trauma with the highest conditional risk of PTSD among HIV-infected South Africans (17.4%) (Morris, Naidoo, Cloete, Harvey, and Seedat, 2013).

Women are more likely to be victims of rape and sexual assault and are more likely, compared to men, to develop PTSD following a traumatic event (Hetzel-Riggin & Roby, 2013; Kessler et al., 2017; Kolltveit, et al., 2012; Olff, Langeland, Draijer, & Berthold, 2007; Tolin & Foa, 2006). It has been suggested that self-blame may contribute to poorer intervention outcomes in PTSD treatment and result in lasting psychological impact (Abrahams, Jewkes, & Mathews, 2013; Hembree, Street, Riggs, & Foa, 2004). Women who have been sexually abused are more likely to develop PTSD compared to survivors of other trauma types (Arata, 2002; Hetzel-Riggen & Roby, 2013; Kessler et al., 2017; Scott et al., 2018). Female rape survivors are therefore at high risk of developing PTSD (Kessler et al., 2017) and therefore an important focus for research. The present study focused on female survivors of rape in KZN.

1.2.4 PTSD/PTSS

A diagnosis of PTSD can be made one month after a person experienced a traumatic event (American Psychiatric Association [APA], 2013). According to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), symptoms include intrusion, avoidance, negative alterations in cognitions and mood, and alterations in arousal and reactivity (APA, 2013). In addition to the presence of these symptoms for more than one month after a traumatic event, a diagnosis of PTSD can be made when these symptoms cause significant distress or impairs functionality, such as social, occupational, or other important areas and is not caused by the physiological effects of a substance or a medical condition (APA, 2013). Within the present dissertation, these symptoms of PTSD are referred to as posttraumatic stress symptomatology (PTSS).

1.2.4.1 Global prevalence of PTSD

According to the World Mental Health (WMH) surveys, the global prevalence of PTSD has been estimated to range between 1.3% (Kawakami, Tsuchiya, Umeda, Koenen, & Kessler 2014) and 8.8% (Ferry et al., 2014) and the WMH surveys published in 2014 revealed a 12-month PTSD prevalence of 1.1% (Karam et al., 2014). Among American adults, the lifetime prevalence of PTSD has been suggested to be 6.8% (Kessler et al., 2005) and in European populations, PTSD prevalence has been estimated to be 7.4% (de Vries, & Olff, 2009). Lifetime prevalence of PTSD in adult women has been estimated to be 9.7% in the United States (Kilpatrick et al., 2003).

1.2.4.2 Prevalence of PTSD in South Africa

PTSD is a disabling disorder that affects 2.3% of the general South African population (Herman et al., 2009). PTSD is one of the most prevalent disorders among people living with HIV (10,4%-42%) (Martinez, Israelski, Walker, & Koopman, 2002; Pingo & Seedat, 2009; Radcliffe et al., 2007). A study conducted in Cape Town revealed that of the 465 HIV-positive participants, sexual assault (17,4%) was the highest contributing factor and significantly correlated with PTSD (Morris et al., 2013). Mbalo et al. (2017) reported that living in Kwa-Zulu Natal (KZN) is a major risk factor for PTSD and that female survivors of rape residing in KZN indicated significantly higher prevalence of PTSD compared to the Western Cape and Limpopo provinces (Mbalo et al., 2017). Police stations in KZN had the highest rape reported since April 2017 to March 2018, which was Inandi (278 reported rape cases) and Umlazi (252 reported rape cases) stations (SAPS, 2018). KZN has high levels of poverty and has been

identified by SAPS to be South Africa's murder capital. This suggests that there could be more trauma in KZN. KZN has the largest population in South Africa (Statistics South Africa, 2019). A study by Mbalo et al. (2017) suggests that, compared to other provinces (Limpopo and Western Cape) in South Africa, female rape survivors from KZN experienced more childhood abuse, and specifically more childhood sexual abuse (Mbalo et al., 2017). However, as mentioned earlier, the study by Mbalo et al. (2017) is only one study, the sample size was small, and no large comparative study is available. Living in KZN under these conditions may further exacerbate symptoms of mental illness, such as posttraumatic stress post rape (Mbalo et al., 2017)

Female rape survivors are at high risk of developing PTSD (Kessler et al., 2017), and considering the above information and that the highest rape is reported in KZN, could possibly be linked to higher trauma in KZN. However, provincial level data on mental health does not exist in this regard. In the absence of provincial level data on mental health in South Africa, it is not clear if there is higher trauma and higher risk of developing PTSD in KZN, compared to other provinces. Given the higher levels of rape reported, murders and poverty in KZN (SAPS, 2018), the available data might suggest that KZN could have higher levels of trauma, however this should be confirmed with future research in this area.

The topic on reasons for women in KZN, also rape survivors, possibly having a higher risk of developing PTSD compared to other provinces, would be a valuable topic for future research, also suggested by Mbalo et al. (2017), as little is known about this.

1.2.4.3 Risk factors associated with the development of PTSD and/or symptoms of PTSD

Several risk factors have been identified for the development of PTSD and symptoms of PTSD. These risk factors include biological, psychological, and social mechanisms connected to the development of PTSD and/or posttraumatic stress symptomatology (PTSS) (Chivers-Wilson, 2006). In the next chapter (Chapter 2), biological mechanisms connected to the development of PTSD/PTSS will be discussed, specifically focusing on maladaptation of the hypothalamic pituitary adrenal (HPA) axis, which is most relevant to the topic of the present study. Psychological and social risk factors that have been studied previously and which are applicable to the present study, are a history of trauma (Delahanty, Raimonde, Spoonster, & Cullado, 2003; Ehring, Ehlers, Cleare, & Glucksman, 2008; Kolassa et al., 2010; Neuner et al., 2004; Resnick, Yehuda, Pitman, & Foy, 1995; Steudte-Schmiedgen, Kirschbaum, Alexander,

& Stalder, 2016; Walsh et al., 2013), including childhood trauma (Gladstone et al., 2004; Hillberg, Hamilton-Giachritsis, & Dixon, 2011; Lang, Stein, Kennedy, & Foy, 2004; Mbalo et al., 2017), perceived stress (Besser, Neria, Haynes, 2009; Heinze, Lin, Reniers, & Wood 2016; Matud, 2004), and alcohol abuse (Back, Sonne, Killeen, Dansky, & Brady 2003; Kilpatrick, Acierno, Resnick, Saunders, & Best, 1997; Rothbaum, Foa, Riggs, Murdock, & Walsh, 1992; Shipherd, Stafford, & Tanner, 2005). Negative social reactions or social support perceived as being negative, have also been connected to increased symptoms of PTSD and a greater risk to develop PTSD (Billette, Guay, & Marchand, 2008; Dworkin & Schumacher, 2018; Elklit & Chrisiansen, 2013; Gutner, Rizvi, Monson, & Resick, 2006; Iob, Kirschbaum & Steptoe, 2018; Koss & Figuerdo, 2004; Ullman & Peter-Hagene, 2014).

1.2.4.3.1 Previous trauma and PTSS/PTSD

Prior trauma history or trauma load is associated with an increased risk for PTSD development when individuals experience a new trauma later in life (Delahanty et al., 2003; Ehring et al., 2008; Resnick et al., 1995; Walsh et al., 2013). Furthermore, previous studies have shown a dose-response relationship between trauma load and symptoms of PTSD, suggesting increased trauma load is associated with increased severity and frequency of PTSD symptoms (Kolassa et al., 2010; Steudte-Schmiedgen et al., 2016; Neuner et al., 2004). In a study by Steudte et al. (2011a), severely traumatised participants with PTSD also had higher trauma load compared to traumatised controls without PTSD.

History of rape strengthened the relationship between previous sexual abuse and psychopathology, such as depression and PTSD in a study by Chen et al. (2010). When females are re-victimised, they have an increased risk to experience trauma-related symptoms, especially symptoms of PTSD and depression (Saunders, Villeponteaux, Lipovsky, Kilpatrick, & Veronen, 1992; Mbalo et al., 2017).

Childhood trauma

The relationship between childhood sexual abuse (CSA) and adult mental health has been researched extensively, suggesting an association between CSA and adult psychopathology, including symptoms of PTSD (Gladstone et al., 2004; Hillberg et al., 2011; Lang et al., 2004). In a study by Mbalo et al. (2017), 10% of survivors of rape also indicated to have experienced childhood sexual abuse. Compared to other provinces (Limpopo and Western Cape) in South Africa, female rape survivors from KZN experienced more childhood abuse, and specifically more childhood sexual abuse (Mbalo et al., 2017).

1.2.4.3.2 Perceived stress and PTSS/PTSD

Heinze et al. (2016) found that perceived stress was higher in those participants diagnosed with psychopathology compared to healthy controls. Compared to men, it has been suggested that women experience significantly higher levels of perceived stress (Matud, 2004). Higher levels of perceived stress have been associated with increased risk for PTSD and increased PTSD symptom severity (Besser et al., 2009).

1.2.4.3.3 Alcohol use and PTSS/PTSD

The use of substances, such as alcohol, as well as the association between substance use disorders and PTSD in the aftermath of sexual assault has been researched extensively (Kilpatrick et al., 1997). Alcohol use has been documented to be higher in the acute aftermath (within weeks) of assault (Rothbaum et al., 1992). Furthermore, alcohol use, instead of abuse, has been associated with victims of sexual assault, and could be used as a coping mechanism to reduce negative affect (Kilpatrick et al., 1997). Alcohol abuse has also been documented a risk factor for the development of PTSD (Back et al., 2003; Shipherd et al., 2005). Furthermore, when PTSD is comorbid with alcohol abuse disorder, more severe symptoms of PTSD are experienced, including increased avoidance and hyperarousal symptoms (Back et al., 2003).

1.2.4.3.4 Social support and PTSS/PTSD

When survivors of sexual assault and specifically rape experience or perceive support and social reactions as negative after disclosing information to friends, family, or professional, they have an increased risk to develop symptoms of PTSD and PTSD (National Center for PTSD; Ullman & Peter-Hagene, 2014). A study by Koss and Figuerdo (2004) confirmed that women who experience negative social reactions report more symptoms of PTSD. Feelings associated with rape trauma, such as anxiety, depression, shame and guilt may also increase when these women receive or perceive support as negative (National Center for PTSD). In a study by Wyatt et el. (2017) about the long-term effects (up to 12 months) of rape on women from rural communities in South Africa, the women's emotional support from family and friends decreased over time. More severe depression was documented in those women who experienced social undermining, especially when they experienced social undermining and victim blaming from individuals who they believed would have been supportive in the aftermath of rape.

Furthermore, in a study by Elklit and Chrisiansen (2013), perceived positive support in women in the early aftermath of sexual assault (two weeks post sexual assault) was associated with less severity of PTSD symptoms three months after sexual assault and therefore positive support is important to recovery (Koss, & Figuerdo, 2004). In a study including female rape victims, increased social support was also associated with decreased symptoms of PTSD (Gutner et al., 2006). More specifically, support from intimate partners have been suggested to decrease symptoms of PTSD and depression (Billette et al., 2008). Furthermore, Dworkin and Schumacher (2018) suggest that rape victims should receive appropriate support and perceive this support positively. Iob et al. (2018) highlighted the importance of the quality of social support to decrease stress and improve health. However, no association between social support and PTSD has also been documented (Mbalo et al., 2017).

Few studies have investigated the longitudinal (more than 3 months) mental health outcomes in women who have been victims of rape. Most studies are cross-sectional in design and therefore limits the understanding of the complicated interrelationships of these factors associated with rape. Prevalence studies are needed to complement or challenge the statistics provided by SAPS and prospective, longitudinal studies are needed to investigate the long-term health outcomes of women who have been victims of rape.

1.3 Novelty

It has been suggested that investigation of hair cortisol in individuals with PTSD/PTSS that aims to address questions about the baseline status of these individuals and investigation of cortisol concentrations before the trauma that may predict psychopathology, is needed (Pacella, Hruska, Steudte-Schmiedgen, George, & Delahanty, 2017; Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, 2013). Furthermore, there is a need for longitudinal studies (Olff, Güzelcan, de Vries, Assies, & Gersons, 2006; Pacella et al., 2017; Staufenbiel et al., 2013; Wosu, Valdimarsdóttir, Shields, Williams, & Williams, 2013) in large samples (Usta, Tuncel, Akbas, Aydin, & Say, 2015) that measure the relationships between symptoms of PTSD and the long-term activity of HPA-axis (Johnson, Delahanty, and Pinna 2008; Staufenbiel et al., 2013).

In the next chapter (Chapter 2), biological mechanisms connected to the development of PTSD/PTSS will be discussed, specifically focusing on maladaptation of the HPA-axis. Hair cortisol and the detection of hormones in hair, as well as the benefits associated with hair

cortisol and its role in understanding the psychopathological consequence of rape, will be discussed in Chapter 2.

Hair cortisol concentrations can be a useful tool biomarker of stress-related illnesses (Staufenbiel et al., 2013) and PTSS (Pacella et al., 2017). Olff et al. (2006) suggested that longitudinal research investigating whether the lower cortisol concentrations in individuals with PTSS are "pre-existing risk factors or a consequence of trauma and whether these alterations are deleterious or adaptive". From the literature, it is still unclear whether pre-trauma cortisol concentrations predict PTSD/PTSS or whether HPA dysregulation as manifested by cortisol concentrations are secondary to a traumatic event (Heinrichs et al., 2005; van Zuiden et al., 2011). There is not much known about how hair cortisol concentrations may change over time, the change of these concentrations before and after a traumatic event and the link between the severity of disorders and hair cortisol concentrations (Wosu et al., 2013). Hair cortisol concentrations may make an important contribution to our knowledge of biomarkers of HPA-axis dysregulation and long-term stress disorders (Pacella et al., 2017; Wosu et al., 2013).

Notably, there is a gap in our knowledge of long-term patterns of cortisol and other stress hormones in women who are victims of rape (Usta et al., 2015). Cross-sectional studies of neuroendocrine biomarkers have been inconsistent and limited by relatively small samples. To our knowledge, no other studies have evaluated other hair steroid hormone concentrations (cortisone, testosterone, progesterone, and dehydroepiandrosterone [DHEA]) in rape-exposed women. Rape-exposed participants were defined as those who recently reported and sought care for a rape that occurred within the past 20 days and controls were defined as participants who did not report a recent (within the past 20 days) rape event. Analysis of these hormones in hair may strengthen our current understanding of the relationship between neuro-endocrine axis dysregulation in trauma and posttraumatic stress symptomatology (Steudte et al., 2013). Cortisol and other steroid hormones (cortisone, testosterone, progesterone, and DHEA) measured over a longer time window in hair may be predictive biomarkers of posttraumatic stress symptomatology, serving to identify individuals at risk so that targeted interventions can be timeously administered to reduce symptomatic distress.

There is a gap in the literature with regard to researching long-term secretion of cortisol, as well as other hormones in hair samples of women with PTSS and who have been victims of rape. Also, no studies within the South African context have been found that evaluated cortisol

or other hormones in hair in people who have been traumatised by rape. The present study is the first of its kind.

1.4 Rationale

Most individuals will be exposed to at least one traumatic event during their lifetime (Benjet et al., 2016), however, only a small percentage will develop PTSD or posttraumatic stress symptoms (Atwoli, Stein, Koenen, & McLaughlin, 2015). Therefore, exposure to a traumatic event may not be the only factor related to PTSD and posttraumatic stress symptoms.

However, research findings related to biomarkers have been inconsistent and are often limited by design and sample size. Using cortisol in hair (Pacella et al., 2017) and other steroid hormones (cortisone, testosterone, progesterone, and DHEA) (Kellner et al., 2010) as predictive biomarkers may help identify those individuals at risk of developing posttraumatic stress symptomatology (Wimalawansa, 2014). By identifying these individuals, clinicians may be able to use targeted interventions (Wester, & Rossum, 2015), including drug development (Wimalawansa, 2014), to reduce stress and symptoms of PTSD (Mbalo et al., 2017; Olff et al., 2007; Wimalawansa, 2014).

1.5 Study aims

1.5.1 Primary aim

(i) To compare hormone (cortisol, cortisone, testosterone, progesterone, and DHEA) concentrations between groups (rape-exposed and controls) at different time-points.
The time-points were:
(1) Baseline (within 20 days after the rape), which yielded hormone concentration from three months before the trauma to baseline;
(2) Three months after trauma, which yielded hormone concentrations from baseline to three months;
(3) Six months after trauma, which yielded hormone concentrations from three months to six months post rape

1.5.2 Secondary aims

(i) To compare posttraumatic stress symptoms (as measured by the Davidson Trauma Scale [DTS]) between groups (rape-exposed and controls) at different time-points.
The time-points were as follow:

(1) Baseline (within 20 days post rape)
(2) Three months after rape trauma
(3) Six months after rape trauma

(ii) To establish if there were significant temporal correlations between posttraumatic stress symptoms (as measured by the DTS) and HPA-axis hormones (cortisol, cortisone, testosterone, progesterone, and DHEA) measured at baseline, three months, and six months after rape.

(iii) To establish if pre-trauma hormone concentrations (as sampled at baseline) were predictive of the development of posttraumatic stress symptoms at baseline (within 20 days after rape exposure), three months, and six months post rape.

1.6 Hypotheses

(i) The present study hypothesised that there would be a significant difference between and within groups (rape-exposed and controls) regarding cortisol concentrations and other neuroendocrine markers (cortisone, testosterone, progesterone, and DHEA) measured at different time-points: (1) baseline, (2) three months and (3) six months, with the rape-exposed group displaying an initial increase in cortisol concentrations and then decrease over time to below the baseline measurement.

(ii) The present study hypothesised that there would be significant differences within and between groups (rape-exposed and controls) regarding posttraumatic stress symptoms at different time-points: (1) baseline, (2) three months, and (3) six months.

(iii) The present study hypothesised that there would be significant correlations between posttraumatic stress symptoms and the HPA-axis hormones (cortisol, cortisone, testosterone, progesterone, and DHEA).

(iv) The present study hypothesised that the aforementioned neuroendocrine markers (cortisol, cortisone, testosterone, progesterone, and DHEA), in particular cortisol, would be predictive of the development of posttraumatic stress symptomatology over time (baseline, three months, six months) in victims who have been traumatised by rape.

1.7 RICE study aims

For a comparison between the present study's aims and that of the RICE study's aims, herewith a description of the RICE study's aims. The RICE study's primary aim was "to describe the

incidence and attributable burden of physical and mental health problems (including HIV acquisition) in adult women over a 2-year post rape period, through comparison with a cohort of women who have not been raped."

1.7.1 Secondary aims of RICE

i. "To determine the incidence, attributable burden and recovery rates of physical and mental health problems that may enhance HIV risk at 3, 6, 9, 12, 18 and 24 months.

ii. To determine the individual, relational, social and criminal justice risk factors for health problems at the different time points that may be HIV risk factors and associated with the persistence of symptoms.

iii. To estimate the relative importance of the different hypothesised pathways to HIV acquisition.

iv. To determine the impact of rape on HIV positive survivors' ability to link to HIV care, retention of treatment and sexual risk-taking behaviour.

v. To evaluate changes in cortisol levels and other steroid hormones (cortisone, testosterone, progesterone, and DHEA) over time, measuring their levels in hair, and to evaluate the stress response of women before and after the traumatic event of rape.

vi. To investigate genetic and epigenetic factors as participating biomarkers in the aetiology and trajectory of PTSD among rape-exposed women.

vii. To determine the incidence and attributable burden of CVD risk factors, markers of increased CVD risk and hypertension-related and diabetes-related renal dysfunction, comparing rape-exposed and non-exposed women.

viii. To explore, qualitatively, the experiences of both rape-exposed and rape non-exposed women and to focus on motivations and experiences of the research process, retention issues and the support provided to them."

1.8 Overview of chapters

Chapter 2 provides an overview of the HPA-axis, maladaptation of the HPA-axis in PTSD and the association between cortisol / cortisone / progesterone / testosterone / DHEA and PTSD and/or PTSS. Benefits of the use of long-term (i.e. hair) hormone concentrations and possible covariates identified from the literature are provided.

Chapter 3 outlines the methodology used within the present study. This includes the research design, sample, ethical considerations, questionnaires and measurements used,

recruitment and study procedures. This is followed by an overview of the statistical analyses performed within the present study.

The results of the study are reported in Chapter 4. Demographic characteristics of the participants are reported, followed by a comparison of hormone concentrations between groups, between visits, and within groups between visits. Thereafter, a comparison of PTSS between groups over time are provided, followed by correlation analyses in the rape-exposed group. Furthermore, results of the regression analyses, as well as the univariate analyses are reported and additional results regarding differences between groups (controls and rape-exposed) are given. To conclude, a summary of the results is provided.

The last chapter (Chapter 5) provides a discussion of the results of the study. The principal study findings, contribution to scientific knowledge, limitation of the present study, as well as the conclusion and recommendations are covered in this chapter.

2. BACKGROUND

2.1 Introduction

In this chapter, the hypothalamic pituitary adrenal (HPA) axis and maladaptation of the HPA-axis in posttraumatic stress disorder (PTSD) and/or posttraumatic stress symptomatology (PTSS) and sexual assault/rape are discussed, as well as the association between cortisol and PTSD/PTSS and sexual assault/rape. Neuroendocrine hormones (cortisol, dehydroepiandrosterone [DHEA], testosterone and progesterone) and their role in PTSD and/or PTSS are discussed. Due to the limited literature available on women with PTSD/PTSS who were victims of rape and the association between PTSS/PTSD and hair hormone concentrations in the context of rape, a broader literature review was performed. Inconsistent findings on the relationship between cortisol concentrations and PTSS/PTSD exist and therefore possible explanations for these inconsistent results are given. This section is followed by a brief discussion of studies about long-term (i.e. hair) cortisol concentrations in participants with other mental disorders (other than PTSD), as well as those who have been traumatised. Thereafter, available literature regarding the relationship between hair cortisol concentrations (HCC) and PTSD are discussed. Furthermore, the benefits of retrieving cortisol concentrations from hair samples, instead of urinary/saliva/blood samples are provided and the chapter is concluded with a section on several factors that may potentially influence hair cortisol concentrations.

2.2 The HPA-axis function

The HPA-axis is one of the key structures involved in the management of the stress response in humans (Mehta & Binder, 2012). In response to a stressor, corticotropin releasing hormone (CRH) is released and stimulates the pituitary gland, which then releases adrenocorticotropic hormone (ACTH) (Pervanidou & Chrousos, 2010; Sapolsky, Romero, & Munck, 2000). The adrenal glands are then stimulated by ACTH to release glucocorticoids (e.g. cortisol) (Pervanidou & Chrousos, 2010). A negative feedback loop is created where cortisol reduces CRH secretion in the hypothalamus and decreases ACTH secretion in the pituitary gland (Oberlander et al., 2008; Radtke et al., 2011; van der Knaap et al., 2014). See Figure 2.

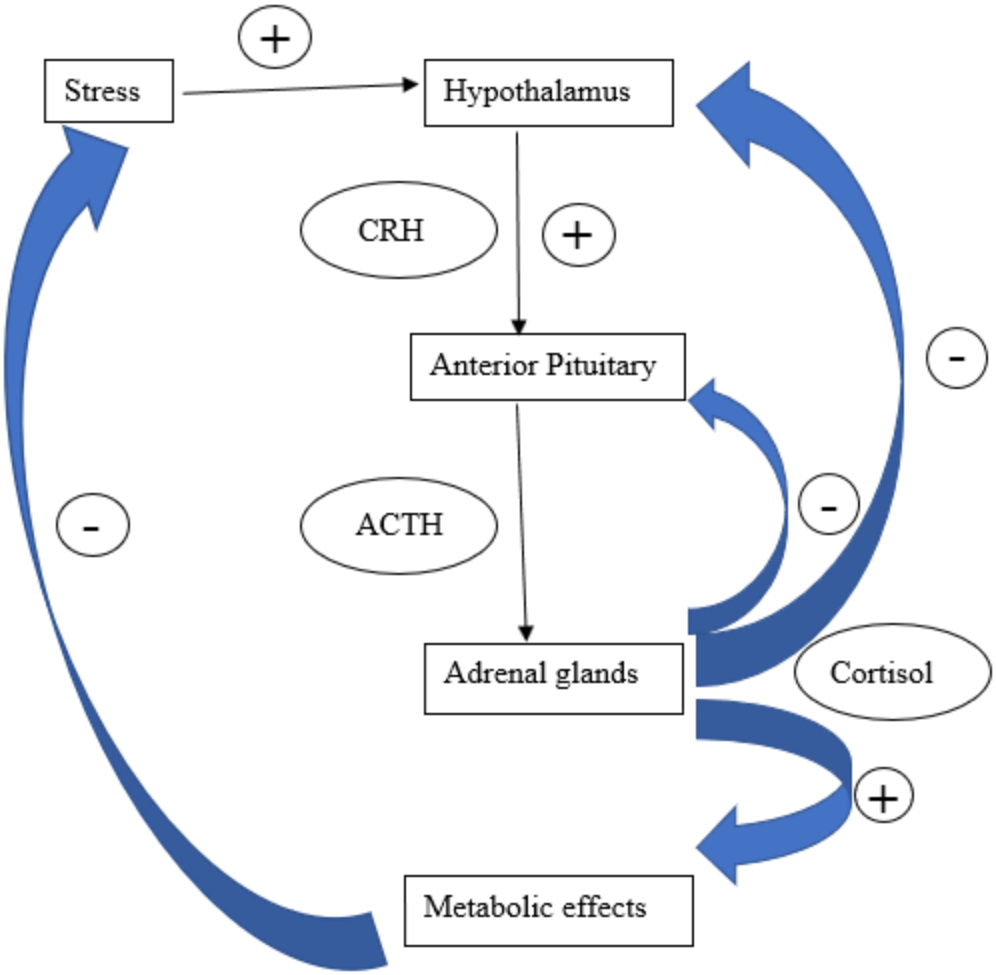

Figure 2 The HPA-axis function (Based on a figure by Herman et al., 2016)

More specifically, in response to a stressor, under normal circumstances, CRH and arginine vasopressin (AVP) are released by the hypothalamus (Pervanidou & Chrousos, 2010). It is believed that AVP plays a role in the regulation of ACTH release (Scott & Dinan, 1998). CRH and AVP bind to receptors on the anterior pituitary and this binding by CRH stimulates corticotropins to synthesise and release beta-endorphins and ACTH into the blood that travels to the adrenal cortex (Pervanidou & Chrousos, 2010). The experience of physical and emotional pain related to traumatic stress can be reduced by beta-endorphins, which produces a pain-relieving effect (Weiss, 2007).

Dehydroepiandrosterone (DHEA) is a steroid hormone that is produced primarily by the adrenal cortex in response to ACTH stimulation (Jones & Moller, 2011). In response to ACTH, the adrenal cortex releases DHEA from the zona reticularis (innermost layer of the adrenal cortex) and cortisol is released from the middle layer of the adrenal cortex (the zona fasciculata) (Maninger, Wolkowitz, Reus, Epel, & Mellon, 2009; Vythilingam et al., 2010).

It has been suggested that DHEA could possibly block or inhibit the effects of cortisol (Maninger et al., 2009; Vythilingam et al., 2010). There is evidence that DHEA may counteract some of the effects of elevated glucocorticoids and play a role in stress-related disorders, such as depression and chronic fatigue (Goodyer, Park, Netherton, & Herbert, 2001; Khorram, 1996; Wolkowitz, Brizendine, & Reus, 2000).

Cortisol influences numerous areas, including brain functions, such as memory, metabolism, immunity, and noradrenaline release from the adrenal medulla. Although cortisol is a major output of the HPA-axis, it is also responsible to end that response (Sapolsky et al., 2000). When enough glucocorticoids have been produced, the negative feedback loop of the HPA-axis is activated. When enough cortisol has been secreted, the release of ACTH and DHEA are inhibited and the hypothalamus slows the release of CRH and vasopressin. It is crucial that the release of glucocorticoids is terminated, otherwise the body may undergo physiological damage that can be caused by the prolonged release of cortisol (Charmandari, Constantine, & Chrousos, 2005; Habib, Gold, & Chrousos, 2001). Excessive cortisol can exacerbate its catabolic, anti-reproductive and immunosuppressive effects (Charmandari et al., 2005; Habib et al., 2001). For example, a decrease in hippocampal volume has been associated with prolonged/hypersecretion of cortisol in patients with PTSD. The hippocampus is not only associated with the inhibition of the HPA-axis, but, plays an important part in learning new information (Squire, 1992).

Cortisol primarily acts by binding to two receptors, namely the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). The MR plays a role in fear appraisal and in the onset of the HPA stress response, whereas GR plays a role in the body's recovery from a stressor, as well as adapting in response to a stressor. Once the stressor has passed, the GR is responsible for triggering the negative feedback loop and returning the system to a normal state. However, for the GRs to function effectively, several other proteins and co-regulators are required. When the GR and its co-regulators do not function properly, the person may be more susceptible to stressor(s) and increase their risk for the development of PTSD (Frodl & O'Keane, 2013; Mehta & Binder, 2012).

Cortisone is the inactive form for cortisol and is converted to cortisol by the enzyme 11β-hydroxysteroid-dehydrogenase type 1 (11β-HSD1). Cortisol is converted to cortisone by the enzyme 11β-hydroxysteroid-dehydrogenase type 2 (11β-HSD2) (Raff and Findling, 2003). Cortisone exhibits no activity at glucocorticoid receptors (GR) and mineralocorticoid receptors

(MR). In the liver and the adipose tissues, 11β-HSD1 is primarily found, and in the kidney and skin, 11β-HSD2 is primarily found. These enzymes regulate active cortisol and inactive cortisone levels. Total glucocorticoid (GC) levels are represented by cortisol and cortisone concentrations (Raff and Findling, 2003). Higher cortisone concentrations, compared to cortisol, are found in hair and saliva samples, whereas lower levels of cortisone, compared to cortisol, is found in blood samples (Perogamvros et al., 2010; Raul et al., 2004). The differences in cortisol-to-cortisone ratios found in hair and saliva, compared to blood, are hypothesized to be due the different locations and functions of to 11β-HS, as well as due to the different corticosteroid-binding globulin (CBG) and albumun affinities for cortisol and cortisone (Perogamvros et al., 2010; Raul et al., 2004). Hair cortisol and hair cortisone concentrations have showed moderate test-retest associations and similar intraindividual stability (Zhang et al., 2017). Incorporating hair cortisol and hair cortisone concentrations may provide a more complete picture of the HPA-axis functioning (Stalder et al., 2013; Staufenbiel et al., 2015).

2.3 Dysregulation in PTSD

In patients with PTSD, dysregulation in several systems of the body has been established, including immune, neural, and endocrine structures (Brunello et al., 2001). Structural and functional abnormalities that contribute to PTSD symptoms are associated not only with HPA-axis maladaptation, but also dysregulation of the glutamatergic, adrenergic, and serotonergic systems. The dysregulation of the HPA-axis can have an influence on the adrenergic system, activating gamma-aminobutyric acid receptor (GABA-R), associated with memory and perception (Davidson et al., 1988; Nutt, 2000). This might shed light on the association between emotions and factual memory. The immune system is regulated by both the neural and endocrine systems and can therefore be influenced by dysregulation of the HPA-axis (Tucker et al., 2004) and be affected by PTSD (Boscarino, 2004). It is known that inflammatory illnesses are commonly reported in patients diagnosed with PTSD, which could probably be due to HPA dysregulation, which affects the immune system (Tucker et al., 2004). Furthermore, most researchers have investigated and found significant connections between the methylation of genes (such as GR gene, Nuclear Receptor Subfamily 3, group C, Member 1 (*NR3C1*) (Boyle, Kolber, Vogt, Wozniak, & Muglia, 2006); FK506 binding protein *(FKBP5)* (Pratt & Toft, 1997); and Pituitary Adenylate Cyclase-Activating Polypeptide (*PACAP*) (Almli, Fani, Smith, & Ressler, 2014) and the HPA-axis in patients with PTSD.

However, the focus of the present study was on hormones associated with the HPA-axis and its association with symptoms of PTSD in females. Therefore, information that follows will focus specifically on maladaptation of the HPA-axis associated with PTSD/PTSS and thereafter its association with rape or sexual assault.

2.4 HPA-axis maladaptation in PTSD

The most common hypothesis is that decreased cortisol concentration is seen in patients with PTSD (Heim, Newport, Mletzko, Miller, & Nemeroff, 2008; Susman, 2006). It has been suggested that the decreased cortisol concentrations observed in patients with PTSD could be explained by allostasis, which is an ongoing process in the body to maintain homeostasis (Seeman, McEwen, Rowe, & Singer, 2001). Allostatic load refers to chronic arousal of the HPA-axis that causes stress on the body (McEwen, 2003). Allostatic load is based on 10 biological markers, namely cortisol, DHEA-S, adrenaline, noradrenaline, high-density lipoprotein (HDL), total cholesterol, systolic and diastolic blood pressure, waist-to-hip ratio, and glycosylated haemoglobin (HbA1c) (Seeman et al., 2001). Within the present study, hair cortisol and DHEA concentrations, as well as systolic and diastolic blood pressure were measured. Under normal circumstances, in response to a stressor, protective factors of other neuroendocrine systems are increased, and the psychological and physiological effect of a stressor is blunted by the activation of the HPA-axis and cortisol that is released (Michaud, Matheson, Kelly, & Anisman, 2008; Sapolsky et al., 2000). When stress is low, allostatic load is small. However, when the body is exposed to prolonged stress or trauma, an allostatic overload is created causing excessive demand on the HPA-axis, which lowers the body's ability to facilitate stress via activation of the HPA-axis and thereby increasing the body's susceptibility to pathology (McEwen, 2000). These systems on how the body (mal)adapts in response to chronic stress is not fully understood, however, it is hypothesised that the body decreases hormones in the HPA-axis, downregulates receptors of the pituitary and also alter glucocorticoid receptor sensitivity (McEwen, 2007; Vidovic et al., 2011). It has been suggested that decreased release of cortisol in response to prolonged exposure to stress or trauma, is associated with a protective adaptation of the body in order to protect the body from harmful effects of cortisol, such as in the hippocampus, frontal cortex, amygdala, cardiovascular system, and immune system (Sapolsky et al., 2000; Trickett, Noll, Susman, Shenk, & Putnam, 2010). The hippocampus, in addition to the frontal cortex and amygdala, plays a role in preventing activation of the HPA-axis. Therefore, hyposecretion of cortisol by the HPA-axis seen in PTSD patients may reflect the HPA-axis's attempt to limit overactivation.

It is known that cortisol may affect other systems in the body, e.g. the mesocorticolimbic dopaminergic system, which is associated with inappropriate reactions to fear and persistent mild depression. Furthermore, increased CRH can cause harm to the hippocampus, which is a central area in the brain linked to learning and memory (Maren, 1999; van Voorhees, & Scarpa, 2004). Decreased hippocampal size has been associated with traumatic experiences (Brunello et al., 2001). Reduced hippocampal size, in addition to HPA hypersensitivity, has been connected to decreased cognitive functioning (Bremner, & Vermetten, 2004).

Cortisol is the end product of the HPA-axis and therefore several studies have focused on cortisol as an indicator of HPA-axis activity in patients with PTSD (Gill, Vythilingam, & Page, 2008; Paris et al., 2010; Shalev et al., 2008).

2.4.1 Cortisol and its association with PTSD/PTSS and sexual assault/rape

Cortisol, also known as the primary stress hormone in the body, is one of the hormones of the hypothalamic pituitary adrenal (HPA) axis that is released in reaction to stress (de Kloet et al., 2007). As mentioned earlier, CRH stimulates the release of cortisol, and cortisol is inhibited through a negative feedback loop. Cortisol levels usually peak during the morning and declines throughout the day to a lower level during the evening (Stalder et al., 2017).

As mentioned previously, although short-term activation of the HPA-axis is needed in stressful situations, chronic activation may be harmful (Stalder et al., 2013; Staufenbiel et al., 2013) and has been associated with several poor health outcomes (De Bellis, Spratt, & Hooper, 2011) and psychiatric disorders, including PTSD and symptoms of PTSD (Daskalakis, Lehrner, & Yehuda, 2013; Ehlert, Gaab, & Heinrichs, 2001; Yehuda, 2009).

Mason, Giller, Kosten, Ostroff, and Harkness (1986) published the first study to differentiate PTSD from other disorders (major depressive disorder [MDD], bipolar disorder [BD], and schizophrenia) based on urinary cortisol levels, documenting lower urinary cortisol levels in PTSD patients compared to other disorders (MDD, BD, and schizophrenia). Later, more evidence emerged that biological changes, specifically in the neuroendocrine system, occur in the aftermath of a traumatic event (American Psychiatric Association, 1994; Boscarino, 1996). Before 2002, most studies were conducted by Yehuda and her colleagues (Yehuda et al., 1990; 1993a; 1993b; 1993c; 1995a; 1995b; 1996; 1998) examining male combat veterans with PTSD. As women are more likely to develop PTSD, they became the focus of

investigation (Breslau, Chilcoat, Kessler, & Davis, 1999; Kessler, Sonnega, Bromet, & Nelson, 1995). The present study only included females.

More recently, the development of PTSD has also been linked to a dysfunctional HPA-axis activity (Bomyea, Risbrough, & Lang, 2012; Daskalakis et al., 2013; Mehta & Binder, 2012). Findings from meta-analyses show that lower cortisol levels are associated with an increase in posttraumatic stress symptomatology in adults (Meewissa, Reitsma, de Vries, Gersons, & Olff, 2007; Morris, Hellman, Abelson, & Rao, 2016; Morris, Compas, & Garber, 2012; Pan, Wang, Wu, Wen, & Liu, 2018), both in terms of a 24-hour cycle, as well as in response to a stressor (Daskalakis et al., 2013).

In response to a traumatic stressor, a blunted cortisol response has been linked to individuals who later develop PTSD (Anisman, Griffiths, Matheson, Ravindran, & Merali, 2001; Delahanty, Raimonde, & Spoonster, 2000; McFarlane, Atchison, & Yehuda, 1997). Findings of lower basal cortisol concentrations in patients with PTSD have been supported by studies investigating plasma (Boscarino, 1996; Olff et al., 2006), urinary (Baker et al., 1999; Glover & Poland, 2002; Yehuda et al., 1990) and salivary (Gill et al., 2008; Rohleder, Joksimovic, Wolf, & Kirschbaum, 2004; Wahbeh, & Oken, 2013; Wessa, Rohleder, Kirschbaum, & Flor, 2006) cortisol concentrations. Several researchers suggest that decreased cortisol in PTSD may be due to an enhanced negative feedback loop of the HPA-axis (Ehlert, Wagner, Heinrichs, & Heim, 1999; Newport & Nemeroff 2000; van der Kolk 1997; Yehuda, 2001), as discussed previously.

Furthermore, evidence also suggest that attenuated cortisol concentrations, as measured at baseline, have been associated with trauma-exposed individuals, regardless of PTSD diagnosis (Horn, Pietrzak, Corsi-Travali, & Neumeister, 2014; Morris et al., 2012). Similarly, trauma exposure in healthy individuals without PTSD have been related to blunted cortisol stress reactivity (Elzinga et al., 2008; Lovallo, Farag, Sorocco, Cohoon, & Vincent, 2012; Trickett, Gordis, Peckins, & Susman, 2014) and to an enhanced HPA feedback inhibition (de Kloet et al., 2007).

Several studies found a negative correlation between cortisol concentrations and severity of posttraumatic stress symptoms (Gill et al., 2008; Olff et al., 2006; Wessa et al., 2006; Witteveen et al., 2010). In the present study, we examined the relationship between cortisol and posttraumatic stress symptoms in the context of rape.

The processing of trauma-related memories has been suggested to be influenced by the effects of cortisol on the limbic and frontal brain (review: Ulrich-Lai, & Herman, 2009). Yehuda (2009) suggested that reduced cortisol concentrations may further create a neuroendocrine environment that leads to poor consolidation of traumatic memories. Deficits in cognitive functioning, such as impairments in memory, have been found in rape victims (Jenkins, Langlais, Delis, & Cohen, 1998). Furthermore, negative correlations have been found between PTSD symptom severity and immediate recall (Lindauer, Olff, van Meijel, Carlier, & Gersons, 2006). Intrusion symptoms of PTSD, such as intrusive trauma-related memories, have also been linked to reduced cortisol levels (Hauer et al., 2014; Holz, Lass-Hennemann, Streb, Pfaltz, & Michael., 2014). In the present study, we examined the relationship between cortisol and the following PTSD symptoms: re-experiencing/intrusion, avoidance/numbing, and hyperarousal (American Psychiatric Association [APA], 2013), as measured with the Davidson Trauma Scale (DTS) (Davidson et al., 1997).

Prolonged symptoms of re-experiencing, post trauma, could cause an increase in CRH concentrations, which can damage the hippocampus, an important area in the brain associated with learning and memory (Maren, 1999; van Voorhees, & Scarpa, 2004). A decrease in hippocampal volume has been associated with psychological trauma (Brunello et al., 2001). A decrease in the volume of the hippocampus, in addition to hypersensitivity of the HPA-axis, has been associated with decreased cognitive functioning (Bremner, & Vermetten, 2004).

Stress, such as sexual abuse, could result in abnormalities in the body's reaction to stress by rising CRH levels and cause dysregulation in the HPA-axis (Nutt, 2000). Decreased reaction to CRH leads to an overactivation of the HPA-axis and can disrupt the negative feedback by cortisol (Nutt, 2000).

2.4.2 DHEA and PTSD/PTSS

As discussed previously, DHEA is a steroid hormone that is produced primarily by the adrenal cortex in response to ACTH stimulation (Jones & Moller, 2011). In response to ACTH, the adrenal cortex releases DHEA from the zona reticulates (innermost layer of the adrenal cortex) and cortisol is released from the middle layer of the adrenal cortex (the zona fasciculata) (Maninger et al., 2009; Vythilingam et al., 2010). In reaction to stress, alterations in DHEA and DHEA-S may occur (Lemieux & Coe, 1995).

It has been suggested that DHEA could inhibit/block the effects of cortisol (Hu, Cardounel, Gursoy, Anderson, & Kalimi, 2000; Kalimi, Shafoagoj, Loria, Padgett, & Regelson, 1994) and other glucocorticoids on the hippocampus and peripheral tissues (Kaminska, Harris, Gijsbers, & Dubrosvsky, 2000; Kimonides, Khatibi, Svendsen, Sofroniew, & Herbert 1998). DHEA have anti-inflammatory, antioxidant (Chen & Parker, 2004; Russo, Murrough, Han, Charney, & Nestler, 2012) as well as anxiolytic effects (Prasad, Imamura, & Prasad, 1997), therefore increasing the body's resilience towards a stressor (Pfau & Russo, 2015).

Although very few studies have focused on DHEA/DHEA-s and its association with PTSD/PTSS, inconsistent findings regarding DHEA and its association with PTSD has been found. Increased DHEA and DHEA-S has been associated with chronic stress (Fuller, Hobson, Reyes, Winter, & Faiman, 1984) and PTSD participants compared to controls (Gill et al., 2008; Kellner et al., 2010; Olff et al., 2006 Yehuda, Brand, Golier, & Yang**,** 2006). Compared to controls, lower DHEA concentrations have been found in PTSD participants (Kanter et al., 2001), as well as no difference in DHEA levels between PTSD participants and controls (Bremner, Vermetten, & Kelley, 2007).

However, most studies confirm higher circulating levels of DHEA/DHEA-S in PTSD, and increased DHEA-to-cortisol ratio (Bremner, et al., 2007; Butterfield, et al., 2005; Pico-Alfonso et al., 2004; Rasmusson, et al., 2004; Spivak et al., 2000; Yehuda, 2009). Very few studies have found low levels of DHEA in PTSD (Kanter et al., 2001; Mulchahey et al., 2001).

Furthermore, Mouthaan et al. (2014) pointed out that the DHEA-to-cortisol ratio may be useful in predicting PTSD symptoms. More consistent results have been seen when researchers compared DHEA-S-to-cortisol ratios, pointing to higher levels in PTSD participants (Butterfield et al., 2005; Rasmusson et al., 2004; Yehuda et al., 2006). An increased ratio of DHEA-cortisol has been connected to the body's resilience associated with the development of PTSD symptom severity. Lipschitz et al. (2003) suggested that there is an increase in DHEA in response to ACTH that is associated with PTSD and negatively correlated with PTSD symptom severity. Furthermore, increased DHEA in PTSD was been associated with improvement in symptoms and increased coping, however, decreased DHEA-cortisol ratio has been connected to increased PTSD symptom severity (Yehuda, 2009; Yehuda et al., 2006). Allostatic load is increased when DHEA-S levels decrease, and cortisol is elevated (Maninger et al., 2009; McEwen, 2004).

When stress is prolonged, higher levels of DHEA/DHEA-S have been found (Fuller et al., 1984). Increased DHEA/DHEA-S has been found to be beneficial, instead of harmful to the body (Maninger et al., 2009). Increased levels of DHEA/DHEA-S has been connected to a decrease in PTSD symptomatology (Rasmusson et al., 2004), improvement in PTSS and improved coping (Yehuda, 2006), as well as a reduction in PTSS in reaction to therapy (Olff et al., 2007). DHEA and DHEA-S has been found to counteract the negative effects of cortisol, particularly in the hippocampus (Kalimi et al., 1994; Kaminska et al., 2000; Kimonides et al., 1998). In healthy individuals, improved coping with the negative effects of acute stress has been associated with a higher DHEA-S-to-cortisol ratio.

Confounding variables may contribute to the differences seen in the results of DHEA concentrations in PTSD patients, like those seen in studies of cortisol concentrations in PTSD patients. However, most confirm a higher DHEA-S-to-cortisol ratio in PTSD patients (Butterfield et al., 2005; Rasmusson et al., 2004; Yehuda et al., 2006). As mentioned earlier, instead of being harmful, higher ratios of DHEA-s-to-cortisol is suggested to be protective and contribute to the body's resilience against negative effects of circulating glucocorticoids. DHEA-S are thought to exhibit protective effects on the neuroendocrine system.

Studies on the potential of DHEA as a treatment option in PTSD have been limited. However, Sageman and Brown (2006) have found positive outcomes with the use of DHEA, using 7-keto DHEA (a metabolite of DHEA), because in this form it cannot be converted to estrogen or testosterone. They found improvements in PTSD avoidance, numbing and dissociation symptoms when they treated five women with PTSD with this metabolite of DHEA. This study is however limited, because the sample size was very small. However, these findings prove potential for future treatment. Treatment studies should consider the role of HPA-axis hormones in improving physical and psychological symptoms in reaction to a stressor.

2.4.3 Testosterone and PTSD/PTSS

Testosterone is also a steroid hormone and classified as an androgen. Testosterone plays an important role in the regulation of aggression, sexuality, cognition, and emotions (Rubinow & Schmidt, 1996). Dysregulation of HPA-axis can influence steroid hormones, namely estrogen and testosterone, also controlled by the HPA-axis.

In females, the middle layer of the adrenal cortex (the zona fasciculata) and the ovaries produce testosterone and its forerunner, androstenedione, both accounting for approximately 50% of the total secretion of testosterone (Tyagi et al., 2017). The rest of testosterone secretion takes place in peripheral cells, such as bone, muscle, fat, and breasts (Burger, 2002). More specifically, the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland is stimulated by gonadotropin releasing hormone (GnRH) from the hypothalamus. Testosterone production by the ovaries, are stimulated by LH. Testosterone then inhibits the release of GnRH from the hypothalamus and LH from the pituitary, in a negative feedback loop (Schally et al., 1971). Testosterone as therapy options have been suggested in women, however there are greater uncertainties in women compared to therapy in men (Tyagi et al., 2017).

Lower urinary testosterone has been found in soldiers anticipating combat in Vietnam compared to controls (Rose et al., 1969), as well as lower plasma testosterone levels in soldiers in a training program (Kreux, Rose, & Jennings, 1972). Testosterone levels may be suppressed by physical or psychological stressors (Francis, 1981; Kreux et al., 1972; Rose et al., 1969). Elevated basal testosterone levels have been found in combat veterans with PTSD compared to individuals with MDD and controls (Mason, Giller, Kosten, & Wahby., 1990). No differences have also been found (Mulchahey et al., 2001). Spivak, Maayan, Mester, and Weizman (2003) was the first to examine testosterone and cortisol levels in individuals with combat related PTSD (CR-PTSD) who had not been treated previously. The authors found no difference in morning plasma testosterone levels between CR-PTSD and controls. A significant correlation was found between avoidance symptoms and plasma testosterone levels in CR-PTSD (Spivak et al., 2003). No studies were found that examined the relationship between testosterone and PTSD/PTSS in females.

2.4.5 Progesterone and PTSD/PTSS

Inslicht et al. (2014) suggested that progesterone may mediate glucocorticoid reactivity in PTSD. No further studies were found to examine progesterone and its relationship to PTSS and/or PTSD.

Steroid hormones, such as testosterone, cortisone, progesterone and DHEA may be important when researching neuroendocrine interactions in the context of trauma and PTSD (Gao et al., 2013; Inslicht et al., 2014). The present study investigated the potential role of

cortisol and these hormones (testosterone, cortisone, progesterone and DHEA) with posttraumatic stress symptoms in women who have been raped.

2.5 Review of hormones in the context of rape and PTSD/PTSS

It has been suggested that PTSD risk may vary according to trauma type and that traumas, including interpersonal violence, may contribute to the highest risk for PTSD (Caramanica, Brackbill, Stellman, & Farfel, 2015). According to the World Mental Health (WMH) surveys, Kessler et al. (2017) reported that globally, a form of interpersonal violence, namely intimate partner sexual violence, which includes rape and sexual assault, has been associated with the highest risk for PTSD (Kessler et al., 2017).

Due to the limited literature available on women with PTSD/PTSS who were victims of rape and the association between PTSS/PTSD and hair hormone concentrations in the context of rape, a broader literature review was performed. A literature review to synthesise the evidence obtained from studies of PTSD in adult women due to interpersonal violence that assessed short-term (urinary/plasma/saliva) cortisol, testosterone, DHEA, or progesterone levels, with the aim of determining the relationship between cortisol and other neurosteroid hormones and PTSD/PTSS, was performed.

2.5.1 Short-term release of cortisol and PTSD in adult women due to interpersonal violence

Most studies of PTSD due to interpersonal violence included in the following mini review examined salivary cortisol levels in females (Altemus, Cloitre, & Dhabhar, 2003; Brand et al., 2010; Cordero et al., 2017; Inslicht et al., 2006; Johnson et al., 2008; Martinson, Craner, & Sigmon, 2016; Pico-Alfonso et al., 2004; Schechter et al., 2004; Young, Tolman, Witkowski, & Kaplan, 2003). Two studies examined urinary cortisol levels. Both were studies of females who experienced interpersonal violence and subsequently developed PTSD (Lemieux & Coe, 1995; Lemieux, Coe, & Carnes 2008). See Table 2.1 for a summary of these studies.

TABLE 2.1 Studies on short-term release of cortisol and DHEA and PTSD in adult women due to interpersonal violence

Name	Participants (n)	Design	M/F	Age Mean(SD) years	Method of collection	Single/Repeated measure	Trauma Type	Time since trauma	Measured Neurosteroids	Results	Main findings
CORTISOL AND PTSD DUE TO INTERPERSONAL VIOLENCE											
Intimate Partner Violence											
Salivary cortisol concentrations											
Johnson, Delahanty, & Pinna (2008)	52 survivors of IPV staying in shelters (32 with PTSD & 20 without PTSD)	Longitudinal	All Female	34.29 (9.50)	Saliva collection. Using the Salivette sampling device, four saliva samples were collected in the first hour after waking to estimate participants' cortisol response to awakening. The first sample was collected immediately upon waking. The latter three samples were taken at 30, 45, and 60 min after waking. Samples were processed using immunoassay with fluorescence detection. **PTSD**: Structured Clinical Interview for the DSM-IV nonpatient version	Repeated (4 measurements)	Intimate partner violence	Not stated	Cortisol	No measurements available	IPV-related PTSD and abuse chronicity have opposite effects on waking salivary cortisol curves in battered women. PTSD severity was associated with significantly greater cortisol output the first hour after awakening. More chronic abuse was associated with lower total cortisol output in the first hour after awakening
Cordero et al. (2017)	36 mothers @ home: (18 survivors of IPV with PTSD, & 18 control mothers) 45 mothers @ lab: (27 survivors of IPV with PTSD. & 18 control mothers)	Longitudinal (over 24hours)	all female	45 Mothers: IPV-PTSD group: 34(1.3) Control group 35(14)	Salivary cortisol @ lab and @ home: 30 min after waking up, between 2–3 pm and at bedtime @ Lab: Four saliva samples for the assessment of cortisol were collected from the mother and the child: baseline (before the procedure begin), immediately after the end of the stress situation (T0), and at 30 (T30) and 60 (T60) min after the end of the experimental condition and during the recovery phase	Repeated (7 measurements)	Intimate partner violence	Not stated	cortisol	**Measurement (units):** nmol/L Measurements not stated	**Home:** Compared to non-PTSD controls, IPV-PTSD mothers — but not their toddlers, had lower morning cortisol and higher bedtime cortisol. **Lab:** IPV-PTSD mothers and their children showed blunted cortisol reactivity to the laboratory stressor. IPV-PTSD mothers showed significantly lower cortisol levels during a laboratory stressor than non-PTSD controls.

Inslicht et al. (2006)	49 survivors of IPV (15 current PTSD, 14 remitted PTSD & 20 no PTSD)	Cross-sectional	All Female	>18yrs	Saliva: Collected at 1, 4, 9, & 11h after awakening PTSD: Structured Clinical Interview for the DSM-IV nonpatient version.	Repeated (4 measurements)	Intimate partner violence	Ongoing/prior IPV	Cortisol	**Measurement units: mg/Dl** **PTSD group (mean/SD):** 1 h after awakening (SD) 489.77 (405.80) 4 h after awakening (SD) 186.27 (116.80) 9 h after awakening (SD) 413.43 (978.14) 11 h after awakening (SD) 290.36 (371.20) **No PTSD Group (mean/SD):** 1 h after awakening (SD) 398.61 (314.79) 4 h after awakening (SD) 136.44 (79.71) 9 h after awakening (SD) 180.89 (387.96) 11 h after awakening (SD) 154.18 (195.09)	Abused women with lifetime PTSD had significantly higher cortisol levels throughout the day compared to abuse-exposed participants without PTSD.
Pico-Alfonso et al. (2004)	162 women from the local community (70 survivors of physical IPV, 46 survivors of psychological IPV, & 46 non-abused controls)	Longitudinal, observational study	All Female	physically abused: 44.77(10.76) psycho-logically abused: 45.39(10.09) Control group 47.57(12.79)	Salivary cortisol & DHEA measured at 8am & 8pm during 4 consecutive days.	Repeated (8 measurements)	Physical and psychological abuse due to intimate partner violence	Past 12 months	Cortisol & DHEA	Not stated	Physically abused women had significantly higher evening levels of cortisol than non-abused. DHEA: Morning DHEA was higher in psychologically abused women than non-abused. Evening DHEA sig. higher in both abused groups compared to non-abused group. No correlation between total PTSD score and hormonal levels.
Plasma cortisol concentrations											
Seedat et al. (2003)	38 participants from the local community (10 survivors of IPV with PTSD, 12 survivors of IPV without PTSD, & 16 non-abused controls).	Cross-sectional	All Female	IPV group: 35.6 (9.6) Control group 40.9 (9.9)	Morning plasma samples were collected. Plasma samples were collected between 09h00 and 12h00. Plasma cortisol concentrations were determined using radioimmunoassay kits. **PTSD:** Structured Clinical Interview for DSM-IV Disorders	Single	Inter-partner physical or sexual abuse	Participants needed to be out of the abusive relationship at least 4 months and no longer than 2 years.	Cortisol	**Measurement units**: µg/dL **Cortisol (SD):** **IPV Controls p** 10.5 (3.1) 13.4 (4.6) <0.034* **IPV with PTSD IPV without PTSD** 10.3 (3.1) 10.6 (3.2)	Mean cortisol levels were significantly lower in IPV subjects compared with controls. No sig. difference between IPV groups with and without PTSD. No sig. relationship was found between cortisol and PTSD symptoms. No sig. difference in the severity of IPV between the PTSD group and the no PTSD group.
Childhood Trauma											
Salivary cortisol concentrations											

Schechter et al. (2004)	41 IPV mothers (24 PTSD)	Cross-sectional	All female	30	Maternal salivary cortisol before & 30 min after a videotaped play paradigm with their children, involving 2 separations and reunions and cortisol reactivity 30min after separation stress. **PTSD:** Structured Clinical Interview for the DSM–IV PTSD Module and Symptom Checklist-Short Version.	Repeated (3 measurements)	Interpersonal violence in childhood or adulthood. Interpersonal violence: physical and sexual abuse, and domestic violence.	lifelong	Cortisol	Not stated	Baseline salivary cortisol levels were significantly negatively correlated with childhood interpersonal violence trauma severity (i.e. trauma severity prior to age 16). Cortisol reactivity was not sig. correlated with interpersonal trauma severity. Baseline salivary cortisol values were negatively correlated with severity of current PTSS and with dissociative symptoms. Direct relationship between cortisol reactivity and PTSD symptoms (SCID). (Mothers with higher PTSD scores = largest increase cortisol post stressor).
Brand et al. (2010)	126 mothers with MDD (15 IPV with PTSD, 38 IPV without PTSD).	Cross-sectional	Moms: All female;	34 (4)	Salivary cortisol. Mother saliva (T0-baseline) were obtained. Next, the mother completed a series of questionnaires, while adjacent to her, the infant was held by a research assistant. After a 20-minute period, the second saliva sample (T1-post-separation stressor) was obtained from both the mother and infant. Saliva samples were taken from the mother and the infant immediately after the lab stressor tasks were completed (T2-post-noise/arm stressor I), and again 20 min later (T3-post-noise/arm stressor II). Three post-stressor cortisol measures (T1, T2, T3) were taken in post-stressor time windows when cortisol levels typically increase (5—40 min).	Repeated (4 measurements)	Childhood Sexual or physical Abuse	Not stated	Cortisol	Not stated	No significant differences in baseline cortisol levels in mothers with a history of childhood abuse, however in response to the lab stressor, lower cortisol concentrations were found in mothers with a history of childhood abuse. In those mothers with childhood experiences of trauma who experienced additional stress, such as maternal PTSD, current MDD and experienced a recent stressful event, cortisol levels were increased.
Altemus et al. (2003)	31 women (16 IPV with PTSD & 15 controls)	Cross-sectional (Brief report)	All Female	IPV/PTSD: 34(8) Controls: 35(9	Circadian salivary cortisol levels every 3 hours between 8am-11pm, and a single time point measurement of plasma cortisol **PTSD**: Structured Clinical Interview for DSM-IV (SCID) and the Clinician- administered PTSD Scale	Repeated (5 saliva samples & 1 blood collection)	Childhood physical or sexual abuse	Age of trauma: <18years	cortisol	**Measurement (units)**: nmol/L Plasma levels: PTSD group (mean/SD): mean=217 nmol/liter (SD=60), No PTSD Group (mean/SD): 293 nmol/liter (SD=171)	Cortisol measures did not significantly differ between PTSD and healthy comparison subjects.

Muhtz et al. (2011)	50 refugees (25 with chronic PTSD, 25 trauma-controlled, without PTSD) Born between 1933 and 1940, were displaced as children (5-12years) during/after WWII	Cross-sectional	64% F	71	Cortisol & DHEA were measured using low-dose-dexamethasone suppression test. **Plasma cortisol & DHEA** **DAY 1** 08H00 Baseline blood sample (BL) 23h00 0.5 mg DEX was administered orally **DAY 2** 08h0 Post DEX blood sample (Post DEX) **Salivary cortisol** Salivette salivary collection device - Collected on day 1 only T1: 08h00; T2: 12h00; T3: 16h00; T4: 20h00 **PTSD:** Posttraumatic Diagnostic Scale	Repeated (6 measurements).	Childhood trauma: Events from being child refugees (e.g. rape)	6 decades ago (trauma between ages 5-12 years)	Cortisol & DHEA	**Measurement units:** plasma cortisol (µg/l); DHEA (ng/ml); salivary cortisol (ng/ml) **Variables PTSD Non-PTSD p** cortisol (day 1) 251.4 (17.6) 251.4(14.5) 0.87 cortisol (day 2) 159.2 (18.7) 170.4 (15.7) 0.68 DHEA 5.24 (0.6) 5.15 (1.0) 0.95 Salivary cortisol* 8:00 a.m. 6.18 (0.7) 5.65 (0.5) 12:00 p.m. 2.9 (0.4) 2.58 (0.2) 4:00 p.m. 2.1 (0.3) 1.8 (0.2) 8:00 p.m. 0.9 (0.2) 0.9 (0.3) *Test of linear trend: F(1,43) = 164.8; p G 0.0001	No significant results regarding hormones.
Young et al. (2018)	266 Gulf veterans	Longitudinal	14% Female	44.45 (9.5) yrs	Eight saliva samples: Day one: 1, 30, 45 and 60-minute increments with sample collection occurring upon the awakening. 0.5 mg of dexamethasone 15 hours after awakening on Day one A second set of saliva samples were collected upon the awakening on Day two using the same incremental pattern described for day one. **PTSD:** CAPS	Repeated (8 measurements)	Childhood abuse	Not stated	Cortisol	Not stated	No relationship was observed between child abuse and basal pre-dexamethasone cortisol levels or any significant main effects of child abuse on percent cortisol suppression after taking dexamethasone.
Plasma cortisol concentrations											
Stein et al. (1997)	40 women from community clinics (19 with IPV & 21 non-abused controls). 13 of 19 with CSA had PTSD.	Cross-sectional	all Female	IPV group 32.2(6.7) Control group 30.8(6.8)	50 mL of whole blood. Plasma samples for cortisol were collected on ice, spun, aliquoted, and stored at 270°C for subsequent assay. At 11 PM subjects took oral doses of 0.50 mg of dexamethasone. Blood samples for cortisol and dexamethasone levels were obtained at 8 AM the following morning. **PTSD:** Clinician-Administered PTSD Scale (CAPS)	Repeated (2 measurements)	Childhood Sexual Abuse	Age of trauma: <18years	Cortisol	**Measurement units:** nmol/L (cortisol) & ng/ml (dexamethasone) nCSA (n=21) CSA (n=19) PTSD (n=13) 8 AM baseline cortisol (nmol/L): Mean (SD): 450 (160) 392 (124) 367 (105) 8 AM postdex cortisol (nmol/L): 89 (92) 46 (38) 40 (40)	The abused women had significantly lower post-DEX cortisol levels compared to the non-abused group. The abused women with PTSD also had significantly lower post-DEX cortisol levels compared to the non-abused women.

Bremner et al. (2007)	43 participants (19 with IPV & PTSD; 11 IPV without PTSD; 13 without PTSD or IPV)	Cross-sectional	All Female	>18yrs	Intravenous (plasma) catheter over 24-hour period Cortisol: 7-10pm, 12-8pm, 8pm-4am, 4am-12pm. DHEA were available for n=34 (13ctrl, 12 abuse + PTSD, 9 abuse only). PTSD: Structured Clinical Interview for DSM-IV (SCID)	Repeated (4 measurements)	Childhood Sexual Abuse (CSA)	Onset of abuse between 4 and 13 years (mean 7)	Cortisol, DHEA	**Measurement units:** microg/dL **PTSD group (mean/SD):** **Cortisol** **DHEA** 12-8pm: 62.5 (21); 4am-2pm: 91 (22.9) 8pm-4am: 35.8 (15.1) 7-10pm 14.5 (7.4) AUC 24h: 184.5 (54.8) 184.1 (85.6) **No PTSD Group (mean/SD):** **Abuse only:** 12-8pm: 92.8 (42); 4am-2pm: 103.5 (37.8) 8pm-4am: 32.6 (17.3) 7-10pm 18.6 (8.1) AUC 24h: 229.0 (90.2) 150.3 (87.9) Controls: 12-8pm: 85.0 (38.5); 4am-2pm: 94.3 (39.4) 8pm-4am: 40.2 (32.8) 7-10pm 20.5 (13.1) AUC 24h: 219.5 (96.3) 123.7 (56.9)	Abused PTSD group had lower concentrations of cortisol during the afternoon hours (12–8 p.m.) compared with women with abuse without PTSD and control group. Cortisol levels were negatively correlated with PTSD symptom level and severity of abuse - cortisol not related dissociative state/ depression. - Therefore reduction in cortisol is specific to trauma and PTSD There were no significant differences between groups in DHEA-S or estradiol. DHEAS higher but NOT significant.
Newport et al. (2004)	64 participants 15 IPV and no MDD 20 IPV/PTSD, 10 MDD only. . 19 Controls ,	Longitudinal	All Female	IPV/NO PTSD: 33.6(6.2); IPV/PTSD: 32.4(7.6); MDD only 33.9(8.3) CTRL: 28.5(6.9);	**PTSD** Structured Clinical Interview for DSM-IV (SCID) **Plasma cortisol & ACTH** Day 1 08H00 Baseline blood sample 23h00 1mg DEX was administered orally Day 2 08H00 Post DEX 1 16H00 Post DEX 2 #ONE WEEK LATER# Day 3 08H00 Baseline blood sample 23h00 0.5 mg DEX was administered orally Day 4 08H00 Post DEX 1 16H00 Post DEX 2	Repeated (6 measurements)	Childhood Abuse (sexual or physical)	Abuse before age 14 years	cortisol	**Measurement (units):** µg/dL CONTROL ELS/NO PTSD ELS/PTSD MDD Cortisol (baseline): 20.7 (8.1) 14.5 (4.0) 15.5 (5.9) 16.7 (9.2) Cortisol (post-Dex 8AM): 4.6 (3.7) 4.9 (6.6) 1.3 (.6) 3.0 (2.6) Ratio: 8AM/baseline: .28 (.29) .31 (.38) .09 (.04) .15 (.08) Cortisol (post-Dex 4PM): 4.5 (3.4) 2.9 (3.2) 2.4 (2.6) 3.6 (2.5) Cortisol (post-Dex 4PM): 4.5 (3.4) 2.9 (3.2) 2.4 (2.6) 3.6 (2.5) Ratio: 4 PM/baseline: .23 (.15) .20 (.17) .17 (.19) .20 (.15)	Significant differences in rates of cortisol super suppression between subject groups with 90.0% (18 of 20) of abuse survivors with PTSD and 66.7% (10 of 15) of abuse survivors without PTSD. Post hoc analysis from the low-dose DST revealed that all post-DEX cortisol measures were significantly lower among abuse survivors with PTSD than healthy volunteer
Urinary cortisol concentrations											
Lemieux & Coe (1995)	28 women (11 IPV with PTSD 8 IPV without PTSD & 9 non-abused controls).	Cross-sectional	All Female	35.3(6.3)	24h Urine samples collection - urine split into 2. U1: Baseline urine U2: Creatine corrected Urinary-free cortisol levels were determined with an iodinated (I25I) radioimmunoassay kit	Single	Childhood Sexual Abuse	Age of trauma: <18years	Cortisol	**Measurement units**: µg/dL Mean(SD): PTSD+ PTSD- CTRL 111.8 (55.8) 83.1 (28.9) 87.8 (21.2) After creatine correction: 5.93 (.93)* 3.13 (0.29) 3.2 (0.3) * Sig. difference between PTSD+ & ctrl groups	The correct values indicated that the women with PTSD, who experienced childhood sexual abuse, had significantly elevated daily levels of norepinephrine, epinephrine, dopamine, and cortisol.
Lemieux et al. (2008)	36 women (11 IPV with PTSD, 13 IPV without PTSD, & 12 controls)	Cross-sectional	All female	CTRL 31.17(1.7), range 20-40yrs. IPV/PTSD 28.2(2.1); range 18-40yrs IPV/ no PTSD 31.3(1.8); range 21-42yrs	24h urine samples for analysis of cortisol. **PTSD:** Impact of Events Scale (IES) to delineate PTSD symptoms	Single	Childhood Abuse	longer than 12 months ago	Cortisol	**Measurement: (units)** µg/dL CTRL PTSD- PTSD+ Cortisol µg /day (n = 35)*: 82.2 (34.4) 48.8 (17.1) 22.2 (9.8) * Data presented as total daily excretion not corrected by creatinine.	No significant difference in cortisol levels between women with PTSD and a history of sexual abuse, women with no PTSD but no history of sexual abuse, and women with no PTSD or sexual abuse.
Adult sexual assault victims											

Salivary cortisol concentrations											
Martinson et al. (2016)	50 women : (26 with history of sexual trauma with and without PTSD & 24 control)	Longitudinal (measurements over +- 60 min)	all female	IPV: 21.38 (7.59); Control groups 18.92 (1.14) All 50 participants: >18yrs 19.7(4.19)	Salivary cortisol samples were collected with Salivette Cortisol with a synthetic swab from Sarstedt, Inc. Participants completed the POMS-A, POMS-D, IOS, and provided a saliva sample following each set of discussion slips (i.e., after 15 min into the task, 30 min into the task, and 45 min into the task). **PTSD:** PTSD checklist—civilian version (PCL-C)	Repeated (5 measurements)	Sexual Trauma	Mean age & SD: First sexual trauma 12.77 (5.12) Second sexual trauma 15.54 (2.70)	Cortisol	No PTSD measurements available **Measurement (units):** nmol/L Cortisol analyses with sexual trauma CTRLs Baseline (-5 min): 8.76 (5.07) 12.09 (9.03) 15 min into task (+15 min): 8.71 (4.61) 14.89 (13.51) 30 min into task (+30 min): 7.42 (3.61) 13.51 (12.35) 45 min into task (+45 min): 6.70 (3.24) 12.54 (11.05) After 15 min relaxation (+60 min): 6.10 (2.92) 10.09 (8.37)	Women with a history of sexual trauma exhibited a blunted cortisol response and greater anxious mood in reaction to the intimacy induction task compared to controls. Results also demonstrated that, unexpectedly, PTSD symptom severity scores among sexual trauma survivors were not associated with differential cortisol responding to the task compared to controls.
Blood plasma and serum cortisol concentrations											
Walsh et al. (2013)	235 IPV (medical rape examination)	Cross-sectional	All Female	Mean: 26.3years (15-71 yrs)	Serum cortisol at the time of a post sexual assault medical exam. Blood samples were collected in an untreated vacutainer tube (10 ml) by routine venipuncture.	Single	Sexual Assault	Victims of sexual assault within previous 72h	Cortisol	Measurements not available	Post assault cortisol level was not related to PTSD symptoms 6, 12 or 24 weeks post assault. Inverse relationship between prior assault and serum cortisol. Prior history of assault was positively related to PTSD 12 weeks after assault
Yehuda et al. (1998)	20 IPV	Cross-sectional	All Female	18-51 yrs Mean(SD) yrs: 29.9 (9.4)	Plasma (blood) samples 27-157 days post rape. **PTSD:** Foa PTSD scale	Single	Rape	27-157 days post rape	Cortisol	**Measurement units**: µg/100mL PTSD group (mean): 18.7 No PTSD Group(mean): 25.6	Women with a history of prior physical or sexual assault showed a significantly attenuated cortisol response to the acute stress of rape compared to women without such a history. Plasma cortisol response to the rape was not associated with rape characteristics (i.e., occurrences immediately before the hormonal responses to the index rape), or symptom severity following the rape in this sample. Cortisol response post rape was not associated with the subsequent diagnosis of PTSD. PTSD status at the 3-month follow-up was predicted by both a prior history of assault and high injury rape, but was not directly predicted by cortisol.
Interpersonal trauma – not specified & other											
Salivary cortisol concentrations											
Heim et al. (2009)	113 with Chronic fatigue syndrome (22 likely to have PTSD) & 124 controls	Cross-sectional	78.1% Female	range: 18-58yrs	Salivary cortisol response to awakening (upon awakening, 30min, 45min, and 60 min after awakening). **PTSD**: The Davidson PTSD Scale	Repeated (4 measurements)	Not specified	Not stated	Cortisol	Not stated	30min after waking, PTSD symptoms were negatively correlated to cortisol levels. The PTSD and non-PTSD participants did not differ in terms of cortisol levels upon waking, 45 and 60 minutes after waking. Additionally, cortisol levels 30min after waking were negatively correlated with emotional neglect, CTQ total, and PTSD symptom scores (all p<.05). They also confirm findings that childhood abuse predicts PTSD symptoms in chronically fatigued individuals.

Young et al. (2003)	171 women with IPV (72 no PTSD, 29 recent PTSD, & 70 past PTSD)	Longitudinal (24hours)	All Female	range: 18-54 yrs	Saliva cortisol was collected at awakening, 30 minutes later, at bedtime, and during a clinic visit.	Repeated (4 measurements)	Interpersonal trauma (not specified)	12 months or in adulthood or in childhood	Cortisol	Measurement: (units) (µg/dL) Table 1. Mean Cortisol _ Standard Error (_g/dL) by Diagnostic Group Awakening AM Bedtime Clinic Never Exposed n=16: (.39+-.05) (.43+-.08) (.15+-.05) (.31+-.07) Exposed, Never PTSD n=72: (.47+-.03) (.57+-.04) (.18+-.02) (.34+-.03) Recent PTSD n=29 (45+-.05) (.50+-.05) (.26+-.07) (.35+-.04) Past PTSD n=70 . (43+-.04) (.48+-.03) (.25+-.07) (.33+-.04) Recent PTSD, No MDD n=17 (.46+-.07) (.49+-.07) (.17+-.05) (.35+-.06) Recent PTSD, Recent MDD n=12 (.44+-.08) (.51+-.12) (.38+-14) (.35_.05) Past PTSD, No MDD n=56: (.44+-.04) (.48+-.04) (.18+-.03*) (.39_.04) Past PTSD, MDD n=14 (.41+-.10) (.48+-.06) (.21+-.06) (.45+-.11) *Sig. difference (p<.05)	Exposure to trauma had no significant effect on saliva cortisol. Women with recent trauma (past 12 months) had significantly higher cortisol levels than those not recently traumatised (n=117)
van der Hal-Van Raalte et al. (2008)	133 IPV (38 with PTSD, 95 without PTSD)	Non-convenience sample, cross-sectional	61% Female	65	Three saliva samples for basal cortisol measurements during a normal day: upon awakening, before lunch and before dinner. Stressful task: Questionnaire with questions about their Holocaust survival experiences and exposure to other shocking life events, ST20: 20 min after the start ST40: 40 min after the start ST60: 60 min after the start Post stress: After a resting period of 40 min PTSD PTSD functional impairment was assessed by means of the Posttraumatic stress diagnostic scale (PDS	Repeated (7 measurements)	Survivors of Holocaust	Born between 1935 and 1944. Aged from several months to 10 years at the end of the Second World War in 1945	Cortisol	Measurement unit not available. **PTSD group (mean/SD/N):** ***(functional impairment)*** **Cortisol** Morning 0.89 (0.34) 36 Noon 0.53 (0.19) 36 Afternoon 0.25 (0.27) 36 ST20 – 0.56 (1.07) 31 ST40: –0.65 (1.16) 34 ST60: –0.69 (1.17) 33 **No PTSD Group (mean/SD/N):** **Cortisol** Morning 0.87 (0.30) 86 Noon 0.47 (0.26) 84 Afternoon 0.18 (0.29) 92 ST20: – 0.60 (1.06) 73 ST40: –0.57 (1.02) 74 ST60: – 0.58 (1.00) 67	PTSD was not associated with differences in basal diurnal cortisol levels. There were no significant difference in post-stressor cortisol levels between groups.
see Heim et al. (2009) above											
DHEA/DHEA-S AND PTSD DUE TO INTERPERSONAL VIOLENCE											
Intimate Partner Violence											
see Pico-Alfonso et al. (2004) above											
Childhood Sexual Abuse											
see Bremner et al. (2007) above											

2.5.1.1 Intimate partner violence

2.5.1.1.1 Salivary cortisol concentrations

Significantly lower levels of salivary cortisol were associated with more chronic abuse during the first hour after awakening (Johnson et al., 2008). In a study by Cordero et al. (2017), lower salivary morning cortisol levels were observed in a group of females who experienced intimate partner violence and developed PTSD (IPV-PTSD) compared to controls. In response to a laboratory stressor, significantly lower salivary cortisol levels were observed in the IPV-PTSD group compared to the non-PTSD group (Cordero et al., 2017).

Significantly higher cortisol levels were measured after the first hour after awakening in a group of women diagnosed with PTSD and who experienced intimate partner violence compared to abused women without PTSD diagnosis (Johnson et al., 2008). Johnson et al., (2008) also found that the severity of PTSD was associated with higher cortisol levels during the first hour after awakening. Inslicht et al. (2006) found that women who experienced intimate partner violence with lifetime PTSD had significantly higher levels of cortisol levels compared to those who were exposed to abuse but did not go on to develop PTSD. Cortisol levels were markedly higher nine hours after awakening (Inslicht et al., 2006).

Night-time cortisol levels were significantly higher in women who experienced physical intimate partner violence compared to non-abused females (Pico-Alfonso et al., 2004). In a study by Cordero et al. (2017), higher evening cortisol levels were observed in a group of females who experienced intimate partner violence and developed PTSD (IPV-PTSD) compared to controls.

No correlation between hormone concentrations (measured at 08h00 and 20h00 over a period of four days) and PTSD were established in a study by Pico-Alfonso et al. (2004). In response to a laboratory stressor, a blunted cortisol response was observed in the IPV-PTSD group compared to controls (Cordero et al., 2017).

2.5.1.1.2 Plasma cortisol concentrations

Significantly lower morning plasma cortisol levels were found in a group of females traumatised by intimate partner physical/sexual abuse with/without PTSD compared to healthy controls (Seedat, Stein, Kennedy, & Hauger, 2003). There was no significant difference between abuse groups with and without PTSD and no relationship was also found between PTSS and cortisol levels (Seedat et al., 2003).

2.5.1.2 Childhood trauma

2.5.1.2.1 Salivary cortisol levels

In a study of mothers who experienced interpersonal violence in childhood or adulthood (physical and sexual abuse, and domestic violence), salivary cortisol levels were significantly negatively correlated with current PTSS severity at baseline (before videotaped play with their children) (Schechter et al., 2004). Maternal salivary cortisol was measured before and 30 min after a videotaped play paradigm with their children, involving two separations and reunions and cortisol were again measured 30min after separation stress (Schechter et al., 2004). In response to a laboratory stressor, females with a history of childhood abuse had lower cortisol concentrations (Brand et al., 2010).

In mothers who experienced childhood physical or sexual abuse and were diagnosed with MDD and comorbid PTSD, as well as experiencing a recent stressful event, levels were increased in response to a stressor. (Brand et al., 2010).

Altemus et al. (2003) found no significant difference in morning salivary cortisol levels (samples taken every three hours from 08h00-23h00) between females with PTSD, who experienced childhood sexual or physical abuse, compared to healthy controls (non-abused and without psychiatric illness). No significant difference in baseline (before a laboratory stressor) salivary cortisol concentrations in females with childhood trauma and those without childhood trauma were observed (Brand et al., 2010). No significant results with regard to daytime (measured at 08h00, 12h00, 16h00 and 20h00) salivary cortisol concentrations were found in male and female adults traumatised as children by events from being refugees, e.g. rape, with and without PTSD (Muhtz et al., 2011). In this study, 64% of participants were female (Muhtz et al., 2011).

No association was found between PTSD and basal diurnal (daytime) salivary cortisol levels in gulf veterans who experienced childhood abuse (Young, Inslicht, Metzler, Neylan, & Ross, 2018). Of these, only 14% were women (Young et al., 2018).

2.5.1.2.2 Plasma cortisol concentrations

In a group of females who experienced childhood sexual abuse (CSA) and were diagnosed with PTSD, significantly lower plasma cortisol levels were measured compared to controls (no CSA and no PTSD) after taking dexamethasone (DEX) (Stein, Yehuda, Koverola, & Hanna, 1997).

Significantly lower levels of afternoon (12pm-8pm) plasma cortisol levels were measured in women exposed to CSA, who developed PTSD, compared to those exposed to CSA, who did not develop PSTD, and controls (non-exposed without PTSD) (Bremner et al., 2007). PTSS and abuse severity were also associated with lower cortisol levels (Bremner et al., 2007).

A significantly lower post-DEX cortisol plasma levels were measured in female victims of childhood trauma diagnosed with MDD comorbid PTSD compared to a control group (no childhood abuse history and no MDD/PTSD) (Newport, Heim, Bonsall, Miller, & Nemeroff, 2004).

2.5.1.2.3 Urinary cortisol concentrations

In the study by Lemieux and Coe (1995), significantly higher daytime urinary cortisol levels were measured in female participants who experienced CSA and were diagnosed with PTSD compared to non-abused participants and those with CSA without PTSD, respectively. Women who experienced CSA, but not diagnosed with PTSD, had the lowest levels of cortisol (Lemieux & Coe, 1995).

In another study, no significant difference in urinary cortisol levels were found between females who experienced childhood abuse and developed PTSD, those who experienced childhood abuse and did not develop PTSD, and controls (non-abuse without PTSD) (Lemieux et al., 2008).

2.5.1.3 Adult sexual assault victims

2.5.1.3.1 Salivary cortisol concentrations

In women with a history of sexual abuse, compared to controls (non-abused), a blunted cortisol response was observed in reaction to an intimacy induction test and no significant relationship between PTSS severity and cortisol levels in response to the abovementioned task were found (Martinson et al., 2016).

2.5.1.3.2 Blood plasma and serum cortisol concentrations

In a group of female sexual abuse victims, lower serum plasma cortisol levels (measured within 72 hours of the assault) and previous assault were associated with higher initial PTSS that decreased at a slower rate compared to those victims with higher cortisol levels without

previous assault (Walsh et al., 2013). In women with a history of prior physical or sexual abuse, the experience of rape was associated with an attenuated cortisol response (concentrations measured from blood plasma) compared to those without a history of abuse (Yehuda et al., 1998).

In a study by Yehuda et al. (1998) of female rape victims, cortisol did not predict PTSD diagnosis and plasma cortisol levels were not associated with symptom severity.

2.5.1.4 Interpersonal trauma – not specified

2.5.1.4.1 Salivary cortisol concentrations

Emotional neglect (measured by Childhood Trauma Questionnaire [CTQ]), CTQ total, and PTSS were significantly negatively correlated with salivary cortisol concentrations taken 30 minutes after awakening in a group (78.1% females) with Chronic Fatigue Syndrome (CFS) and comorbid PTSD diagnosis (Heim et al., 2009).

Young et al. (2003) found that in a group of women exposed to trauma (interpersonal trauma not specified), significantly higher levels of cortisol were measured in those who recently (past 12 months) experienced a traumatic event compared to those who were not recently exposed to trauma. Saliva samples were taken at awakening, 30 minutes later, bedtime and during a clinic visit (Young et al., 2003).

No significant relationship was established between trauma exposure and cortisol concentrations in a group of females (Young et al. 2003). No association was found between PTSD and basal diurnal cortisol levels (diurnal slope refers to levels from morning to evening) in a group of Holocaust child survivors (mean age 65 years at time of study of whom 61% were female) (van der Hal-van Raalte, Bakermans-Kranenburg, & van Ijzendoorn, 2008). In the study by Heim et al. (2009), of whom 78.1% females, the CFS with co-morbid PTSD and non-PTSD participants did not differ in terms of salivary cortisol levels upon waking, 45 and 60 minutes after waking.

2.5.2 Short-term release of DHEA/DHEA-S and PTSD due to interpersonal violence

One study was found that examined salivary DHEA levels in participants who experienced intimate partner violence (Pico-Alfonso et al., 2004) and one study measured these hormone concentrations in plasma among participants who experienced CSA (Bremner et al., 2007).

2.5.2.1 Intimate partner violence

Significantly higher morning and evening salivary DHEA concentrations (as measured over four consecutive days at 08:00 and 20:00) were measured in women who experienced psychological abuse compared to non-abused women (Pico-Alfonso et al., 2004). No correlation was found between DHEA and total PTSD score (Pico-Alfonso et al., 2004).

2.5.2.2 Childhood sexual abuse (CSA)

Not significantly higher plasma levels of daytime DHEA-S (as measured over a 24-hour period) were measured in women who experienced CSA and diagnosed with PTSD compared to those who experienced CSA, but who did not go on to develop PTSD (Bremner et al., 2007).

2.5.3 Short-term release of progesterone and/or testosterone and PTSD due to interpersonal violence

No studies were found that examined testosterone or progesterone concentrations in women with PTSD/PTSS due to interpersonal violence.

2.5.4 Summary of studies regarding the short-term release of cortisol and DHEA and PTSD/PTSS in adult women due to interpersonal violence

Most studies examined salivary cortisol levels in females with PTSD due to IPV. Studies found lower morning salivary (Johnson et al., 2008) in trauma-exposed groups compared to non-traumatised women. Studies also suggested differences in salivary cortisol levels in PTSD between traumatised groups (traumatised by IPV) and those who did not experience IPV (Cordero et al., 2017). Cordero et al. (2017) found lower morning cortisol levels in females with IPV-PTSD compared to controls (no abuse/trauma and no PTSD) and higher evening levels. Lower salivary morning cortisol levels are congruent with findings by Johnson et al. (2008), but the authors did not assess evening levels. The study by Johnson et al. (2008) collected four samples within the first hour of awakening and did not collect comparative data on separate days or throughout the day. Higher salivary levels of cortisol throughout the day in IPV-PTSD women is in congruence with findings of a study by Inslicht et al. (2006) who compared IPV-PTSD women to IPV-non-PTSD participants. Notably, Inslicht et al. (2006) compared two traumatised groups whereas Cordero et al. (2017) compared a traumatised group with PTSD to a non-traumatised group without PTSD. Johnson et al. (2008) also compared two traumatised groups, like Inslicht et al. (2006), and found higher salivary morning cortisol levels

were linked to the severity of PTSD symptoms. In addition, Cordero et al. (2017) compared cortisol levels between the groups in response to a laboratory stressor and here found that the IPV-PTSD group exhibited significantly lower salivary cortisol levels than the non-PTSD group, as well as a blunted response in the IPV-PTSD group compared to controls. No correlation was found between total PTSD score and salivary cortisol concentrations in a study by Pico-Alfonso et al. (2004).

Studies comparing cortisol levels between women with PTSD due to childhood trauma and controls, were identified in this mini review. In comparing women with CSA and PTSD to those with CSA without PTSD, it is suggested that evening plasma levels are significantly lower in those with PTSD who experienced CSA (Bremner et al., 2007). PTSS and the severity of abuse were also associated with lower cortisol levels (Bremner et al., 2007). Significantly lower plasma morning levels were also established in women with CSA with PTSD compared to those without CSA and without PTSD (Stein et al., 1997), showing a difference in cortisol levels between childhood trauma-exposed and non-traumatised women. In a study examining PTSD co-morbid with MDD, lower plasma cortisol levels were found in women with PTSD and MDD compared to controls (no childhood trauma and no MDD/PTSD (Newport et al., 2004).

In response to a laboratory stressor, females with a history of childhood abuse showed decreased salivary cortisol levels, but when additional stressors were added (maternal PTSD, MDD, and a recent stressful event), an elevated cortisol response was seen (Brand et al., 2010), compared to abused women without PTSD. Although short-term activation of the HPA-axis is needed in stressful situations, chronic activation may be harmful and has been associated with several poor health outcomes (Sapolsky et al., 1986; Schnurr and Green, 2004) and psychiatric disorders, including PTSD (Ehlert, Gaab, & Heinrichs, 2001).

In a group of participants with PTSD with co-morbid CFS there was a negative correlation between childhood trauma and salivary cortisol levels 30 min after awakening (Heim et al., 2009), but no difference in cortisol levels were seen between these groups at awakening, 15min and 60min after awakening. However, the time since the trauma is not provided in this study and the samples were only collected for one morning (within 60 minutes of awakening), i.e. no comparison data over time (days or weeks) were collected in this study sample (Heim et al., 2009). Higher daytime urinary cortisol levels were seen in females who experienced CSA and diagnosed with PTSD compared to non-abused participants and those

with CSA without PTSD (Lemieux and Coe, 1995). There was no significant difference between the traumatised without PTSD compared to the control group, suggesting that the diagnosis of PTSD in addition to CSA could be related to increased cortisol levels.

These studies draw our attention to the possibility that cumulative trauma may be associated with differences in cortisol levels when a stressful event occurs (Brand et al., 2010), as well as the presence of co-morbid disorders (Brand et al., 2010; Heim et al., 2009), and that differences in cortisol concentrations might exist between abused women with PTSD compared to abused women without PTSD (Bremner et al., 2007; Lemieux and Coe, 1995), as well as between traumatised versus non-traumatised women (Stein et al., 1997; Newport et al., 2004).

Contrary to these findings, several researchers have found no significant difference in salivary cortisol levels between those with PTSD and childhood trauma compared to non-abused without PTSD participants (Altemus et al., 2003; Lemieux et al., 2008), or compared to those traumatised by childhood trauma who did not go on to develop PTSD (Lemieux et al., 2008). In mixed gender studies, no differences were also seen between PTSD and non-PTSD participants traumatised in childhood by experiences from being refugees, e.g. rape, (Muhtz et al., 2011) and no association between PTSD and salivary cortisol levels in women (gulf veterans) traumatised during childhood (Young et al., 2018). Due to mixed results from studies, future research in this area is needed.

In the context of recent trauma (past 12 months), higher cortisol levels were measured in women compared to those not recently traumatised (Young et al., 2003). Additionally, Young et al. (2003) found no statistically significant difference in cortisol levels in women with trauma exposure of more than 12 months compared to non-traumatised controls. This study draws our attention to the possible effect of recent trauma on cortisol levels compared to no significant effect of trauma that occurred more than 12 months ago, on cortisol levels. However, Young et al. (2003) did not measure and compare cortisol levels over time in the same sample, e.g. within 12 months since the trauma compared to after 12 months of the trauma and therefore could not conclusively say that cortisol levels attenuate with time since trauma.

With regard to DHEA/DHEA-S levels, only two studies were found that met inclusion criteria for this review. Salivary DHEA levels in women who experienced interpersonal violence, higher morning and evening levels were evident in women who experienced psychological abuse compared to non-abused women (Pico-Alfonso et al., 2004). Morning and evening samples were collected over four consecutive days in non-abuse women and from

women recently (within the past 12 months) traumatised by physical and/or psychological abuse due to intimate partner violence. Bremner et al. (2007) found no significant difference in plasma DHEA levels between abused with or without PTSD versus non-abused women without PTSD. However, the type of trauma (intimate partner violence vs. CSA) was different between these two studies (Pico-alfonso et al., 2004 vs Bremner et al., 2007), the time since the trauma (within past 12 months versus childhood), method of collection (saliva vs. plasma), and comparison groups (traumatised vs non-traumatised, compared to PTSD vs CSA without PTSD vs controls [no CSA and no PTSD]), and therefore makes comparison of the findings between these studies difficult. Bremner et al. (2007) compared women who experienced CSA and developed PTSD to women who experienced CSA and did not develop PTSD, and women without CSA and without PTSD.

This mini review highlights the need for additional studies of cortisol, DHEA/DHEA-S, progesterone and testosterone in interpersonal violence-related PTSD and specific trauma types in women. The development of PTSD and symptoms of PTSD (Altemus, Cloitre, & Dhabhar, 2003; Bomyea, Risbrough, & Lang, 2012) has been associated with HPA axis abnormalities. Therefore, future research in this area is needed.

To conclude, there are several limitations to comparing studies of neurosteroid levels in traumatised participants with or without PTSD and non-traumatised without PTSD participants, which are discussed in the next section "possible explanations for the inconsistent findings in the literature".

2.6 Possible explanations for the inconsistent findings in the literature

A possible explanation given by Yehuda (1999) for the inconsistent findings in the literature is that different studies may use different assay techniques, or different data collection procedures that yield different results (Yehuda, 1999). Examples of possible explanations of inconsistencies found in cortisol concentrations in PTSD populations may be due to the time of day samples were collected, severity of the illness, sex, the time since the traumatic event, inpatient vs outpatient status, and comorbidity (Meewissa et al., 2007; Yehuda, 2002a, 2002b). Many studies assessed baseline cortisol sampled during the morning or the cortisol awakening response (CAR), but these results may be unreliable, because it does not accurately reflect cortisol secretion (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010; Edwards, Clow, Evans, & Hucklebridge, 2001). Another reason for inconsistent findings, specifically for salivary samples, is participant non-compliance. Compliance should be monitored (Broderick, Arnold,

Kudielka, & Kirschbaum, 2004), but the use of a monitoring device has not been mentioned in studies of PTSD research.

Other authors suggest that comorbid depression, current versus lifetime PTSD, the severity of PTSD symptoms, time since the traumatic event, the duration of the trauma exposure and a history of trauma in the control group may influence results and contribute to inconsistencies in the literature (Meewisse et al., 2007; Wessa & Rohleder, 2007). Meewissa et al., (2007) also suggest that females with PTSD, individuals with a history of physical and sexual abuse, and samples collected later in the day (e.g. afternoons may have lower cortisol levels) may influence results.

2.7 Limitation of studies focusing on the short-term release of hormone concentrations and shift to hair cortisol concentrations

One of the limitations of these studies is that all measured short-term release of cortisol. Although there have been countless studies measuring urinary, saliva and plasma cortisol concentrations in traumatised individuals and people with PTSD, very few have studied cortisol in hair. It has been argued that cortisol can be reliably measured in human hair (Stalder & Kirschbaum, 2012; Steudte et al., 2013). Measuring cortisol in hair can provide a pattern of the body's release of cortisol over a longer period of time (Stalder & Kirschbaum, 2012; Steudte et al., 2013; Wester & Rossum, 2015), enabling the analysis of cortisol levels of up to three months before trauma (in this case rape). Thus, the analysis of cortisol from hair samples may strengthen our current understanding of the relationship between PTSD and the neuro-endocrine axis (Steudte et al., 2013).

2.8 Benefits of retrieving cortisol concentrations from hair samples

Some of the benefits of retrieving cortisol concentrations from hair samples are that hair samples can be easily stored at room temperature (Gow, Thomson, Rieder, can Uum, & Koren, 2010), sample collection is not invasive (Pacella et al., 2017; Gow et al., 2010), and hair can be used to evaluate concentrations over periods of time (Pragst & Balikova, 2006), as well as provide hormone concentrations before an event (Pereg et al., 2001). However, hair sampling being suggested as not invasive, is a recommendation by research conducted outside of South Africa and therefore not representative of different cultures and women with different hair styles. Also, according to the free hormone hypothesis by Mendel (1989), only the unbound

hormones will be in hair. Furthermore, HCC will not be influenced by non-compliance (Stalder & Kirschbaum, 2012; Staufenbiel et al., 2013).

Compared to other measures (saliva, blood, urine), and in addition to providing long-term data on cortisol concentrations, hair samples can be stored at room temperature (Wosu et al., 2013). Furthermore, hair cortisol concentrations are not easily affected by short-term factors (e.g. individual or situational variability) (Wosu et al., 2013), however previous research has suggested that hair cortisol concentrations may be influenced by hair products and practices. Recent literature on hair cortisol concentrations have been inconsistent regarding hair washing, e.g. van den Heuvel et el. (2019) found a significant relationship between HCC and hair product use and frequency of hair washing, whereas Morris et al. (2017) found no significant association between HCC and hair colour/ structure, hair treatment or frequency of hair washing.

Wosu et al. (2013) hypothesized that smoking could impact on hair cortisol concentrations, however a study by Stalder et al. (2017) found no relation between HCC and smoking. Obtaining a hair sample has been regarded to be not invasive compared to e.g. a blood sample (Gow et al., 2010; Pacella et al., 2017). Only a small amount of hair is needed, compared to saliva/urine/blood and only one sample is needed to reflect a three-month window (i.e. no repeated measures are needed within a three-month period), compared to saliva/urine/blood. However, as mentioned earlier, this is a recommendation by research conducted outside of South Africa and therefore not representative of different cultures and women with different hair styles. Saliva and urine samples are also less invasive, however often repeated measures are needed per participant visit and within a shorter timeframe compared to a three-month follow-up visit for hair samples (Wosu et al., 2013). Obtaining blood samples can be more invasive and painful compared to collection of hair samples (Wosu et al., 2013.

Less repeated measures are needed with hair samples; however, participants may be unwilling to provide a sample at a follow-up visit (Wosue et al., 2013). With urine/saliva/blood samples, often repeated measures are required, which can be expensive, a burden for participants, and may increase the likelihood of incomplete sample collection, or a loss to follow-up (Wosu et al., 2013).

2.9 Review of relevant studies focusing on hair cortisol concentrations

2.9.1 HCC and other mental health studies

Studies have researched hair cortisol concentrations in people diagnosed with mental illness (Heinze et al., 2016), such as depression (Dettenborn et al., 2012; Dowlati et al., 2010; Hinkelmann et al., 2013; Pochigaeva et al., 2017; Wei et al., 2015), bipolar disorder (Coello, Munkholm, Nielsen, Vinberg, & Kessing, 2019; Manenschijn et al., 2012), and generalised anxiety disorder (GAD) (Steudte et al., 2011b). Inconsistent results have been found, including no alterations in people with depression (Dowlati et al., 2010) and bipolar disorder (Manenschijn et al., 2012) compared to controls, higher hair cortisol levels in depressed individuals (Dettenborn et al., 2012; Wei et al., 2015), and lower cortisol levels in people with GAD (Steudte et al., 2011b) and depression (Hinkelmann et al., 2013; Pochigaeva et al., 2017), compared to controls.

In the study by Heinze et al. (2016) increased HCC of the past three months were elevated in participants diagnosed with mental illness compared to healthy controls. No difference between groups were established in the past three to six months. In the past month, significantly higher levels of perceived stress were measured in the diagnosed group compared to the controls, but perceived stress was not significantly correlated with HCC. Heinze et al. (2016) concluded that in the early phase (three months) of mental illness, it appears that HCC are elevated, but that longitudinal studies are needed to investigate the relationship between cortisol and mental illness, as well as the progression of mental illness.

In participants diagnosed with bipolar disorder, HCC were higher compared to healthy controls, when adjusted for age and sex (Coello et al., 2019). In congruence with the findings of Heinze et al. (2016), Coello et al. (2019) also found elevated levels of HCC in the early phase of mental illness, namely BD. They suggest that these findings may indicate physiological stress during the early phase of BD (Coello et al., 2019).

2.9.2 HCC in traumatised populations

Cortisol concentrations in hair have also been examined in studies of people who have experienced other traumatic events, such as death of a close relative or serious illness (Karlen, Ludvigsson, Frostell, Theodeorsson, & Faresjo, 2011), earthquake survivors (Gao et al., 2014), intimate partner violence (Boeckel et al., 2017) and adverse childhood experiences (Groer, Kane, Williams, & Duffy, 2015; Hinkelmann et al., 2013; Kalmakis, Meyer, Chiodo, & Leung,

2015; Morris, Abelson, Mielock, & Rao, 2017; Schalinski, Elbert, Steudte-Schmiedgen, & Kirschbaum, 2015).

In students who have experienced major life stressors, higher levels of hair cortisol concentrations have been found compared to students who have not experienced such stressors (Karlen et al., 2011).

A study by Gao et al. (2014) found similar results to the study by Luo et al. (2012). This was also a longitudinal study and hair was sampled at 13, 24 and 45 weeks after the Wenchuan earthquake from two cohorts. The first cohort included 12 male adults and 8 females who survived the earthquake and 23 non-exposed controls. The second cohort included 22 adolescent males and 29 controls. There was an initial increase (during the first 6 and 22 weeks after the earthquake) in HCC seen in survivors of the earthquake compared to non-traumatised controls. No difference was seen between groups at a later stage (43 weeks after the earthquake). HCC decreased in the group of survivors during this later stage.

2.9.2.1 Intimate partner violence

One cross-sectional study including females who experienced intimate partner violence, examined hair cortisol concentrations (Boeckel et al., 2017). Women traumatised by intimate partner violence had higher HCC and PTSS severity compared to controls (non-abused) (Boeckel et al., 2017).

2.9.2.2 Childhood trauma

The study by Kalmakis et al. (2015) was cross-sectional in design and assessed the possible correlation between childhood trauma (as measured by the adverse childhood experiences [ACEs] questionnaire) and HCC. Participants included 55 students aged 18-24 years. ACE scores were significantly, inversely associated with HCC.

The study by Groer et al. (2015) included 81 female veterans (aged 18-70 years) from the U.S Armed Services. Of these, 30% reported childhood sexual abuse, of which 38.5% experienced military-related sexual assault and 70.3% experienced sexual assault outside of the military environment. Amongst other variables, those with CSA had higher levels of perceived stress. When controlling for perceived stress, those with CSA had lower HCC. They concluded that female veterans with a history of CSA may be at increased risk of developing mental illness later in life and they may have an increased allostatic load (Groer et al. (2015).

Hinkelmann et al. (2013) found that a history of childhood abuse may be associated with decreased HCC in participants diagnosed with major depression (MD), as well as in healthy controls. They found that those participants with a history of childhood abuse had lower levels of HCC than non-exposed participants (Hinkelmann et al., 2013).

Schalinski et al. (2015) included 43 women with stress-related disorders. Higher HCC was measured in those who experienced childhood trauma,. Furthermore, they also found that traumatic experiences and depression also contributed to higher levels of HCC (Schalinski et al., 2015)

Morris et al. (2017) found that greater stress exposure was predicted by lower HCC in women who experienced childhood trauma, namely childhood abuse or neglect, compared to healthy controls (women without a history of childhood trauma). They concluded that their study contributes to the literature about the link between HCC as a risk marker for stress exposure in women with a history of childhood trauma, particularly in the context of recent traumatic experience(s) (Morris et al., 2017).

2.9.3 Hair cortisol studies in PTSD patients

Studies have increased their focus on the long-term secretion of hair cortisol and its connection to PTSD. Four studies have specifically focused on hair cortisol concentrations in individuals with PTSD (Luo et al., 2012; Steudte et al., 2011a; Steudte et al., 2013; Steudte-Schmiedgen et al., 2015). Two of these were longitudinal analyses (Luo et al., 2012; Steudte-Schmiedgen et al., 2015) and the other two cross-sectional studies (Steudte et al., 2013; Steudte et al., 2011a). See Table 2.2 for a summary of these studies.

TABLE 2.2 Hair cortisol studies in PTSD patients

Name	Participants (n)	Design	M/F	Age Mean(SD) years	Method of collection	Single/Repeated measure	Trauma Type	Time since trauma	Main findings
Steudte et al. (2011a)	10 participants with PTSD and 17 traumatised controls from a civil war area in Northern Uganda	Cross-sestional	PTSD: 4 (40%) M, Controls: 11 (64.7%) M	PTSD: 19.2 (3.2), controls: 20.1 (5.7)	3cm hair, ball mill, CLIA, scalp-near hair segment (≤3cm) PTSD: posttraumatic diagnostic scale (PDS) & CAPS	Single. After recent traumatisation	Not specified	Age of worst trauma: PTSD (12.5yrs), controls (16.38yrs)	PTSD participants had higher levels of HCC than traumatised controls. Positive relationship between HCC and number of different lifetime traumatic events.
Steudte-Schmiedgen et al. (2015)	Pre-Deployment: 113 traumatised and 129 non-traumatised soldires. Predictive sample: 90 soldiers	Longitudinal	All male	27.68 (6.11)	2cm hair segment (LC-MS/MS)	Repeated. Pre-deployment and 12 months after deployment	Military	Recent	Lower HCC were correlated with an increase in PTSD symptoms in those participants who experienced new-onset traumatic events. Increase of HCC over time and a negative correlation between cortisol concentrations and the amount of new-onset traumatic events

Steudte et al. (2013)	28 participants with PTDS, 27 traumatised without PTSD, 32 non-traumatised controls	Cross-sestional	PTSD: 24 (96%) F, Traumatised controls: 23 (92%) F, non traumatised: 25 (89.3%)	PTSD: 36.84 (11.25), Traumatised controls: 41.72 (12.32), non traumatised: 37.61 (14.05)	6cm hair (2 x 3cm segments), LC_MS/MS PTSD: Munich Composite International Diagnostic Interview	Single. After distant traumatisation	Severe accidents (n=9), natural disasters (n=6), sexual assaults (n=12), physical assaults (n=11), life-threatening illnesses (n=6), and other traumatic events (e.g. sudden death/suicide of close relative) (n=11).	Recent	Inverse relationship between hair cortisol concentrations and the number of lifetime traumatic events, the frequency of traumatic events, the time since the traumatic event, and the severity of the intrusion symptoms of PTSD
Luo et al. (2012)	Adolescent girls. 32 with PTSD, 32 trauma-exposed without PTSD, 20 non-exposed controls	Longitudinal	All female	PTSD: 13.81, traumatised without PTSD: 13.84, Controls: 14.40	12cm hair (x4 3 cm segments), ball mill, ECL immunoassay (ECLIA)	Repeated. 4 measurements. (1) Baseline, (2) 2 months befre and 1 month after earthquake, (3) 2-4 months after earthquake, (4) 5-7 months after earthquake	Earthquake	Recent	No differences between the groups at baseline, but a decline in cortisol over time since the trauma (earthquake) in individuals with PTSD. Higher cortisol levels in the groups with PTSD and traumatised non-PTSD participants compared to the control group was seen two months before the trauma and one month after. Two months after the trauma, the non-PTSD traumatised group had higher cortisol levels compared to the PTSD group and the PTSD group had higher cortisol levels than controls. Five to seven months after the trauma the non-PTSD trauma-exposed group still had the highest cortisol levels compared to the PTSD group

Steudte et al. (2011a) included 10 participants with PTSD and 17 traumatised controls from a civil war area in Northern Uganda. In this study, higher hair cortisol was found in the PTSD group compared to traumatised controls, as well as a significant positive correlation between the number of traumatic events and cortisol (Steudte et al., 2011a). The authors concluded that individuals with PTSD who live in stressful environments have elevated hair cortisol concentrations (Steudte et al., 2011a). A limitation of this study was that the traumatic events were not specified and as these individuals were exposed to stressful situations daily, this may have confounded the relationship between trauma exposure and cortisol levels.

Steudte-Schmiedgen et al. (2015) included male soldiers, who were examined pre-deployment, and 12-month after deployment. They found that lower hair cortisol levels were correlated with an increase in PTSD symptoms in those participants who experienced new-onset traumatic events. There was an increase of hair cortisol concentrations over time and a negative correlation between cortisol concentrations and the amount of new-onset traumatic events. They concluded that reduced cortisol secretion is a risk factor for the development of PTSD symptoms after exposure to a traumatic event. They suggested that future studies should investigate their findings in different samples.

Another study included 28 participants with PTSD, 27 traumatised, and 32 non-traumatised controls matched for age, sex, body mass index, smoking status, and use of oral contraceptives (Steudte et al., 2013). Patients with PTSD had the following comorbidities: depressive disorder (n=20), specific phobia (n=9), GAD (n=4), panic disorder with/without agoraphobia (n=3), and obsessive-compulsive disorder (OCD) (n=2). Traumatic events included severe accidents in nine participants, natural disasters (n=6), sexual assaults (n=12), physical assaults (n=11), life-threatening illnesses (n=6), and other traumatic events (e.g. sudden death/suicide of close relative) (n=11). Steudte et al. (2013) found an inverse relationship between hair cortisol concentrations and the number of lifetime traumatic events, the frequency of traumatic events, the time since the traumatic event, and the severity of the intrusion symptoms of PTSD. This study is limited in that it included participants who had PTSD that was not keyed to a specific traumatic event (but to different events) and several comorbidities. Also, both studies (Steudte et al., 2013; Steudte et al., 2011a) had very small samples compared to the sample of the present study.

A study by Luo et al. (2012) included adolescent girls of whom 32 had PTSD, 32 were trauma-exposed without PTSD and 20 were non-exposed controls. The authors found no

differences between the groups at baseline but a decline in cortisol over time since the trauma (earthquake) in individuals with PTSD. According to Kirschbaum, Tietze, Skoluda, and Dettenborn (2009), hair cortisol levels reach a plateau in hair segments of 10-12cm and may explain the blunted effect at baseline (before the trauma). Higher cortisol levels in the groups with PTSD and traumatised non-PTSD participants compared to the control group was seen two months before the trauma and one month after. Two months after the trauma, the non-PTSD traumatised group had higher cortisol levels compared to the PTSD group and the PTSD group had higher cortisol levels than controls. Five to seven months after the trauma the non-PTSD trauma-exposed group still had the highest cortisol levels compared to the PTSD group (Luo et al., 2012). A limitation of this study (Luo et al., 2012) is that it only included children (average age 14 years). In the present study we included adult women.

2.10 Factors that may influence hair cortisol concentrations

A recent meta-analysis by Stalder et al. (2017) found significant positive correlations between HCC and the following variables: age, physical stressors (52% HCC increase), chronic stress (22% HCC increase), body mass index (BMI), and systolic blood pressure, respectively. Examples of physical stressors included pregnancy or acute alcohol withdrawal, excluding mental illness. They also established a significant sex difference in HCC, with males exhibiting a 21.4% higher HCC than women. HCC was correlated negatively with oral contraceptives, hair treatment, washing frequency, general anxiety disorder, and PTSD. Stalder et al. (2017) found no relation between HCC and smoking, perceived stress, social support, or any other mental disorder. Van den Heuvel et al. (2019) found a significant relationship between HCC and the following variables in a sample of South African women from mixed ethnicity: age, level of education, hair product use, frequency of hair washing. However, HCC was not significantly associated with alcohol use, BMI, income, or employment status (van den Heuvel et al., 2019).

A review by Wosu et al. (2013) suggested that the following factors may influence HCC: hair washing, treatment of the hair, and position where the hair segment is taken from (see Wosu et al. 2013 for review). Morris et al. (2017) found no significant association between HCC and hair colour/ structure, hair treatment or frequency of hair washing.

Other stress-related factors have also been identified, including demographic information, such as income, educational level, occupation, characteristics of the

neighbourhood, age, and sex. The findings regarding sex have been inconsistent, with findings of higher cortisol levels in both men and women. Other demographic information includes race and ethnicity which may influence the texture and growth of the hair. For example, it has been suggested that the growth of hair in Africans may be slower than in Caucasians (see Wosu et al., 2013 for review).

Other factors that have been hypothesised to influence hair cortisol concentrations are psychiatric symptoms and disorders, medical conditions such as Cushing's disease and Addison's disease (see Wosu et al., 2013 for review), chronic pain, cardiovascular disease and metabolic syndrome. It has also been well established that pregnancy influences hair cortisol levels. To conclude, factors such as alcohol and drug abuse, cigarette smoking, oral contraceptives, other medications, and physical activity have been hypothesised to influence hair cortisol concentrations and should therefore be accounted for in studies (see Wosu et al., 2013 for review). Wosu et al. (2013) has suggested that these factors be considered in the design, analysis, and interpretation of data in future studies. The abovementioned variables were, therefore, considered as possible covariates within the present study.

2.10.1 BMI and cortisol

As pointed out in the above section, a significant association between BMI and HCC has been found (Stalder et al., 2017), however no significant association been BMI and HCC has also been found (van den Heuvel et al., 2019).

In a study by Farag et al. (2008), cortisol and stress were associated with BMI, with BMI predicting 31% of the variability in cortisol concentrations. Obese women also reported the highest stress levels. In individuals who experience chronic stress, dysfunction of the HPA axis has been documented (Kudielka, & Kirschbaum, 2005). Obesity is a contributing factor to increased blood pressure, glucose, triglycerides, and low-density lipoprotein cholesterol (LDL-C), and decreases in high-density lipoprotein (HDL-C) (Van Gaal et al., 2006).

Cortisol (the end-product of the HPA axis) influences numerous areas, including energy metabolism. Under normal circumstances, cortisol levels usually peak during the morning (at the time of awakening) and declines throughout the day to a lower level during the evening (Stalder et al., 2017). The morning peak of cortisol provides a signal to other cells of the body, cortisol regulates gene expression in many cell types and entrain their activity (Buijs et al., 2003). When the quality of this signal is reduced, i.e. a smaller peak difference during the

morning, this would represent a reduced daily signal to the body which may indicate a decrease in the function of integrated systems. In diabetes and hypertension, disturbances in this circadian rhythm is seen (Buijs et al., 2003).

In obesity, lower levels of morning cortisol levels (plasma) are seen, as well as blunted diurnal variation (Rosmond, Dallman, & Bjorntorp, 1998; Walker et al., 2000), and therefore obesity is associated with HPA axis function disturbances (Rosmond et al., 1998; Walker et al., 2000). Obesity may therefore contribute to dysregulation in cortisol; however, stress-induced elevations of cortisol may also contribute to overeating, which may lead to obesity, type 2 diabetes, and Cardiovascular disease (CVD). Dallman et al. (2004) suggested that frequent activation of the HPA axis (by extrinsic or intrinsic factors), resulting in excessive cortisol secretion, may contribute to obesity and type 2 diabetes (Rosmond, 2005).

Studies have found that in obese participants, there is an increase in cortisol production and secretion from the adrenal glands, acute hyperresponsivity and elevated 24-hour urinary free cortisol (UFC), however normal or decreased blood serum and saliva cortisol conentraions have also been seen (Pasquali et al., 2006; Bose et al., 2009; Müssig et al., 2010) It has been demonstrated that weight gain and the accumulation of fat cells are promoted by cortisol (Björntorp and Rosmond, 2000; Bjorntorp, 2001). Glucocorticoids, such as cortisol, promotes the conversion of preadipocytes to mature adipocytes (Peckett et al., 2011). There are numerous researches showing evidence between stress and weight gain through increased cortisol concentrations (e.g., Björntorp and Rosmond, 2000; Björntorp et al., 2000; Bjorntorp, 2001; Peeke and Chrousos, 1995; Wallerius et al., 2003).

Cortisol's role in fat physiology is quite complex (Incollingo et al., 2015) and has been connected to weight loss as well, through enhancing lipolysis and triglycedride uptake. Cortisol is also involved in insulin resistance (Andrews and Walker, 1999) through proliferation of adipokines and the secretion of proinflammatory cytokines (Antuna-Puente et al., 2008)".

It is not clear whether HPA axis dysfunction contributes to obesity or if obesity contributes to HPA dysregulation (Incollingo et al., 2015). Currently, more research is needed to investigate the role of the connection between hair cortisol concentration and dysregulation of the HPA axis in obese individuals. It is not yet clear how studies on hair cortisol concentrations contribute to the current research on dysregulation of the HPA axis in obese individuals and therefore further research in this area is needed. (Stalder et al., 2012).

Due to high levels of obesity around the world, and in South Africa, it is important that obesity in relation to physiological dysregulation are investigated (Farag et al., 2008). Although this was not the focus of the present study, it was however important to include variables such as BMI and blood pressure in the context of cortisol.

2.10.2 Blood pressure and cortisol

As mentioned earlier, Stalder et al. (2017) found a significant positive correlation between HCC systolic blood pressure. In a study by Gold et al. (2005), a significant associated was found between hypertension and impaired glucocorticoid feedback. They controlled for age, gender, and BMI, however they looked at the effect of hypertension on the brain and suggested future studies in this area should include an assessment of cortisol (Gold et al., 2005).

HPA axis dysregulation has been hypothesized to be involved in the pathological process of high blood pressure (Whitworth, Mangos, & Kelly, 200). Clinical disease involving elevated levels of cortisol, such as Cushing's disease and glucocorticoid resistance syndrome, are often associated with hypertension (Kino et al., 2002; Torpy et al., 2002).

Despite the knowledge that elevated cortisol concentrations as a result from disorders in the endocrine system are associated with increased blood pressure, the association between dysregulation of the HPA axis and hypertension is less clear and requires further investigation (Gold et al., 2005).

2.10.3 Smoking and cortisol

Research has indicated that nicotine has a strong effect on the central nervous system, binds to nicotinic acetylcholinergic receptors and encourages significant changes in many brain systems. The HPA axis is one of the systems affected by nicotine. Single doses of nicotine activate the HPA axis to secrete CRH in the paraventricular nucleus of the hypothalamus, which is followed by ACTH secretion from the pituitary gland and ultimately cortisol secretion by the adrenal glands. Reactions to stress and HPA axis activity is changed with chronic nicotine exposure and research has suggested that the HPA axis could be altered in smokers (Rohleder, & Kirschbaum, 2006). Kirshbaum et al. (1992) reported that two or more cigarettes is associated with an increase in cortisol concentration in saliva.

Nicotinic binds to acetylcholinergic receptors, which are widely distributed in the CNS, however the HPA axis can be activated by different pathways (Rosecrans and Karin, 1998). More specifically, Matta et al. (1998) suggested a dose-response release of noradrenaline after

smoking and correlated this to ACTH release. A more recent study, by Badrick, Kirschbaum and Kumari (2007) found an association between smoking and salivary cortisol, showing increased cortisol concentrations in current smokers. However, different results have been found regarding the relationship between smoking and cortisol, which may be due to small sample size, different samples (saliva / plasma / urine) (Steptoe, & Ussher, 2006), and timing in collection (Field et al., 1994). The benefits of retrieving cortisol concentrations from hair samples have been discussed previously, however these benefits could be applicable in investigating the relationship between smoking and cortisol.

Wosu et al. (2013) hypothesized that smoking could impact on hair cortisol concentrations, however a study by Stalder et al. (2017) found no relation between HCC and smoking.

2.11 Ethics and acceptability of collecting hair samples

As mentioned earlier in section 2.8 “benefits of retrieving cortisol concentrations from hair samples”, obtaining a hair sample is seen to be non-invasive compared to, e.g. a blood sample, as suggested by researchers (Gow et al., 2010; Pacella et al., 2017). Only a small amount of hair is needed, compared to saliva/urine/blood and only one sample is needed to reflect a three-month window (i.e. no repeated measures are needed within a three-month period), compared to saliva/urine/blood. However, as mentioned earlier, this is a recommendation by research conducted outside of South Africa and therefore not representative of different cultures and women with different hair styles.

The only study about the experiences of women to provide hair samples for research purposes in South Africa, was a study by Coetzee et al. (2012). The purpose of this qualitative study was to acquire information, through interviews, from 21 Xhosa-speaking women living with HIV in the Western Cape, about their cultural beliefs, perspectives, attitudes, and concerns with regard to providing hair samples for medical testing (Coetzee et al., 2012). The medical testing was related to monitoring their ARV drug exposure to improve treatment. Coetzee et al. (2012) found that women had several cultural beliefs which could have an effect on their decision to provide hair samples for research purposes, however, almost all of these participants agreed that, if they received enough information (e.g. positive impact on their health, the reason for taking a hair sample) from the researchers, that they would provide samples for research reasons. However, this is only one study and does not represent the Black, Zulu-speaking women in KZN. Future research in this area would be beneficial.

In a pilot study by Kader et al. (2012), information obtained from the AUDIT and DUDIT were compared to alcohol biomarkers in hair and urine, as well as drugs in urine samples, respectively. They also investigated the feasibility of using the AUDIT and DUDIT compared to tests done with hair and urine samples. A practical challenge regarding obtaining hair samples was that hair was often too short (less than 1cm) from Black and Coloured participants. Kader et al. (2012) found that most participants wore their hair in braids which made it difficult to obtain a natural hair strand.

Future research should however take the above into account when wanting to obtain hair samples from Black African women. Qualitative investigation of the acceptability and cultural meaning of hair sampling should be conducted in future research, specifically in Black South African women.

3. METHODOLOGY

3.1 Research Design

This longitudinal study (Stellenbosch Health Research Ethics Committee [HREC] ID S15/08/166, first approval 11 September 2015) was nested within a South African Medical Research Council (SAMRC) Flagship project entitled: "The impact of rape in women on HIV acquisition and retention and linkages to care: a longitudinal study" (also known as Rape Intervention Cohort Evaluation or RICE) (Ethics reference: EC019-10/2013) (*see protocol paper*: Abrahams et al., 2017) which was led by the Gender and Health Research Unit team (Principal Investigator [PI]: Prof. Naeemah Abrahams). The primary aim of the RICE study was to "determine the incidence and attributable burden of HIV acquisition in adult women up to 12 months post-rape and compare these to a cohort of women who have not been raped" (follow-up for some participants in RICE were more than 12 months).

3.2 Sample

For the present study, all participants were females and ranged in age from 18-40 years (inclusive). Rape-exposed (RE) participants were defined as those who recently reported and sought care for a rape that occurred within the past 20 days and controls were defined as participants who did not report a recent (within the past 20 days) rape event. Rape-exposed participants were matched for age (+- 1 year) with controls.

3.2.1 Inclusion criteria

Women aged 18-40 years were included for the RICE, as well as the nested study. For the rape-exposed group, only participants with penetrative forced sex, who reported to a sexual assault service clinic, were included. Care and treatment for those reporting rape included a medical examination by a doctor to confirm the rape. Participants with PTSD, diagnosed with comorbid depression, were included. In addition, for the present study, participants willing and able to give hair samples, as well as those whose hair samples were 3cm or longer, were included.

3.2.2 Exclusion criteria

Attempted rape cases were excluded, as were women who were participating in other studies. Participants under 18 years of age were excluded from the RICE study in view of the ethical considerations related to consent for research participation required from a parent or legal guardian. Participants presenting with severe emotional distress were also excluded. For the present study, women under 18 years were excluded (Gunnar & Quevedo, 2007; Nelson,

Leibenluft, McClure, & Pine, 2005) in view of the developmental effects of age on the hypothalamic pituitary adrenal (HPA) axis. Pregnant women were excluded because of the effects of pregnancy on the HPA-axis (D'Anna-Hernandez, Ross, Natvig, & Laudenslager, 2011; Kirschbaum et al., 2009).

Women with hair shorter than 3cm in length at the posterior vertex region of the scalp were excluded as hair shorter than 3cm in length would not be adequate for the determination of cortisol concentrations over a three-month period (Wenning, 2000). From proximal to distal segments of hair, there is a decline in cortisol concentrations referred to as a 'wash out' effect (Stalder & Kirschbaum, 2012). Stalder and Kirschabaum (2012) suggested that the most valid reflections of cortisol concentrations are yielded from those hair segments reflecting a period of three to six months. As physical illness or the use of certain medications can affect HPA-axis activity and, as such, influence cortisol secretion (Steudte et al., 2013), other exclusion criteria with regard to hair sampling were: any severe physical disease in the past five years (e.g. cancer, adrenocortical dysfunction [e.g. Cushing's disease, Addison's disease] which result in an overproduction of cortisol or insufficient secretion of cortisol, respectively (Wester et al., 2017)), and/or use of glucocorticoid-containing medications or psychotropic medications (e.g. antidepressants) within the past six months (based on self-report) (Steudte et al., 2013).

3.2.3 Sample size

The sample size for the present study was determined with the assistance of a senior statistician (Prof. Kidd) at Stellenbosch University's Centre for Statistical Consultation. As there were no previous trauma-focused studies with a similar design, our sample size was informed by a study by Steudte et al. (2013) that included ±25 participants per group (28 participants with PTSD, 27 traumatised without PTSD, and 32 non-traumatised controls). These group sizes were able to discriminate hair cortisol concentration (HCC) in posttraumatic stress disorder (PTSD) and trauma-exposed groups from a non-trauma control group, however, the study was not able to discriminate HCC between PTSD and trauma-exposed groups.

The present study included 323 participants at baseline who met inclusion criteria, 228 participants at the three-month follow-up visit (97 RE and 131 control), and 196 participants at six-month follow-up (77 RE and 119 controls). It was determined that approximately 150 participants per group (rape-exposed and controls) would provide enough discriminatory power of HCC and posttraumatic stress symptomatology (PTSS) in rape-exposed participants and controls. Of these, the present study had access to hair samples at baseline from 323

participants (160 RE and 163 controls), 108 participants at the three-month follow-up visit (55 RE and 53 control), and 62 participants at six-month follow-up (32 RE and 30 controls) (see Figure 3).

3.3 Ethical Considerations

3.3.1 Ethical approval procedures

The broader RICE study and the present study were submitted to the South African Medical Research Council's Ethics Committee (SA MRC REC) and received ethical clearance (Ethics reference: EC019-10/2013). The RICE study was also submitted to Stellenbosch University's Health Research Ethics Committee and received acknowledgement of approval. The MRC REC was the oversight committee of the RICE project. The present study was submitted separately to SU HREC and received approval (HREC # S15/08/166, first approved 11 September 2015). Access to Thuthuzela Care Centres (TCCs) was approved by the National Prosecuting Authority (NPA).

3.3.2 Informed consent

Participation in the RICE study, as well as in the present study, was completely voluntary and written informed consent (IC) was obtained from all participants before the initiation of study procedures (see Addenda A1-A5 for RICE study's approved informed consent documents that were used for the present study). Separate consent was sought for the use of a biometric system BiCeps which prevented co-enrolment into other studies [A1], permission for collection of biological samples for genetic analysis [A2], permission to do HIV testing [A3] and permission to collect biological samples that will be stored for future research. Separate consent forms were developed for the women recruited from rape services [A4] and the control participants recruited at family planning clinics [A5]. The procedures of consent and confidentiality adhered to the principles of the Belmont Report (National Commission for the Protection of Human Subjects of Biomedical and Behavioural Research, 1978).

Trained research assistants (from the same ethnic background as participants) performed the informed consent procedures in isiZulu, including information on the nature and purpose of the RICE study, as well as the present study, interview procedures, collection of biological samples, need for follow-up interviews, the potentially sensitive nature of questions, confidentiality, payment for participation and travel, risks and benefits (including referral to other services if needed) and the freedom to withdraw participation at any time,

without penalty. When respondents verbally indicated an understanding of these issues, they signed a consent form.

The following informed consent documents are of relevance to the present study:

1. Biometric informed consent (Addendum A1) [Used by RICE study staff to obtain permission for a finger-print scan from participants to ensure that there was no duplication in participation and data entry. Most participants were also part of the present study and this consent is, therefore, included in the present study for completeness].

2. RICE study IC taken at rape service clinics (Addendum A4)

3. RICE study IC taken at family clinics (Addendum A5)

3.3.3 Compensation

Compensation, as discussed in the present study, was for both studies (i.e. the main RICE study, as well as the present study). Participants were compensated at each interview for time and inconvenience (covered by MRC funding). This amount was similar to the minimum daily wage. Furthermore, participants were asked about their actual travel costs for which they received additional compensation to cover the cost of travel to and from the research site.

3.3.4 Protection of participant anonymity and confidentiality

The study was introduced to potential participants as the *Women's Health and Wellbeing Study,* in order to protect women from being identified as individuals who had been raped. The safety guidelines of the United States Agency for International Development (USAID) and the World Health Organisation (WHO) on research regarding gender violence were followed (WHO, 2001; WHO, 2016).

Protection of participant confidentiality was essential to ensure both safety and data quality. All information was kept on secure computers or servers and protected by firewall and passwords. To ensure that data was kept confidential, participants were given a study identification number. Contact details were entered into a database that was password protected and not stored on the same network as the study data, so that data could not be linked to individual participants. The only database linking women to their study ID numbers, was kept securely on a non-networked machine and password protected. All information was kept anonymous and findings were reported as such.

3.3.5 Risks

Approaching this vulnerable population so close to the occurrence of the traumatic event involves some inherent risks. There is also the possibility of therapeutic misconception. In order to minimise these risks, the RICE research assistant approached the victim after they had received the standard care at the TCC. TCCs are one-stop centres based at public hospitals providing 24-hour integrated care to rape survivors including access to police, counselling and medical care (UNICEF, 2012).

The RICE study involved minimal harm to participants, however as questions may have been perceived as intrusive and may have resulted in emotional distress, all RICE staff were trained to respond to these reactions and provide containment. A trauma counsellor was employed by RICE and available on site to provide support to all participants on an ongoing basis. The research team followed the World Health Organisation's Ethical and Safety Guidelines for research on violence against women where these were applicable. The RICE research team was trained on how to approach rape victims and if they had concerns about participants, they could refer them back to TCC staff for consultation with the legal team, assessment and care. All RICE staff were trained not to ask about the details of the rape at baseline.

Furthermore, the RICE team tried to provide women with a pleasant experience at the clinic. Frequent breaks were provided in between questionnaires to minimise questionnaire fatigue and women were given snacks in between. The research assistants received two weeks of extensive training, which included training them to recognise fatigue and to intervene at such point, e.g. stop / have a break / provide a cooldrink/tea or a snack. The RICE team followed the ethical guidelines by the Belmont report, which includes the team to make sure that the research does not harm women and minimise risks for participants.

The main risk associated with taking a hair sample from the scalp is that the area from where the hair is cut may leave a visible patch. However, the RICE staff were careful only to take hair from the back of the head and as close as possible to the scalp to minimise this risk. Obtaining a hair sample was seen to be non-invasive compared to, e.g. a blood sample, as suggested by researchers (Gow et al., 2010; Pacella et al., 2017). However, this is a recommendation by research conducted outside of South Africa and therefore not representative of different cultures and women with different hair styles. Only a small amount

of hair is needed, and only one sample is needed to reflect a three-month window (i.e. no repeated measures are within a three-month period), compared to saliva/urine/blood. Saliva and urine samples are also less invasive, however often repeated measures are needed per participant visit and within a shorter timeframe compared to three months. Obtaining blood samples can be more invasive and painful compared to collection of hair samples. The research team did not foresee any other risks involved in participants providing these samples.

3.4 Questionnaires and measurements

At each visit, face-to-face interviews were conducted by trained RICE staff (from the same ethnic background as the participants) in isiZulu with responses recorded on personal digital assistants (PDAs). All questionnaires were translated into isiZulu and back translated into English. All questionnaires were pre-tested with 20 cognitive interviews with control women over a period of two months. These interviews involved asking the women each question to test their understanding. The interview process and participants' understanding of sample collection (including taking hair samples) were also tested. The questionnaires were then finalised for the research process. It took a total of three months to optimise and pre-test the questionnaires in isiZulu.

3.4.1 Demographic questionnaire

Demographic details were collected with a questionnaire that asked about age, income, education, and employment (Jewkes et al., 2006) (see Addendum B). The demographic questionnaire was developed for a previous study by Jewkes et al. (2006), where it was administered to participants in the rural areas of the Eastern Cape, South Africa.

Participants were screened for psychiatric disorders with the following instruments: The Mini International Neuropsychiatric Interview (MINI) for DSM-IV (Pinninti, Madison, Musser, & Rissmiller, 2003; Sheehan et al., 1998), Center for Epidemiologic Studies Depression Scale (CES-D) (Knight, Williams, McGee, & Olaman, 1997), a shortened version of the Drug Use Disorders Identification Test (DUDIT) (Berman, Bergman, Palmstierna, and Schlyter, 2005), Alcohol Use Disorders Identification Test - Consumption (AUDIT-C) (Dawson, Grant, Stinson, & Zhou, 2005; Morojele et al., 2017), and the Davidson Trauma Scale (DTS) (Davidson et al., 1997). Furthermore, traumatic life events were measured with an adapted version of the Life Events Checklist (LEC) (Gray, Litz, Hsu, & Lombardo, 2004; Mollica et al., 1993), a modified version of the Childhood Trauma Questionnaire – Short Form

(CTQ-SF) (Bernstein, Ahluvalia, Pogge, & Handelsman, 1997; Chirwa et al., 2018; Jewkes, Dunkle, Nduna, Jama, & Puren, 2010), the Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983), and the Multidimensional Scale of Perceived Social Support (MPSS) (Zimet, Dahlem, Zimet, & Farley, 1988).

3.4.2 The Mini International Neuropsychiatric Interview (MINI)

The MINI (Pinninti at al., 2003; Sheehan et al., 1998) was used to screen for psychosis, anxiety disorders and suicidality. The MINI was administered by RICE clinical staff as a structured psychiatric interview used to screen for 17 DSM-IV Axis-I psychological disorders and took approximately 15 minutes to complete (Pinninti et al., 2003; Sheehan et al., 1998). Good test-retest and inter-rater reliability was found by Lecrubier et al. (1997). Kappa values have ranged from 0.65 and 0.85 in a study in which the MINI was administered to general practitioners by medicine residents (de Azevedo Marques & Zuardi, 2008). Furthermore, specificity and sensitivity ranged between 0.90 and 0.99, and 0.75 and 0.92, respectively. Also, positive predictive values were found to be between 0.60 and 0.86, whereas negative predictive values were found to range between 0.92 and 0.99. The kappa coefficient for accuracy of the MINI ranged between 0.88 and 0.98 (de Azevedo Marques & Zuardi, 2008).

3.4.3 Center for Epidemiologic Studies Depression Scale (CES-D)

The CES-D (Knight et al., 1997) was used to screen for depression. The CES-D is a short, self-administered questionnaire consisting of 20 items that measure current (during the last week) symptoms of depression (Knight et al., 1997). The study by Knight et al. (1997) provides support for the construct validity of the subscales of the CES-D as well as for the total score. Furthermore, they established good reliability of the scale (Knight et al., 1997). The criterion validity of the CES-D has also been found to be satisfactory (Knight et al., 1997). The internal consistency of this measure has been found to be high in the general population (coefficient alpha 0.85) and even higher in patients (coefficient alpha 0.9) (Radloff, 1977). The scale has been found to have good test-retest stability, very good concurrent validity (clinical and self-report criteria), a high internal consistency and good construct validity (Radloff, 1977).

In South Africa, the CES-D has been used (Vythilingum et al., 2012) previously in community-based research (Smit et al., 2006; Myers et al., 1980) and has, for example, been validated among Black South Africans (Pretorius, 1991). In a study by Nduna et al. (2010), which was done in the Eastern Cape, South Africa, excellent reliability was calculated, with a

Cronbach alpha of .90 for women. In the present study, the internal reliability (Cronbach's alpha) of the CES-D was 0.92.

3.4.4 Drug Use Disorders Identification Test (DUDIT)

The shorter version of the DUDIT was used to screen for drug abuse (Berman et al., 2005). The DUDIT is a self-report questionnaire consisting of 11 items to assess drug abuse (Berman et al., 2005). For the RICE study, as well as the present study, the following six items were used (Berman et al., 2005): "How often do you use drugs other than alcohol?", "How many times do you take drugs on a typical day when you use drugs?", "Over the past year, have you felt your longing for drugs was so strong that you could not resist it?", "Over the past year, have you felt that you have not been able to stop taking drugs once you started?", "How often over the past year have you taken drugs and then neglected to do something you should have done such as work or family responsibilities?", "Has a relative or friend or a doctor or another health worker been worried about your drug use or said to you that you should stop using drugs?".

In a study by Berman et al. (2005), the DUDIT was found to predict drug dependence with a sensitivity of 90% and specificity of 78% for DSM-IV criteria. With regard to reliability, a Cronbach's alpha of 0.80 was documented (Berman et al., 2005). Additionally, a study that measured the reliability of the DUDIT of drug users in Hungary, reported a Cronbach's alpha of 0.92 across their sample groups (outpatient treatment program participants, drug treatment program participants, young adults at risk of drug use, controls), indicating good reliability (Matuszka et al., 2013). In a population of inpatient and outpatient substance abusers, the DUDIT was found to have a Cronbach's alpha of 0.94 and a high convergent reliability (r=0.85), when compared with the Drug Abuse Screening Test (DAST-10). Furthermore, its sensitivity was 0.90 and specificity 0.85. Also, Voluse et al. (2012) found the DUDIT to have good discriminant validity as it was able to identify drug abusers compared to alcohol abusers.

3.4.5 Alcohol Use Disorders Identification Test- Consumption (AUDIT-C)

The AUDIT-C was used to screen for alcohol abuse (Dawson et al., 2005). The AUDIT-C is a shortened version of the AUDIT, developed by the World Health Organisation to assess alcohol consumption and consists of three items (Dawson et al., 2005). A cut-off score of 2 was used to determine excessive alcohol drinking (Morojele et al., 2017). In a South

African HIV group, studied by Morojele et al. (2017), the AUDIT-C showed high sensitivity (0.98) and low specificity (0.23).

The AUDIT is a self-report scale, developed by the WHO to measure alcohol consumption, drinking behaviour, and alcohol-related problems (Saunders, Aasland, Babor, Fuente, & Grant, 1993). It consists of 10 items and takes approximately 2 minutes to complete. A total score ranging from 0-40 is obtained, and a cut-off score of 8 is usually accepted (Hays, Merz, & Nicholas, 1995). The AUDIT has been used in several settings and across countries and cultures (Babor, Higgins-Biddle, Saunders, & Monteiro, 2001). It has high internal consistency and very good test-retest reliability (Babor et al., 2001). Furthermore, an American study found that the AUDIT had very high internal consistency (Cronbach alpha of 0.83). In another study, the internal reliability of the AUDIT was 0.77 (Schmidt, Barry, & Fleming, 1995). Within the South African context, the AUDIT has been tested in a sample using HIV treatment and compared to the MINI (Myer et al., 2008). The AUDIT showed good sensitivity and specificity across all groups in the sample, identifying 100% of participants with MINI classified alcohol dependence/abuse (sensitivity), and 79% without MINI classified alcohol abuse and dependence. The AUDIT-C showed good reliability in the present study with a Cronbach alpha score of 0.86 at each visit.

3.4.6 Davidson Trauma Scale (DTS)

The DTS were used to screen for PTSD symptoms/criteria. The DTS is a self-report questionnaire that consists of 17 items consistent with the 17 DSM-IV symptoms of PTSD that have occurred during the past week (Davidson et al., 1997). The participant is asked to recognise their most traumatic event and rate the symptom frequency and severity experienced in the past week. Items are rated from 0 to 4 on a 5-point Likert scale that measures both the frequency, where 0 = not at all and 4 = every day, and the severity of the symptoms (0 = not at all distressing and 4 = extremely distressing). The severity and frequency scores range from 0 to 68 with a total score for the scale ranging from 0 to 136. A cut-off score of 40 was used to delineate PTSD diagnosis (Davidson et al., 1997; McDonald, Beckham, Morey, & Calhoun, 2009). Also, the scale can be used to calculate a subtotal score for each of the PTSD symptom clusters. Within the present study, cluster A refers to re-experiencing/intrusion symptoms (items 1-5), cluster B refers to avoidance/numbing symptoms (items 6-12), and cluster C refers to persistent symptoms of increased arousal / hyperarousal (items 13-17) (American Psychiatric Association [APA], 2013).

The scale demonstrated good test-retest reliability (r = 0.86) and internal consistency (r = 0.99) in a sample of war veterans, survivors of rape or hurricane and a mixed trauma group in a clinical trial. With comparison to the Structured Clinical Interview for DSM-III-R (SCID), the DTS showed a diagnostic accuracy of 83%. The DTS has shown good convergent and divergent validity (Davidson et al., 1997).

In a sample of sexual abuse survivors, the DTS has shown good internal consistency, with Cronbach alpha coefficients of .93 (full scale), .85 (re-experiencing subscale), .83 (avoidance subscale), and .87 (hyperarousal subscale) (Zlotnick et al., 1996). The DTS also showed adequate concurrent validity with the CAPS, IES (impact events scale), and the crime-related posttraumatic stress scale (CR-PTSD) (Zlotnick et al., 1996).

In the present study, participants with both severity and frequency scores (i.e. those participants without missing data in the DTS scale) had either the same score for severity than for frequency or very close to their frequency score. Missing severity scores were imputed based on a regression analysis (see Results chapter, section "*4.3 Comparison of posttraumatic stress symptoms (PTSS) between groups over time"* and Figure 4.3).

In the present study, the DTS showed excellent reliability with the following Cronbach alpha scores at each visit: Visit 1 frequency (0.95) and severity (0.97); Visit 2 frequency (0.93) and severity (0.92); Visit 3 frequency (0.94) and severity (0.94).

3.4.7 Life Events Checklist (LEC) - modified

An adapted version of the LEC was used for the RICE study, as well as the present study, to identify lifetime traumatic events (Mollica et al., 1993) at baseline, traumatic events since the last visit at three months follow-up and at six months follow-up. Responses were either "yes" or "no" and consisted of 12 items. The RICE PI reported that two items ("torture" and "sexual assault") of the LEC-modified version were not completed accurately by participants. Torture is not well translated into isiZulu and the meaning of "torture" was therefore misunderstood and misinterpreted. Sexual assault was also not well understood and responses to this item did not reflect past sexual assault (excluding the recent rape event, +- 20 days before). Participants found it difficult to distinguish between a recent event and sexual assault prior to the recent event. These two items were, therefore, excluded from the calculation of the total score on the LEC-modified version. Scores were summed to create a total score out of 10 (Mollica et al., 1993). Excluding these variables may have impacted on the validity of the LEC in the present

study. The following 10 items were used to calculate the total score: imprisonment, civil unrest/war, serious injury, being close to death, murder of family or friend, unnatural death of family/friend, murder of stranger/strangers, robbed/carjacked at gunpoint or knifepoint, kidnapped and/or any other event not listed. The total score calculated for the LEC-modified version was used as an indicator of previous trauma and referred to in the present study as trauma load. The baseline LEC-modified version was included in the present study.

The LEC is used to identify potential traumatic experiences (Gray et al., 2004). It was developed by the National Center for PTSD in accordance with the Clinician Administered PTSD Scale (CAPS). The LEC consists of 17 items and measures different types of exposure to an event, with scores 1 (happened to me), 2 (witnessed), 3 (learned about it), 4 (not sure), and 5 (does not apply) (Gray et al., 2004). Acceptable test-retest values have been calculated for the total score, as well as the separate item of the LEC. With regard to the reliability of the LEC, the kappa coefficients of the items were all above 0.5, with seven items having kappa values above 0.6, and only one below 0.4. The mean value of the kappa coefficient for all items was 0.6, and a retest correlation of r = 0.82 was calculated (Gray et al., 2004).

3.4.8 Childhood Trauma Questionnaire – Short Form (CTQ-SF) - modified

Childhood trauma (before the age of 18 years) was measured using a modified version of the CTQ-Short Form (CTQ-SF) (Bernstein & Fink, 1998; Chirwa et al., 2018). The modified version consisted of 14 items and measured five types of childhood trauma, namely witness of abuse of mother, sexual abuse, physical abuse, emotional abuse and parental neglect (Chirwa et al., 2018; Jewkes et al., 2010). The modified version consisted of the following seven modified items of the CTQ-SF: “I did not have enough to eat”, “I was told I was lazy or stupid or weak by someone in my family”, “Someone touched my buttocks or genitals or made me touch them when I did not want to”, “I was insulted or humiliated by someone in my family in front of other people”, “I was beaten at home with a belt or stick or whip”, “One or both of my parents were too drunk or drugged to take care of me”, “I had sex with someone because I was threatened or frightened or forced”. The questions were rated on a 4-point Likert scale, ranging from 1 = never, 2 = sometimes, 3 = often, 4 = very often.

The CTQ-SF is a self-report questionnaire that takes approximately 10 minutes to complete and consists of 28 questions (Bernstein et al., 1997). These questions are rated on a 5-point Likert scale, ranging from 1 = never true, to 5 = very often true. The scale assesses five

childhood traumas, namely physical abuse, sexual abuse, emotional abuse, physical neglect and emotional. Each subscale consists of five questions. The scale also has three questionnaires that are not included in the total score, but measures denial or minimisation and these questions are aimed at detecting false-negative traumatic reports, leading to a total of 28 questions. A total of one for these three questions are acceptable, but a total from 2-3 reflects response bias (Bernstein et al., 1997). The CTQ-SF has been found to have an internal consistency of 0.91 (Cronbach's alpha). The coefficients for the subscales were as follows: physical neglect (0.58), physical abuse (0.69), emotional abuse (0.83), emotional neglect (0.85), and sexual abuse (0.94) (Scher, Stein, Asmundson, McCreary, & Forde, 2001). The total score of the CTQ-SF gives an indication of the severity of the trauma, with a score ranging from 25-36 reflecting no to minimal trauma, 41-51 indicating low to moderate, 56-68 indicating severe trauma, and a score between 73 and 125 referring to severe/extreme traumatic experience (Bernstein et al., 1997; Bernstein & Fink, 1998). A brief screening version of the CTQ has been validated by Bernstein et al. (2003).

In South Africa, the CTQ has been used widely in research studies in different populations, for example, the CTQ was used in a study by Jewkes et al. (2006) where it was administered to participants in the rural areas of the Eastern Cape. The CTQ was recently validated in South Africa in African Black, Xhosa speaking, HIV positive and negative females with and without a history of childhood trauma (Spies, Kidd, & Seedat, 2019). With regards to reliability, the Cronbach alpha coefficients for the subscales were as follows: physical neglect (0.64), physical abuse (0.78), emotional abuse (0.67), emotional neglect (0.83), and sexual abuse (0.89) (Spies et al., 2019).

3.4.9 Perceived Stress Scale (PSS)

The PSS is a 10-item self-report scale used to determine an individual's appraisal of stressful situations and investigates elements of anxiety related to personal control, predictability and overload (Cohen et al., 1983). Responses are measured on a 4-point Likert scale with response options: 0 (never), 1 (almost never), 2 (Sometimes), 3 (Fairly often) and 4 (very often). The scale has shown good reliability and validity (Cohen & Williamson, 1988). The PSS showed good reliability in the present study with the following Cronbach alpha scores: at Visit 1 (0.82), Visit 2 (0.86), and Visit 3 (0.82).

3.4.10 Multidimensional Scale of Perceived Social Support (MPSS)

The MSPSS is a 12-item self-report scale used to determine the individual's perception of available levels of social support obtained from family and friends (Zimet et al., 1988). Responses are measured on a 4-point Likert scale with response options: 1 (strongly agree), 2 (agree), 3 (disagree), 4 (strongly disagree). This scale has shown good reliability, factorial validity and construct validity (Zimet et al., 1988). In the present study, good reliability for the MPSS was calculated at each visit: Visit 1 (0.89), Visit 2 (0.88), and Visit 3 (0.89).

3.4.11 Hair Questionnaire

A questionnaire specific to hair was used that asked about natural hair features, hair care practices, any scalp/hair problems, diagnosed hair conditions and other medications used in the past three months.

3.4.12 Health assessments

In order to address the aims of the RICE study, participants were screened for HIV, pregnancy, cardio-metabolic risks (blood pressure, measures of adiposity, blood glucose, lipid panels to monitor their trajectories over time and explain relationships). In HIV positive women, viral loads and CD4 counts were measured. Participants were asked about their medical history.

3.4.13 Past rape (excluding the recent event)

Since the item on sexual assault in the LEC-modified version was not accurately responded to by participants, the investigators of the RICE study decided to identify past rape by other questions that were asked during the first interview. Past rape (more than 20 days before the baseline visit, excluding the recent rape event) was identified with three questions where participants indicated one/more of the following: intimate partner rape and/or non-partner rape and/or first sexual intercourse that was forced/rape.

3.5 Recruitment and study procedures

3.5.1 Recruitment

Participants were recruited in Kwa-Zulu Natal (KZN). The study employed recruiters, who were based at the rape service centres and who worked closely with the rape service centre staff, to identify potential participants. Initially, information was shared about the study and if participants were interested, they were invited to the RICE clinic for full consent procedures. Rape-exposed participants were recruited from three dedicated sexual assault services known

as Thuthuzela Care Centres (TCCs: Umlazi Thuthuzela based at Prince Mshiyeni Memorial Hospital (PMMH), Mahatma Ghandi Memorial Hospital (MGMH) TCC in Phoenix, and the TCC at R K Khan Hospital in Chatsworth) and from a crisis centre based at Addington Hospital. RICE staff used posters to assist rape service centre staff to inform potential participants of the study. The rape service staff were trained by RICE project staff on the ethical issues that were pertinent to research procedures for the present study. Recruitment of controls was undertaken daily by RICE study staff at family planning clinics near the rape service centres.

Participants in the present study were recruited from August 2014 until June 2017 and follow-up data collection was completed in February 2018. The broader RICE study recruited 1284 participants during this period (August 2014 - June 2017) of which 593 were recently (within the past 20 days) exposed to rape (rape-exposed [RE]) and 691 were controls (see figure 3). The RICE study stopped recruitment end of March 2019 and follow-up data collection stopped on 25 March 2020. The present study was a sub-study while the RICE study was still ongoing. The present study's data collection was completed two years before the main study's completion.

For the present study, an average of 4.57 rape-exposed and 4.66 controls were recruited per month, with a minimum of 0 and a maximum of 16 participants per month. A total of 160 RE and 163 controls met inclusion criteria for the present study at the baseline visit. At the three-month follow-up visit 97 RE and 131 controls were retained and at six-month follow-up visit 77 RE and 119 control participants were retained. Overall, RICE recruited a total of 1799 participants by the end of March 2019, of which 947 were RE and 852 were controls.

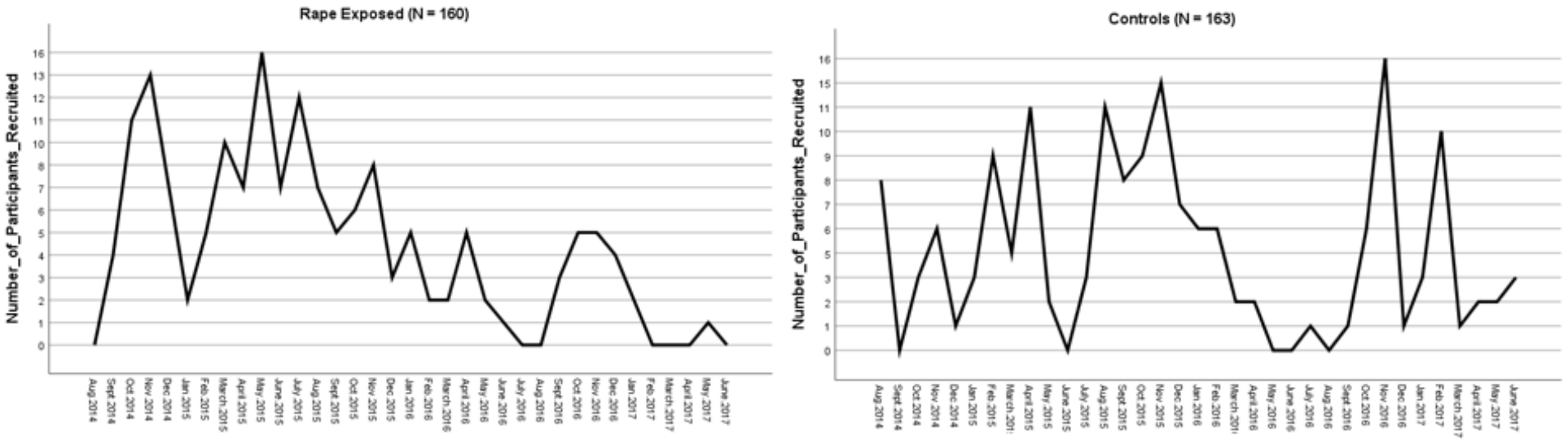

Figure 3.1 Participant Recruitment/Baseline visits completed August 2014 – June 2017 (RE and Controls)

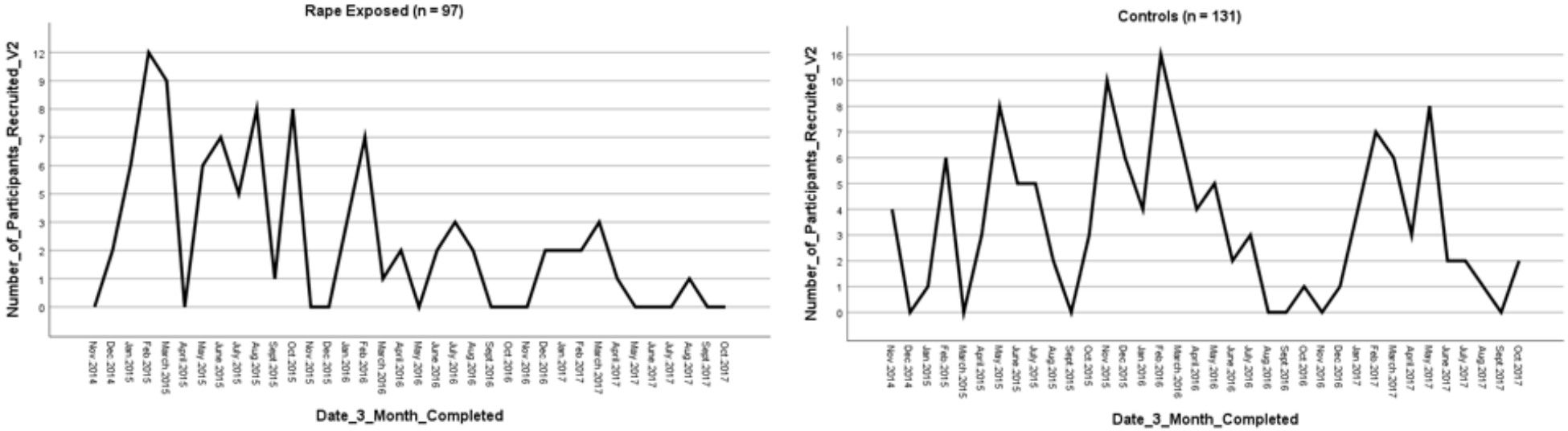

Figure 3.2 Visit 2: 3 Month Follow-up visits completed November 2014 – October 2017 (RE and Controls)

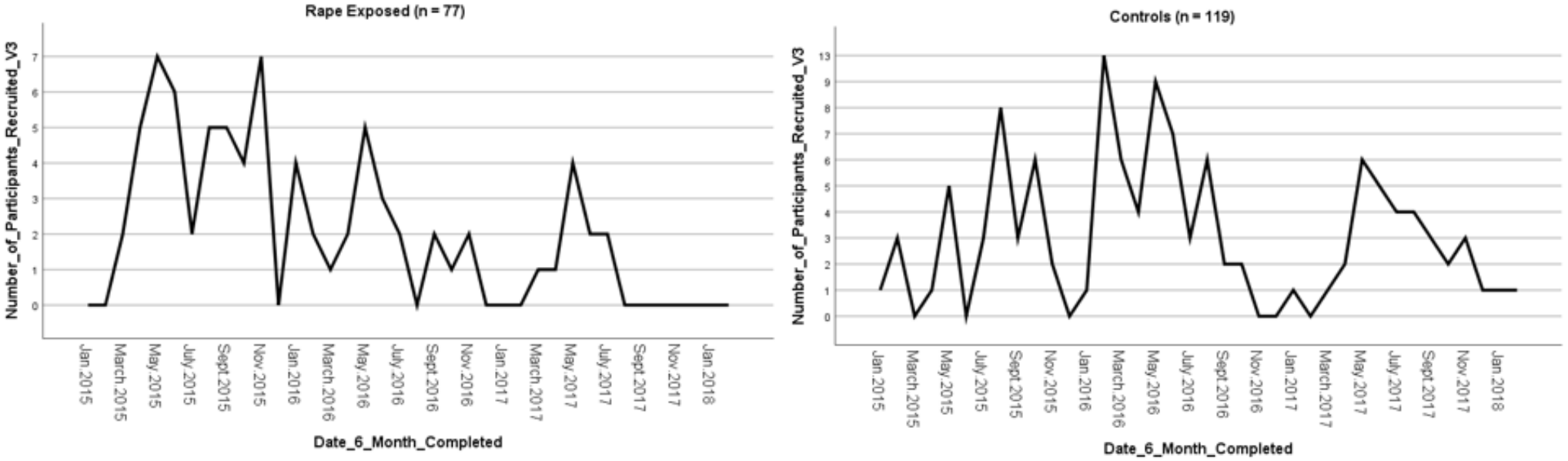

Figure 3.3 Visit 3: 6 Month Follow-up visits completed January 2015 – February 2018 (RE and Controls)

3.5.2 Study procedures

First, the rape service centres' staff informed potential participants of the RICE study. If they were interested in participating, they were asked if their contact details (see Addendum C) could be forwarded to project staff. They completed a form with their contact details and project staff contacted interested participants within 20 days to initiate consent procedures (see Addendum C). Eligible participants attended the RICE clinic and trained research assistants completed informed consent procedures, which included details on the hair sample collection. They were given the opportunity to decide if they wished to participate in the RICE study as well as in the hair cortisol sub-study (i.e. the present study). Participants were invited to return to the research clinic for research interviews every three months.

The two informed consent documents (Addenda A4 & A5) each included a section on hair sampling and a separate page for participants to sign specifically to agree to give a hair sample. Participants who signed consent documents participation in the RICE study were able to opt-out of consenting to blood specimens and/or hair samples.

In the informed consent document of the RICE study, it was explained to participants that they would be invited to visit the research clinic and have interviews every three months. Each interview took about two hours and at each visit hair samples were collected from participants who consented to giving a hair sample and where hair strands of 3cm or longer could be cut. Data for the present study was collected at three visits (baseline, three months follow-up and six months follow-up).

Participants who were diagnosed with a psychiatric disorder or showed moderate to high suicidality risk were referred to the trauma counsellor within the RICE, who provided immediate support and referral to services for further assessment or treatment, which may include referral back to the sexual assault services or other services for the control group. The RICE trauma counsellor provided short-term support only and participants could have up to three sessions with her while a referral was made for long-term counselling.

3.5.3 Hair sampling

For the present study, hair samples were collected at baseline (within 20 days after the rape), three months and at six months. Hair strands of approximately 3mm in diameter were cut with fine scissors as close as possible to the scalp from the posterior vertex position by RICE clinical staff. Hair was collected from this location because it shows the least inconsistency among

different strands (Sauvé, Koren, Walsh, Tokmakejian, & Van Uum, 2007) and hair from other parts of the body has shown inconsistent results (Sharpley, Kauter, & McFarlene, 2010). The end closest to the scalp was marked and hair was then secured in a bundle using a rubber band or string (See Addendum D1 & D2). The hair sample was then placed in an aluminium foil and placed into an envelope that was coded and sealed. First, samples were sent to Stellenbosch University, where they were stored in a dark, dry place at room temperature at the MAGIC laboratory at the Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Tygerberg Campus. Samples were batched and sent to the laboratory at Technische Universität Dresden for analysis.

3.5.4 Data and hair storage and analysis

Data was uploaded from personal digital assistants (PDAs) immediately and electronically transmitted to a secure server at the MRC in Cape Town. Hair samples were stored and analysed at the Technische Universität Dresden, Germany, under the supervision of Prof. Clemens Kirschbaum (see Addendum E for material transfer agreement).

A highly specific and sensitive liquid chromatography-tandem mass spectroscopy method was used to analyse the hair samples. This method allowed for the simultaneous detection of six hormones in hair (Gao et al., 2013). Samples were washed in 2.5ml isopropanol at room temperature for 3 minutes, after which it could dry under a fume hood, for 12 hours. The steroid hormones were extracted from 10mg whole, nonpulverized hair using 1.8ml methanol for 18 hours at room temperature. Samples were centrifuged in a bench top centrifuge at 10 000 rpm for 12 minutes. Afterwards 1ml of supernatant was transformed into a new 2ml tube. At 65°C the alcohol would have evaporated under a constant stream of nitrogen until completely dried. The dried residue was reconstituted with 250µl double-distilled water and 200µl of the reconstituted liquid was then injected into a Shimadzu HPLC-tandem mass spectrometry system (Shimadzu, Canby, Oregon) coupled to an ABSciex API 5000 Turbo-ion-spray triple quadrupole tandem mass spectrometer (AB Sciex, Foster City, California) with purification by on-line solid-phase extraction.

3.5.5 Retention

3.5.5.1 Retention rates

The retention rate was 61% for RE and 80% for controls from Visit 1 to Visit 2. The retention rate from Visit 2 to Visit 3 was 79.4% for RE and 90.8% for controls. Overall, the retention

rate (from baseline to the six-month follow-up) was 48% for RE and 73% for controls (see Table 3.1 and Figure 3).

Table 3.1 Participants (RE vs controls) completing each visit with hair samples

	Baseline (Visit 1)	**3-month follow-up (Visit 2)**	**6-month follow-up Visit 3**	**Overall retention rate**
Rape-Exposed	160	97	77	48%
Controls	163	131	119	73%

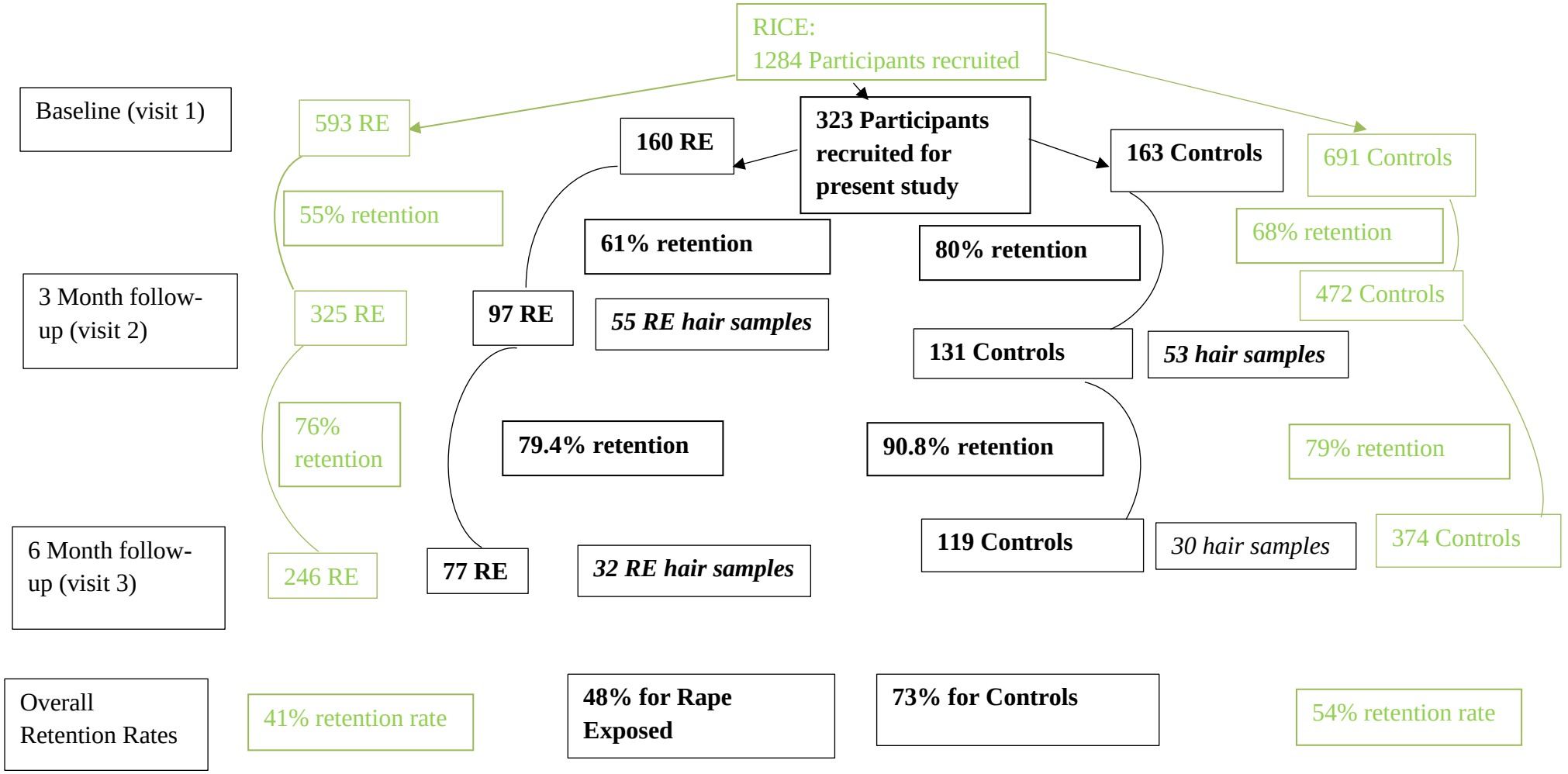

Figure 3 Study flow of participants and retention rates for RICE and present study

3.5.5.2 Retention strategies

RICE initiated several strategies to enable follow-up. The collection of extended contact details from study participants was important and changes in the contact details were updated at each visit. Contact details of family or friends were also collected. See Robinson, Dennison, Wayman, Pronovost, and Needham (2007) regarding strategies for retaining participants. Further retention strategies included the following: a retention counsellor was a member of the RICE study team. The most common barriers to attending follow-up visits was not having money for transportation, relocation to an area far from the clinic and some of the rape-exposed participants expressed that the study reminded them of the rape event. Home visits and assisting with transport to attend the clinic were also retention strategies implemented by RICE. Incremental increase of reimbursement for each follow-up visit was another way to improve retention (baseline visit = R100, R20 increase per follow-up visit). In addition, pampering sessions were provided to participants while they were waiting, e.g. having their nails varnished and hand massages. Participants also received grocery hampers for attending the 12-month follow-up visit.

3.6 Role of PhD candidate

The present study (*participants in the present study were recruited from August 2014 until June 2017 and follow-up data collection was completed in February 2018*) was a sub-study of the RICE main study and data collection was completed two years before the main study's completion. The RICE study stopped data collection on 25 March 2020. The PhD candidate made substantial contributions to the conception and design of the present study. The PhD candidate participated in meetings with the MRC/RICE research team, as well as contributing to the decision of questionnaires used in overall (RICE) research project, such as the mental health and hair questionnaire. The hair questionnaire was adjusted by the PhD candidate from a questionnaire from the Dresden team. Furthermore, the PhD candidate had a main role in the management of the hair samples and took full responsibility for the sub-study, however little responsibility for the parent study. Specifically, the candidate was responsible for organizing and setting up the transfer of hair samples from Stellenbosch University to the laboratory at Technische Universität Dresden. The PhD candidate visited the research sites in KZN and acquainted herself with the data collection process. Data collection was completed in isiZulu by RICE team members. The PhD candidate did quality checking for the present sub-study, including cross-checking all hair samples and matching these with the raw data. The candidate

created a PhD SPSS database, including entering all data applicable to PhD, as well as quality checking the database. Furthermore, the candidate was responsible for acquisition of data, analysis, interpretation of data, and performed statistical analyses with the assistance of Prof. Kidd. The candidate ensured that questions related to the accuracy of the work were appropriately resolved, worked independently in drafting, and revising the dissertation, and drafting manuscripts for publication.

3.7 Statistical analyses

Data was analysed using Statistica Version 13.5, with the assistance of a senior statistician (Prof. Kidd) at Stellenbosch University's Centre for Statistical Consultation. Descriptive statistics, analysis of variance (ANOVA) and group mean differences were performed between groups (rape-exposed and controls) for demographic, clinical and health variables. For binary response variables, generalised estimating equation (GEE) analyses were conducted with group, times, and group*time as the interaction term. Participants were included as the identification variable. Fisher's least significant difference (LSD) post-hoc analyses were done. All tests were two-tailed, and significance was set at $p < .05$.

3.7.1 Statistical analyses (outlined for each aim)

Herewith a description of the statistical analyses performed with regard to each aim:

Aim 1: To compare hormone (cortisol, cortisone, testosterone, progesterone, and DHEA) concentrations between groups (rape-exposed and controls) at different time-points.

The time-points were:

(1) Baseline (within 20 days after the rape), which yielded hormone concentration from three months before the trauma to baseline;

(2) Three months after trauma, which yielded hormone concentrations from baseline to three months;

(3) Six months after trauma, which yielded hormone concentrations from three months to six months post rape.

Mixed Model Repeated Measures ANOVAs were conducted to establish significant differences in hormone concentrations within and between groups at different time-points (baseline, three months follow-up and six months follow-up). LSD post-hoc testing was done.

Values outside the range of mean+3*standard deviation were excluded (Miller & Plessow, 2013). A trimmed mean (5% of smallest values and 5% of highest values were excluded) and a trimmed standard deviation (SD) were used to calculate the range. A graphical representation of hormone concentrations over time were created.

Only hormone concentrations that were detectable in laboratory analysis were included in statistical analyses and therefore participant numbers differ for each analysis. See Table 3.2 for participants included in each analysis.

Table 3.2 Number of participants included for each analysis (Aim 1)

	Cortisol		Cortisone		Testosterone		Progesterone		DHEA	
Visit	**RE**	**Controls**	**RE**	**Controls**	**RE**	**Controls**	**RE**	**Controls**	**RE**	**Controls**
Visit 1	139	130	146	143	72	85	127	115	144	155
Visit 2	50	46	47	52	55	53	53	53	48	50
Visit 3	31	28	31	28	33	30	31	29	32	28

Aim 2: To compare posttraumatic stress symptoms (as measured by the DTS) between groups (rape-exposed and controls) at different time-points.

The time-points were as follow:

(1) Baseline

(2) Three months after trauma

(3) Six months after trauma

A Mixed Model Repeated Measures ANOVA was conducted to establish significant differences in DTS total scores within and between groups at different time-points. LSD post-hoc testing was done. Furthermore, a cut-off score of 40 was used to delineate PTSD diagnosis in the DTS total score at three months and six months post rape. Generalised estimating equation (GEE) analyses were conducted with group, time, and group*time as the interaction term. A graphical representation of posttraumatic stress symptoms over time was also created.

See Table 3.3 for participants included in each analysis.

Table 3.3 Number of participants included for each analysis (Aim 2)

	DTS Imputed Total scores	
Visit	**RE**	**Controls**
Visit 1	160	163

Aim 3: To establish if there were significant temporal correlations between posttraumatic stress symptoms (as measured by the DTS) and HPA-axis hormones (cortisol, cortisone, testosterone, progesterone, and DHEA) measured at baseline, three months, and six months after rape.

Pearson's tests were performed to establish possible significant correlations between DTS total scores, as well as DTS subscales and cortisol, cortisone, testosterone, progesterone and DHEA concentrations in the rape-exposed group. See Table 3.4 for participants included in each analysis.

Table 3.4 Participants included for each correlation analysis with DTS in the RE group (Aim 3)

Hormone	Visit	DTS Visit 1	DTS Visit 2	DTS Visit 3
Cortisol	Visit 1 (Pre-trauma concentrations to baseline)	139	87	73
	Visit 2 (Baseline to 3 months post-rape concentrations)	50	50	37
	Visit 3 (3 Months to 6 months post-rape concentrations)	30	28	30
Cortisone	Visit 1 (Pre-trauma concentrations to baseline)	140	84	69
	Visit 2 (Baseline to 3 months post-rape concentrations)	52	45	34
	Visit 3 (3 Months to 6 months post-rape concentrations)	31	29	29
Testosterone	Visit 1 (Pre-trauma concentrations to baseline)	64	60	47
	Visit 2 (Baseline to 3 months post-rape concentrations)	53	50	38
	Visit 3 (3 Months to 6 months post-rape concentrations)	31	29	-
Progesterone	Visit 1 (Pre-trauma concentrations to baseline)	123	81	67
	Visit 2 (Baseline to 3 months post-rape concentrations)	53	49	38
	Visit 3 (3 Months to 6 months post-rape concentrations)	31	29	29
DHEA	Visit 1 (Pre-trauma concentrations to baseline)	141	85	72
	Visit 2 (Baseline to 3 months post-rape concentrations)	52	47	35
	Visit 3 (3 Months to 6 months post-rape concentrations)	31	29	28

Aim 4: To establish if pre-trauma hormone concentrations (as sampled at baseline) were predictive of the development of posttraumatic stress symptoms at baseline (within 20 days after rape exposure), three months, and six months post rape.

A linear regression analysis was performed in the RE group, with posttraumatic stress symptomatology (PTSS) (measured by DTS-total) as the dependent variable, as measured at baseline (n = 51), three- month (n = 48) and 6 month follow-up (n = 39), and the following independent variables (as measured at baseline) included in the model: cortisol, cortisone, testosterone, progesterone, and DHEA. Only participants with all the abovementioned variables were included in each regression model.

Next, univariate analyses (Pearson correlations and one-way ANOVAs) were conducted. Pearson moment correlation analyses were conducted in order to find any possible associations between baseline (Visit 1) demographic characteristics (age and education), baseline clinical data (depression, alcohol use, perceived stress, childhood abuse, social support, trauma load) and baseline hormone concentrations (cortisol, cortisone, testosterone, progesterone, DHEA) and the dependent variable (PTSS, as measured by the DTS total score), as measured at baseline (Visit 1), Visit 2 (three-months post rape), and Visit 3 (Six months post rape), within the RE group. A one-way ANOVA was conducted to establish significant associations between HIV and past rape (excluding the recent rape trauma), as measured at baseline, and posttraumatic stress symptoms (DTS Total), as measured at visits 1, 2 and 3, in the RE group.

Furthermore, regression analyses with cortisol and the abovementioned covariates were performed. A linear regression analysis was performed in the RE group, with PTSS (measured by the DTS-total score at baseline) as the outcome variable and cortisol (as measured at baseline) as predictor variable. The following variables (as measured at baseline) were considered possible covariates: Age, education, HIV, past rape (excluding the recent event), childhood abuse, trauma load, perceived stress (PSS total), alcohol use (AUDIT-C Total), depression (CES-D-total), and social support (MPSS total). The regression analysis was done with baseline (n = 136), three-month (n = 84) and six-month follow-up (n = 70) PTSS data, and baseline cortisol and covariate data, in the RE group. Only participants with all the abovementioned variables were included into each regression model.

Multicollinearity assumptions in regression analyses were checked by reviewing tolerance indexes, and the guideline of tolerances <0.2 were satisfied in all regression analyses.

Univariate analyses (Pearson correlations and one-way ANOVAs) were conducted to establish possible significant associations between cortisol and the following variables in the RE group: BMI, systolic blood pressure, diastolic blood pressure, smoking, age, depression, alcohol use, hair wash frequency, use of hormone containing products, medicated shampoo or scalp treatment, and steroid containing medication, as measured at baseline, three months and six months post rape.

3.7.2 Additional differences between groups

In order to provide more clarity of possible differences between the rape-exposed and control group, the following variables were further explored: (1) experiences of trauma (including trauma load, childhood trauma, and previous rape [excluding the recent rape trauma]), (2) HIV status, (3) psychopathology, as measured by the MINI (including depression, as measured by the CES-D), (4) alcohol use, as measured by the AUDIT-C, (5) BMI, (6) perceived stress, (7) social support, (8) blood pressure, (9) smoking, and (10) hair characteristics and practices.

Mixed model repeated measures ANOVAs were conducted to establish whether there were significant group differences (RE vs controls) in depression (CES-D total score), alcohol use (AUDIT-C total score), BMI, perceived stress (PSS total score), social support (MPSS total score), average systolic and diastolic blood pressure (three systolic and three diastolic blood pressure measurements were taken for each participant at each visit and the average of the three measurements at each visit were used for analyses), between visits, and within groups at the three different visits (visit 1 which constituted the baseline, Visit 2 which constituted the three-month follow-up, and Visit 3 which constituted the six-month follow-up).

An ANOVA was conducted to test for significant group differences with regard to trauma load and childhood trauma, as well as on the subscales of childhood trauma, as measured by the adapted version of the CTQ-SF. Levene's Test for Homogeneity of Variances was performed. Welch's t-test was performed for “witnessing the abuse of a mother” and “childhood sexual abuse”.

For binary response variables, generalised estimating equation (GEE) analyses were conducted with group, time, and group*time as the interaction term. Participants were included as the identification variable.

LSD post-hoc analyses were done. All tests were two-tailed, and significance was set at $p < .05$.

4. RESULTS

4.1 Demographic characteristics of participants

All participants (N = 323) were female (rape-exposed [RE]: n = 160, controls: n = 163). There were no statistically significant differences in demographic characteristics between groups (RE vs controls). The mean age of participants was 25.5 years (*SD* = 5.4, range 18-40 years, *p* = 0.43). Almost all participants (n = 316, 98%) were from African Black ethnicity (RE: 155 [96.9%]; controls 161 [98.8%]), and isiZulu (n = 290, 90%) was their home language* in the majority (RE: 149 [90.6%]; controls: 145 [89%]). With regard to educational level, almost all participants had completed secondary education (ranging from Gr.8-Gr.12) (RE: 153 [95.7%]; controls: 151 [92.7%]) with no statistically significant difference in the number of years of education between groups (RE vs controls) (*p* = 0.52). The source of income for most (n = 105, 33%; RE: 52 [32.5%]; controls: 53 [32.5%]) was family support (parents/grandparents), followed by a child support grant (n = 102, 32%; RE: 48 [30.0%]; controls: 54 [33.1%]), and then part-time employment (n = 34, 11%; (RE: 15 [9.4%]; controls: 19 [11.7%]). See Table 4.1 for a summary of the demographic details of participants.

4.1.1 Demographic characteristics of participants from the RICE study

Compared to the present study, participants recruited during the same time period (August 2014 – June 2017), all participants (N = 1284) in RICE were also female (RE: n = 593, controls: n = 691). At this time (June 2017), only 46.2% of the total RE participants were recruited, and 53.8% of the controls in the overall RICE study. The RICE study stopped recruitment March 2019 and follow-up data collection stopped on 25 March 2020, i.e. two years after data collection was completed for the present study. The mean age of RE participants was 24.9 years (*SD* = 5.4, range 16-40 years), and for controls was 25.5 (*SD* = 5.5, range 18-40 years, *p* = 0.03). In the present PhD sub-study there was no significant difference in age between groups (RE vs controls) (*p* = 0.43). In congruence with the present PhD sub-study, almost all participants (n = 1273, 99%) were from African Black ethnicity (RE: 587 [99%]; controls 686 [99.3%]), and isiZulu (n = 1152, 89.7%) was their home language in the majority (RE: 526 [88.7%]; controls: 626 [90.6%]). With regard to educational level, almost all participants had completed secondary education (ranging from Gr.8-Gr.12) (RE: 531 [89.5%]; controls: 636 [92%]) with a statistically significant difference in the number of years of education between groups (RE vs controls) (*p* = 0.04). In the present PhD sub-study, almost all participants also completed secondary education, however there was no significant difference in the number of

years of education between groups (RE vs controls) (p = 0.52). In congruence with the demographics of the participants from the present PhD sub-study, the source of income for most (n = 489, 38%; RE: 214 [36.1%]; controls: 275 [39.8%]) was family support (parents/grandparents), followed by a child support grant (n = 412, 32%; RE: 161 [27.2%]; controls: 251 [36.3%]), and then part-time employment (n = 116, 9%; (RE: 56 [9.4%]; controls: 60 [8.7%]). See Table 4.1.1 for a summary of the demographic details of participants from the RICE study.

Table 4.1 Socio-Demographic Details (N=323)

		Rape-exposed (N=160)	**Controls (N=163)**	***p*-value**
Age	Mean (SD)	25.2 (5.4)	25.7 (5.3)	0.43
	Range	18-40	18-40	
Ethnicity	Black	155 (96.9%)	161 (98.8%)	
	Coloured	2 (1.3%)	0	
	Asian/Indian	3 (1.9%)	2 (1.2%)	
Home Language*	English	4 (2.5%)	3 (1.8%)	
	isiZulu	145 (90.6%)	145 (89%)	
	Sesotho	1 (0.6%)	4 (2.5%)	
	Xhosa	8 (5.0%)	11 (6.7%)	
	Afrikaans	1 (0.6%)	0	
	Other	1 (0.6%)	0	
Years of Education	Mean (SD)	11.39 (1.41)	11.29 (1.55)	0.52
Educational Level	Primary Grade (Grade 1-7)	4 (2.5%)	6 (3.7%)	
	Secondary Grade (Grade 8-12)	153 (95.7%)	151 (92.7%)	
	Tertiary Qualification	3 (1.9%)	6 (3.7%)	
Source of Income				0.56
	Self-employed	6 (3.8%)	5 (3.1%)	
	Full-time employment	17 (10.6%)	8 (4.9%)	
	Part-time employment	15 (9.4%)	19 (11.7%)	
	Child support grant	48 (30.0%)	54 (33.1%)	
	Social grant for disability	3 (1.9%)	8 (4.9%)	
	Partner support	8 (5.0%)	8 (4.9%)	
	Family support (parents/grandparents)	52 (32.5%)	53 (32.5%)	
	No Income	11 (6.9%)	8 (4.9%)	

**Mother tongue*

Table 4.1.1 Baseline socio-demographic details of overall RICE study (N=1284)

		Rape-exposed n=593 (46.2%)	Controls n=691 (53.8%)	*p* -value
Age	Mean (SD)	24.9 (5.4)	25.5 (5.5)	0.03
	Range	16-40	18-40	
Ethnicity	Black	587 (99.0%)	686 (99.3%)	0.15
	Coloured	3 (0.5%)	0 (0.0%)	
	Asian/Indian	3 (0.5%)	5 (0.7%)	
Home Language	English	9 (1.5%)	11 (1.6%)	0.47
	isiZulu	526 (88.7%)	626 (90.6%)	
	Sesotho	4 (0.7%)	8 (1.2%)	
	Xhosa	50 (8.4%)	42 (6.1%)	
	Afrikaans	1 (0.2%)	0 (0.0%)	
	Other	3 (0.5%)	4 (0.6%)	
Years of Education	Mean (SD)	11.1 (1.5)	11.2 (1.3)	0.04
Education Level	Primary Grade (Grade 1-7)	19 (3.2%)	15 (2.2%)	0.28
	Secondary Grade (Grade 8-12)	531 (89.5%)	636 (92.0%)	
	Tertiarty Qualification	43 (7.3%)	40 (5.8%)	
Source of Income	Self-employed	13 (2.2%)	26 (3.8%)	0.10
	Full-time employment	86 (14.5%)	26 (3.8%)	<0.01
	Part-time employment	56 (9.4%)	60 (8.7%)	0.64
	Child support grant	161 (27.2%)	251 (36.3%)	<0.01
	Social grant for disability	10 (1.7%)	14 (2.0%)	0.65
	Partner support	34 (5.7%)	39 (5.6%)	0.95
	Family support (parents/grandparents)	214 (36.1%)	275 (39.8%)	0.17
	No Income	47 (7.9%)	40 (5.8%)	0.13

Aim 1: To compare hormone (cortisol, cortisone, testosterone, progesterone, and DHEA) concentrations between groups (rape-exposed and controls) at different time-points.

4.2 Comparison of hormone concentrations between groups

Mixed Model Repeated Measures ANOVAs were conducted to establish significant differences in hormone concentrations (cortisol, cortisone, testosterone, progesterone, and DHEA) within and between groups (rape-exposed and controls) at different time-points (baseline, three-month follow-up and six months follow-up). LSD post-hoc testing was done. Values outside the range of mean+3*standard deviation were excluded. A trimmed mean (5% of smallest values and 5% of highest values were excluded) and a trimmed SD were used to calculate the range.

4.2.1 Cortisol

There was no significant interaction effect for group*time (p = 0.92), implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group) (see Figure 4.2.1). Furthermore, there was no significant group main effect (p = 0.68) and no significant time main effect (p = 0.15), implying that there were no significant differences between groups or between visits. Although not significant, the RE group showed slightly lower cortisol concentrations as measured at Visit 2 and at Visit 3 compared to controls, and mean cortisol concentration decreased over time in both groups. See tables 4.2.1.1 and 4.2.1.2, as well as Figure 4.2.1.

Table 4.2.1.1 Cortisol concentrations

	Rape-exposed			Controls		
	N	**Mean (pg/mg)**	**SEM**	**N**	**Mean (pg/mg)**	**SEM**
Visit 1	139	9.58	0.87	130	9.66	0.89
Visit 2	50	7.63	1.39	46	8.58	1.45
Visit 3	31	7.12	1.74	28	7.74	1.83

Table 4.2.1.2 Results of ANOVAs for main and interaction effects regarding cortisol concentrations

	***F* value**	***p* value**
Main group effect	0.18	0.68
Main time effect	1.91	0.15
Interaction effect (Group*time)	0.09	0.92

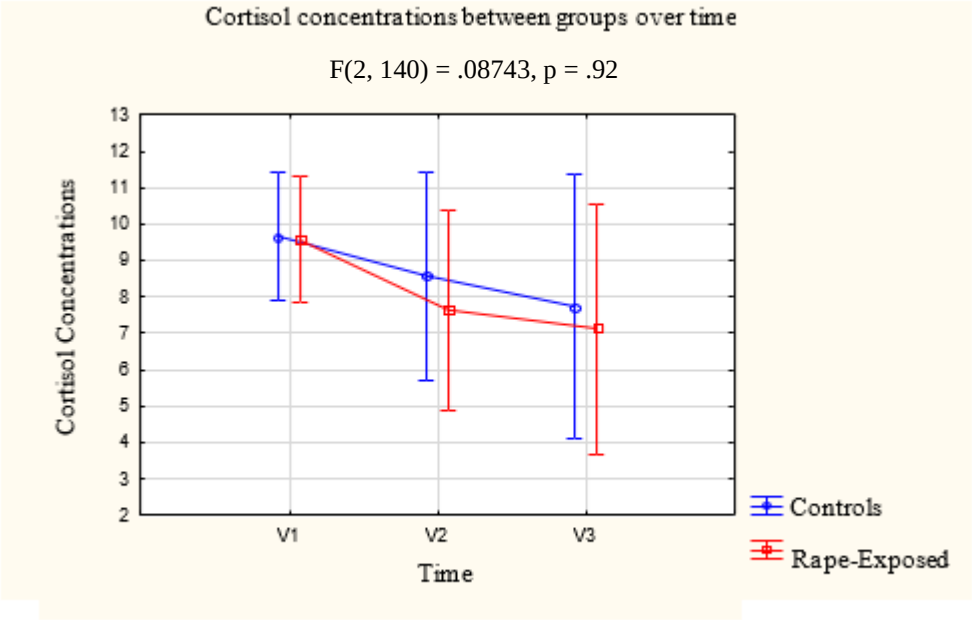

Figure 4.2.1 Cortisol concentration between groups (RE and Controls)

4.2.2 Cortisone

There was no significant interaction effect for group*time (p = 0.33), implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group) (see Figure 4.2.2). Furthermore, there was no significant group main effect (p = 0.22) and no significant time main effect (p = 0.73), implying that there were no significant differences between groups or between visits. Although not significant, mean cortisone concentration in the RE group was higher as measured at Visit 2 compared to measurement at Visit 1 and decreased at Visit 3. The RE group also had slightly (non-significant) higher mean cortisone concentration than the control group at Visit 2 and at Visit 3. See tables 4.2.2.1 and 4.2.2.2, as well as Figure 4.2.2.

Table 4.2.2.1 Cortisone concentrations

	Rape-exposed			**Controls**		
	N	**Mean (pg/mg)**	**SEM**	**N**	**Mean (pg/mg)**	**SEM**
Visit 1	146	8.59	0.63	143	8.63	0.64
Visit 2	47	10.38	1.08	52	8.10	1.03
Visit 3	31	9.49	1.31	28	8.17	1.38

Table 4.2.2.2 Results of ANOVAs for main and interaction effects regarding cortisone concentrations

	***F* value**	***p* value**
Main group effect	1.51	0.22
Main time effect	0.31	0.73
Interaction effect (Group*time)	1.11	0.33

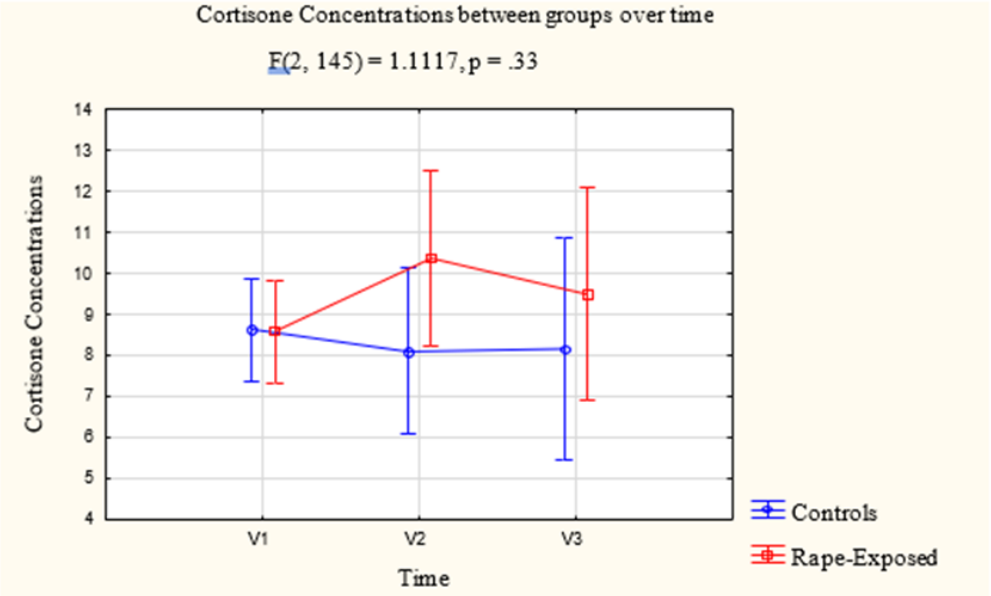

Figure 4.2.2 Cortisone concentration between groups (RE and Controls)

4.2.3 Testosterone

There was no significant interaction effect for group*time (p = 0.10), implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group). Furthermore, there was no significant group main effect (p = 0.09) and no significant time main effect (p = 0.42), implying that there were no significant differences between groups or between visits. Although not significant, mean testosterone concentration was lower in the RE group compared to the control group at all three time-points. See tables 4.2.3.1 and 4.2.3.2, as well as Figure 4.2.3.

Table 4.2.3.1 Testosterone concentrations

	Rape-exposed			**Controls**		
	N	**Mean (pg/mg)**	**SEM**	**N**	**Mean (pg/mg)**	**SEM**
Visit 1	72	0.44	0.49	85	1.55	0.46
Visit 2	55	0.39	0.49	53	1.65	0.46
Visit 3	33	0.42	0.49	30	1.46	0.46

Table 4.2.3.2 Results of ANOVAs for main and interaction effects regarding testosterone concentrations

	***F* value**	***p* value**
Main group effect	2.9	0.09
Main time effect	0.88	0.42
Interaction effect (Group*time)	2.3	0.1

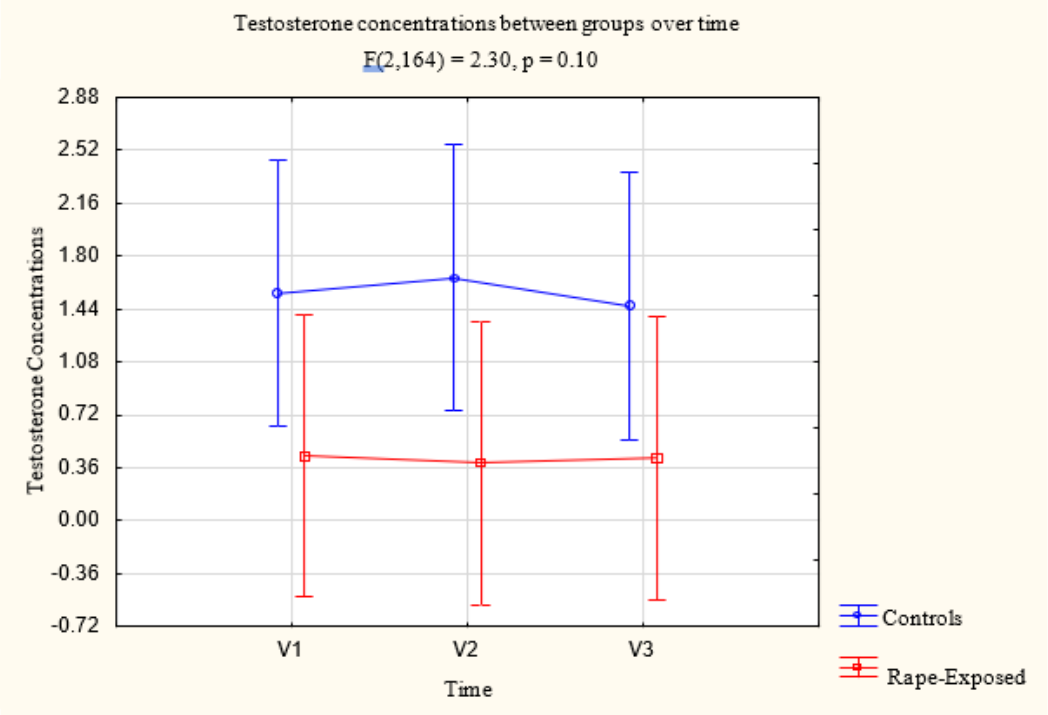

Figure 4.2.3 Testosterone concentration between groups (RE and Controls)

4.2.4 Progesterone

There was no significant interaction effect for group*time (p = 0.36), implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group). Furthermore, there was no significant group main effect (p = 0.97), implying that there were no differences between groups. However, a significant time main effect was found (p < 0.01), implying significant changes between visits. LSD post-hoc testing revealed the following significant differences: The RE group at Visit 1 had a higher mean progesterone concentration than at Visit 2 (p < 0.01), and the RE group at Visit 1 had a higher mean progesterone concentration than at Visit 3 (p = 0.04). However, controls at Visit 1 also had a higher mean progesterone concentration than controls at Visit 3 (p = 0.03). See tables 4.2.4.1, 4.2.4.2. as well as Figure 4.2.4.

Table 4.2.4.1 Progesterone concentrations

	Rape-exposed			Controls		
	N	**Mean (pg/mg)**	**SEM**	**N**	**Mean (pg/mg)**	**SEM**
Visit 1	127	3.96	0.25	115	3.74	0.26
Visit 2	53	2.76	0.38	53	3.34	0.38
Visit 3	31	2.88	0.49	29	2.55	0.51

Table 4.2.4.2 Results of ANOVAs for main and interaction effects regarding progesterone concentrations

	***F* value**	***p* value**
Main group effect	0.00	0.97
Main time effect	6.57	0.00**
Interaction effect (Group*time)	1.03	0.36

**$p < 0.01$

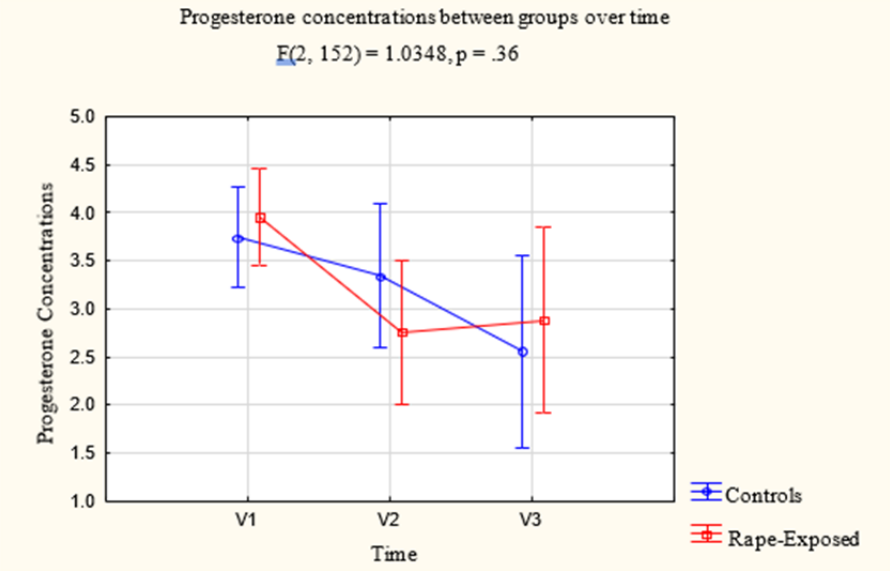

Figure 4.2.4 Progesterone concentration between groups (RE and Controls)

4.2.5 DHEA

There was no significant interaction effect for group*time (p = 0.84), implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group). Furthermore, there was no significant group main effect (p = 0.40) and no significant time main effect (p = 0.16), implying that there were no significant differences between groups or between visits. Although not significant, mean DHEA concentration were slightly higher in the RE group compared to the controls at all three time-points. See tables 4.2.5.1 and 4.2.5.2, as well as Figure 4.2.5.

Table 4.2.5.1 DHEA concentrations

	Rape-exposed			Controls		
	N	**Mean (pg/mg)**	**SEM**	**N**	**Mean (pg/mg)**	**SEM**
Visit 1	144	32.2	1.79	155	28.31	1.74
Visit 2	48	30.53	2.83	50	26.64	2.77
Visit 3	32	26.07	3.38	28	24.75	3.56

Table 4.2.5.2 Results of ANOVAs for main and interaction effects regarding DHEA concentrations

	***F* value**	***p* value**
Main group effect	0.71	0.40
Main time effect	1.88	0.16
Interaction effect (Group*time)	0.18	0.84

$^{**}p < 0.01$

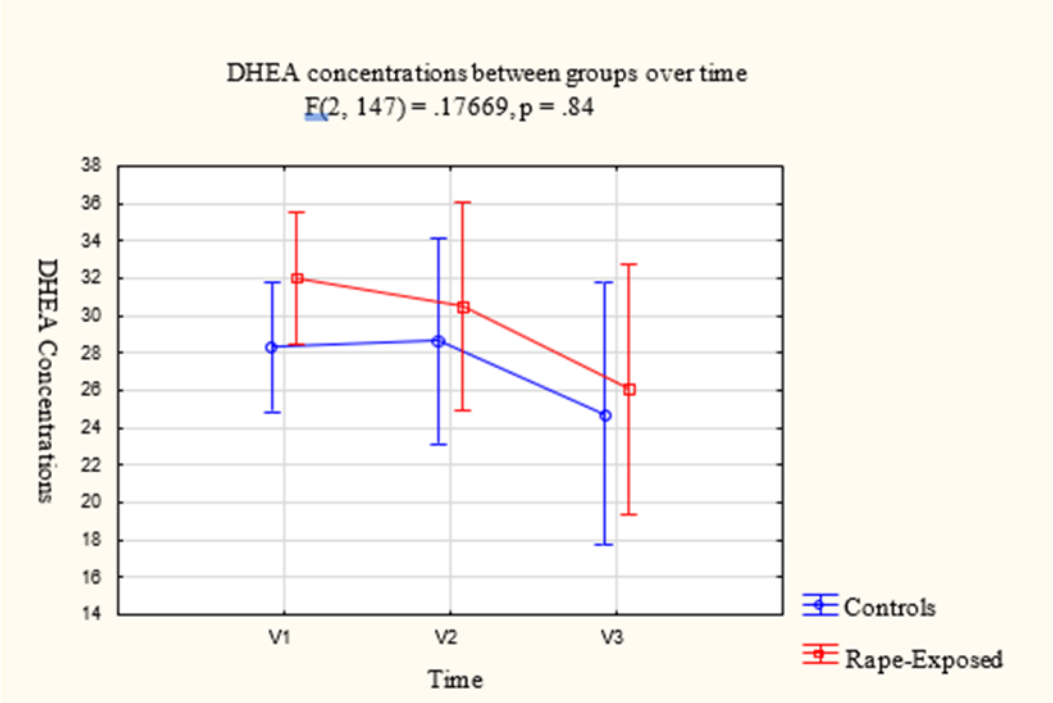

Figure 4.2.5 DHEA concentration between groups (RE and Controls)

Aim 2: To compare posttraumatic stress symptoms (as measured by the Davidson Trauma Scale [DTS]) between groups (rape-exposed and controls) at different time-points.

4.3 Comparison of posttraumatic stress symptoms (PTSS) between groups over time

Regression analyses revealed a linear association between the Davidson Trauma Scale (DTS) severity and DTS frequency scores, with a gradient very close to 1 (m = 0.99) and y-intercept very close to zero (c = 0.02) (see scatterplot below, Figure 4.3).

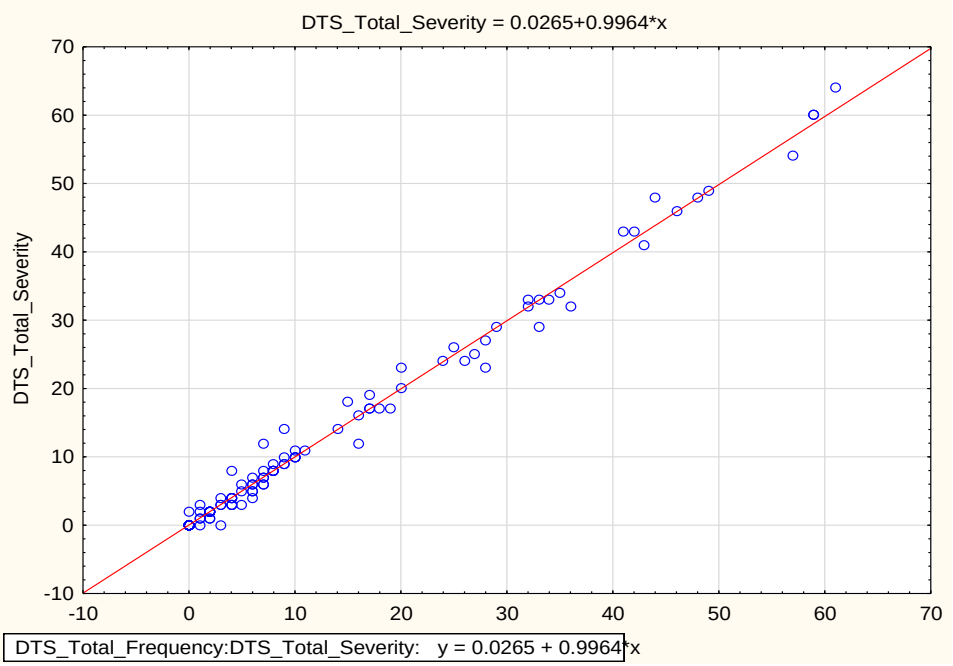

Figure 4.3 DTS severity and frequency

Participants with both severity and frequency scores (i.e. those participants without missing data in the DTS scale) had either the same score for severity than for frequency or very close to their frequency score. Missing severity scores were imputed based on the regression analysis (as indicated in the scatterplot above, see Figure 4.3).

A Mixed Model Repeated Measures ANOVA was conducted to establish significant differences in DTS total imputed mean scores within and between groups at different time-points. A significant interaction effect for group*time ($p < 0.01$) was found, implying that changes from Visit 1 through Visit 2 to Visit 3 were not the same for controls and cases (RE group). Furthermore, a significant group main effect ($p < 0.01$) and a significant time main effect ($p < 0.01$) was found, implying that there were differences between groups and between visits. LSD post-hoc testing revealed the following significant differences:

At visits 1 (V1), Visit 2 (V2), and Visit 3 (V3), the RE group had significantly higher mean DTS scores than controls ($p < 0.01$). The RE group had significantly higher mean DTS scores at visit 1 compared to Visit 2 ($p < 0.01$) and visit 3 ($p < 0.01$), respectively. The RE group also had significantly higher mean DTS scores at Visit 2 compared to Visit 3 ($p = 0.02$). See tables 4.3.1 and 4.3.2, as well as Figure 4.4.

Table 4.3.1 DTS Imputed Total Scores for Rape Exposed and Control Participants

	Rape-Exposed			Controls		
	N	**Mean**	**SD**	**N**	**Mean**	**SD**
Visit 1	160	67.67	35.3	163	17.94	24.32
Visit 2	98	37.04	31.71	134	12.32	17.89
Visit 3	78	29.36	31.98	120	12.04	19.11

Table 4.3.2 Results of ANOVAs for main and interaction effects regarding posttraumatic stress symptomatology (PTSS)

	***F* value**	***p* value**
Main group effect	142.47	0.00**
Main time effect	63.25	0.00**
Interaction effect (Group*time)	36.44	0.00**

***p < 0.01*

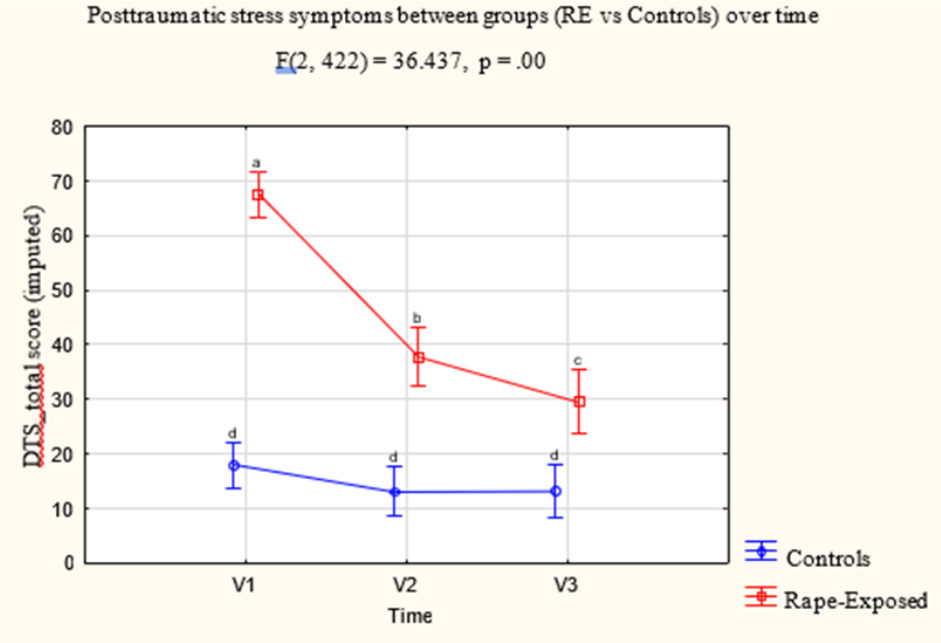

Figure 4.4 Posttraumatic stress symptomatology (DTS total score) between groups (RE vs Controls)

4.3.1 PTSD with DTS cut-off scores: differences between groups

A cut-off score of 40 was used to delineate PTSD diagnosis in the DTS total score at three months and six months post rape (Davidson et al., 1997; McDonald et al., 2009). Generalised estimating equation (GEE) analyses were conducted with group, time, and group*time as the interaction term. Participant identification was included as the identification variable for two outcomes (Visit 2 and Visit 3) over time per subject.

There was no significant interaction effect for group*time (p = 0.08), implying that changes from Visit 2 to Visit 3 were the same for controls and cases (RE group). However, a significant group main effect (p < 0.01) and a significant time main effect (p = 0.04) was found (see Table 4.3.4), implying changes between visits and between groups, respectively. LSD post-hoc analysis revealed the following significant differences: Higher rates of PTSD diagnosis was found in the RE group at Visit 2 compared to controls at Visit 2 (p < 0.01), as well higher rates of PTSD diagnosis in the RE group at Visit 3 compared to controls at Visit 3 (p < 0.01). In the RE group, there was a significant decrease in rates of PTSD diagnosis from Visit 2 to Visit 3 (p = 0.02).

Table 4.3.3 PTSD diagnosis between groups according to DTS cut-off scores

	Rape-exposed	**Controls**
	N (%)	N (%)
Visit 2 (3-month follow-up)	45 (46%)	11 (8%)
Visit 3 (6-month follow-up)	23 (29%)	11 (9%)

Table 4.3.4 Results of GEE for main and interaction effects regarding PTSD diagnosis according to DTS cut-off score

	Wald	***p* value**
Main group effect	39.45	0.00**
Main time effect	4.02	0.04*
Interaction effect (Group*time)	3.01	0.08

* p < 0.05
** p < 0.01

Aim 3: To establish if there were significant temporal correlations between posttraumatic stress symptoms (as measured by the DTS) and HPA-axis hormones (cortisol, cortisone, testosterone, progesterone, and DHEA) measured at baseline (Visit 1), three months (Visit 2), and six months (Visit 3) after rape.

4.4 Correlation analyses in rape-exposed group

Correlation analysis (Pearson's tests) of DTS total and subscale scores (Cluster A, Cluster B, and Cluster C) and cortisol / cortisone / testosterone / progesterone / DHEA concentrations, as measured at Visit 1 (baseline), Visit 2 and Visit 3 was performed in the rape-exposed group. Within the present study, Cluster A refers to re-experiencing/intrusion symptoms, Cluster B refers to avoidance/numbing symptoms, and Cluster C refers to persistent symptoms of increased arousal / hyperarousal (American Psychiatric Association [APA], 2013).

4.4.1 Cortisol and PTSS

In the RE group, a significant positive correlation was found between DTS total (as measured at visit 1) and pre-trauma cortisol concentrations (as measured at visit 1) ($r = 0.22$, $p < 0.01$) (see Table 4.4.1 and Figure 4.5). When pre-trauma cortisol concentrations were relatively low (between 0 -10 pg/mg), more variance was observed in DTS total scores (as measured at visit 1). However, when the pre-trauma cortisol concentrations increased above 20 pg/mg, DTS total scores correlated positively with pre-trauma cortisol concentrations (see scatterplot in Figure 4.5). Significant positive correlations were also found between pre-trauma cortisol concentrations (as measured at Visit 1) and the following subscales of the DTS (as measured at visit 1): DTS Cluster A (re-experiencing/intrusion symptoms) ($r = 0.21$, $p < 0.05$), DTS Cluster B (avoidance/numbing symptoms) ($r = 0.18$, $p < 0.05$), and DTS Cluster C (hyperarousal) ($r = 0.20$, $p < 0.05$). See Table 4.4.1.

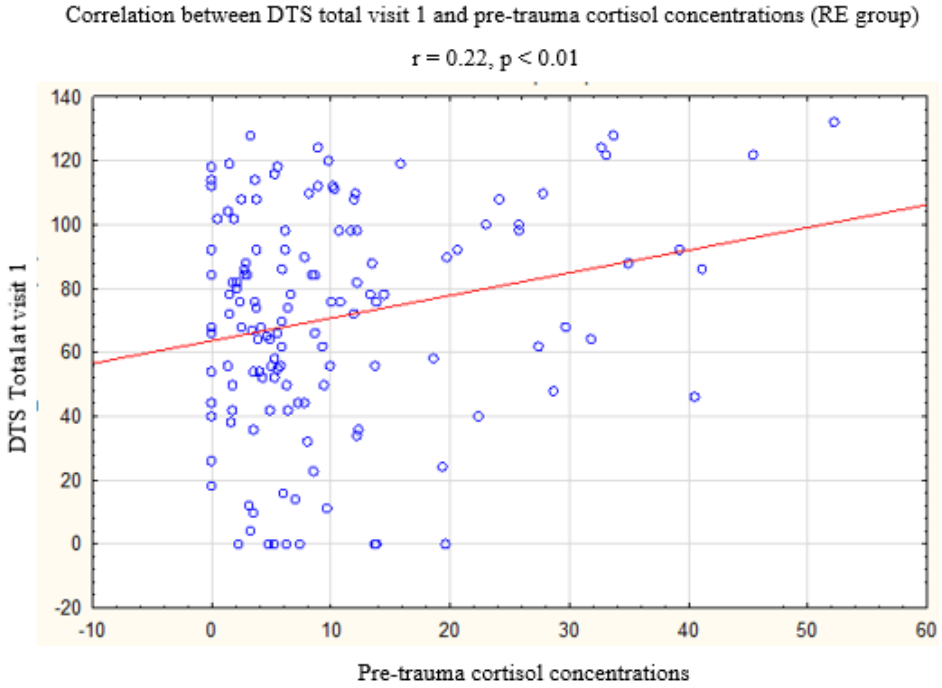

Figure 4.5 Scatterplot of DTS total (v1) and pre-trauma cortisol concentration (RE group)

Table 4.4.1 Correlation analyses for cortisol and posttraumatic stress symptoms in the rape-exposed group

	Visits	**Baseline cortisol measurement (pre-trauma)**			**Cortisol V2 (0-3months)**			**Cortisol V3 (3-6months)**		
		n	**r-value**	***p*-value**	**n**	**r-value**	***p*-value**	**n**	**r-value**	***p*-value**
DTS Total	V1	139	0.22	0.00**	50	0.01	0.97	30	-0.18	0.34
	V2	87	0.04	0.71	50	0.05	0.74	28	-0.18	0.35
	V3	73	0.05	0.65	37	0.03	0.85	30	0.22	0.24
Re-experiencing / intrusion	V1	139	0.21	0.01*	50	0.07	0.64	30	-0.03	0.89
	V2	87	0.03	0.77	50	0.10	0.49	28	-0.10	0.61
	V3	73	0.02	0.87	37	0.05	0.76	30	0.23	0.23
Avoidance / numbing symptoms	V1	139	0.18	0.03*	50	-0.09	0.53	30	-0.23	0.21
	V2	87	0.02	0.82	50	0.04	0.79	28	-0.19	0.33
	V3	73	0.06	0,6	37	-0.07	0.69	30	0.19	0.33
Hyperarousal	V1	139	0.20	0.02*	50	0.07	0.64	30	-0.19	0.32
	V2	87	0.06	0.61	50	-0.01	0.96	28	-0.19	0.34
	V3	73	0.04	0.76	37	0.11	0.5	30	0.17	0.36

*$p < 0.05$
**$p < 0.01$

4.4.2 Cortisone and PTSS

A significant positive correlation was found between DTS Cluster B (avoidance / numbing symptoms) v2 and cortisone, as measured at v3 (r = 0.39, *p* = 0.04) (see Table 4.4.2).

Table 4.4.2 Correlation analyses for cortisone and posttraumatic stress symptoms in the rape-exposed group

	Visits	**Baseline cortisone measurement (pre-trauma)**			**Cortisone v2 (0-3 months)**			**Cortisone v3 (3-6 months)**		
		n	**r-value**	***p*-value**	**n**	**r-value**	***p*-value**	**n**	**r-value**	***p*-value**
DTS Total	V1	140	-0.02	0.78	52	-0.24	0.09	31	-0.04	0.84
	V2	84	-0.13	0.22	45	0.05	0.77	29	0.31	0.10
	V3	69	0.04	0.74	34	0.05	0.78	29	0.12	0.55
Re-experiencing / intrusion	V1	140	-0.01	0.94	52	-0.21	0.14	31	-0.01	0.97
	V2	84	-0.10	0.36	45	-0.02	0.92	29	0.28	0.13
	V3	69	0.06	0.64	34	0.02	0.93	29	-0.03	0.89
Avoidance / numbing symptoms	V1	140	-0.06	0.46	52	-0.25	0.07	31	-0.11	0.56
	V2	84	-0.12	0.27	45	0.10	0.52	29	0.39	0.04*
	V3	69	0.08	0.54	34	-0.04	0.81	29	0.03	0.87
Hyperarousal	V1	140	0.02	0.80	52	-0.16	0.27	31	0.04	0.84
	V2	84	-0.13	0.24	45	0.01	0.94	29	-0.13	0.52
	V3	69	-0.05	0.68	34	0.15	0.40	29	0.18	0.34

*$p < 0.05$
**$p < 0.01$

4.4.3 Testosterone and PTSS

No significant correlations were found between DTS total and subscale scores and testosterone concentrations in the RE group (see Table 4.4.3).

Table 4.4.3 Correlation analyses for testosterone and posttraumatic stress symptoms in the rape-exposed group

	Visits	**Baseline testosterone measurement (pre-trauma)**			**Testosterone v2 (0-3 months)**			**Testosterone v3 (3-6 months)**		
		n	**r-value**	***p*-value**	**n**	**r-value**	***p*-value**	**n**	**r-value**	***p*-value**
DTS Total	V1	64	0.11	0.39	53	0.09	0.53	31	-0.20	0.28
	V2	60	0.05	0.70	50	-0.01	0.94	29	0.03	0.89
	V3	47	-0.01	0.93	38	0.14	0.42	-	-	-
Re-experiencing / intrusion	V1	64	0.12	0.36	53	0.08	0.56	31	-0.13	0.48
	V2	60	0.02	0.89	50	-0.07	0.64	29	0.22	0.26
	V3	47	0.02	0.87	38	0.01	0.94	-	-	-
Avoidance / numbing symptoms	V1	64	0.12	0.34	53	-0.01	0.92	31	-0.31	0.09
	V2	60	0.04	0.76	50	-0.04	0.81	29	-0.07	0.72
	V3	47	0.03	0.84	38	0.21	0.20	-	-	-
Hyperarousal	V1	64	0.03	0.80	53	0.14	0.31	31	-0.02	0.91
	V2	60	0.07	0.59	50	0.08	0.56	-	-	-
	V3	47	-0.08	0.61	38	0.07	0.66	-	-	-

*$p < 0.05$
**$p < 0.01$

4.4.4 Progesterone and PTSS

No significant correlations were found between DTS total and subscale scores and progesterone concentrations in the RE group (see Table 4.4.4).

Table 4.4.4 Correlation analyses for progesterone and posttraumatic stress symptoms in the rape-exposed group

	Visits	**Baseline DHEA measurement (pre-trauma)**			**DHEA v2 (0-3 months)**			**DHEA v3 (3-6 months)**		
		N	**r-value**	***p*-value**	**n**	**r-value**	***p*-value**	**n**	**r-value**	***p*-value**
DTS Total	V1	123	0.12	0.20	53	0.12	0.40	31	-0.03	0.89
	V2	81	0.22	0.05	49	-0.04	0.79	29	-0.03	0.89
	V3	67	0.08	0.53	38	-0.04	0.82	29	0.11	0.56
Re-experiencing / intrusion	V1	123	0.15	0.09	53	0.08	0.56	31	0.11	0.57
	V2	81	0.28	0.01	49	0.03	0.82	29	0.10	0.60
	V3	67	0.05	0.69	38	0.04	0.80	29	0.05	0.81
Avoidance / numbing symptoms	V1	123	0.04	0.64	53	0.04	0.77	31	-0.21	0.25
	V2	81	0.16	0.14	49	-0.08	0.57	29	-0.03	0.86
	V3	67	0.13	0.29	38	0.03	0.86	29	0.10	0.61
Hyperarousal	V1	123	0.14	0.12	53	0.20	0.14	31	0.09	0.63
	V2	81	0.15	0.18	49	-0.03	0.84	29	-0.01	0.97
	V3	67	0.02	0.86	38	-0.16	0.35	29	0.12	0.54

*$p < 0.05$
**$p < 0.01$

4.4.5 DHEA and PTSS

In the RE group, a significant negative correlation was found between DTS Cluster A V2 and DHEA, as measured at v2 (r = -0.40, $p < 0.01$). A significant positive correlation was found between DTS total V2 and DHEA, as measured at v3 (r = 0.40, $p = 0.03$), as well as between DTS cluster A V2 and DHEA, as measured at v3 (r = 0.49, $p < 0.01$). See Table 4.4.5.

Table 4.4.5 Correlation analyses for DHEA and posttraumatic stress symptoms in the rape-exposed group

	Visits	**Baseline DHEA measurement (pre-trauma)**			**DHEA v2 (0-3 months)**			**DHEA v3 (3-6 months)**		
		N	**r-value**	***p*-value**	**n**	**r-value**	***p*-value**	**n**	**r-value**	***p*-value**
DTS Total	V1	141	-0.01	0.90	52	0.12	0.39	31	-0.20	0.28
	V2	85	0.04	0.74	47	-0.35	0.01	29	0.40	0.03*
	V3	72	-0.04	0.75	35	-0.02	0.89	28	-0.21	0.28
Re-experiencing / intrusion	V1	141	-0.06	0.49	52	0.19	0.17	31	-0.24	0.19
	V2	85	0.01	0.95	47	-0.40	0.00**	29	0.49	0.00**
	V3	72	-0.09	0.44	35	-0.05	0.77	28	-0.24	0.22
Avoidance / numbing symptoms	V1	141	0.00	0.97	52	0.05	0.71	31	-0.15	0.42
	V2	85	-0.01	0.96	47	-0.30	0.04	29	0.33	0.08
	V3	72	-0.06	0.61	35	-0.12	0.50	28	-0.10	0.63
Hyperarousal	V1	141	0.03	0.69	52	0.09	0.50	31	-0.12	0.52
	V2	85	0.10	0.35	47	-0.21	0.16	29	0.05	0.75
	V3	72	0.03	0.80	35	0.11	0.54	28	0.02	0.94

*$p < 0.05$
**$p < 0.01$

Aim 4: To establish if pre-trauma hormone concentrations (as sampled at baseline) are predictive of the development of posttraumatic stress symptoms at baseline (within 20 days after rape exposure), three months, and six months post rape.

4.5 Regression Analyses

4.5.1 Visit 1: Baseline

At baseline, a linear regression analysis was performed in the RE group (n = 51), with posttraumatic stress symptomatology (PTSS) (measured by DTS-total) as the dependent variable and the following independent variables (as measured at baseline): cortisol, cortisone, testosterone, progesterone, and DHEA. Only participants with all the abovementioned variables were included in the regression model. See Table 4.5.1.

At baseline, we found that the abovementioned independent variables together explained 4% of the variance in PTSS ($R^2 = 0.04$). No significant predictors of PTSS were identified ($p > 0.01$).

Table 4.5.1 Baseline predictors of PTSS in RE participants (n=51) (p = 0.89)

	Standardised Beta Coefficients	**Standard Error**	***t(45)***	***p*-value**
Cortisol	0.04	0.2	0.2	0.84
Cortisone	-0.03	0.18	-0.16	0.87
Testosterone	0	0.16	0	1
Progesterone	0.14	0.17	0.84	0.4
DHEA	0.07	0.16	0.42	0.68

*$p < 0.05$
**$p < 0.01$

Multicollinearity assumptions in regression analyses were checked by reviewing tolerance indexes, and the guideline of tolerances <0.2 was satisfied in all regression analyses.

4.5.2 Visit 2: three-month follow-up

At Visit 2 (three-month follow-up), a linear regression analysis was performed in the RE group (n = 48), with posttraumatic stress symptomatology (PTSS) (measured by DTS total at Visit 2) as the dependent variable and the following as independent variables (as measured at baseline): cortisol, cortisone, testosterone, progesterone, and DHEA. Only participants with all the abovementioned variables were included in the regression model. See Table 4.5.2.

We found that the abovementioned independent variables (as measured at baseline) together explained 6% of the variance in PTSS (as measured at three months post rape) ($R^2 = 0.06$).

No significant baseline predictors of three months post rape posttraumatic stress symptomatology were identified. See Table 4.5.2.

Table 4.5.2 Visit 2: Three-month prediction of PTSS in RE participants (n=48) (p = 0.73)

	Standardised Beta Coefficient	**Standard Error**	***t*(42)**	***p*-value**
Cortisol	0.15	0.2	0.76	0.45
Cortisone	-0.18	0.18	-0.98	0.33
Testosterone	0.03	0.16	0.17	0.86
Progesterone	0.17	0.18	0.97	0.34
DHEA	-0.05	0.16	-0.29	0.78

*$p < 0.05$
**$p < 0.01$

Multicollinearity assumptions in regression analyses were checked by reviewing tolerance indexes, and the guideline of tolerances <0.2 was satisfied in all regression analyses.

4.5.3 Visit 3: Six-month follow-up

At Visit 3 (six-month follow-up), we performed a linear regression analysis in the RE group (n = 39), with posttraumatic stress symptomatology (PTSS) (measured by DTS-total at Visit 3) as dependent variable and the following as independent variables (as measured at Visit 1): cortisol, cortisone, testosterone, progesterone, and DHEA. Only participants with all the abovementioned variables were included in the regression model. See Table 4.5.3.

We found that the abovementioned independent variables together explained 2% of the variance in the dependent variable ($R^2 = 0.02$).

No significant baseline predictors of six months post rape PTSS were identified in this model. See Table 4.5.3.

Table 4.5.3 Visit 3: Six-month follow-up: Prediction of PTSS in RE participants (n=39) (p = 0.99)

	Standardised Beta Coefficient	**Standard error**	***t*(33)**	***p*-value**
Cortisol	-0.04	0.27	-0.13	0.9
Cortisone	-0.06	0.23	-0.28	0.78
Testosterone	0.03	0.18	0.14	0.89
Progesterone	0.1	0.21	0.46	0.65
DHEA	-0.04	0.19	-0.22	0.82

*$p < 0.05$

**$p < 0.01$

Multicollinearity assumptions in regression analyses were checked by reviewing tolerance indexes, and the guideline of tolerances <0.2 was satisfied in all regression analyses.

4.6 Results of univariate analyses

4.6.1 Correlation analyses in the rape-exposed group

Pearson moment correlation analyses were conducted in order to find any possible associations between baseline (Visit 1) demographic characteristics (age and education), baseline clinical data (depression, alcohol use, perceived stress, childhood abuse, social support, trauma load) and baseline hormone concentrations (cortisol, cortisone, testosterone, progesterone, DHEA) and the dependent variable (PTSS, as measured by the DTS total score), as measured at baseline (Visit 1), Visit 2 (3 months post rape), and Visit 3 (6 months post rape), within the RE group.

The following significant correlations with PTSS were found: education and DTS at visit 1 (r = -0.17, $p = 0.03$); depression (CES-D total) and DTS at visit 1 (r = 0.65, $p < 0.01$), DTS Visit 2 (r = 0.29, $p < 0.01$), and DTS at Visit 3 (r = 0.24, $p = 0.03$), perceived stress (PSS) and DTS at visit 1 (r = 0.37, $p < 0.01$); trauma load and DTS at visit 1 (r = 0.36, $p < 0.01$), and DTS visit 2 (r = 0.32, $p < 0.01$), cortisol and DTS at visit 1 (r = 0.24, $p < 0.01$); and progesterone at Visit 2 and DTS at Visit 2 (r = 0.30, $p < 0.01$). See Table 4.6.1

Table 4.6.1 Correlation analyses for demographics, clinical data, and hormone concentrations, as measured at baseline, three months and six months posttraumatic stress symptoms, in the rape-exposed group

	Baseline PTSS (DTS total, as measured at Visit 1)			**3 months PTSS (DTS total, as measured at Visit 2)**			**6 months PTSS (DTS total, as measured at Visit 2)**		
	n	**r-value**	***p*-value**	**n**	**r-value**	***p*-value**	**n**	**r-value**	***p*-value**
Age	157	0.10	0.21	97	-0.04	0.66	79	0.13	0.25
Education	157	-0.17	0.03*	97	-0.11	0.29	79	0.16	0.16
Depression (CES-D Total)	157	0.65	0.00**	97	0.29	0.00**	79	0.24	0.03*
Alcohol Use (AUDIT-C Total)	157	0.00	0.96	97	0.08	0.44	79	0.02	0.85
Perceived Stress (PSS Total)	157	0.37	0.00**	97	0.18	0.07	79	0.20	0.07
Childhood Abuse	157	0.10	0.21	97	0.13	0.22	79	0.11	0.34
Social Support (MPSS Total)	157	0.03	0.70	97	0.14	0.16	79	0.05	0.69
Trauma Load	157	0.36	0.00**	97	0.32	0.00**	79	0.14	0.22
Cortisol	136	0.24	0.00**	84	0.07	0.53	70	0.10	0.41
Cortisone	143	-0.03	0.72	87	-0.13	0.22	72	0.05	0.68
Testosterone	66	0.09	0.46	62	0.07	0.60	49	0.08	0.61
Progesterone	126	0.14	0.12	84	0.30	0.00**	70	0.15	0.21
DHEA	144	0.00	0.99	88	0.06	0.57	75	0.08	0.49

*$p < 0.05$
**$p < 0.01$

4.6.2 One-way ANOVAs in the rape-exposed group

A one-way ANOVA was conducted to establish significant associations between Human Immunodeficiency Virus (HIV) status and past rape (more than 20 days before the baseline visit, excluding the recent rape event), as measured at baseline, and posttraumatic stress symptoms (DTS Total), as measured at visits 1, 2 and 3, in the RE group.

No significant differences in PTSS, as measured at Visit 1 (n = 157, F(1, 155) = 3.4, p = 0.07), Visit 2 (n = 97, F(1, 95) = 0.28, p = 0.60), or Visit 3 (n = 79, F(1, 77) = 0.11, p = 0.74) were found between HIV positive and negative groups. See Table 4.6.2.

In the RE group with previous rape (excluding the recent rape trauma), a significantly higher DTS total score, as measured at Visit 2 (three months post recent rape) was found compared to the RE group without previous rape trauma (N = 97, F(1, 95) = 4.57, p = 0.04). No significant differences in DTS total scores were found between RE groups with/without a previous rape at visit 1 (N = 157, F(1, 155) = 3.74, p = 0.06) or at Visit 3 (N = 79, F(1, 77) = 0.15, p = 0.70).

Table 4.6.2 Summary of baseline, three months and six months posttraumatic stress symptom total mean scores between HIV and past rape, in the rape-exposed group

	Baseline PTSS (DTS total, as measured at Visit 1) (N = 160)			**3-month PTSS (DTS total, as measured at Visit 2) (N = 100)**			**6-month PTSS (DTS total, as measured at Visit 2) (N = 82)**		
	n	**Mean**	***SD***	**N**	**Mean**	***SD***	**n**	**Mean**	***SD***
HIV negative	76	61.47	35.01	50	36.5	34.31	41	31.83	32.18
HIV positive	81	71.74	34.52	47	40.04	31.92	38	29.42	31.91
Without past rape	96	62.5	37.36	61	32.8	31.40	52	31.67	32.31
With past rape	61	73.49	30.07	36	47.39	34.19	27	28.74	31.52

4.7 Regression analyses results with cortisol and covariates

4.7.1 Visit 1: Baseline

A linear regression analysis was performed in the RE group (n = 136), with PTSS (measured by the DTS-total score at baseline) as the outcome variable and cortisol (as measured at baseline) as predictor variable. The following variables (as measured at baseline) were considered possible covariates: Age, education, HIV, past rape (excluding the recent event), childhood abuse, trauma load, perceived stress (PSS total), alcohol use (AUDIT-C Total), depression (CES-D total), and social support (MPSS total). Only participants with all the abovementioned variables were included in the regression model. See Table 4.7.1.

We found that the abovementioned independent variables together explained 50% of the variance in the dependent variable (Adjusted $R^2 = 0.50$).

Three significant predictors of PTSS were identified. The strongest predictor of PTSS was depression (CES-D total score) (standardised $\beta = 0.56$, $p < 0.01$), followed by trauma load (standardised $\beta = 0.28$, $p < 0.01$), and perceived stress (standardised $\beta = 0.18$, $p = 0.01$). See Table 4.7.1.

Table 4.7.1 Baseline cortisol as predictor of PTSS with covariates in RE group (n=136) (p < 0.01)

	Standardised Beta Coefficient	**Standard Error**	***t*(124)**	***p*-value**
Cortisol	0.03	0.07	0.38	0.7
Age	0.06	0.06	0.94	0.35
Education	-0.04	0.07	-0.58	0.57
HIV status	0.08	0.07	1.1	0.27
Past rape (excluding recent rape)	0.08	0.06	1.32	0.19
Childhood Abuse	-0.11	0.07	-1.54	0.13
Trauma Load	0.28	0.07	4.03	0.00**
Perceived Stress (PSS Total)	0.18	0.07	2.57	0.01*
Alcohol Use (AUDIT-C Total)	-0.05	0.06	-0.84	0.4
Depression (CES-D Total)	0.56	0.06	8.82	0.00**
Social Support (MPS Total)	-0.07	0.06	-1.18	0.24

**p<0.05*
***p<0.01*

Multicollinearity assumptions in regression analyses were checked by reviewing tolerance indexes, and the guideline of tolerances <0.2 was satisfied in all regression analyses.

4.7.2 Visit 2: three-month follow-up

A linear regression analysis was performed in the RE group (n = 84), with posttraumatic stress symptomatology (PTSS) (measured by DTS-total at the three-month follow-up) as the outcome variable and cortisol (as measured at baseline) as predictor variable. The following variables (as measured at baseline) were considered possible covariates: Age, education, HIV, past rape (excluding the recent event), childhood abuse, trauma load, perceived stress (PSS total), alcohol use (AUDIT-C Total), depression (CES-D total), and social support (MPSS total). Only participants with all the abovementioned variables were included in the regression model. See Table 4.7.2.

We found that the abovementioned independent variables together explained 12% of the variance in the dependent variable (Adjusted $R^2 = 0.12$).

Two significant predictors of PTSS were identified. The strongest predictor of PTSS was trauma load (standardised $\beta = 0.29$, $p = 0.02$), followed by depression (CES-D Total score) (standardised $\beta = 0.27$, $p = 0.02$). See Table 4.7.2

Table 4.7.2 Baseline cortisol as predictor of three months PTSS with covariates in RE group (n = 84) (p < 0.05)

	Standardised Beta Coefficient	**Standard Error**	***t*(72)**	***p*-value**
Cortisol	-0.08	0.11	-0.68	0.5
Age	-0.11	0.11	-1.03	0.31
Education	-0.04	0.12	-0.3	0.77
HIV status	0.05	0.12	0.4	0.69
Past rape (excluding recent rape)	0.15	0.11	1.27	0.21
Childhood Abuse	-0.03	0.13	-0.22	0.83
Trauma Load	0.29	0.12	2.41	0.02*
Perceived Stress (PSS Total)	0.02	0.12	0.2	0.84
Alcohol Use (AUDIT-C Total)	-0.01	0.12	-0.06	0.95
Depression (CES-D Total)	0.27	0.11	2.49	0.02*
Social Support (MPS Total)	0.1	0.11	0.94	0.35

**p < 0.05*
***p < 0.01*

Multicollinearity assumptions in regression analyses were checked by reviewing tolerance indexes, and the guideline of tolerances <0.2 was satisfied in all regression analyses.

4.7.3 Visit 3: six-month follow-up

A linear regression analysis was performed in the RE group (n = 70), with posttraumatic stress symptomatology (PTSS) (measured by DTS-total at six-month follow-up) as the outcome variable and cortisol (as measured at baseline) as the predictor variable. The following variables (as measured at baseline) were considered possible covariates: Age, education, HIV, past rape (excluding the recent event), childhood abuse, trauma load, perceived stress (PSS total), alcohol use (AUDIT-C Total), depression (CES-D total), and social support (MPSS total). Only participants with all the abovementioned variables were included in the regression model. See Table 4.7.3.

We found that the abovementioned independent variables together explained 2% of the variance in the dependent variable (Adjusted $R^2 = 0.02$).

No significant predictors of PTSS were identified in this model. See Table 4.7.3.

Table 4.7.3 Baseline cortisol as predictor of 6 months PTSS with covariates in RE group (n = 70) (p = 0.35)

	Standardised Beta Coefficient	**Standard Error**	***t*(58)**	***p*-value**
Cortisol	0.06	0.14	0.41	0.68
Age	0.12	0.13	0.94	0.35
Education	0.2	0.14	1.5	0.14
HIV status	-0.05	0.14	-0.37	0.71
Past rape (excluding recent rape)	-0.18	0.13	-1.37	0.18
Childhood Abuse	0.02	0.14	0.16	0.88
Trauma Load	0.11	0.16	0.74	0.46
Perceived Stress (PSS Total)	0.13	0.15	0.88	0.38
Alcohol Use (AUDIT-C Total)	0.06	0.14	0.45	0.65
Depression (CES-D Total)	0.18	0.12	1.44	0.16
Social Support (MPS Total)	0.09	0.13	0.73	0.47

**p < 0.05*
***p < 0.01*

Multicollinearity assumptions in regression analyses were checked by reviewing tolerance indexes, and the guideline of tolerances <0.2 was satisfied in all regression analyses.

4.8 Univariate analysis results of possible covariates with cortisol

Possible associations between cortisol and the following variables were explored in the RE group: age, educational level, Body Mass Index [BMI], systolic blood pressure, diastolic blood pressure, smoking, depression, trauma load, childhood trauma, perceived stress, social support, alcohol use, HIV status, previous rape, hair wash frequency, use of hormone containing products, medicated shampoo or scalp treatment, and steroid containing medication, as measured at baseline, at three months and at six months post rape.

4.8.1 Questions regarding hair practices

Regarding the question on hair wash frequency ("*On average how often did you wash your hair in the last three months?*"), 156 participants responded to this question. Most responded that they wash their hair monthly.

With regard to the use of hormone containing products ("*In the last three months, have you used any hormone containing products in your hair (e.g. placenta/growth enhancers?*"): at Visit 1 and 2, only one participant responded "yes" to this variable, and at Visit 3, none responded "yes". Therefore, this variable was excluded in subsequent analyses.

Regarding the use of medicated shampoo or scalp treatment ("*In the last three months, have you used any medicated shampoo or scalp treatments*?"): at visit 1, only two participants

responded "yes" to this variable, and therefore it was excluded in subsequent analyses. At visit 2 and 3, enough participants responded "yes" to this variable and therefore this variable was included in the univariate analyses done at Visit 2 and 3.

Regarding the use of steroid containing medication ("*In the last three-six months have you used any medication that contains steroids?"):* at visit 1, all participants responded "no", and at Visit 2 and 3 only 1 participant responded "yes". Therefore, this variable was excluded in subsequent analyses.

4.8.2 Correlation analyses

Pearson moment correlation analyses were conducted for cortisol and the following baseline variables: age, educational level, depression, trauma load, childhood trauma, perceived stress, social support, alcohol use, BMI, systolic blood pressure, diastolic blood pressure, and hair wash frequency, in the RE group. A significant correlation was found between trauma load and cortisol (r = 0.21, p = 0.01), as measured at visit 1. A significant correlation was also found between educational level and baseline cortisol (r = -0.19, p = 0.03) and three-month follow-up cortisol concentration (r = -0.62, p < 0.01), respectively. Furthermore, no significant correlations were found. See Table 4.8.1.

Table 4.8.1 Correlation analyses for possible covariates and cortisol at baseline, three months and six months post-rape measurements, in the rape-exposed group

	Baseline cortisol (measured at Visit 1)			**3 months cortisol measurement**			**6 months cortisol measurement**		
	N	**r-value**	***p*-value**	**N**	**r-value**	***p*-value**	**n**	**r-value**	***p*-value**
Age	139	0.1	0.22	50	0.17	0.23	29	-0.12	0.54
Educational level	134	-0.19	0.03**	48	-0.62	<0.01*	28	0.06	0.75
Depression (CES-D Total)	139	0.16	0.07	50	0.06	0.66	29	-0.12	0.54
Trauma load	139	0.21	0.01*	50	0.23	0.11	30	-0.17	0.36
Childhood trauma	134	-0.02	0.84	48	0.22	0.14	28	0.05	0.81
Perceived stress	134	0.13	0.14	48	0.19	0.20	28	0.29	0.13
Social support	134	0.14	0.10	48	0.02	0.87	28	0.11	0.57
Alcohol use (AUDIT-C Total)	139	-0.04	0.64	50	0.21	0.14	29	-0.37	0.05
Systolic blood pressure	139	0.11	0.18	50	0.17	0.24	29	-0.2	0.31
Diastolic blood pressure	139	0.07	0.4	50	0.03	0.81	29	-0.08	0.66
Body mass index	139	0.03	0.76	50	0.00	1.00	29	-0.08	0.69
Hair washing frequency	135	0.12	0.16	48	-0.14	0.35	28	0.09	0.65

*p < 0.05
**p < 0.01

4.8.2 One-way ANOVAs

A one-way ANOVA was conducted to assess the association between smoking, HIV status, previous rape (excluding the recent rape trauma), and the use of medicated shampoo or scalp

treatment and cortisol concentrations (as measured at baseline, three months and six months post rape), respectively, in the RE group.

No significant difference in mean cortisol concentration was found between the smoking and non-smoking subgroups in the RE group at baseline (n = 139, F(1, 137) = 0.17, p = 0.68), at three months (n = 50, F(1, 48) = 3.44, p = 0.07), or at 6 months (n = , F(1,26) = 1.24, p = 0.28) post rape. See Table 4.8.2.

A significant difference in mean baseline (i.e. pre-trauma) cortisol concentration was found between HIV positive and negative participants (n = 134, F(1, 132) = 4.95, p = 0.05), with significantly higher cortisol concentrations in the HIV positive group. There was no significant difference in mean cortisol concentration between HIV positive and HIV negative participants at three months (n = 48, F(1, 46) = 6.04, p = 0.08) and six months post rape (n = 28, F(1, 26) = 1.14, p = 0.41). See Table 4.8.2.

There was no significant difference in baseline (i.e. pre-trauma) (n = 134, F(1, 132) = 1.44, p = 0.31) and three months post rape (n = 48, F(1, 46) = 2.49, p = 0.40) mean cortisol concentration between participants that previously experienced rape trauma (excluding the recent trauma event) and those who have not previously experienced a rape trauma. However, there was a significant difference in mean cortisol concentration, as measured at six months post rape between participants who experienced past rape (excluding the recent rape trauma) and those without experiencing a past rape (excluding the recent rape trauma) (n = 28, F(1, 26) = 2.52, p = 0.05), with significantly lower cortisol concentrations in the group who experienced previous rape. See Table 4.8.2.

No significant difference in cortisol concentrations were found between participants who used medicated shampoo or scalp treatment in the past three months compared to those who did not, as measured at Visit 2 (three months post rape) (n = 48, F(1, 46) = 0.06, p = 0.80). See Table 4.8.2.

Table 4.8.2 Summary of baseline, three months and six months mean cortisol concentrations and smoking, HIV status, past rape, and use of medicated shampoo/scalp treatment in the rape-exposed group (n = 139)

	Baseline cortisol			**3- month cortisol**			**6- month cortisol**		
	N	**Mean**	***SD***	**N**	**Mean**	***SD***	**n**	**Mean**	***SD***
Non-smoking	118	9.68	10.49	44	6.08	8.62	23	5.16	6.27
Smoking	21	10.72	11.39	6	13.83	15.66	5	1.97	2.12
HIV positive	69	11.97	12.35	23	10.79	12.42	11	3.09	3.49
HIV negative	65	7.91	8.23	25	4.1	5.41	17	5.51	6.91
With past rape	53	11.37	12.11	21	9.83	12.45	11	2.43	3.62

Without past rape	81	9.11	9.67	27	5.34	7.05	17	5.93	6.67
Non-Use of Medicated shampoo or scalp treatment	-	-	-	43	7.12	10.18	-	-	-
Use of Medicated shampoo or scalp treatment	-	-	-	5	8.3	8.78	-	-	-

4.9 Additional results between RE and control group

In order to shed light on other differences between the rape-exposed and control groups, the following variables were further analysed: (1) lifetime trauma experiences (including trauma load, childhood trauma, and previous rape [excluding the recent rape trauma]), (2) HIV status, (3) psychopathology, as measured by the MINI (including depression, as measured by the Center for Epidemiologic Studies Depression Scale [CES-D]), (4) alcohol use, as measured by the Alcohol Use Disorders Identification Test- Consumption [AUDIT-C], (5) Body Mass Index [BMI], (6) perceived stress, (7) social support, (8) blood pressure, (9) smoking, and (10) hair characteristics and practices.

4.9.1 Experiences of trauma

At baseline (Visit 1), as measured by the modified version of the Life Events Checklist (LEC), the three most frequently reported lifetime traumatic experiences among the RE participants (n = 160), excluding the recent rape, were: being robbed and/or carjacked at gunpoint and/or knifepoint (n = 72 [45%]), being close to death (n = 54 [33.8%]) and serious injury (n = 35 [21.9%]). The following were the three most frequently reported lifetime traumatic experiences for controls (n = 163): being robbed and/or carjacked at gunpoint and/or knifepoint (n = 51 [31.3%]), unnatural death of family member or friend (n = 36 [22.1%]), and murder of family or friend (n = 29 [17.8]).

A significantly greater proportion of RE participants endorsed the following traumatic experiences compared to controls: serious injury (chi-square [χ^2]= 9.01, $p < 0.01$), being close to death ($\chi^2 = 15.08$, $p < 0.01$), kidnapped ($\chi^2 = 17.20$, [$p < 0.01$), being robbed and/or carjacked at gunpoint and/or knifepoint ($\chi^2 = 6.46$, $p = 0.01$), and murder of a stranger ($\chi^2 = 4.03$, $p = 0.045$). See Table 4.9.1 for a summary of experiences of trauma (excluding the recent rape trauma).

Table 4.9.1 Experiences of trauma as measured at baseline (visit 1) (N=323)

	Rape-exposed (N=160)	**Controls (N=163)**	**χ^2 (df=1)**	***p* value**
Imprisonment	12 (7.5%)	9 (5.5%)	0.52	0.47
Civil unrest or war	10 (6.3%)	9 (5.5%)	0.08	0.78
Serious injury	35 (21.9%)	16 (9.8%)	9.01	0.00**

Being close to death	54 (33.8%)	25 (15.3%)	15.08	0.00**
Murder of family or friend	33 (20.6%)	29 (17.8%)	0.42	0.52
Unnatural death of family or friend	30 (18.8%)	36 (22.1%)	0.55	0.46
Murder of stranger	34 (21.3%)	21 (12.9%)	4.03	0.045*
Robbed and/or carjacked at gunpoint and/or knifepoint	72 (45.0%)	51 (31.3%)	6.46	0.01*
Kidnapped	19 (11.9%)	2 (1.2%)	17.20	0.00**
Other	8 (5.0%)	5 (3.1%)	0.79	0.38

** $p < 0.05$*
***$p < 0.01$*

4.9.1.1 Trauma load

An ANOVA to compare differences in trauma load (total mean scores) between groups as measured at baseline (Visit 1), indicated a significant group main effect ($F(1, 321) = 12.29$, $p < 0.01$), implying a significant difference between groups, with significantly higher trauma load scores in the RE group compared to controls (see Table 4.9.1.1 and Figure 4.9.1.1).

Table 4.9.1.1: Trauma Load as measured at baseline (visit 1)

Group	N	Mean	SD
Rape-exposed	160	1.92	1.78
Controls	163	1.25	1.67

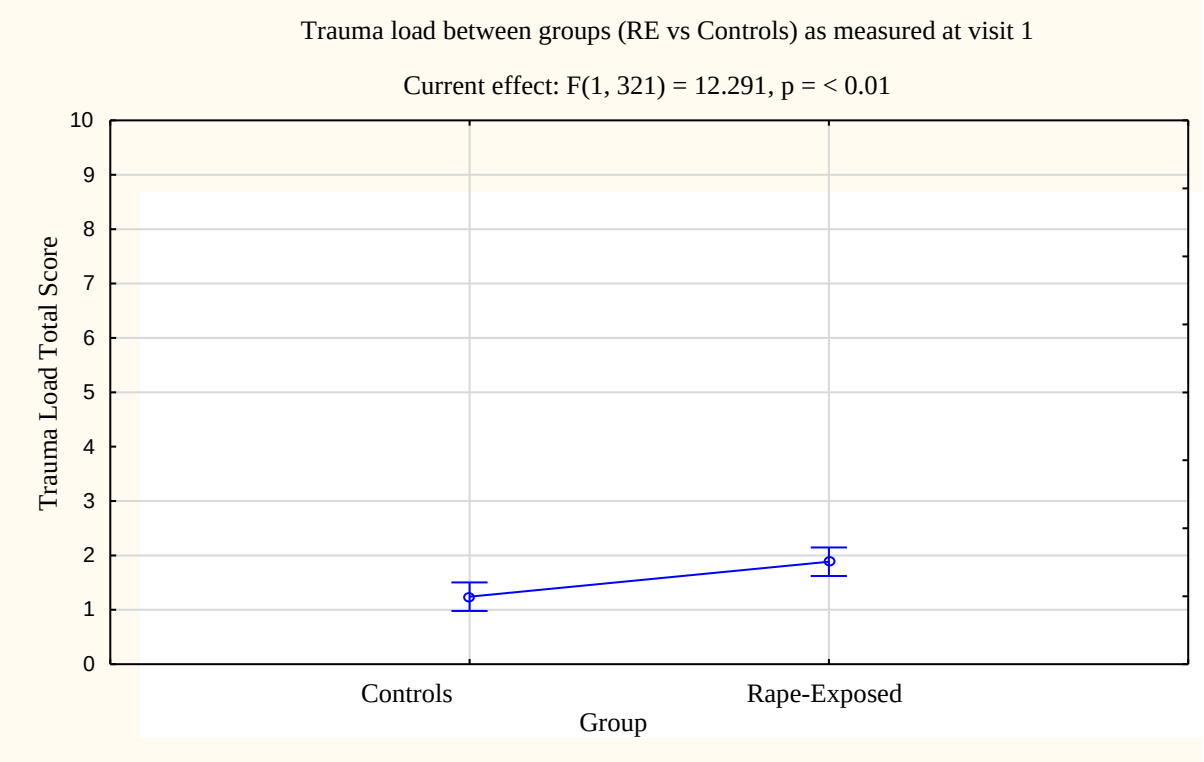

Figure 4.9.1.1 Trauma load between groups (RE vs controls) as measured at Visit 1

4.9.1.2 Childhood trauma

There was no significant difference in childhood trauma (total mean scores) between groups (RE vs controls) ($F(1, 321) = 1.71$, $p = 0.19$). Although not significant, the RE group had higher total childhood trauma scores compared to the controls. There was also no significant difference between groups (RE vs controls) on the following subscales of childhood trauma: emotional abuse ($p = 0.89$), parental neglect ($p = 0.96$), and physical abuse ($p = 0.45$). The RE group had a significantly higher score for childhood sexual abuse ($p < 0.01$) and witnessing abuse of their mother ($p = 0.01$), compared to controls. See tables 4.9.2 and 4.9.3, as well as Figure 4.9.2.

Table 4.9.2 Childhood trauma (RE and controls)

	RE (N=160)		**Controls (N=163)**	
	Mean	**SD**	**Mean**	**SD**
Witness abuse of mother	1.21	0.6	1.07	0.25
Childhood sexual abuse	4.37	0.84	4.17	0.58
Childhood physical abuse	3.84	1.19	3.37	1.48
Childhood emotional abuse	2.36	0.93	2.35	0.8
Childhood parental neglect	4.75	1.26	4.74	1.18
Total childhood abuse	16.53	3.37	16.06	3.18

Table 4.9.3 Results of ANOVAs for differences between groups with regard to childhood trauma (N= 323)

	***F* value**	***p* value**
Witness abuse of mother	7.20	<0.01**
Childhood sexual abuse	6.41	0.01*
Childhood physical abuse	.58	0.45
Childhood emotional abuse	.02	0.89
Childhood parental neglect	.00	0.96
Total childhood abuse	1.71	0.19

* $p < 0.05$
** $p < 0.01$

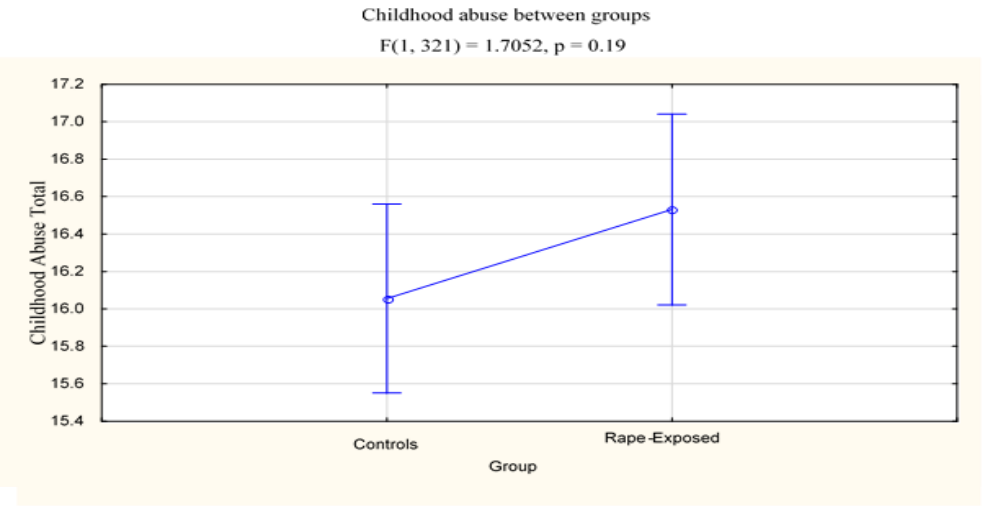

Figure 4.9.2 Childhood abuse (CTQ-SF adapted version) total score between groups (RE vs Controls)

4.9.1.3 Previous rape exposure

As measured at baseline, a significantly greater proportion of RE participants (n = 62, 38.8%) endorsed previous rape (more than 20 days before the baseline visit, excluding the recent rape trauma) compared to controls (n = 13, 8%) (χ^2 (df = 1) = 45.76, $p < 0.01$). A significantly greater proportion of RE participants endorsed the following compared to controls: first intercourse being forced/raped (χ^2 (df = 2) = 11.27, $p < 0.01$), forced sexual intercourse with a partner (χ^2 (df = 1) = 9,74, $p < 0.01$), forced intercourse with a non-partner (χ^2 (df = 1) = 44.62, $p < 0.01$), frequency of sexual intercourse against will when drunk/drugged (χ^2 (df = 1) = 4.01, $p = 0.045$), and frequency of sexual intercourse against will with more than one male at the same time (χ^2 (df = 1) = 5.57, $p = 0.02$).

4.9.2 HIV

At baseline, 51.3% in the RE group (n = 160) and 40.5% (n = 66) of the control group (n = 163) were HIV positive with no significant between group difference (χ^2 (df = 1) = 3.77, $p = 0.52$). LSD-post-hoc analyses also revealed no significant difference between groups ($p = 0.06$).

4.9.3 Psychopathology as measured by the MINI

In the RE group (n = 160) at Visit 1, current suicidality (n = 119, 74.4%) was the most common, with low risk (n =59, 36.9%) and high risk (n = 45, 28.1%). Moderate risk for suicidality at Visit 1 was 6.9% (n = 11). Current major depressive disorder (MDD) (n = 62, 39.2%), and past

MDD (n = 52, 32.7%) was the commonest diagnoses. In the RE group at Visit 2 (n = 95), current suicidality (n = 56, 58.9%) was the commonest, with low risk (n = 41, 43.2%) and high risk (n = 11, 11.6%). Moderate risk was at 2.1% (n = 2). Past MDD (n = 34, 35.8%) and current agoraphobia (n = 27, 28.4%) were the commonest diagnoses at Visit 2. In the RE group at Visit 3 (n = 69), current suicidality (n = 28, 40.6%) was the most common, with low risk (n = 24, 35.3%). Moderate risk was 0 and high risk was 4.4% (n = 3).

In the control group at baseline (visit 1) (n = 162), past MDD (n = 38, 23.5%), and current MDD (n = 25, 15.4%) were the commonest diagnoses. Current suicidality (n = 72, 44.4%) was also common with more than a third with low risk of suicidality (n = 61, 37.7%), 0.6% (n = 1) with moderate risk and 3.1% (n = 5) with high risk. In the control group at Visit 2 (n = 131), current suicidality (n = 33, 25.2%) was the most common with 22.9% (n = 30%) with low risk, and 0 for moderate and high risk. The most common diagnosis at Visit 2 was current MDD (n = 12, 9.2%). At Visit 3 (n = 116), low risk current suicidality (n = 17, 14.7%) was the most common. See Table 4.9.4 for a summary of MINI diagnoses.

Participants with a psychiatric diagnosis and/or suicidality were referred for further assessment and counselling as needed. At baseline, 115 (71.9%) of RE participants and 47 (28.8%) of control participants were referred. At Visit 2 (three-month follow-up), 48 (50.5%) of RE participants and 16 (12.2%) of controls were referred for counselling. At Visit 3 (six-month follow-up), 16 (23.5%) of RE and 4 (3.4%) of controls were referred. See Table 4.9.4.

Table 4.9.4 MINI Diagnoses/clinical problem

		Controls			Rape Exposed		
		Visit 1 (n=162)	**Visit 2 (n=131)**	**Visit 3 (n=116)**	**Visit 1 (n=160)**	**Visit 2 (n=95)**	**Visit 3 (n=69)**
Diagnosis/Clinical Problem							
Major Depressive Disorder	Current	25 (15.4%)	12 (9.2%)	4 (3.4%)	62 (39.2%)	26 (27.4%)	12 (17.4%)
	Past	38 (23.5%)	8 (6.1%)	7 (6.0%)	52 (32.7%)	34 (35.8%)	13 (18.8%)
	Recurrent	19 (11.7%)	0	4 (3.4%)	25 (15.6%)	16 (16.8%)	7 (10.1%)
Suicidality	Current	72 (44.4%)	33 (25.2%)	17 (14.7%)	119 (74.4%)	56 (58.9%)	28 (40.6%)
	Low Risk	61 (37.7%)	30 (22.9%)	17 (14.7%)	59 (36.9%)	41 (43.2%)	24 (35.3%)
	Moderate Risk	1 (0.6%)	0	0	11 (6.9%)	2 (2.1%)	0

	High Risk	5 (3.1%)	2 (1.5%)	0	45 (28.1%)	11 (11.6%)	3 (4.4%)
Panic	Lifetime	6 (3.7%)	2 (1.5%)	0	11(6.9%)	12 (12.6%)	6 (8.8%)
Disorder	Current	1 (0.6%)	2 (1.5%)	0	8 (5.0%)	9 (9.5%)	3 (4.4%)
	With Agoraphobia Current	1 (0.6%)	1 (0.8%)	0	6 (3.8%)	5 (5.3%)	3 (4.4%)
	Without Agoraphobia Current	1 (0.6%)	1 (0.8%)	0	3 (1.9%)	1 (1.1%)	0
Agoraphobia	Current	9 (5.6%)	8 (6.1%)	2 (1.7%)	39 (24.5%)	27 (28.4%)	10 (14.7%)
	Current without a history of Panic Disorder	7 (4.3%)	7 (5.3%)	0	28 (17.5%)	16 (16.8%)	0
SAD (Current)		0	0	1 (0.9%)	6 (3.8%)	10 (10.5%)	2 (2.9%)
PTSD (Current)		10 (6.2%)	3 (2.3%)	1 (0.9%)	30 (18.8%)	3 (3.2%)	2 (2.9%)
Anorexia Nervosa (Current)		0	0	0	0	1 (1.1%)	0
Bulimia Nervosa (Current)		0	0	0	0	0	0
Anorexia Nervosa (Binge Eating/purging type) (Current)		0	0	0	0	0	0
Generalised Anxiety Disorder (Current)		1 (0.6%)	0	0	2 (1.3%)	1 (1.1%)	1 (1.5%)
Referral made*		47 (28.8%)	16 (12.2%)	4 (3.4%)	115 (71.9%)	48 (50.5%)	16 (23.5%)

** Participants with a psychiatric diagnosis and/or suicidality were referred for further assessment and counselling as needed.*

Generalised estimating equation (GEE) analyses were conducted with group, visits, and group*time as the interaction term. Participant identification was included as the identification variable for three outcomes (Visit 1, Visit 2, Visit 3) over time per subject.

4.9.3.1 Current diagnosis of MDD

There was no significant interaction effect for group*time (df = 2, Wald = 0.94, $p = 0.62$) regarding a ***current diagnosis of MDD***, implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group). There was a decline in the rates of current diagnosis of MDD from Visit 1 through Visit 2 to Visit 3 in both groups. However, a significant group main effect was found (df = 1, Wald = 36.56, $p < 0.01$), implying differences between groups (controls and RE). There was also a significant time main effect (df = 2, Wald = 23.71, $p < 0.01$), implying a difference between visits. LSD post-hoc analysis revealed higher rates in the RE group at Visit 1, Visit 2, and Visit 3 compared to controls at Visit 1, Visit 2, and Visit 3 (all $p < 0.01$), respectively. Furthermore, there was a significant decline in rates of current diagnosis of MDD from Visit 1 to Visit 2 ($p = 0.03$), as well as from Visit 1 to Visit 3 ($p < 0.01$) in the RE group. There was also a significant decline in rates of current diagnosis of MDD from Visit 1 to Visit 3 ($p < 0.01$), as well as from Visit 2 to Visit 3 in the control group ($p = 0.04$).

4.9.3.2 Past diagnosis of MDD

There was a significant interaction effect for group*time (df = 2, Wald = 16.68, $p < 0.01$) with regard to a ***past diagnosis of MDD***, implying that changes from Visit 1 through Visit 2 to Visit 3 were not the same for controls and cases (RE group). LSD post-hoc analysis revealed significantly higher rates of past diagnosis of MDD in the RE group compared to controls at Visit 2 ($p < 0.01$) and at Visit 3 ($p = 0.01$). In the RE group, significantly higher rates of past diagnosis of MDD at Visit 1 compared to Visit 3 ($p = 0.03$), as well as higher rates at Visit 2 compared to Visit 3 ($p = 0.01$), was found. In the control group, higher rates of past diagnosis of MDD was found at Visit 1 compared to Visit 2 ($p < 0.01$), as well as higher rates at Visit 1 compared to Visit 2 ($p < 0.01$).

4.9.3.3 Recurrent MDD

There was a significant interaction effect for group*time (df = 2, Wald = 9.05, $p = 0.01$) with regard to ***recurrent MDD***, implying that changes from Visit 1 through Visit 2 to Visit 3 were not the same for controls and cases (RE group). LSD post-hoc analysis revealed significantly

higher rates of recurrent MDD diagnosis in the RE group compared to controls at Visit 2 ($p < 0.01$). In the RE group, that there was a slight (non-significant) increase from Visit 1 to Visit 2 ($p = 0.9$) and a slight (non-significant) decrease from Visit 2 to Visit 3 ($p = 0.15$). In the control group, there was a significant decrease in rate of MDD recurrent diagnosis from Visit 1 to Visit 2 ($p < 0.01$) and a slight increase from Visit 2 to Visit 3 ($p = 0.11$). There was also a significant decrease in rate of MDD diagnosis from Visit 1 to Visit 3 ($p < 0.01$) in the control group.

4.9.3.4 Current suicidality

There was no significant interaction effect for group*time (df = 2, Wald = 0.25, $p = 0.88$) regarding ***current suicidality***, implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group). There was a decline in the rates of current suicidality from Visit 1 through Visit 2 to Visit 3 in both groups. However, a significant group main effect was found (df = 1, Wald = 51.02, $p < 0.01$), implying differences between groups (controls and RE). There was also a significant time main effect (df = 2, Wald = 68.14, $p < 0.01$), implying a difference between visits. LSD post-hoc analysis revealed significantly higher rates of current suicidality at Visit 1 compared to Visit 2 ($p < 0.01$) and Visit 3 ($p < 0.01$), respectively, in the RE group. There were also significantly higher rates of current suicidality in the RE group at Visit 2 compared to Visit 3 ($p < 0.01$). Furthermore, there were significantly higher rates of current suicidality in the RE group compared to controls at Visit 1 ($p < 0.01$), Visit 2 ($p < 0.01$), and at Visit 3 ($p < 0.01$). In the control group, significantly higher rates of current suicidality were found at Visit 1 compared to Visit 2 ($p < 0.01$) and Visit 3 ($p < 0.01$), respectively. Significantly higher rates were also found at Visit 2 compared to Visit 3 ($p = 0.01$), in the control group.

4.9.3.5 Suicidality low risk

There was a significant interaction effect for group*time (df = 2, Wald = 10.72, $p < 0.01$) with regard to ***low risk suicidality***, implying that changes from Visit 1 through Visit 2 to Visit 3 were not the same for controls and cases (RE group). LSD post-hoc analyses revealed significantly higher rates of low risk suicidality in the RE group compared to controls at Visit 2 ($p < 0.01$), and at Visit 3 ($p < 0.01$). There was no significant difference in rates of low risk suicidality between Visit 1 and Visit 2 ($p = 0.4$), Visit 1 and Visit 3 ($p = 0.49$), or between Visit 2 and Visit 3 ($p = 0.14$), in the RE group. In the control group, there was significantly higher rates of low risk suicidality at Visit 1 compared to Visit 2 ($p < 0.01$), higher rates at Visit 1 compared to Visit 3 ($p < 0.01$), as well as higher rates at Visit 2 compared to Visit 3 ($p = 0.03$).

4.9.3.6 Suicidality high risk

There was no significant interaction effect for group*time (df = 2, Wald = 0.68, $p = 0.71$) regarding ***high risk suicidality***, implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group). However, a significant group main effect (df = 1, Wald = 30.6, $p < 0.01$) and a significant time main effect was found (df = 2, Wald = 29.55, $p < 0.01$). LSD post-hoc analyses revealed significantly higher rates of suicidality (high risk) in the RE group at Visit 1 compared to Visit 2 ($p < 0.01$), as well as higher rates at Visit 1 compared to Visit 3 ($p < 0.01$). However, a non-significant decrease was found from Visit 2 to Visit 3 ($p = 0.16$), in the RE group. Compared to controls, significantly higher rates of suicidality (high risk) was found in the RE group at Visit 1 ($p < 0.01$) and at Visit 2 ($p < 0.01$).

4.9.3.7 Panic disorder lifetime

There was no significant interaction effect for group*time (df = 2, Wald = 4.31, $p = 0.12$) regarding ***lifetime panic disorder*** diagnosis, implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group). There was also no significant time main effect (df = 2, Wald = 3.42, $p = 0.18$), implying no significant difference between visits was found. However, a significant group main effect (df = 1, Wald = 9.16 $p < 0.01$) was found. LSD post-hoc analyses revealed higher rates of lifetime panic disorder in the RE group compared to controls at Visit 2 ($p < 0.01$) and at Visit 3 ($p = 0.03$). In the RE group, there was a significant increase in rates of lifetime panic disorder from Visit 1 to Visit 2 ($p = 0.04$) and a slight (non-significant) decrease from Visit 2 to Visit 3 ($p = 0.13$).

4.9.3.8 Current panic disorder

With regard to rates of ***current panic disorder***, there was no significant interaction effect for group*time (df = 2, Wald = 0.16, $p = 0.92$), implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group). There was also no significant time main effect (df = 2, Wald = 3.76, $p = 0.15$), implying no changes between visits. However, there was a significant group main effect (df = 1, Wald = 10.28, $p < 0.01$). LSD post-hoc analysis revealed significantly higher rates of current panic disorder in the RE compared to controls at Visit 1 ($p = 0.04$), and at Visit 2 ($p = 0.02$). No significant difference in rates of current panic disorder was found in the RE group at Visit 1 and Visit 2 ($p = 0.15$), Visit 1 and Visit 3 ($p = 0.61$), or Visit 2 and Visit 3 ($p = 0.15$).

4.9.3.9 Current agoraphobia

There was no significant interaction effect for group*time (df = 2, Wald = 0.27, p = 0.88) regarding ***current agoraphobia*** diagnosis, implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group). However, a significant group main effect (df = 1, Wald = 42.5, $p < 0.01$) and a significant time main effect (df = 2, Wald = 11.49, $p < 0.01$) was found. LSD post-hoc analysis revealed significantly higher rates of current agoraphobia in the RE group compared to controls at Visit 1 ($p < 0.01$), Visit 2 ($p < 0.01$), and at Visit 3 ($p < 0.01$). In the RE group, there was a significant decrease in rates of current agoraphobia from Visit 1 to Visit 3 ($p = 0.04$), as well as a significant decrease from Visit 2 to Visit 3 ($p < 0.01$). A non-significant increase in rates of current agoraphobia was seen from Visit 1 to Visit 2 ($p = 0.49$), in the RE group.

4.9.3.10 Current agoraphobia without panic disorder

There was no significant interaction effect for group*time (df = 1, Wald = 0.2, p = 0.65) regarding ***current agoraphobia without panic disorder***, implying that changes from Visit 1 to Visit 2 were the same for controls and cases (RE group). There was also no significant time main effect (df = 1, Wald = 0.01, p = 0.94), implying no change between visits. However, a significant group main effect (df = 1, Wald = 19.27, $p < 0.01$) was found. LSD post-hoc analysis revealed significantly higher rates of current agoraphobia without panic disorder in the RE group compared to controls at Visit 1 ($p < 0.01$) and at Visit 2 ($p < 0.01$). No significant difference in rates of current agoraphobia without panic disorder was seen in the RE group between Visit 1 and Visit 2 ($p = 0.84$). There was insufficient data (too few cases) at Visit 3 to conduct statistical analyses and therefore a comparison was only done between Visit 1 and Visit 2.

4.9.3.11 Current social anxiety disorder (SAD)

There was no significant interaction effect for group*time (df = 2, Wald = 1.11, p = 0.57) regarding ***current SAD***, implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group). There was also no significant time main effect (df = 2, Wald = 3.67, p = 0.16), implying no significant change in rates of current SAD between visits. However, a significant group main effect (df = 1, Wald = 12.56, $p < 0.01$) was found, implying significant differences between groups. LSD post-hoc analysis revealed significantly higher rates of current SAD in the RE group compared to controls at Visit 1 ($p = 0.03$) and at Visit 2 ($p = 0.01$). There was no significant difference in rates of current SAD between groups

at Visit 3 (p = 0.37). In the RE group, there was a slight (non-significant) increase in rates of current SAD from Visit 1 to Visit 2 (p = 0.19), and a significant decreased from Visit 2 to Visit 3 (p = 0.05). There was a non-significant decrease from Visit 1 to Visit 3, in the RE group (p = 0.26).

4.9.3.12 Current PTSD

There was a significant interaction effect for group*time (df = 2, Wald = 10.72, p < 0.01) with regard to rates of ***current PTSD***, implying that changes from Visit 1 through Visit 2 to Visit 3 were not the same for controls and cases (RE group). LSD post-hoc analysis revealed significantly higher rates of current PTSD in the RE group compared to controls at Visit 1 (p < 0.01), Visit 2 (p < 0.01), and at Visit 3 (p < 0.01). In the RE group, there was a slight (non-significant) increase in rates of current PTSD from Visit 1 to Visit 2 (p = 0.22), and a slight (non-significant) decrease from Visit 2 to Visit 3 (p = 0.06).

4.9.3.13 Other psychopathology

There was insufficient data (too few cases) in other pathology to conduct statistical analyses and therefore only the abovementioned psychopathologies were included in statistical analyses.

4.9.4 Depression, as measured by the CES-D

A mixed model repeated measures ANOVA revealed a significant difference in mean depression (Center for Epidemiologic Studies Depression Scale [CES-D]) total score between groups (controls vs RE) (p < 0.01), with significantly higher mean depression scores in the RE group compared to controls. LSD post-hoc analyses revealed the following significant differences between groups (see tables 4.9.5 and 4.9.6, as well as Figure 4.9.5):

At all three visits (Visit 1 (baseline), Visit 2, Visit 3), the RE group had significantly higher mean depression scores than controls (p < 0.01). Within the RE group, the mean CES-D score was significantly higher at Visit 1 compared to visits 2 and 3, respectively (p < 0.01). See tables 4.9.5 and 4.9.6, as well as Figure 4.9.5.

Table 4.9.5 CES-D Total Score in Rape-exposed and Control Participants

	Rape-exposed			Controls		
	N	**Mean**	**SD**	**N**	**Mean**	**SD**
Visit 1	160	33.71	13.54	163	15.31	10.38
Visit 2	98	19.02	13.10	134	12.93	9.33
Visit 3	78	17.37	13.26	120	13.33	9.55

Table 4.9.6 Results of ANOVAs for main and interaction effects regarding depression symptoms (as measured with the CES-D)

	F value	*p* value
Main group effect	75.3	0.00**
Main time effect	73.4	0.00**
Interaction effect (Group*time)	45.08	0.00**

**$p < 0.01$

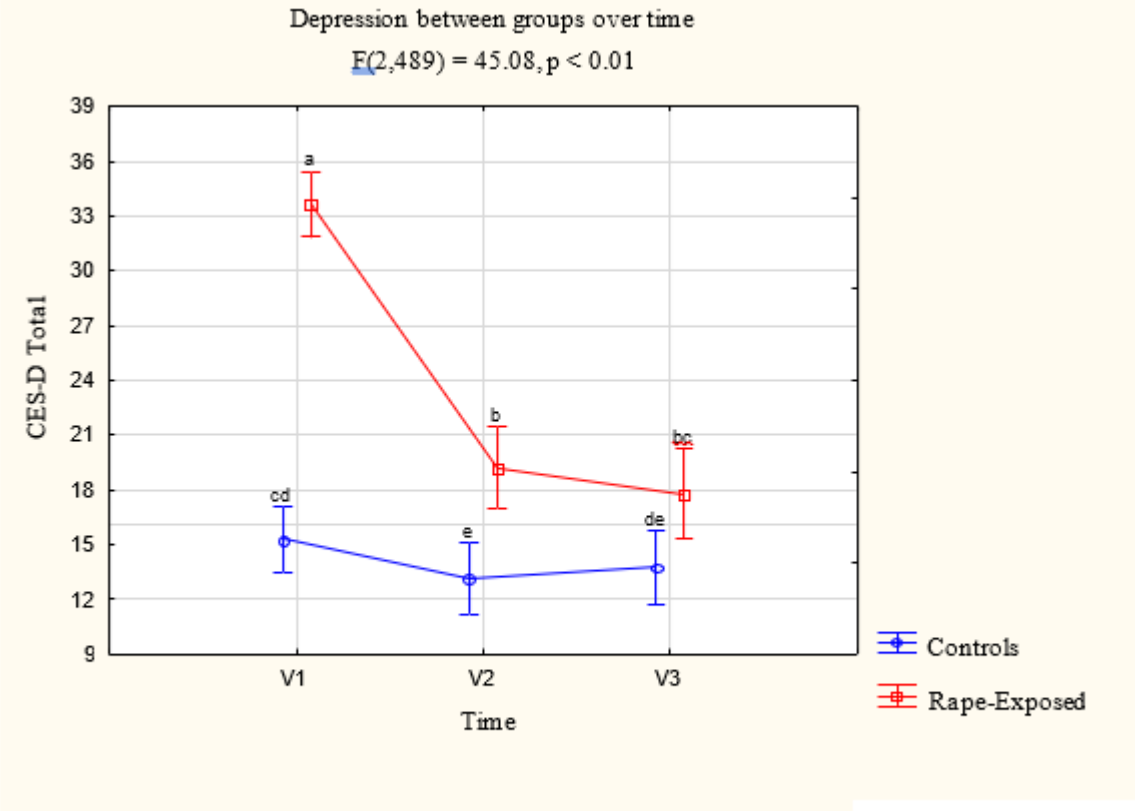

Figure 4.9.5 Depression (CES-D total) between groups (RE vs Controls)

4.9.5 Alcohol use

A mixed model repeated measures ANOVA revealed no significant interaction effect for group*time ($p = 0.36$), with regard to alcohol use (AUDIT-C), implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group). However, a significant group main effect ($p = 0.02$) and time main effect ($p < 0.01$) was found, implying

differences between groups and between visits, respectively. LSD post hoc analyses revealed the following significant differences (see tables 4.9.7 and 4.9.8, as well as Figure 4.9.6):

At Visit 1, the RE group had significantly higher mean scores of alcohol use than controls at Visit 1 (p = 0.01). In the RE group, significantly higher mean alcohol use scores were found at Visit 1 (p < 0.01) compared to Visit 2, as well as higher scores at Visit 1 compared to Visit 3 (p = 0.01). There was a slight (non-significant) increase in mean alcohol use scores in the RE group from Visit 2 to Visit 3 (p = 0.17). Controls at Visit 1 had higher mean alcohol use scores than controls at both Visit 2 (p < 0.01) and Visit 3 (p < 0.01), respectively.

Table 4.9.7 AUDIT-C Total Scores Rape-exposed and Control participants

	Rape-exposed			Controls		
	N	**Mean**	**SD**	**N**	**Mean**	**SD**
Visit 1	160	2.18	2.63	163	1.51	2.36
Visit 2	98	1.28	2.36	134	0.82	1.95
Visit 3	78	1.41	2.32	120	0.86	1.99

Table 4.9.8 Results of ANOVAs for main and interaction effects regarding alcohol use

	***F* value**	***p* value**
Main group effect	5.18	0.02*
Main time effect	17.92	0.00**
Interaction effect (Group*time)	1.03	0.36

*p<0.05
**p<0.01

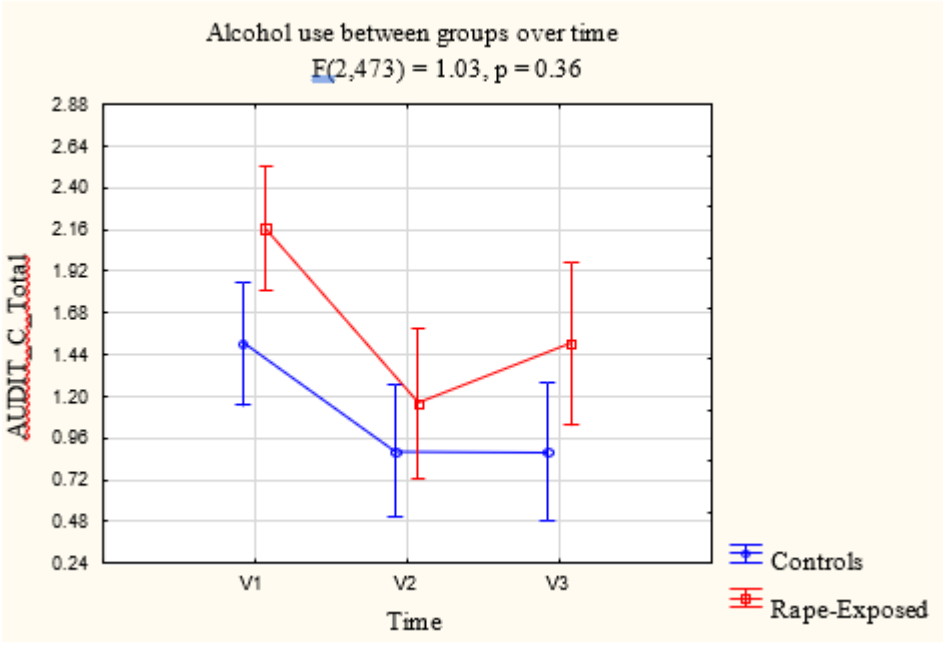

Figure 4.9.6 Alcohol use (AUDIT-C total) between groups (RE vs controls)

4.9.6 BMI

A mixed model ANOVA revealed no significant interaction effect for group*time (p = 0.29), with regard to BMI, implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group) (see Figure 4.9.7). However, a significant group main effect (p < 0.01) and time main effect (p = 0.01) was found, implying differences between groups and between visits, respectively. LSD post hoc analyses revealed significantly lower mean BMI scores in the RE group compared to controls at Visit 1 (p = 0.01), visit 2 (p = 0.01), and at Visit 3 (p < 0.01) (see tables 4.9.9 and 4.9.10, as well as Figure 4.9.7).

Table 4.9.9 BMI in Rape-exposed and Control Participants

	Rape-Exposed			Controls		
	N	**Mean**	**SD**	**N**	**Mean**	**SD**
Visit 1	160	25.76	5.49	163	27.70	7.30
Visit 2	98	26.45	6.17	134	27.53	7.37
Visit 3	78	26.60	6.21	120	28.38	7.61

Table 4.9.10 Results of ANOVAs for main and interaction effects regarding BMI

	F value	*p* value
Main group effect	8.21	0.00**
Main time effect	17.92	0.01**
Interaction effect (Group*time)	1.03	0.29

***p < 0.01*

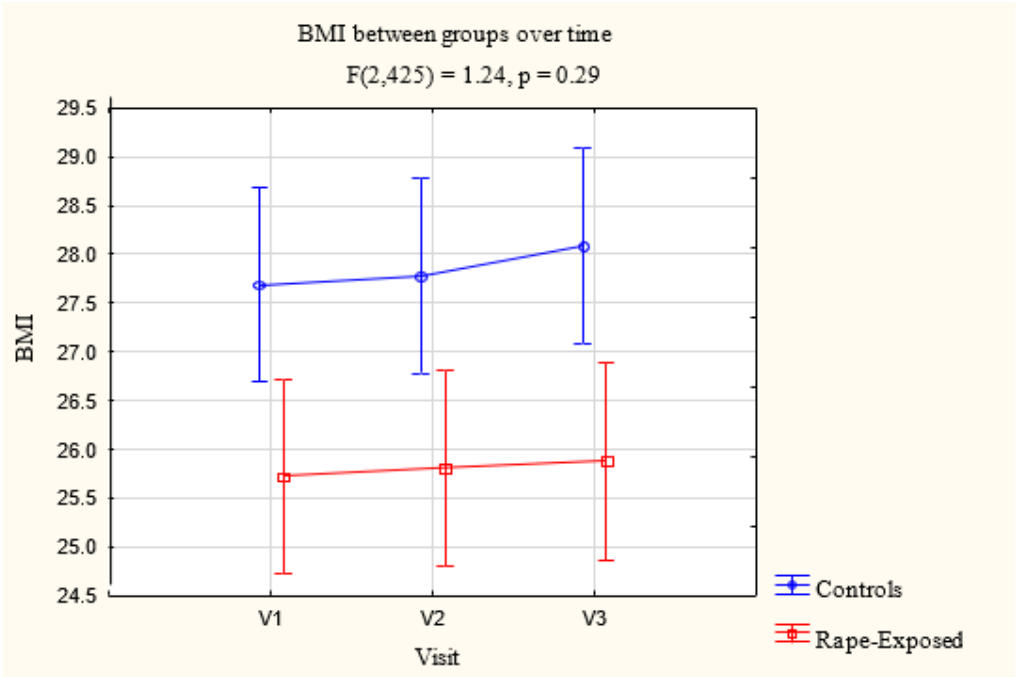

Figure 4.9.7 BMI between groups (RE vs Controls)

4.9.7 Perceived Stress

A mixed model ANOVA revealed no significant interaction effect for group*time ($p = 0.13$), with regard to perceived stress (PSS total score), implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group) (see Figure 4.9.8). However, a significant group main effect ($p < 0.01$) and time main effect ($p < 0.01$) was found, implying differences between groups and between visits, respectively.

LSD post-hoc analysis revealed significantly higher mean perceived stress scores in the RE group compared to controls at Visit 1 ($p < 0.01$), Visit 2 ($p < 0.01$), and at Visit 3 ($p = 0.02$) (see tables 4.9.11 and 4.9.12, as well as Figure 4.9.8). Significantly higher mean perceived stress scores were found in the RE group at Visit 1 compared to Visit 2 ($p = 0.02$), higher scores at Visit 1 compared to Visit 3 ($p < 0.01$), as well as higher scores at Visit 2 compared to Visit 3 ($p = 0.05$). Controls at Visit 1 had significantly higher mean perceived stress scores than controls at Visit 2 ($p < 0.01$), and controls at Visit 3 ($p = 0.03$), respectively.

Table 4.9.11 Perceived stress total scores in Rape-exposed and Control Participants

	Rape-exposed			**Controls**		
	N	**Mean**	**SD**	**N**	**Mean**	**SD**
Visit 1	160	25.02	5.88	163	21.00	7.30
Visit 2	98	23.52	6.85	134	19.00	6.80
Visit 3	78	21.90	7.17	120	19.53	5.52

Table 4.9.12 Results of ANOVAs for main and interaction effects regarding perceived stress

	***F* value**	***p* value**
Main group effect	32.4	0.00**
Main time effect	12.46	0.00**
Interaction effect (Group*time)	2.08	0.13

p < 0.01

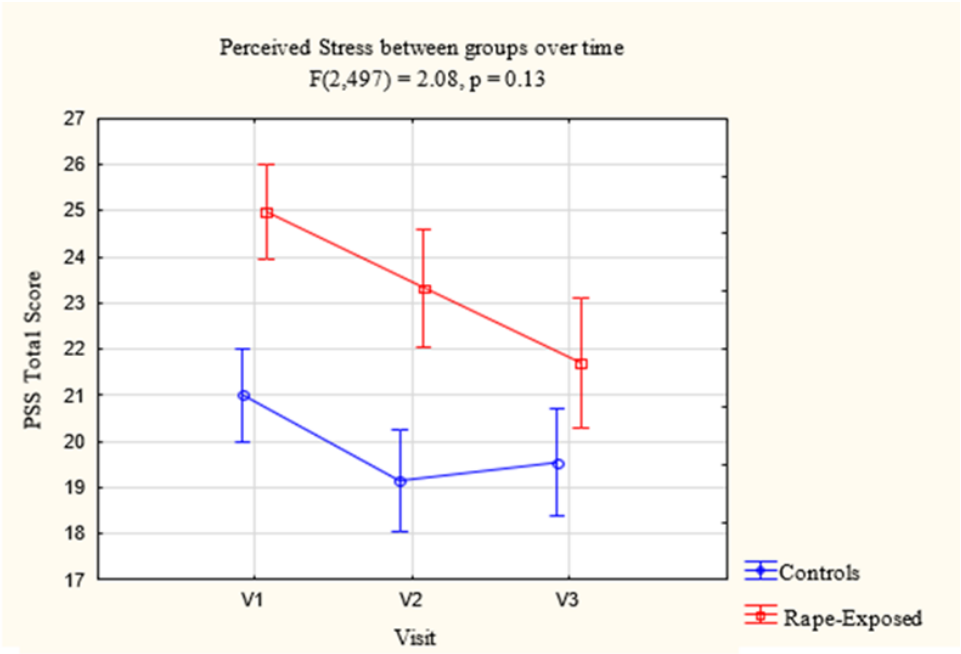

Figure 4.9.8 Perceived stress between groups (RE vs Controls)

4.9.8 Social Support

A mixed model ANOVA revealed no significant interaction effect for group*time (p = 0.85) with regard to mean social support scores (as measured by the Multidimensional Scale of Perceived Social Support [MPSS]), implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group). There was also no significant group main effect (p = 0.66) and no significant time main effect (p = 0.07). See tables 4.9.13 and 4.9.14, as well as Figure 4.9.9.

Table 4.9.13 Social Support in Rape-exposed and Control Participants

	Rape-Exposed			Controls		
	N	**Mean**	**SD**	**N**	**Mean**	**SD**
Visit 1	160	25.22	5.82	163	25.26	3.81
Visit 2	98	24.60	5.39	134	24.43	4.77
Visit 3	78	24.83	6.07	120	24.58	4.92

Table 4.9.14 Results of ANOVAs for main and interaction effects regarding social support

	***F* value**	***p* value**
Main group effect	0.19	0.66
Main time effect	2.68	0.07
Interaction effect (Group*time)	0.16	0.85

$*p < 0.05$
$**p < 0.01$

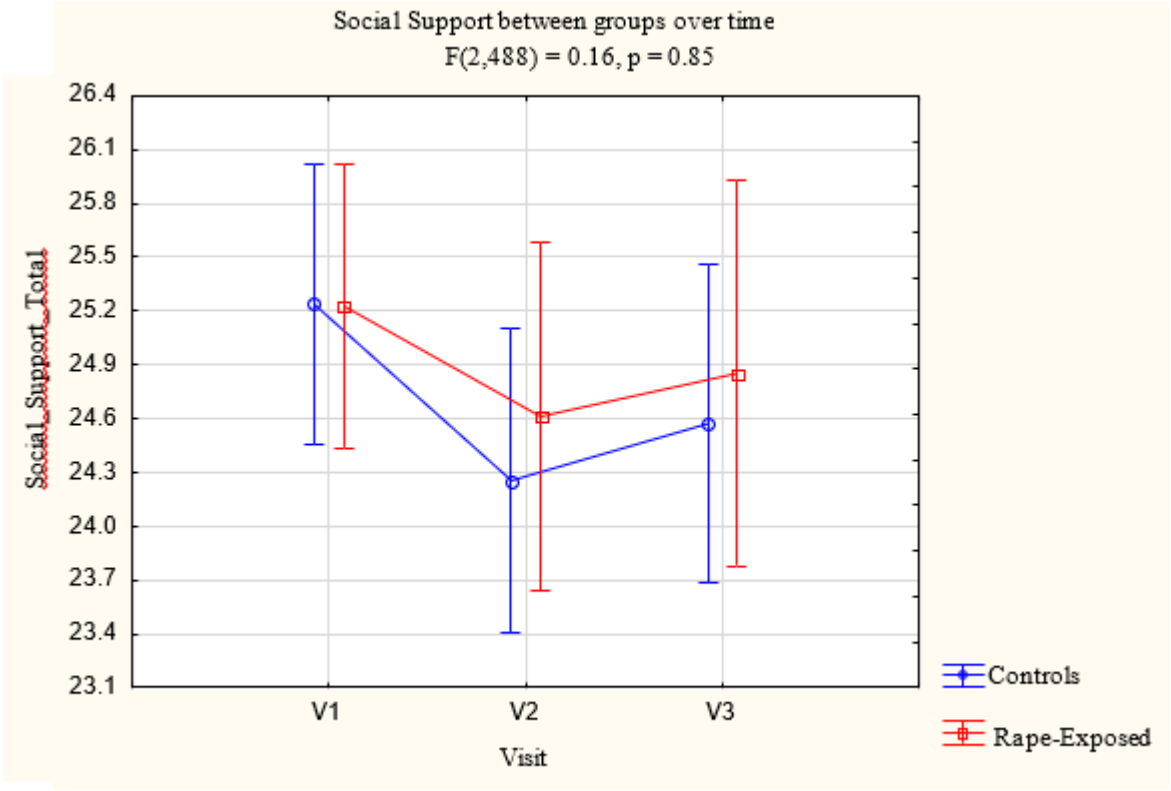

Figure 4.9.9 Social support between groups (RE vs Controls)

4.9.9 Mean blood pressure

4.9.9.1 Mean systolic blood pressure

A mixed model repeated measures ANOVA revealed no significant interaction effect for group*time ($p = 0.29$) with regard to mean systolic blood pressure, implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group). There was also no significant group main effect ($p = 0.88$), implying no differences between groups. However, a significant time main effect ($p < 0.01$) was found, implying differences between visits. LSD post hoc analyses revealed the following significant differences (see tables 4.9.15 and 4.9.16 as well as Figure 4.9.10):

The RE group at Visit 1 had significantly higher mean systolic BP than the RE group at Visit 3 ($p = 0.04$). Controls at Visit 1 had higher mean systolic BP than controls at Visit 2 ($p < 0.01$) and controls at Visit 3 ($p < 0.01$), respectively.

Table 4.9.15 Mean Systolic Blood Pressure Rape-exposed and Control Participants

	Rape-Exposed			Controls		
	N	**Mean**	**SD**	**N**	**Mean**	**SD**
Visit 1	160	106.48	10.12	163	107.43	10.34
Visit 2	98	105.58	8.68	134	104.77	8.90
Visit 3	78	105.21	7.73	120	104.86	7.82

Table 4.9.16 Results of ANOVA for differences between groups, between visits and within groups across visits with regard to average systolic blood pressures

	***F* value**	***p* value**
Main group effect	0.02	0.88
Main time effect	8.87	0.00**
Interaction effect (Group*time)	1.25	0.29

***p < 0.01*

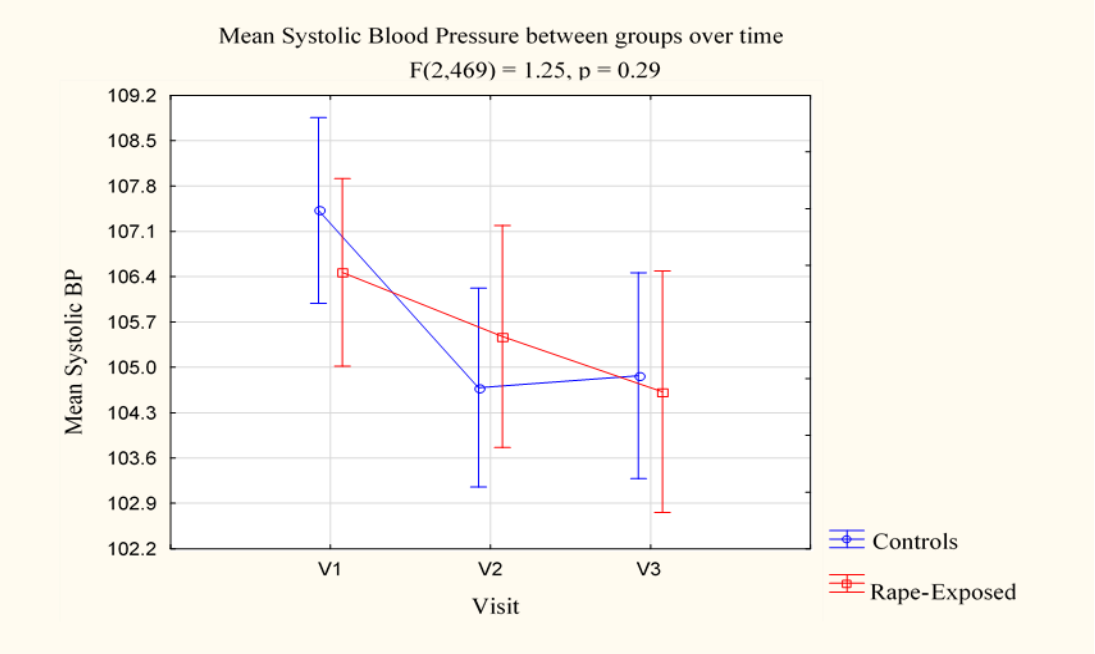

Figure 4.9.10 Mean Systolic BP between groups (RE vs Controls)

4.9.9.2 Mean diastolic blood pressure

A mixed model repeated measures ANOVA revealed no significant interaction effect for group*time (p = 0.86) with regard to mean diastolic blood pressure, implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group). There was also no significant group main effect (p = 0.10), implying no significant differences

between groups. However, a significant time main effect ($p < 0.01$) was found, implying differences between visits. LSD post hoc analyses revealed the following significant differences (see tables 4.9.17 and 4.9.18, as well as Figure 4.9.11):

Table 4.9.17 Mean Diastolic Blood Pressure for Rape-exposed and Control Participants

	Rape-exposed			Controls		
	N	**Mean**	**SD**	**N**	**Mean**	**SD**
Visit 1	160	70.57	8.39	163	72.11	9.17
Visit 2	99	68.67	6.98	134	69.79	8.30
Visit 3	78	69.85	7.34	120	70.46	7.44

Table 4.9.18 Results of ANOVAs for main and interaction effects regarding mean diastolic blood pressures

	***F* value**	***p* value**
Main group effect	2.71	0.10
Main time effect	6.91	0.00**
Interaction effect (Group*time)	0.15	0.86

***p < 0.01*

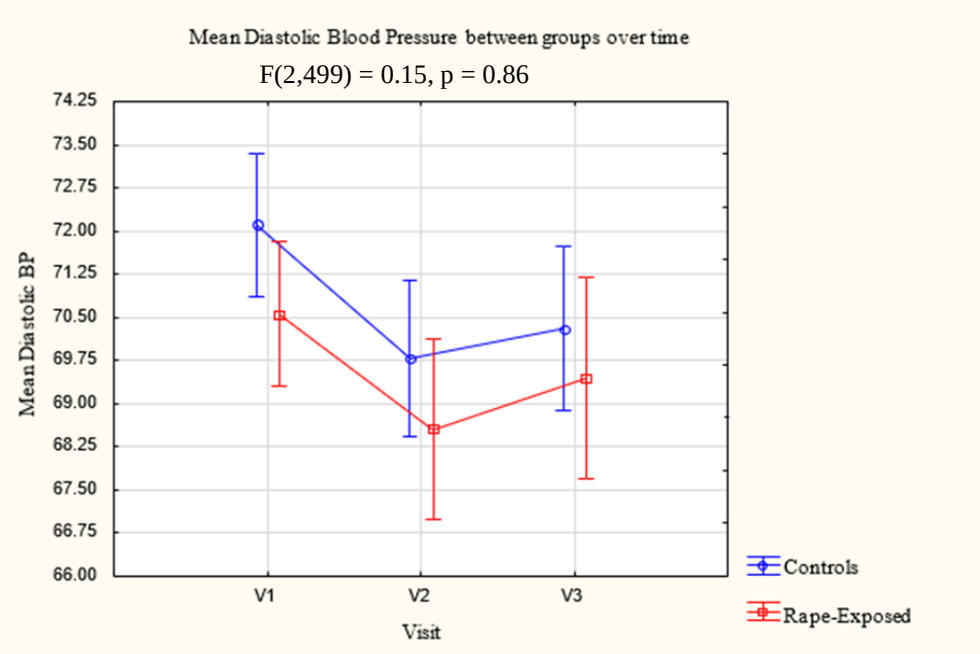

Figure 4.9.11 Mean Diastolic BP between groups (RE vs Controls)

In the RE group, significantly higher mean diastolic blood pressure was found at Visit 1 compared to Visit 2 ($p = 0.03$). Controls at Visit 1 had a significantly higher mean diastolic blood pressure than controls at Visit 2 ($p < 0.01$) and controls at Visit 3 ($p = 0.03$), respectively.

4.9.10 Smoking

GEE analyses were conducted with group, time, and group*time as the effect. Participant identification was included as the identification variable for three outcomes (Visit 1, Visit 2, Visit 3) over time per subject. GEE analyses revealed no significant interaction effect for group*time (p = 0.68), implying that that changes from Visit 1 through Visit 2 to visit 3 were the same for controls and cases (RE group). Furthermore, there was no significant group main effect (p = 0.07) and no significant time main effect (p < 0.27), implying that there were no significant differences between groups or between visits. See Table 4.9.19.

Table 4.9.19 Results of GEE for main and interaction effects regarding smoking

	Wald	***p* value**
Main group effect	3.4	0.07
Main time effect	2.59	0.27
Interaction effect (Group*time)	0.77	0.68

***p < 0.01*

4.9.11 Hair characteristics and practices

At baseline, most participants indicated that their natural hair colour is black (RE n = 101, 63.1%; controls n = 108, 66.3%). Most of the hair was coarse/thick (RE n = 106, 66.3%; controls n = 84, 51.5%). Most participant used only shampoo, and no other products, when washing their hair (RE n = 96, 60%; controls n = 98, 60.1%) and their most recent hair wash was a month before their visit (RE n =77, 48.1%; controls n =89, 54.6%). Most participants used heating devices on their hair (RE n = 109, 68.1%; controls n = 93, 57.1%) and these were mostly hair dryers (RE n = 106, 66.3%; controls n = 91, 55.8%), used monthly (RE n = 66, 41.3%; controls n = 53, 32.5%).

4.10 Summary of results

Participants (RE and controls) were matched for age and there were no significant between-group differences in education or source of income. Furthermore, there were no significant between-group differences in HIV status, systolic or diastolic BP, smoking, childhood trauma, or social support.

A significantly greater proportion of RE participants endorsed previous trauma (total trauma load scores) and the following traumatic experiences compared to controls: serious injury, being close to death, murder of stranger, being robbed and/or carjacked at gunpoint/knifepoint, kidnapped. as well as higher scores on trauma load were found compared to controls. A significantly greater proportion of RE participants endorsed previous rape (excluding the recent rape trauma [+- 20 days]) compared to controls.

Significantly higher rates of the following disorders (as measured with the MINI) were found in the RE group compared to controls: MDD, suicidality, panic disorder, agoraphobia, SAD, and PTSD. Furthermore, there was significantly higher mean depression scores (CES-D), alcohol use (AUDIT-C) within 20 days post rape and mean perceived stress scores in the RE group compared to controls. To conclude, significantly lower mean BMI scores were found in the RE group compared to controls. See Section 4.9.3 above for more detail.

Aim 1:

1.1 Cortisol: There were no significant interaction or main effects.

1.2 Cortisone: There were no significant interaction or main effects.

1.3 Testosterone: There were no significant interaction or main effects.

1.4 Progesterone: There were no significant interaction or group main effect, however a significant time main effect was found. Within the RE group, a significant decrease in mean progesterone concentration was seen from pre-trauma to three months (i.e. from trauma to three months).

1.5 DHEA: There were no significant interaction or main effects.

Aim 2:

2.1 At all three time-points, significantly more (frequency and severity) symptoms of PTSS was found in the RE group compared to controls. Highest PTSS was seen within 20 days post rape in the RE group. These symptoms decreased to three months post rape and the lowest symptoms of PTSD was seen at six months post rape in the RE group.

2.2 Significantly higher rates of PTSD were established in the RE group at three- and six months post rape compared to controls. In the RE group, the highest rates of PTSD were seen at three months post rape, compared to six months post rape.

Aim 3:

3.1 Cortisol: Weak positive correlations were seen between pre-trauma cortisol and PTSS (total, as well as re-experiencing/intrusion, avoidance/numbing, and hyperarousal symptoms) within 20 days post rape.

3.2 Cortisone: A weak positive correlation was seen between cortisone three-six months post rape and avoidance/numbing three months post rape

3.3 Testosterone: No significant correlations between testosterone concentrations and DTS total or subscales.

3.4 Progesterone: No significant correlations between progesterone concentrations and DTS total or subscales.

3.5 DHEA: A significant negative correlation was seen between zero-three months DHEA concentrations and re-experiencing/avoidance symptoms at three months. Furthermore, a significant weak positive correlation between PTSD symptoms at three months post rape and DHEA three-six months post rape was seen, as well as between re-experiencing/intrusion symptoms three months post rape and DHEA concentrations three-six months post rape.

Aim 4:

4.1 Pre-trauma hormone concentrations were not predictive of symptoms of PTSD within 20 days, three- or six months post rape.

4.2 At baseline measurement (within 20 days post rape), three significant predictors of PTSS were identified. The strongest predictor of PTSS was depression, followed by previous trauma (trauma load / cumulative trauma), and perceived stress. At three-month follow-up, two significant predictors of PTSS were identified. The strongest predictor of PTSS was trauma load, followed by depression. At six-month follow-up, no significant predictors of PTSS were identified.

5. DISCUSSION

5.1 Introduction

The present study was conducted firstly, to examine the differences in mean cortisol concentrations and the concentrations of other neuroendocrine hormones (cortisone, testosterone, progesterone, and DHEA), between and within groups (rape-exposed [RE] and controls) at different time-points. The first time-point was at the baseline visit, which provided an approximate three-month window of hormone concentrations, preceding the rape trauma. The second sampling was at three months post rape and this covered the window between the baseline assessment and three months post rape. The last time-point was at six months post rape, providing concentrations in the window between three- and six months post rape.

Second, the present study sought to examine differences in post-traumatic stress symptomatology (PTSS) between and within groups at different time-points (baseline [data and samples collected within 20 days post rape], three months and six months post rape). Third, an analysis of temporal correlations between PTSS and hormones, as measured at baseline, three months, and six months post rape was undertaken. Lastly, the study sought to establish whether pre-trauma hormone concentrations were predictive of the development of PTSS at baseline, three months, and six months post rape.

The present study was the first of its kind to examine hair cortisol, cortisone, testosterone, progesterone, and DHEA concentrations in rape-exposed participants. No previous studies have compared differences in hair cortisol or other hair neurohormones in rape-exposed participants and controls. Therefore, the findings of the present study are compared to findings in other traumatised (other than rape) groups, and hair findings of these hormones are compared to findings in other tissue samples (urine, saliva, and plasma).

In the sections that follow, findings are discussed separately for each aim.

5.2 Comparison of hormone (cortisol, cortisone, testosterone, progesterone, and DHEA) concentrations between and within groups (rape-exposed and controls) at different time-points

5.2.1 Power calculation

For the realized data, the power of the sample was dependent on each analysis that was conducted, and therefore not only one power calculation could be done. However, power

calculations were conducted with regard to the primary aim: "To compare hormone (cortisol, cortisone, testosterone, progesterone, and DHEA) concentrations between groups (rape-exposed and controls) at different time-points".

A further complication of the power calculations is that it depends on "effect size". The current calculations were based on effect sizes from the results, which has the implication that the more non-significant a result was (p-values closer to 1), the smaller the effect size and the lower the power will be.

5.2.2 Differences in cortisol between groups

No significant differences were found in hair cortisol concentrations between groups (rape-exposed vs. controls) or within each group at the different time-points. This is not the first study to show no difference in cortisol concentrations between trauma exposed and control groups (e.g. Luo et al., 2012). In the present study, a history of family violence, and information regarding the context of the rape (e.g. number of perpetrators, relationship to perpetrator, coercion, weapon used, physical force, threatened murder, perceived life threat, repeat perpetrator, number of sexual acts, rape reported to police) were not included. However, in the present study, lifetime traumatic events were measure with the LEC-modified version.

There is evidence for attenuated cortisol concentrations as measured at baseline in trauma-exposed individuals, regardless of a PTSD diagnosis (Horn et al., 2014; Morris et al., 2012). In the present study, rape-exposed females were compared to controls.

At baseline (concentrations representing levels of before the traumatic event), Luo et al (2012), also found no difference in HCC between groups with PTSD, trauma-exposed without PTSD, and non-exposed controls. In those with PTSD, a decline in cortisol was seen over time since the trauma (earthquake). However, one month after the trauma, HCC was higher in the PTSD and traumatised without PTSD groups compared to controls. Two months after the trauma, both the PTSD and traumatised without PTSD groups had higher HCC than controls. At five to seven months, the non-PTSD traumatised group had the highest HCC (Luo et al., 2012). Previous research (Luo et al., 2012) has shown a decline in hair cortisol concentrations in traumatised participants. In women with a history of prior physical or sexual abuse, the experience of rape has been associated with an attenuated cortisol response (concentrations measured from blood plasma) compared to those without a history of abuse (Yehuda et al., 1998). Furthermore, evidence also suggest that attenuated cortisol concentrations, as measured

at baseline, have been associated with trauma-exposed individuals, regardless of PTSD diagnosis (Horn et al, 2014; Morris et al., 2012). Similarly, trauma exposure in healthy individuals without PTSD has been related to blunted cortisol stress reactivity (Elzinga et al., 2008; Lovallo et al., 2012; Trickett, Gordis, Peckins, & Susman, 2014) and to an enhanced HPA feedback inhibition (de Kloet et al., 2007).

In the present study, the group*time interaction effect of cortisol was not significant ($p = 0.92$). This resulted in a low power of 6%, because it was based on a small effect size.

5.2.3 Differences in cortisone between groups

No significant differences between groups, between visits, or within group between visits were found in the present study. It was important to investigate possible differences in cortisone between groups, because cortisone is the inactive form for cortisol and is converted to cortisol by the enzyme 11β-hydroxysteroid-dehydrogenase type 1 (11β-HSD1). Cortisol is converted to cortisone by the enzyme 11β-hydroxysteroid-dehydrogenase type 2 (11β-HSD2) (Raff and Findling, 2003). It has been suggested that higher cortisone concentrations, compared to cortisol, are found in hair and saliva samples, whereas lower levels of cortisone, compared to cortisol, is found in blood samples (Perogamvros et al., 2010; Raul et al., 2004). Incorporating hair cortisol and hair cortisone concentrations may provide a more complete picture of the HPA-axis functioning (Stalder et al., 2013; Staufenbiel et al., 2015). The present study was the first to examine hair cortisone concentrations in females and therefore no comparative research (including human urinary/saliva/plasma/hair cortisone concentrations) was available. These results should be interpreted with caution, because the results were not statistically significant.

The group*time interaction effect of cortisone was not significant ($p = 0.33$). This resulted in a low power of 16%, because it was based on a small effect size.

5.2.4 Differences in testosterone between groups

There were no significant group differences in testosterone concentrations at any of the three time-points. Although not significant, mean testosterone concentration was lower in the RE group compared to the control group at all three time-points. Previous research has suggested that testosterone levels may be suppressed by physical or psychological stressors (Francis, 1981; Kreux et al., 1972; Rose et al., 1969). These studies were done in males, whereas the present study was conducted with females. In the present study, the sample size was small in

the testosterone analyses, mostly due to undetectable results. Furthermore, the results were not significant at the 0.05 level and therefore interpretation of these results should be done with caution. Notably, in the present study, all participants were female and testosterone concentrations were often not detectable. Concentrations that were not detectable, were excluded from the analyses of the present study. In other studies of traumatised individuals regarding testosterone concentrations, only studies of males were found. Lower urinary testosterone has been found in soldiers anticipating combat in Vietnam compared to controls (Rose et al., 1969), as well as lower plasma testosterone concentrations in soldiers in a training program (Kreux et al., 1972). As mentioned earlier, testosterone levels may be suppressed by physical or psychological stressors (Francis, 1981; Kreux et al., 1972; Rose et al., 1969). However, in the present study, testosterone levels were not significantly different between groups (RE and controls) at any of the three time-points. No studies were found that examined the difference between testosterone concentrations in RE females compared to controls and therefore future research should be conducted in this area.

The group*time interaction effect of testosterone was non-significant ($p = 0.1$). This resulted in a low power of 5%, because it was based on a small effect size.

5.2.5 Differences in progesterone between groups

Between groups (RE and controls), no significant difference in progesterone concentrations were established at any of the three time-points. However, baseline (pre-trauma) mean progesterone concentration were slightly higher in the RE compared to the control group, slightly lower at three months (i.e. from trauma to three months) compared to controls, and slightly higher six months (i.e. from three to six months) post rape.

Within the RE group, there was a significant decrease in progesterone concentrations over time. Baseline (pre-trauma) mean progesterone concentration was significantly higher compared to the other two time-points. However, in the control group, there was also a significant decrease from the first time point (baseline) to the second (three months follow-up) and third (six months follow-up) time-points. Notably, pregnant women were excluded from all analyses. Further exclusions were any severe physical disease in the past five years, and/or use of glucocorticoid-containing medications or psychotropic medications within the past six months (based on self-report) (Steudte et al., 2013). Other factors may have contributed to the changes in progesterone concentrations seen in these groups (RE and controls) over time. We

did not consider the stage of the menstruation cycle, and infrequent or absent ovulation of these women, which may have influenced progesterone concentrations. However, each measurement reflected a three-month window and the average progesterone concentration for that three-month period was used in the statistical analyses. Therefore, the stage of the cycle might not have had such a big impact on the results that were generated.

No studies were found to examine progesterone concentrations and its relationship to PTSS in rape-exposed (or other traumatised) women compared to controls.

The main time effect of progesterone was significant ($p < 0.01$) and the power was high (95%), indicating that there was as significant difference in progesterone concentrations over time. However, the group*time interaction effect of progesterone was not significant ($p = 0.36$), implying that changes from Visit 1 through Visit 2 to Visit 3 were the same for controls and cases (RE group). The power for the group*time interaction effect of progesterone was low (31%).

5.2.6 Differences in DHEA between groups

No significant differences between groups, between visits, or within groups across the different measurement points were established. The present study is not the first to show no difference in DHEA between groups (Bremner et al., 2007), however Bremner et al. (2007) compared PTSD participants with controls, and in the present study rape-exposed participants were compared to controls. It was important to investigate DHEA between groups in the present study, because it has been suggested that DHEA could possibly block or inhibit the effects of cortisol (Maninger et al., 2009; Vythilingam et al., 2010). There is evidence that DHEA may counteract some of the effects of elevated glucocorticoids and play a role in stress-related disorders, such as depression and chronic fatigue (Goodyer, Park, Netherton, & Herbert, 2001; Khorram, 1996; Wolkowitz, Brizendine, & Reus, 2000). In reaction to stress, alterations in DHEA and DHEA-S may occur (Lemieux & Coe, 1995). It has been suggested that DHEA could inhibit/block the effects of cortisol (Hu, Cardounel, Gursoy, Anderson, & Kalimi, 2000; Kalimi, Shafoagoj, Loria, Padgett, & Regelson, 1994) and other glucocorticoids on the hippocampus and peripheral tissues (Kaminska, Harris, Gijsbers, & Dubrosvsky, 2000; Kimonides, Khatibi, Svendsen, Sofroniew, & Herbert 1998). DHEA have anti-inflammatory, antioxidant (Chen & Parker, 2004; Russo, Murrough, Han, Charney, & Nestler, 2012) as well as anxiolytic effects (Prasad, Imamura, & Prasad, 1997), therefore increasing the body's resilience towards a stressor (Pfau & Russo, 2015). Maninger et al. (2009) suggested that

allostatic load is increased when DHEA-S levels decrease, and cortisol are elevated. Allostatic load refers to chronic arousal of the HPA-axis that causes stress on the body (Seeman et al., 2001).

The group*time interaction effect of DHEA was not significant (p = 0.84). This resulted in a low power of 8%.

5.3 Comparison of posttraumatic stress symptoms between and within groups (rape-exposed and controls) across different time-points.

According to the DSM-5 criteria, a diagnosis of PTSD can be made at the earliest one month after a traumatic event (American Psychiatric Association [APA], 2013). In the present study, we assessed for symptoms of PTSD (PTSS, as measured by the DTS) within 20 days after the trauma, however a diagnosis of PTSD with reference to the recent rape-trauma could not be made at baseline (within 20 days post rape). Although a PTSD diagnosis is made after the presentation of these symptoms for more than one month after a traumatic event, in addition to clinically significant distress/impairment in social, occupational, or other important areas of functioning and not caused by the physiological effects of a substance or another medical condition, the DSM-5 points out that symptoms of PTSD may be present before the 1-month post-trauma time point (American Psychiatric Association [APA], 2013). Therefore, we examined differences in PTSS between groups (RE and controls) at 3 time-points, namely baseline (within 20 days post rape), at three months, and at six months post rape.

5.3.1 Comparison of posttraumatic stress symptoms (PTSS) between groups over time

A significant difference was found between groups (RE and controls) with regard to PTSS (as measured by the DTS total imputed mean scores), between time-points, and within groups across time-points. Females recently exposed to rape, had significantly more posttraumatic stress symptoms (frequency and severity) in the aftermath of rape (within 20 days), and at three and six months after rape, compared to controls. This indicates that in comparison to a control group, who had not recently (within the past 20 days) experienced a rape trauma, rape-exposed women experienced more symptoms of PTSD (total symptom frequency and severity).

In the RE group, PTSS symptoms decreased significantly from baseline (within 20 days post rape) to three months post rape and from three months to six months post rape, with the lowest mean PTSS scored at six months post rape. This indicates that in the acute period post

rape, symptoms of PTSD are the highest (more severe and more frequent), compared to three- and six months post rape. In the present study, rape-exposed participants were recruited from three dedicated sexual assault services known as Thuthuzela Care Centres and from a crisis centre. Attending these support centres could have contributed to the decrease in posttraumatic stress symptoms seen over time in the present study. A trauma counsellor was also part of the RICE team to support participants. According to the National Center for PTSD, in one study, 94% of women who experienced sexual assault presented with symptoms of PTSD during the first two weeks post rape. In the immediate aftermath of sexual assault, MacGregor et al. (2019) pointed out that symptoms for PTSD were highest, consistent with the findings of the present study. In a review of MacGregor et al. (2019), PTSD symptoms decreased three months post trauma (Feiring et al., 2002; Mouilso, Calhoun, & Gidycz, 2011), supporting the results of the present study. However, in one study (Khadr et al., 2018), PTSD symptomatology did not decrease/increase over time. However, these studies (Feiring et al., 2002; Khadr et al., 2018; Mouilso et al., 2011) focused on adolescents and young adults, whereas the present study included women above the age of 18 with a mean age of 25 years.

5.3.2 Probable diagnosis of PTSD based on DTS cut-off scores: differences between groups

Statistically significant higher rates of PTSD (according to the DTS, using a cut-off score of 40 to delineate PTSD diagnosis at three- and six months post rape), were seen in the RE group, compared to the controls at three months post rape, as well as six months post rape. Within the RE group, rates of PTSD diagnosis decreased significantly from three to six months post rape. At three months post rape, significantly higher rates of PTSD were seen in the RE group, compared to six months post rape.

In a study by Morris et al. (2013), sexual assault (including rape) was the trauma associated with the highest conditional risk for PTSD among HIV-infected South African adults. Results of the present study showed that half of the rape-exposed women had HIV, and 45 of those that were followed-up at three months (n = 97) met PTSD criteria (according to the DTS) at three months post rape. Among people living with HIV/AIDS, one of the most prevalent disorders is PTSD (10,4%-42%) (Martinez et al., 2002; Pingo & Seedat, 2009; Radcliffe et al., 2007). A study conducted in Cape Town revealed that of the 465 HIV-positive participants, sexual assault (17,4%) was the highest contributing factor and significantly correlated with PTSD (Morris et al., 2013).

According to the World Mental Health (WMH) surveys, Kessler et al. (2017) reported that globally, intimate partner sexual violence, including rape and sexual assault, were associated with the highest risk for PTSD (Kessler et al., 2017). Sexual assault is associated with a broad spectrum of psychopathology and not only PTSD. However, the strongest association in a study by Dworkin et al (2017) was found between sexual assault and PTSD, compared to other psychopathologies. According to the National Center for PTSD, 45% of women who experienced rape trauma meet diagnostic criteria for PTSD. Women are more likely to be victims of rape and sexual assault and are more likely, compared to men, to develop PTSD following a traumatic event (Hetzel-Riggin & Roby, 2013; Kessler et al., 2017; Kolltveit, et al., 2012; Olff et al., 2007; Tolin & Foa, 2006). It has been suggested that self-blame may contribute to poorer intervention outcomes in PTSD treatment and result in lasting psychological impact (Abrahams et al., 2013; Hembree et al., 2004). Women who have been sexually abused are at higher risk for psychiatric disorders than those who have not been sexually abused (Campbell, 2002; Ellsberg et al., 2008) and are more likely to develop PTSD compared to survivors of other trauma types (Arata, 2002; Hetzel-Riggen & Roby, 2013; Kessler et al., 2017; Scott et al., 2018). Female rape survivors are therefore at high risk of developing PTSD (Kessler et al., 2017). In the present study, we investigated female survivors of rape. The rape-exposed women were not compared to persons with other trauma types, however, the present study is the first study to follow-up a cohort of rape-exposed and control women over time and to assess PTSD outcomes.

PTSD is a disabling disorder that affects 2.3% of the general South African population (Herman et al., 2009). According to the WMH surveys, the global prevalence of PTSD has been estimated to range between 1.3% (Kawakami et al., 2014) and 8.8% (Ferry et al., 2014) and the WMH surveys published in 2014 revealed a 12-month PTSD prevalence of 1.1% (Karam et al., 2014). Among American adults, the lifetime prevalence of PTSD has been suggested to be 6.8% (Kessler et al., 2005) and in European populations, PTSD prevalence has been estimated to be 7.4% (de Vries, & Olff, 2009).

Among survivors of sexual assault, PTSD prevalence has been reported to be 20.2% (Scott et al., 2018). The Australian National Survey suggested that the lifetime prevalence of PTSD in women who have been sexually abused is 50% (Creamer et al., 2001).

Mbalo et al. (2017) reported that living in KZN is a major risk factor for PTSD and that female survivors of rape residing in KZN indicated significantly higher prevalence of PTSD compared to the Western Cape and Limpopo provinces (Mbalo et al., 2017).

In the present study, there was a significant difference in the rate of PTSD diagnosis between recently rape-exposed and control women at three- and six months post rape. The literature also suggests that women living in KZN are at a higher risk to develop PTSD, compared to living in other provinces in South Africa (Mbalo et al., 2017). Police stations in KZN had the highest rape reported since April 2017 to March 2018, which was Inandi (278 reported rape cases) and Umlazi (252 reported rape cases) stations (SAPS, 2018). KZN has high levels of poverty and has been identified by SAPS to be South Africa's murder capital. This suggests that there could be more trauma in KZN. KZN has the largest population in South Africa (Statistics South Africa, 2019). A study by Mbalo et al. (2017) suggests that, compared to other provinces (Limpopo and Western Cape) in South Africa, female rape survivors from KZN experienced more childhood abuse, and specifically more childhood sexual abuse (Mbalo et al., 2017). Living in KZN under these conditions may further exacerbate symptoms of mental illness, such as posttraumatic stress post rape (Mbalo et al., 2017).

Female rape survivors are at high risk of developing PTSD (Kessler et al., 2017), and considering the above information and that the highest rape is reported in KZN, could possibly be linked to higher trauma in KZN. However, provincial level data on mental health does not exist in this regard. In the absence of provincial level data on mental health in South Africa, it is not clear if there is higher trauma and higher risk of developing PTSD in KZN, compared to other provinces. Given the higher levels of rape reported, murders and poverty in KZN (SAPS, 2018), the available data might suggest that KZN could have higher levels of trauma, however this should be confirmed with future research in this area.

In the present study, almost all participants had completed secondary education, however very few participants (5.6%) had completed tertiary education and nearly a quarter of the RE participants were employed (23.8%) part-time / full-time / self-employed, compared to an unemployment rate of 27% in 2017 and 2018 in South Africa (Statistics South Africa, 2019). Furthermore, lower levels of education and unemployment has been linked to higher symptoms of PTSD (Mbalo et al., 2017), however this is based on only one study with a small sample size and without comparative data. A direct comparison in this regard is however not possible.

The present study did not compare the risk of developing PTSD in different regions, and therefore conclusions regarding differences in PTSD rates between provinces cannot be done.

The topic on reasons for women in KZN, also rape survivors, possibly having a higher risk of developing PTSD compared to other provinces, would be a valuable topic for future research, also suggested by Mbalo et al. (2017), as little is known about this. The results of the present study make a substantial contribution to the literature on women in KZN who experience rape trauma and the development of PTSD/PTSS.

In the present study, according to the results of the MINI, fewer diagnoses of PTSD were made compared to PTSD diagnosis according to the DTS (cut-off of 40) in each group (RE and controls). It is not clear why there were these differences in PTSD diagnosis between the MINI and the DTS, and therefore future research in this area is needed.

5.4 Correlations between posttraumatic stress symptoms and HPA-axis hormones (cortisol, cortisone, testosterone, progesterone, and DHEA) measured at baseline, three months, and six months after rape.

Within the literature, there is a debate between whether pre-trauma cortisol concentrations are associated with PTSD/PTSS post trauma, or whether traumatisation has a later influence on cortisol concentrations (Heinrichs et al., 2005; van Zuiden et al., 2011). Therefore, for the present study, correlational tests within the RE group of hormone concentrations (cortisol, cortisone, testosterone, progesterone, & DHEA) and PTSD symptomatology (as measured by the DTS total score, as well as cluster scores) at all three time-points (as measured within 20 days post rape, three months, and six months post rape), were conducted. Significant correlations are discussed. In order to understand and discuss significant correlations that were found within the present study, it is important to understand the meaning of the correlation coefficient. The correlation coefficient indicates the strength of the association between variables, ranging from +1.00 to -1.00 (Howell, 2008). The closer this value is to these limits, the stronger the association between variables (Howell, 2008).

5.4.1 Cortisol and PTSS

In the rape-exposed group, a significant, but weak, positive correlation was found between total baseline (within 20 days post rape) PTSD symptoms and baseline (concentrations representing levels of before the traumatic event) cortisol concentrations. Results from univariate analyses

also showed a significant positive correlation between total baseline (within 20 days post rape) PTSD symptoms and baseline (concentrations representing levels of before the traumatic event) cortisol concentrations. This indicates that the higher the pre-cortisol concentrations were before being exposed to the rape trauma, the more PTSS were experienced within 20 days post rape. Within the rape-exposed group, when the pre-trauma cortisol concentrations were relatively low (between 0 -10 pg/mg), more variance were observed in DTS total scores (as measured at baseline [within 20 days post rape]). However, when the pre-trauma cortisol concentrations increased to above 20 pg/mg, DTS total scores correlated positively with pre-trauma cortisol concentrations.

Furthermore, significant, but weak, positive correlations between baseline (concentrations representing levels of before the traumatic event) cortisol concentrations and the following subscales of the DTS (as measured at baseline, i.e. within 20 days post rape) were also found: DTS cluster A (re-experiencing/intrusion symptoms), DTS cluster B (avoidance/numbing symptoms), and DTS cluster C (hyperarousal) (American Psychiatric Association [APA], 2013). This indicates that the higher the pre-trauma cortisol concentrations were, the more overall PTSS, as well as higher severity and frequency within 20 days post rape of re-experiencing/intrusion symptoms, avoidance/numbing symptoms, and hyperarousal symptoms. However, these results should be interpreted with caution, due to weak correlations. In the present study, as discussed earlier, greater PTSS (as measured by the DTS) were experienced in the acute phase after rape (within 20 days post rape) compared to three- and six months post rape.

In contrast to these findings, several studies found a negative correlation between cortisol concentrations and severity of posttraumatic stress symptoms (Gill et al., 2008; Olff et al., 2006; Wessa et al., 2006; Witteveen et al., 2010). Findings from meta-analyses show that lower cortisol levels are associated with an increase in posttraumatic stress symptomatology in adults (Meewissa et al., 2007; Morris et al., 2016; Morris et al., 2012; Pan et al., 2018), both in terms of a 24-hour cycle, as well as in response to a stressor (Daskalakis et al., 2013). However, in a study by Yehuda et al. (1998) of female rape victims, plasma cortisol was not associated with posttraumatic symptom severity. Research suggests mixed results and therefore future studies in this area should be conducted. In the present study, as mentioned earlier, cortisol concentrations were lower in the RE group compared to controls at three months (i.e. from

trauma to three months) and at six months (i.e. from three to six months) post rape, however, these findings were not significant and should therefore be interpreted with caution.

Intrusion symptoms of PTSD, such as intrusive trauma-related memories, have also been linked to reduced cortisol levels (Hauer et al., 2014; Holz et al., 2014). Yehuda (2009) suggested that reduced cortisol concentrations may further create a neuroendocrine environment that leads to poor consolidation of traumatic memories. Lemieux and Coe (1995) found no significant correlation between intrusion and avoidance symptoms (as measured by the Impact of Event Scale [IES]), as well as total IES score and cortisol. In studies of combat veterans, it has been reported that elevated levels of cortisol may be observed at times when intrusive memories or psychopathology are reactivated (Yehuda, 1993).

Deficits in cognitive functioning, such as impairments in memory, have also been found in rape victims (Jenkins et al., 1998). Furthermore, negative correlations have been found between PTSD symptom severity and immediate recall (Lindauer et al., 2006). Future studies should consider assessing cognitive performance, including memory, in rape victims in the immediate aftermath of rape as well as over time.

5.4.2 Cortisone and PTSS

A significant, but weak ($r = 0.39$), positive correlation was found between avoidance/numbing symptoms at three months post rape and cortisone concentrations at 6 months (i.e. three to six months post rape). This indicates that as mean cortisone concentration increased, at three to six months post rape, RE women experienced more avoidance/numbing symptoms. In the present study, as mentioned earlier, cortisone concentrations were highest at three months (i.e. from trauma to three months). However, a significant difference in cortisone concentrations over time in the RE group was not seen. The present study is the first to examine hair cortisone concentrations in females and therefore no comparative studies (including human urinary/saliva/plasma/hair cortisone concentrations) are available. Future research should further investigate the findings of the present study.

5.4.3 Testosterone and PTSS

No significant correlations were found between total PTSS symptoms and individual PTSS symptom clusters and testosterone concentrations in the RE group. However, as mentioned earlier, differences in testosterone concentrations in the RE group was seen over time in the present study, and testosterone concentrations were slightly lower in RE compared to controls

over time. No studies were found that examined the relationship between testosterone and PTSD/PTSS in females. However, in a study by Spivak et al. (2003), a significant positive correlation was established between plasma testosterone concentrations and PTSD avoidance symptoms, as measured by the Impact Events Scale (IES) in combat-related PTSD participants.

5.4.4 Progesterone and PTSS

No significant correlations were found between DTS total and subscale scores and progesterone concentrations in the RE group. Univariate analyses revealed a significant positive correlation between progesterone (0-3 months post rape) and PTSS three months post rape. This indicates that the higher progesterone concentrations were 0-3 months post rape, the more PTSS (frequency and severity) were experienced three months post rape. However, this was a weak correlation and therefore the results should be interpreted with caution.

Furthermore, as mentioned earlier, in the RE group, mean progesterone concentration decreased significantly from baseline (pre-trauma) to three months (i.e. from trauma to three months) and from baseline (pre-trauma) to six months (i.e. from three to six months) post rape. Inslicht et al. (2014) suggested that progesterone may mediate glucocorticoid reactivity in PTSD. No other studies examining progesterone and its relationship to PTSS and/or PTSD were found.

5.4.5 DHEA and PTSS

In the RE group, a significant negative correlation ($r = -0.40$) was found between re-experiencing/intrusion symptoms at three months post rape and DHEA (0-3 months post rape). This indicates that the higher the DHEA concentrations at 0-3 months post rape, the less re-experiencing/intrusions symptoms experienced at three months post rape. DHEA concentrations (0-3 months post rape) may have an inhibiting effect on re-experiencing/intrusion symptoms of PTSD at three months post rape. Lipschitz et al. (2003) suggested that an increase in DHEA in response to ACTH could be negatively correlated with PTSD symptom severity. Increased DHEA/DHEA-S has been found to be beneficial, instead of harmful to the body (Maninger et al., 2009). Increased levels of DHEA/DHEA-S has been connected to a decrease in PTSD symptomatology (Rasmusson et al., 2004), improvement in PTSS and improved coping (Yehuda, 2006), as well as a reduction in PTSS in reaction to therapy (Olff et al., 2007). DHEA and DHEA-S has been found to counteract the negative effects of cortisol, particularly in the hippocampus (Kalimi et al., 1994; Kaminska et al., 2000;

Kimonides et al., 1998). In healthy individuals, improved coping with the negative effects of acute stress has been associated with a higher DHEA-S-to-cortisol ratio.

A significant, however weak, positive correlation (r = 0.40) was found between total PTSS three months post rape and DHEA concentrations (three to six months post rape), as well as between re-experiencing/intrusion symptoms three months post rape and DHEA concentrations three to six months post rape (r = 0.49). This indicates that the higher scores for PTSS were obtained, as well as more re-experiencing/intrusion symptoms at three months post rape, the higher the mean DHEA concentration were three-six months post rape, by these women. From the literature, increased DHEA and DHEA-S have been associated with chronic stress (Fuller et al., 1984) and in PTSD participants compared to controls (Gill et al., 2008; Kellner et al., 2010; Olff et al., 2007, Yehuda et al., 2006). However, increased levels of DHEA/DHEA-S has been connected to a decrease in PTSD symptomatology (Rasmusson, et al., 2004), improvement in PTSS (Yehuda, 2006), as well as a reduction in PTSS in reaction to therapy (Olff et al., 2007). In a study by Pico-Alfonso et al (2004), no correlation was found between salivary DHEA concentrations (as measured over 4 consecutive days at 08:00 and 20:00) and total PTSD symptom score.

Confounding variables may contribute to the differences seen in the results of DHEA, like those seen in studies of cortisol concentrations. However, most studies confirm a higher DHEA-S-to-cortisol ratio in PTSD patients (Butterfield et al., 2005; Rasmusson et al., 2004; Yehuda et al., 2006). As mentioned earlier, instead of being harmful, higher ratios of DHEA-s-to-cortisol is suggested to be protective and contribute to the body's resilience against negative effects of circulating glucocorticoids. DHEA-S are thought to exhibit protective effects on the neuroendocrine system.

Studies on the potential of DHEA as a treatment option in PTSD and reduction in PTSS have been limited. However, Sageman and Brown (2006) have found positive outcomes with the use of DHEA, using 7-keto DHEA (a metabolite of DHEA), because in this form it cannot be converted to estrogen or testosterone. They found improvements in PTSD avoidance, numbing and dissociation symptoms when they treated five women with PTSD with this metabolite of DHEA. This study is however limited, because the sample size was very small. However, the findings of Sageman and Brown (2006) prove potential for future treatment. Treatment studies should consider the role of HPA-axis hormones in improving physical and psychological symptoms in reaction to a stressor.

5.5 Pre-trauma hormone concentrations as possible predictors of posttraumatic stress symptoms

In the present study, pre-trauma hormone concentrations (cortisol, cortisone, testosterone, progesterone, and DHEA) were not predictive of PTSS at baseline, three months, or six months post rape. As mentioned earlier, although a diagnosis of PTSD is made one month post trauma (in this case rape), symptoms of PTSD may be present before this time-point (APA, 2013) and therefore we examined PTSS at baseline (within 20 days post rape), and at three- and six months post rape.

In keeping with the results of the present study, studies have not provided conclusive evidence that pre-trauma cortisol concentrations predict PTSD symptomatology after a traumatic event (Heinrichs et al., 2005; van Zuiden et al., 2011; van Zuiden et al., 2012). From the literature, it is still unclear whether pre-trauma cortisol concentrations predict PTSD or whether HPA dysregulation as manifested by cortisol concentrations are secondary to a traumatic event (Heinrichs et al., 2005; van Zuiden et al., 2011). It appears that, from the results of the present study, cortisol and other hormone concentrations may change in response to a traumatic event (in this case rape trauma), while pre-trauma (concentrations before the trauma) cortisol and other hormone concentrations may not be predictive biomarkers of PTSS after a traumatic event. These findings should be investigated further in future research.

5.6 Identified predictors of PTSS

Although pre-trauma hormone concentrations were not predictive of PTSS, other predictors of PTSS were identified. At baseline measurement (within 20 days post rape), three significant predictors of PTSS were identified. The strongest predictor of PTSS was depression, followed by previous trauma (trauma load / cumulative trauma), and perceived stress. At three-month follow-up, two significant predictors of PTSS were identified. The strongest predictor of PTSS was trauma load, followed by depression. At six-month follow-up, no significant predictors of PTSS were identified. Baseline depression was a predictor of PTSS at baseline and at three months post rape, in the rape-exposed group. These results should be interpreted with caution, because the baseline regression had a low adjusted R^2 (Adjusted $R^2 = 0.50$), the three month regression had a lower adjusted R^2 (Adjusted $R^2 = 0.12$), and the six month regression had the lowest Adjusted R^2 (Adjusted $R^2 = 0.02$). This indicates that these variables only explained a small percentage of the variance in the outcome variable.

5.6.1 Depression as a predictor of PTSS

According to the National Center for PTSD, depression is one of the effects that should be considered in the aftermath of sexual assault. A decrease of depression scores over time were established in previous research (Feiring et al., 2002, Khadr et al., 2018; Mutavi et al., 2018). In the present study, in univariate analyses, a significant positive correlation was found between depression (CES-D total) and DTS within 20 days post rape, three months- and six months post rape, respectively. This indicates that when depression scores were high on the CES-D scale, the more (frequency and severity) PTSS were experienced within 20 days post rape, three- and six months post rape. The strongest correlation ($r = 0.65$) was found between depression symptoms and PTSS within 20 days post rape. As discussed earlier, within the present study, scores for symptoms of PTSD were highest at this time-point (within 20 days post rape). Within the literature, it is evident that participants with PTSD are frequently diagnosed with comorbid depression (Perkonigg et al., 2000).

In the present study, when depression (as measured by the CES-D) was compared between groups, at all three visits, the RE group had significantly higher mean depression scores than controls. The present study found that females who have recently been exposed to rape, showed higher depression within 20 days after the traumatic event, three and six months after the traumatic event, compared to controls. With regard to the results of the MINI, at baseline, three months, and six months post rape, higher rates of current MDD and past MDD diagnoses were made in the RE group compared to controls. In the RE group, slightly higher rates of MDD were seen at three-month follow-up compared to baseline, and a slight decrease at six-month follow-up. In the present study, in both groups (RE and controls), according to the results from the CES-D, more diagnoses of depression were made, compared to the findings of the MINI. It is not clear why there were differences between the results obtained from the MINI compared to the CES-D, and therefore future studies could investigate this finding further.

Although we did not compare women with depression in KZN to other provinces in South Africa, research has found that women who experienced rape trauma in KZN are seven times more likely to experience depression symptoms compared to other provinces in South Africa (Mbalo et al., 2017). Furthermore, Mbalo et al. (2017) found that female survivors of rape are exposed to an increased risk to develop depression and PTSD.

Within the RE group, the mean CES-D score was significantly higher within 20 days post rape, compared to three- and six months post rape. Symptoms of depression decreased significantly in the RE group within 20 days after the rape to three- and six months post rape. Of note, the RE group also experienced significantly more posttraumatic stress symptoms within 20 days after the rape, compared to three months and six months after the traumatic event. Higher rates of depression were also experienced in the RE group at three months compared to six months post rape. A decrease of depression scores over time were established in previous research (Feiring et al., 2002, Khadr et al., 2018; Mutavi et al., 2018), however, these were all studies of adolescents or children. In congruence with the results of the present study, a review of MacGregor et al. (2019) suggested that anxiety and PTSD symptoms were highest in the acute phase after a traumatic event and these symptoms reduced over time (Feiring et al., 2002; Khadr et al., 2018, Mutavi et al., 2018). However, in a study by Oshodi et al. (2020) of adolescent girls, depressive symptoms remained the same over time.

Depression and posttraumatic stress symptoms followed the same trend over time in the RE group: a steep decrease from within 20 days post rape to three months post rape, and a steady decrease from 3 to six months post rape, with the highest scores in the acute aftermath of rape (within 20 days post rape). Within the literature, it is evident that participants with PTSD are frequently diagnosed with comorbid depression (Perkonigg et al., 2000) and therefore we included participants with comorbid depression in the present study. A systematic review by Deering et al. (1996), suggested the following possible reasons for the comorbidity between depression and PTSD: (1) pre-existing depression could contribute to the develop of PTSD post trauma, (2) grief and loss that is associated with the trauma could trigger the development of depression, and (3) PTSD and trauma could trigger the development of depression (Deering et al., 1996). Furthermore, Breslau et al. (2000), also provided several suggestions for the comorbidity of PTSD and depression. In congruence with Deering et al. (1996), they propose that pre-existing depression could increase the person's vulnerability to develop psychopathology, such as PTSD, after a traumatic event (Breslau et al. 2000). Another suggestion is that PTSD could increase the risk of depression. In a longitudinal study by Nickerson et al. (2013), participants with PTSD who experienced sexual assault, developed depression secondary to PTSD. These findings could suggest an underlying vulnerability for both PTSD and depression. The traumatic event could also increase the person's susceptibility to develop depression. The one disorder could develop after the other, or the two disorders could have a common underlying vulnerability. In a study by Breslau et al. (2000), pre-existing

depression increased the person's risk for developing PTSD after a traumatic event (Breslau et al., 2000). They also found that participants who developed PTSD after being exposed to a traumatic event, was 2.8 times more likely than those not exposed to a traumatic event, to develop depression. There was no significant association between those exposed to a traumatic event, without developing PTSD, to develop depression (Breslau et al., 2000), suggesting that being exposed to a traumatic event and being diagnosed with PTSD could make the person more susceptible to develop other psychopathology, including depression.

Comorbid depression and PTSD diagnoses could be due to an overlap in symptoms (e.g. alterations in arousal and negative alterations in cognitions) (APA, 2013). However, in a study by Taft et al. (2009), including 162 adult female rape survivors (32% of participants were African American), all met criteria for PTSD (according to the CAPS) and 52% met criteria for depression (according to the SCID). Taft et al. (2009) found that the comorbidity was not due to a mere overlap between symptoms.

Maladaptive beliefs could be associated with the comorbid diagnosis of depression in participants with PTSD. In a study by Nishith et al. (2005), the treatment for individuals with PTSD and depression were more effective if it targeted problematic trauma-related cognitions (Nishith et al., 2005), suggesting that the trauma-related cognitions could play a role in the comorbidity seen in depression and PTSD. In a cross-sectional study by Nixon et al. (2004) regarding female victims of intimate partner violence (physical abuse), which included 59% African American women, suggested that distorted cognitions and schemas could play a role in understanding the comorbidity between depression and PTSD. Dissociative experiences could also be linked to avoidance behaviours, associated with the trauma and/or maladaptive cognitions that could lead to social isolation. Social isolation may lead to the development of comorbid depression.

Dissociative experiences have been linked to PTSD severity and comorbid depression (Lemos-Miller & Kearney, 2006). Trauma processing could be hindered by dissociative experiences and interfere with the integration of the traumatic experiences into memory, causing individuals to be at risk of developing psychiatric disorders after a trauma and cause prolonged stress reactions (Foa & Rothbaum, 1998). However, the mechanism of how dissociative experiences could possibly lead to a comorbid diagnosis of PTSD and depression, is unclear and needs further investigation (Taft et el. 2009).

Prospective studies investigating the complex interrelationship between these two disorders are needed. Furthermore, prospective studies investigating the long-term mental health outcomes of women who have been raped or sexually assaulted is needed. This knowledge could benefit and guide interventions in female survivors of sexual assault and rape.

5.6.2 Previous trauma as a predictor of PTSS

In addition to the findings of the regression analyses showing that baseline trauma load predicts PTSS within 20 days post rape, as well as three months post rape, univariate analyses revealed a significant positive correlation between trauma load and PTSS within 20 days post rape, as well as PTSS three months post rape, respectively. This indicates that when the participant experienced more trauma previously (before the recent rape trauma), they experienced more symptoms of PTSD within 20 days, as well as 3 months post rape. These results are aligned with research showing that prior trauma history is associated with lower cortisol levels after traumatisation, which in turn are related to an increased risk for PTSD development when individuals experience a new trauma later in life (Delahanty et al., 2003; Ehring et al., 2008; Resnick et al., 1995; Walsh et al., 2013). Furthermore, previous studies have shown a dose-response relationship between trauma load and symptoms of PTSD, suggesting increased trauma load is associated with increased symptoms of PTSD severity and frequency (Kolassa et al., 2010; Steudte-Schmiedgen et al., 2016; Neuner et al., 2004).

Furthermore, in the present study, when trauma load was compared between groups (controls and RE), females who recently experienced rape trauma had significantly higher trauma load than controls. A significantly greater proportion of RE participants endorsed previous trauma (total trauma load scores) and the following traumatic experiences compared to controls: serious injury, being close to death, kidnapped, being robbed and/or carjacked at gunpoint and/or knifepoint, and murder of a stranger. In a study by Steudte et al. (2011a), severely traumatised participants with PTSD also had higher trauma load compared to traumatised controls without PTSD. However, in the present study, traumatised participants were compared to controls.

5.6.2.1 Previous rape

Although previous rape was not predictive of PTSS within 20 days, three- or six months post rape in the regression analysis, a significant difference was found in PTSD symptoms three months post rape, between the RE participants who experienced a previous rape (excluding the

recent event) and those without experiencing a previous rape (excluding the recent rape trauma). The RE group who experienced previous rape (in addition to the recent rape trauma), had significantly higher scores for PTSS than RE who did not previously (excluding the recent rape trauma) experience a rape. This indicates that in those participants who had been raped previously, increased symptoms (frequency and severity) of PTSD three months after the most recent rape were experienced. The findings of the present study confirm previous research referring to the building block effect of trauma and the association with increased PTSD symptomatology (Kolassa et al., 2010; Steudte-Schmiedgen et al., 2016; Neuner et al., 2004). Furthermore, in another study, a history of rape strengthened the relationship between previous sexual abuse and psychopathology, such as depression and PTSD (Chen et al., 2010).

Furthermore, when previous rape was compared between groups (controls and RE), the RE group indicated more experiences of rape (excluding the recent event) compared to the control group. More specifically, the findings of the present study showed a significant difference in past rape between rape and controls, as well as first intercourse between groups, a difference in being forced/raped in RE group compared to controls, forced sexual intercourse with a partner, forced intercourse with a non-partner, frequency of sexual intercourse against will when drunk/drugged, and frequency of sexual intercourse against will with more than one male at the same time. These findings suggest that when women experience rape, they are more likely than controls (who have not experienced recent rape), when re-victimised, to experience increased symptoms of PTSD. This is in congruence with previous research showing that when females are re-victimised, they have an increased risk to experience trauma-related symptoms, especially symptoms of PTSD and depression (Mbalo et al., 2017; Saunders et al., 1992).

5.6.2.2 Childhood trauma

Childhood trauma was not predictive of PTSS in the regression analysis and there was also no significant univariate correlation between childhood trauma and PTSS. When childhood trauma was compared between groups (RE and controls), no significant difference was also found. The RE group had significantly higher scores on childhood sexual abuse and witnessing abuse of their mother, compared to controls. In a study by Mbalo et al. (2017), 10% of survivors of rape also indicated to have experienced childhood sexual abuse. Compared to other provinces (Limpopo and Western Cape) in South Africa, female rape survivors from KZN experienced more childhood abuse, and specifically more childhood sexual abuse (Mbalo et

al., 2017). The present study was conducted in KZN and therefore comparative to the study of Mbalo et al. (2017).

Although childhood trauma was not a significant predictor of PTSS in the present study, the relationship between CSA and adult mental health has been researched extensively, suggesting an association between CSA and adult psychopathology, including symptoms of PTSD (Gladstone et al., 2004; Hillberg et al., 2011; Lang et al., 2004). The findings of the current study add to the literature in this regard.

5.6.3 Perceived stress as predictor of PTSS

The finding that perceived stress predicted PTSS within 20 days post rape, was further confirmed with univariate analyses showing a significant positive correlation (r = 0.37) between perceived stress and PTSS within 20 days post rape. This indicates that the more the participant perceived stress after rape trauma, the more PTSS (frequency and severity) they experienced within 20 days post rape. When perceived stress was compared between groups (RE and controls) in the present study, overall (from within 20 days post rape to three- and six months post rape), the RE group experienced significantly higher perceived stress than controls. Heinze et al. (2016) also found that perceived stress was higher in those participants diagnosed with psychopathology compared to healthy controls. In the RE group, perceived stress decreased from within 20 days post rape to three months and six months after the rape. Perceived stress followed the same trend as depression and posttraumatic stress symptoms, by decreasing over time.

5.6.4 Education and age and PTSS

Between groups (RE and controls) there was no significant difference in educational level and age. Within the results from the regression analyses, age and education were not predictive of PTSS. However, from the results of the univariate analyses done in the present study, a significant, however weak, negative correlation (r = - 0.17) was found between the level of education achieved by the participant and PTSD symptoms within 20 days post rape. As noted in earlier results of the present study, within the RE group, the highest scores for PTSS were obtained within 20 days post rape. This indicates that the higher the educational level of the participants were, the less PTSS symptoms were experienced within 20 days post rape. However, these results should be interpreted with caution, because the correlation was very weak, and the results of the regression analyses did not reveal a significant association between education and PTSS.

5.6.5 Social support and PTSS

Social support was not a significant predictor of PTSS in the regression analysis. Mbalo et al. (2017) also found no association between social support and PTSD. Although social support was not a significant predictor of PTSS in the present study, previous research suggests that when survivors of sexual assault and specifically rape experience or perceive support and social interactions as negative after disclosing information to friends, family, or professional, they have an increased risk to develop symptoms of PTSD (Ullman & Peter-Hagene, 2014; National Center for PTSD). A study by Koss and Figuerdo (2004) confirmed that women who experience negative support report more symptoms of PTSD. Feelings associated with rape trauma, such as anxiety, depression, shame and guilt may also increase when these women receive or perceive support as negative (National Center for PTSD). Furthermore, in a study by Elklit and Chrisiansen (2013), perceived positive support in women in the early aftermath of sexual assault (two weeks post sexual assault) was associated with less severity of PTSD symptoms three months after sexual assault and therefore positive support is important to recovery (Koss, & Figuerdo, 2004). In a study including female rape victims, increased social support was also associated with decreased symptoms of PTSD (Gutner et al., 2006). More specifically, support from intimate partners have been suggested to decrease symptoms of PTSD and depression (Billette et al., 2008). Furthermore, Dworkin and Schumacher (2018) suggest that rape victims should receive appropriate support and perceive this support positively. Iob et al. (2018) highlighted the importance of the quality of social support in order to decrease stress and improve health.

When social support was compared between groups (RE and controls), no significant difference was seen.

5.6.6 Alcohol use and PTSS

The RE group had used significantly more alcohol (according to the AUDIT-D total) within 20 days post rape, compared to controls. The use of alcohol in the RE group decreased significantly from within 20 days to three months post rape and increased slightly to six months. The use of substances, such as alcohol, as well as the association between substance use disorders and PTSD in the aftermath of sexual assault has been researched extensively (Kilpatrick et al., 1997). In congruence with the findings of the present study, alcohol use has been documented to be higher in the acute aftermath (within weeks) of assault (Rothbaum et al., 1992). Furthermore, alcohol use, instead of abuse, has been associated with victims of

sexual assault, and could be used as a coping mechanism to reduce negative affect (Kilpatrick et al., 1997).

As mentioned earlier, in the present study, alcohol use was higher in the RE group compared to controls within 20 days post rape. Furthermore, it appears that victims of sexual assault are at lower risk for the development of substance use disorders compared to other disorders (Kilpatrick et al., 1997). In the present study, alcohol use in recently raped females were compared to controls. Please also see Section 5.8 “psychopathology between groups”. In this comparison (Section 5.8), not enough diagnoses of alcohol use disorder (according to the MINI) were made in order to statistically compare the rates of this disorder between RE groups and controls. Future research should consider further examining differences in alcohol use / alcohol use disorder of women recently exposed to rape trauma compared to controls.

5.7 Identified associations with cortisol

Possible associations between cortisol and the following variables were further explored with univariate analyses in the RE group: age, educational level, BMI, systolic blood pressure, diastolic blood pressure, smoking, depression, trauma load, childhood trauma, perceived stress, social support, alcohol use, HIV status, previous rape, hair wash frequency, use of hormone containing products, medicated shampoo or scalp treatment, and steroid containing medication, as measured at baseline, three months and six months post rape.

No significant correlations were found between cortisol and age, depression, childhood trauma, perceived stress, social support, alcohol use, BMI, systolic blood pressure, diastolic blood pressure, and hair wash frequency.

Furthermore, there was insufficient data (too few cases) for use of hormone containing products, medicated shampoo or scalp treatment, and steroid containing medication and therefore statistical analyses could not be undertaken for these variables. No significant difference in cortisol concentrations were observed between those participants who used medicated shampoo or scalp treatment in the past three months compared to those who did not use this treatment.

Furthermore, no significant difference in cortisol concentrations were observed between the smoking and non-smoking subgroups in the RE group at baseline, three- or six months post rape. Wosu et al. (2013) hypothesized that smoking could impact on hair cortisol concentrations, however a study by Stalder et al. (2017) found no relation between HCC and

smoking. The present study also did not find a significant association between smoking and HCC. However, the effect of smoking on cortisol concentrations is beyond the scope of the present study and therefore future research could investigate this possible association.

In contradiction with the findings of the present study, in a meta-analysis by Stalder et al. (2017), a significant positive correlation was found between HCC and the following variables: age, physical stressors (52% HCC increase), chronic stress (22% HCC increase), body mass index (BMI), and systolic blood pressure, respectively. Examples of physical stressors included pregnancy, excluding mental illness. Within the current study, pregnant women were excluded. Van den Heuvel et al. (2019) also found a significant negative association between HCC and age.

In congruence with the findings of the present study, Stalder et al. (2017) also found no relation between HCC and smoking, perceived stress, or social support. Van den Heuvel et al. (2019) also found no relationship between HCC and alcohol use. A review by Wosu et al. (2013) suggested that the following factors may influence HCC: hair washing, treatment of the hair (see Wosu et al. 2013 for review). In congruence with the findings of the present study, Morris et al. (2017) found no significant association between HCC hair treatment or frequency of hair washing, as well as no correlation between age and HCC. In a comparison of healthy controls and participants with mental illness, no association was found between perceived stress and cortisol concentrations (Heinze et al., 2016).

In a study by Iob et al. (2018), that investigated the possible relationship between hair cortisol and cortisone concentrations and positive / negative social support, higher HCC and cortisol-cortisone ratio was found in participants who experienced negative social support, confirming an association between cortisol and social support. This suggest that negative social support may lead to more psychosocial stress, which could result in hyperactivity of the HPA-axis and thereby increasing the person's vulnerability to poor health (Iob et al., 2018).

5.7.1 BMI and cortisol

Although there was no significant association between BMI and cortisol concentrations, when BMI was compared between groups (RE and controls) at each time-point measurement (within 20 days, three and six months post rape), the control group had higher BMI scores than the RE group. Within the RE group, no significant differences were seen in BMI scores over time. Van den Heuvel et al. (2019) also found no relationship between HCC and BMI. In a meta-analysis

by Stalder et al. (2017), a significant positive relationship was found between BMI and cortisol concentration, which is in contradiction with the findings of the present study.

In a study by Farag et al. (2008), cortisol and stress were associated with BMI, with BMI predicting 31% of the variability in cortisol concentrations. Obese women also reported the highest stress levels. In individuals who experience chronic stress, dysfunction of the HPA axis has been documented (Kudielka, & Kirschbaum, 2005). Obesity is a contributing factor to increased blood pressure, glucose, triglycerides, and low-density lipoprotein cholesterol (LDL-C), and decreases in high-density lipoprotein (HDL-C) (Van Gaal et al., 2006).

Cortisol (the end-product of the HPA axis) influences numerous areas, including energy metabolism. Under normal circumstances, cortisol levels usually peak during the morning (at the time of awakening) and declines throughout the day to a lower level during the evening (Stalder et al., 2017). The morning peak of cortisol provides a signal to other cells of the body, cortisol regulates gene expression in many cell types and entrain their activity (Buijs et al., 2003). When the quality of this signal is reduced, i.e. a smaller peak difference during the morning, this would represent a reduced daily signal to the body which may indicate a decrease in the function of integrated systems. In diabetes and hypertension, disturbances in this circadian rhythm is seen (Buijs et al., 2003).

In obesity, lower levels of morning cortisol levels (plasma) are seen, as well as blunted diurnal variation (Rosmond, Dallman, & Bjorntorp, 1998; Walker et al., 2000), and therefore obesity is associated with HPA axis function disturbances (Rosmond et al., 1998; Walker et al., 2000). Obesity may therefore contribute to dysregulation in cortisol; however, stress-induced elevations of cortisol may also contribute to overeating, which may lead to obesity, type 2 diabetes and CVD. Dallman et al. (2004) suggested that frequent activation of the HPA axis (by extrinsic or intrinsic factors), resulting in excessive cortisol secretion, may contribute to obesity and type 2 diabetes (Rosmond, 2005).

Studies have found that in obese participants, there is an increase in cortisol production and secretion from the adrenal glands, acute hyperresponsivity and elevated 24-hour urinary free cortisol (UFC), however normal or decreased blood serum and saliva cortisol conentraions have also been seen (Pasquali et al., 2006; Bose et al., 2009; Müssig et al., 2010) It has been demonstrated that weight gain and the accumulation of fat cells are promoted by cortisol (Björntorp and Rosmond, 2000; Bjorntorp, 2001). Glucocorticoids, such as cortisol, promotes the conversion of preadipocytes to mature adipocytes (Peckett et al., 2011). There are numerous

researches showing evidence between stress and weight gain through increased cortisol concentrations (e.g., Björntorp and Rosmond, 2000; Björntorp et al., 2000; Bjorntorp, 2001; Peeke and Chrousos, 1995; Wallerius et al., 2003).

Cortisol's role in fat physiology is quite complex (Incollingo et al., 2015) and has been connected to weight loss as well, through enhancing lipolysis and triglycedride uptake. Cortisol is also involved in insulin resistance (Andrews and Walker, 1999) through proliferation of adipokines and the secretion of proinflammatory cytokines (Antuna-Puente et al., 2008)".

It is not clear whether HPA axis dysfunction contributes to obesity or if obesity contributes to HPA dysregulation (Incollingo et al., 2015). Currently, more research is needed to investigate the role of the connection between hair cortisol concentration and dysregulation of the HPA axis in obese individuals. It is not yet clear how studies on hair cortisol concentrations contribute to the current research on dysregulation of the HPA axis in obese individuals and therefore further research in this area is needed. (Stalder et al., 2012).

Due to high levels of obesity around the world, and in South Africa, it is important that obesity in relation to physiological dysregulation are investigated (Farag et al., 2008). Although this was not the focus of the present study, it was important to include variables such as BMI and blood pressure in the context of cortisol.

5.7.2 Blood pressure and cortisol

As mentioned earlier, no significant association was established between blood pressure (diastolic / systolic) and cortisol concentrations. Further examination revealed no significant differences within or between groups regarding blood pressure. In contrast to these findings, Stalder et al. (2017) found a significant positive correlation between HCC and systolic blood pressure. In a study by Gold et al. (2005), a significant associated was found between hypertension and impaired glucocorticoid feedback. They controlled for age, gender, and BMI, however they looked at the effect of hypertension on the brain and suggested future studies in this area should include an assessment of cortisol (Gold et al., 2005).

HPA axis dysregulation has been hypothesized to be involved in the pathological process of high blood pressure (Whitworth, Mangos, & Kelly, 200). Clinical disease involving elevated levels of cortisol, such as Cushing's disease and glucocorticoid resistance syndrome, are often associated with hypertension (Kino et al., 2002; Torpy et al., 2002).

Despite the knowledge that elevated cortisol concentrations as a result from disorders in the endocrine system are associated with increased blood pressure, the association between dysregulation of the HPA axis and hypertension is less clear and requires further investigation (Gold et al., 2005).

However, several significant associations with cortisol was found and only statistically significant findings are discussed in the following sections.

5.7.3 Education and cortisol

There was a significant negative correlation between educational level and cortisol concentrations. This indicates that the higher the educational level of participants, the lower cortisol concentrations, as measured at baseline (pre-trauma) ($r = -0.19$), and at three months (i.e. from trauma to three months) ($r = -0.62$). Previous research has suggested a significant association between education and cortisol concentrations. For example, van den Heuvel et al. (2019) found a significant negative association between educational level and cortisol, showing that higher educational level was associated with lower HCC, which is in line with the present study's finding on the association between education and cortisol. In a study by Cohen et al. (2006), lower levels of education have been linked to higher cortisol concentrations, however the study by Cohen et al. (2006) included both males and females and did not collect hair samples, but saliva and blood samples.

In the present study, most of the rape-exposed participants had completed secondary education (95.7%), which may contribute to the finding that higher education is correlated with lower cortisol or stress. In the present study, in the acute phase after a rape (i.e. from trauma to three months), a relatively strong correlation was found between cortisol concentration at three months (i.e. from trauma to three months) and baseline educational level ($r = -0.62$), however the correlation between baseline educational level and baseline cortisol concentration was weak ($r = -0.19$). Education has been linked to better mental and physical health (Adler et al., 1994; Lupie et al., 2001; Sapolsky, 2005). It has been found that more educated individuals make more use of mental health services (Das et al., 2007) and seeking professional help in the acute phase of trauma, in this case rape, could be related to better stress management. In the present study, rape-exposed participants were recruited from three dedicated sexual assault services known as Thuthuzela Care Centres and from a crisis centre. Only those participants who sought care after the rape-incident at these centres were informed of the RICE study and ultimately the present hair study.

5.7.4 Trauma load and cortisol

There was a significant, but weak, positive correlation between trauma load and baseline (pre-trauma) cortisol concentrations. This indicates that the more previous trauma experienced by the rape-exposed females, the higher the baseline (pre-trauma) mean cortisol concentrations were. This was however a weak ($r = 0.21$) correlation and therefore should be interpreted with caution. However, Steudte-Schmiedgen et al. (2016) have suggested increased trauma load could be related to altered HCC.

5.7.5 Past rape and cortisol

There was a significantly lower mean cortisol concentration, as measured at six months post rape (i.e. from 3 to six months) in the RE group who experienced previous rape (excluding the rape trauma) compared to the RE participants who did not previously experience rape trauma. No comparative studies were found that investigated the association between HCC and past rape.

5.7.6 HIV and cortisol

There was a statistically significant difference in mean baseline (i.e. pre-trauma) cortisol concentration between HIV positive and HIV negative participants, with higher cortisol concentration in the HIV positive group compared to HIV negative participants. No comparative studies were found that investigated the association between HCC and HIV/AIDS. However, HIV infection affects different components of the endocrine system (Bhatia, 2018). It has been suggested that this could be due to infiltration of the affected glands by infections or malignancies, alterations of various HPA axes due to immune activation and pro-inflammatory cytokines (Bhatia, 2018). Effects of HIV on the endocrine system includes hypothyroidism, insulin resistance, impaired glucose tolerance, diabetes, metabolic syndrome, lipodystrophy, lipid abnormalities (e.g. low HDL-cholesterol and elevated triglycerides), and dysfunction of the adrenal glands (Brown, 2011). The most frequent abnormalities are manifested in dysfunction in the HPA axis, which is applicable to the findings of the present study. Disorders seen in HIV participants with HPA dysfunction include adrenal insufficiency (AI), due to HIV and destruction by HIV, opportunistic infections, or distortions. These may include either the hypothalamus, or pituitary or the adrenal glands (Glasgow et al., 1985). Furthermore, what is seen most is increased cortisol concentrations in blood and increased ACTH. This happens due to various complex systems (Membreno et al., 1987), which includes

the HPA axis being activated either due to HIV itself or pro-inflammatory cytokines, conversion of cortisone to cortisol (due to activation of 11β-HSD1) in the adipose cells or a decrease in cortisol metabolism (Bons et al., 2013). This may explain why pre-trauma hair cortisol concentration were higher in the HIV positive group, compared to the HIV negative group (Bhatia, 2018).

However, the reason for no significant difference between cortisol concentrations at three- and six months post rape between the HIV positive and negative groups, is not clear. In the present study, it could be due to the change in the sample size. These findings are important in the field of hair cortisol concentration and HIV, and therefore further research should be conducted in this area.

5.8 Psychopathology between groups

The following results of rates of psychopathology between groups (controls and RE), in addition to PTSD and depression (discussed previously), are not discussed in detail, as these were not directly part of the primary or secondary aims of the present study. However, these findings contributed substantially to understanding the psychological differences between the groups of recently rape-exposed women compared to controls, and therefore worth including in the discussion chapter. Only significant results are discussed.

Between groups, higher rates of psychopathology were detected in the RE group compared to controls at different time-points. The findings of the present study are in congruence with the literature. In a review by Dworkin et al. (2017), people who experienced sexual assault, were at a higher risk to develop psychopathology than those who have not experienced sexual assault. Sexual assault seems to not only be related to PTSD, but to other psychopathologies as well (Dworkin et al., 2017). The strongest link between sexual abuse and the following psychopathologies have been identified: depression, PTSD, eating disorders, and suicide attempts (Chen et al., 2010; Gilbert et al., 2009). In the present study, several psychiatric disorders and clinical problems, in addition to depression and PTSD, have been identified in women who experienced rape trauma compared to controls. In the following section, results of psychopathology identified in the RE group compared to controls are briefly discussed.

5.8.1 Suicidality

Within the whole group (RE and controls) *current suicidality* was the most diagnosed than any other pathologic behaviour (according to the MINI). Overall (at all three time points) significantly higher rates of current suicidality was seen in RE group compared to controls. Within the RE group, 74% of participants indicated signs of current suicidality (from low to high risk). In the RE group, there was a significant decrease from baseline (within 20 days post rape) to three months post rape, as well as a significant decrease from three months to six-month follow-up. Also, in the RE group, a significant decrease was seen in current suicidality from baseline to six months post rape.

Overall, significantly higher rates of *low- and high-risk suicidality* were seen in the RE compared to controls. Within the RE group, no significant difference was established between visits (within 20 days post rape, three- or six months post rape) with regard to low risk suicidality. However, there was a significant decrease from baseline to three-month follow-up, as well as a significant decrease from baseline to six-month follow-up regarding *high-risk suicidality* in the RE group.

In a study by Dworkin et al. (2017), sexual assault survivors were at an increased risk to develop suicidal behaviour (ideation and attempts) compared to other conditions. Sexual assault was associated with increased risk for suicidality (Dworkin et al., 2017). When controlling for other risk factors, these findings are in congruence with previous epidemiological studies of the association between sexual assault and suicidality (Ullman & Brecklin, 2002; Stein et al., 2010). Furthermore, Chen et al. (2010) have also identified a link between sexual abuse and suicide attempts.

5.8.2 Panic Disorder

Overall (at all three time points [within 20 days, three-, and six months post rape]), rates of *lifetime panic disorder* (PD) was significantly higher in the RE group compared to controls. In the RE group, there was a significant increase in lifetime panic disorder from within 20 days post rape to three-month follow-up, followed by a slight (non-significant) decrease to six-month follow-up.

Rates of *current panic disorder* was also significantly higher in the RE group compared to controls at all three time-points (within 20 days, three-, and six months post rape). In the RE

group, there was no significant difference between rates of current panic disorder between visits (within 20 days post rape and three- and six months post rape).

5.8.3 Agoraphobia

Overall, significantly higher rates of *current agoraphobia* were seen in the RE group compared to controls. In the RE group, a slight (non-significant) increase was seen from within 20 days post rape to three months and then there was a significant decrease from three months to six months post rape. There was also a significant decrease from baseline to six months post rape, in the RE group. Rates of current agoraphobia was lowest at six months post rape.

Within 20 days and three- months post rape, higher rates of *current agoraphobia without PD* was seen in the RE group compared to controls. At six- month follow-up there were too few cases for statistical analysis. In the RE group, there was no significant difference in rates of current agoraphobia without PD within 20 days post rape compared to three months post rape.

5.8.4 Social Anxiety Disorder

Significantly higher rates of current social anxiety disorder (SAD) were seen in the RE group compared to controls at all three time points (within 20 days, three- and six months post rape). In the RE group, no significant difference was seen between visits (within 20 days post rape, three- or six months post rape).

5.9 Principal study findings

(1) There were no significant differences between groups at different time-points (baseline, three months, and six months) with regard to hormone concentrations. Within the RE group, baseline (pre-trauma) mean progesterone concentration was significantly higher compared to the other two time- points (three- and six months post rape).

(2) There were significant differences in PTSS (severity and frequency) between groups (controls and RE) at all three time-points (baseline, three months, and six months), with the RE group having more PTSS than controls at all three visits, and PTSS decreasing significantly within the RE group from baseline to three months and six months post rape, as well as from three months to six months post rape. Higher rates of PTSD were also seen in the RE group compared to controls at three and six-months post rape.

(3) Several, but weak, significant correlations were found between hormone concentrations and PTSS, as well as PTSD symptom clusters (as measured by the DTS). The following positive correlations were found with pre-trauma cortisol concentration: baseline (within 20 days post rape) total PTSS symptoms, re-experiencing/intrusion symptoms, avoidance/numbing symptoms, hyperarousal. Cortisone concentrations, as measured at six months (i.e. from three to six months post rape) significantly correlated with avoidance/numbing symptoms at three months post rape. No significant correlations between testosterone or progesterone and PTSS were found. A significant, but weak, negative correlation was found between DHEA concentrations as measured at three months (i.e from baseline to three months post rape) and re-experiencing/intrusions at three months post rape. A significant, but weak, positive correlation was found between DHEA as measured at six months (i.e. from 3 to six months post rape) and total PTSS, as well as re-experiencing/intrusion symptoms at three months post rape.

(4) Within the present study, pre-trauma hormone concentrations were not predictive of the development of PTSS within 20 days, three- and six months post rape. However, at baseline measurement (within 20 days post rape), three significant predictors of PTSS were identified. The strongest predictor of PTSS was depression, followed by previous trauma (trauma load / cumulative trauma), and perceived stress. At three-month follow-up, two significant predictors of PTSS were identified. The strongest predictor of PTSS was trauma load, followed by depression. At six-month follow-up, no significant predictors of PTSS were identified.

5.10 Contribution to scientific knowledge

Findings of cortisol concentrations in PTSD and PTSS have been inconsistent. This is the first study to examine hair cortisol and other hair hormone concentrations in female rape victims with PTSS compared to controls.

A major benefit of the present study is that all participants were female, most from black ethnicity, matched for age, and there was no significant difference in education or source of income. Furthermore, there were no significant difference between groups with regard to HIV status, social support, systolic or diastolic BP, smoking, or childhood trauma, making the groups comparable on these variables. Given that most of the sample was black female survivors of rape living in poverty, this study extends the understanding of these women (Mbalo et al., 2017).

Another benefit of this study is that females included in the rape-exposed group certainly experienced rape trauma, because rape was not measured by self-report questions, but these women attended a TCC and rape was confirmed by a doctor at the TCC.

Another benefit of the present study is that it was a longitudinal study that collected data at three time points, namely within 20 days, three- and six months post rape. Furthermore, this is the first study to investigate long-term (hair) hormone concentrations in females with PTSS in the context of rape compared to controls. This is also the first study to investigate long-term (hair) hormone concentrations in South African females in KZN with PTSS compared to controls.

This is the first study to explore other hormone concentrations (cortisone, DHEA, testosterone, progesterone), in addition to cortisol, with PTSS in the context of rape. Studies of PTSD/PTSS in females that have been traumatised by rape has been limited. There is vast literature on cortisol in PTSD, but much less in the context of rape. This is the first study to investigate cortisol and other neurohormones in the context of rape and exploring the relationship with posttraumatic stress symptoms.

For the examination of the HPA-axis activity, cortisol concentrations from bodily fluids are often measured, but these concentrations do not always give a reflection of long-term concentrations. The measurement of hair cortisol concentrations provide levels over months or years and HCC can potentially be used in clinical treatment as a treatment target. Some of the benefits of retrieving cortisol concentrations from hair samples, are that hair samples can be easily stored at room temperature (Gow, Thomson, Rieder, can Uum, & Koren, 2010), sample collection is not invasive (Pacella et al., 2017; Gow et al., 2010), and hair can be used to evaluate concentrations over periods of time (Pragst & Balikova, 2006), as well as provide hormone concentrations before an event (Pereg et al., 2001). Also, according to the free hormone hypothesis by Mendel (1989), only the unbound hormones will be in hair. Furthermore, HCC will not be influenced by non-compliance (Stalder & Kirschbaum, 2012; Staufenbiel et al., 2013).

Compared to other measures (saliva, blood, urine), and in addition to providing long-term data on cortisol concentrations, hair samples can be stored at room temperature (Wosu et al., 2013). In the present study, hair samples were easily stored in separate zip-lock bags and batched together in envelopes at room temperature, which did not take up a lot of space and

was easily transported, whereas saliva/blood/urine samples would require specific storage (e.g. freezer or refrigerator) and transport might be more complicated (Wosu et al., 2013).

Furthermore, hair cortisol concentrations are not easily affected by short-term factors (e.g. individual or situational variability) (Wosu et al., 2013), however previous research has suggested that hair cortisol concentrations may be influenced by hair products and practices. Recent literature on hair cortisol concentrations have been inconsistent regarding hair washing, e.g. van den Heuvel et el. (2019) found a significant relationship between HCC and hair product use and frequency of hair washing, whereas Morris et al. (2017) found no significant association between HCC and hair colour/ structure, hair treatment or frequency of hair washing. In the present study, there was no significant correlations found between cortisol and hair wash frequency, and there was insufficient data (too few cases) for use of hormone containing products, medicated shampoo or scalp treatment, and steroid containing medication and therefore statistical analyses could not be undertaken for these variables. Blood/saliva/urine samples are more easily influenced by factors such as smoking, time of day of collection, and acute stress. Wosu et al. (2013) hypothesized that smoking could impact on hair cortisol concentrations, however a study by Stalder et al. (2017) found no relation between HCC and smoking. In the present study, no significant difference in cortisol concentrations were observed between the smoking and non-smoking groups.

Obtaining a hair sample was seen to be non-invasive compared to, e.g. a blood sample, as suggested by researchers (Gow et al., 2010; Pacella et al., 2017). However, this is a recommendation by research conducted outside of South Africa and therefore not representative of different cultures and women with different hair styles. Only a small amount of hair is needed, compared to saliva/urine/blood and only one sample is needed to reflect a three-month window (i.e. no repeated measures are needed within a three-month period), compared to saliva/urine/blood. Saliva and urine samples are also less invasive, however often repeated measures are needed per participant visit and within a shorter timeframe compared to a three-month follow-up visit for hair samples. Obtaining blood samples can be more invasive and painful compared to collection of hair samples.

It should be noted that participants may be unwilling to provide a sample at the follow-up (Wosue et al., 2013). One of the challenges of the present study was that hair was too short at the follow-up visit and therefore a sample could not be collected. Some participants were unwilling to provide a sample at a follow-up visit and this was accepted by the RICE team.

With urine/saliva/blood, often repeated measures are required, this can be expensive, be a burden for participants, and increase the likelihood of incomplete sample collection, or a loss to follow-up (Wosu et al., 2013).

Olff et al. (2007) concluded that the HPA dysregulation can be altered with clinical therapy for PTSD, although the diagnosis of co-morbid depression should be considered. Furthermore, research on the potential role of DHEA in PTSD treatment is limited and therefore the present study contributes to the existing literature. Research on the association between DHEA and PTSD/PTSS in females are of great importance.

5.11 Limitations

With regard to the literature included in the present study, it should be noted that all relevant research may not have been included. Only those studies identified by the researcher in search engines and judged as applicable to the present study were included. Therefore, the possibility remains that certain important studies may not have been included and could have contributed to bias in the inferences drawn.

The results cannot be generalised to the South African population. All participants were female, recruited from a specific area (KZN) and were isiZulu speaking.

Furthermore, the sample size may have influenced the statistical power of the study and therefore limited the results generated in the present study with regard to differences in hormone concentrations within and between groups. The results that were not statistically significant should be interpreted with caution. It is suggested that future investigation includes bigger sample sizes in order to further investigate the findings of the present study. Most significant correlations within the present study were weak and therefore further investigation is needed to reject or confirm these findings.

According to the South African Stress and Health study, very few people with mental disorders are making use of mental health services to treat their disorder(s) (Seedat et al., 2009). In the present study, only females who reported the rape incident to the clinics where recruitment took place, were included.

We did not consider the menstruation cycle of these women, which may have influenced progesterone concentrations. Future studies could include a questionnaire regarding information about the participants' menstrual cycle over the past three months. However, in

the present study, each measurement reflected a three-month window and the average progesterone concentration for that three-month period was used in the statistical analyses. Therefore, the stage of the cycle might not have had such a big impact on the results that were generated.

Two items ("torture" and "sexual assault") of the LEC-modified version were not completed accurately by participants. Torture is not well translated into isiZulu and the meaning of "torture" was therefore misunderstood and misinterpreted. Sexual assault was also not well understood and responses to this item did not reflect past sexual assault (excluding the recent rape event, +- 20 days before). Participants found it difficult to distinguish between a recent event and sexual assault prior to the recent event. These two items were, therefore, excluded from the calculation of the total score on the LEC-modified version. Scores were summed to create a total score out of 10 (Mollica et al., 1993). The removal of these items may have impacted the validity of the questionnaire and is therefore a limitation of the present study. The interpretation of the results should therefore be done with caution. However, only two items were removed, therefore the questionnaire was included in statistical analyses. Instead of a total score out of 12, a score out of 10 was used. Since the item on sexual assault in the LEC-modified version was not accurately responded to by participants, the investigators of the RICE study decided to identify past rape by other questions that were asked during the first interview. Past rape (more than 20 days before the baseline visit, excluding the recent rape event) was identified with three questions where participants indicated one/more of the following: intimate partner rape and/or non-partner rape and/or first sexual intercourse that was forced/rape.

The results from the regression analyses should be interpreted with caution. All had low adjusted R^2 values: Baseline regressions (Adjusted $R^2 = 0.50$; $R^2 = 0.04$), three-month follow-up regression (Adjusted $R^2 = 0.12$; $R^2 = 0.06$), and six-month follow-up regression (Adjusted $R^2 = 0.02$; $R^2 = 0.02$). This indicates that these variables only explained a small percentage of the variance in the outcome variable.

Furthermore, future research should focus on the meaning that Black South African women attach to their hair to not breach ethical guidelines. The RICE study found that some women did not want to provide a hair sample on return visit(s) and this was accepted by the RICE team. The RICE team discussed the sampling and required separate consent for hair samples at each return visit. Given the responses, the RICE PI planned to do a qualitative study to understand participants' experiences and perceptions of providing hair samples. However,

since funding for the study was limited, the PI was unable to conduct this study. It has remained an area of interest for the study team and should funding become available this will be one of the first qualitative studies to be conducted.

Data was not collected on how the women perceived having their hair taken for research purposes, and therefore this is a limitation of the present study and recommended for future research in this area. Qualitative investigation of the acceptability and cultural meaning of hair sampling should be conducted in future research.

A full and separate consent process was completed for hair sample collection at each visit. Ethics renewal was done annually by the SAMRC's Ethics Committee. A brochure and information sheets were provided to participants. The RICE team's previous experience highlighted the importance of providing full information, time to reflect and testing the understanding of consent before consent forms are signed. Participants were given a clear explanation of voluntariness of participation, and the option of withdrawal was given at each visit.

Another limitation is that other traumatic experiences in Black South African women, such as measuring previous discrimination about race and considering a history of family violence in context of the present study, were not measured. The LEC-modified version was used to measure lifetime traumatic events. In the present study, detailed information regarding the context of the rape (e.g. number of perpetrators, relationship to perpetrator, coercion, weapon used, physical force, threatened murder, perceived life threat, repeat perpetrator, number of sexual acts, rape reported to police) were not included, which could have contributed to heightened trauma in these women. Future studies focusing on predictive factors of PTSD, PTSS and/or depression in the context of rape or sexual assault should consider including these variables. However, in the RICE and present study, the researchers were advised by the NPA and the police not to ask detailed questions regarding the circumstances of the rape, because it could interfere with their ongoing legal case and disclosing such information to the researchers could have compromised the case, may have reduced the likelihood of a successful conviction, and influence the rape survivor's testimony.

The topic on reasons for women in KZN, also rape survivors, possibly having a higher risk of developing PTSD compared to other provinces, would be a valuable topic for future research, also suggested by Mbalo et al. (2017).

All participants included in the present study sought help from a rape crisis centre, and therefore these women could not be compared to those who did not sought help.

To conclude, some of the controls (n = 13) experienced previous rape trauma and trauma load was also not accounted for at three- and six months post rape.

5.12 Conclusion and recommendations

Developing more effective treatment for PTSD and reducing symptoms of PTSD is of great importance. Improving knowledge of the biological, psychological, and social mechanisms connected to the development of PTSD/PTSS may contribute to improving the efficacy of treatment for PTSD (Chivers-Wilson, 2006). In the present study, in addition to examining biological differences (possible differences in hormone concentrations) within and between groups (RE and controls), we also examined the psychological and social differences between females who recently (within the last 20 days) experienced rape trauma compared to controls. Treatment of psychopathology, including PTSD and symptoms of PTSD, in the aftermath of rape is an important target for intervention, especially in the acute phase post rape.

Treatment studies that include HPA-axis parameters as endpoints of physical and psychological health outcomes would be particularly useful (Jones & Moller, 2011). Mouthaan et al. (2014) pointed out that the DHEA-to-cortisol ratio may be useful in predicting PTSD symptoms. More consistent results have been seen when researchers compared DHEA-S-to-cortisol ratios, pointing to higher levels in PTSD participants (Butterfield et al., 2005; Rasmusson et al., 2004; Yehuda et al., 2006). Future studies should consider exploring DHEA-S-to-cortisol ratios and its relationship with PTSS/PTSD.

A benefit of the information acquired from the present study, is that it examines, over time, mental health outcomes, specifically symptoms of PTSD, of South African women who have been raped, which may facilitate future research.

As an alternative to hair samples, nail clippings might be used in future studies (Doan et al., 2018).

When victims of rape experience negative social reactions when they disclose information to friends, family, or professionals, increased risk for PTSD has been identified (Ullman & Peter-Hagene, 2014). Furthermore, negative experience(s) in the aftermath of sexual assault, including rape, may increase symptoms of PTSD in these victims, leaving them

more prone to develop PTSD (Ozer, Best, Lipsey, & Weis, 2003). The stigma associated with rape may also cause victims to not report the incident (Kennedy & Prock, 2016). Victims of rape may internalise this stigma, leaving them to blame themselves, feeling ashamed and thereby not seeking help (Kennedy & Prock, 2016). Therefore, it is important that rape victims receive positive support and understanding when they disclose that they have been raped and interventions should include focusing on improving quality social support (Iob et al., 2018).

Prospective studies investigating the long-term mental health outcomes of women who have been victims of rape or sexually assaulted, is needed. This knowledge could benefit and guide interventions in female survivors of sexual assault and rape.

Future research could benefit from including bigger sample sizes to confirm or reject the findings of the present study. Future research should further investigate the role of hormones (cortisol, cortisone, testosterone, progesterone and DHEA) in hair in participants who have been traumatised and these hormones' connection to the development of PTSD/PTSS.

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Addendum A1
Biometric Informed Consent

A STUDY ON WOMEN'S HEALTH AND WELL-BEING
USE OF BIOMETERIC SYSTEM
INFORMED CONSENT

RICE - WOMEN'S HEALTH AND WELLBEING STUDY

INTRODUCTION

You have decided to participate in the Women's Health and Well Being Study. In addition to the tests taken as part of the study, we are now asking you permission to take a finger print scans for our biometric systems. We have two systems the one is to ensure that there is no duplication of your results in our study and the second scan is to check if you are not participating in another study. It is important for us to know if you are with another study as it is not always safe to do so.

You are free to ask questions about this study at any time and, if you agree, you will be asked to sign this consent form. You will get a copy to keep. Your participation is entirely voluntary and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. Remember you are also free to withdraw from the study at any point, even if you do agree to take part initially.

This research study has been approved by the Medical Research Council of South Africa (MRC) Ethics Committee.

WHAT PROCEDURES WILL BE INVOLVED IN THIS RESEARCH?

We are asking you if we can do two scans of your finger prints. This procedure is harmless and will not hurt you in any way. For the finger print scan, we will check to see if you are already on another study. The second finger print scan will be saved and it will be linked to your unique identifier on the computer for the study. There will not be a picture of your thumb print but the computer will change the thumb print into a code. The code will then be printed into barcodes and this will be linked to your unique identifier number. These barcodes will then be placed on all your blood samples and your study file. In addition, whenever you return for follow-up visits, we will use the scanning of your thumb print to ensure we do not mix you up with someone else and also to make sure you are not with another study.

This is a longitudinal study, meaning that this study will be taking place over a long period of time, and sometimes studies that take place over a long period of time, patient samples and information gets misplaced. Using this systems, we are making sure we link you to the samples without using your name. This is a precautionary measure that we will be taking in this study.

WHAT ABOUT CONFIDENTIALITY?

There will never be a picture of your thumb print. All finger prints done for each participant will be stored as a code and under a unique identifier number. No one will know what your unique identifier is except the researchers working on the study.

WHAT IF I DO NOT WANT TO USE THE BIOMETRIC SYSTEM?

If you chose not to want to use the system, we will make an ID card for you for the study.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study, contact:

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For questions about your rights as the research participant, contact:

Chairperson: The MRC Research Ethics Committee
Prof. Moodley
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USE OF BIOMETRIC SYSTEM SIGNATURE PAGE

I declare that: -

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable. I have had a chance to ask questions and all my questions have been adequately answered.

- I understand that taking part in this study is voluntary and I have not been pressurized to take part.

I DO AGREEE to have my fingerprint used for the two biometric systems.

_____
Participant's Signature/Initial

OR

I DO NOT AGREE to have my fingerprint used for the two biometric systems.

_____
Participant's Signature/Initial

_____	_____	_____
Participant's Name	Signature	Date
_____	_____	_____
Name of Study Staff Conducting Consent Discussion	Signature	Date
_____	_____	_____
Witness Name	Signature	Date

Addendum A2
Genetics Informed Consent

A STUDY ON WOMEN'S HEALTH AND WELL-BEING RESEARCH INVOLVING GENETIC STUDIES INFORMED CONSENT

INTRODUCTION

You have decided to participate in the Women's Health and Wellbeing study**.** In addition to the tests taken as part of the study, we are now asking you permission to collect some of your blood for a study that involves DNA (Genetic) analysis.

You are free to ask questions about this study at any time and, if you agree, you will be asked to sign this consent form. You will get a copy to keep. Your participation is entirely voluntary and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part initially.

This research study has been approved by the ethics Committee of the Medical Research Council of South Africa (MRC).

WHAT PROCEDURES WILL BE INVOLVED IN THIS RESEARCH?

A small blood sample (about 10-20 ml, equivalent to 1-2 tablespoons) will be taken from you by a trained nurse. Bloods will not be taken every time you visits us. The blood will be transported to a laboratory, where researchers will examine and identify your DNA from the blood sample. This sample will not be stored for later use but will be processed for immediate analysis and destroyed. The DNA sample will then help us to look for genetic differences and changes in your DNA. Findings these changes could tell us a lot about the development of stress-related mental health problems.

WHAT ABOUT CONFIDENTIALITY?

All blood samples collected will be identified only by a coded number and not your name to ensure to maintain confidentiality. The research records for this study will be kept in a secured area only accessible to the research team involved.

WHAT DOES THIS PARTICULAR RESEARCH STUDY INVOLVE?

In this study, we hope to be able to find genes that put a person at a higher risk for developing stress-related mental health problems, such as post-traumatic stress disorder (PTSD), depression and anxiety. We will do this by looking for changes in the genes and this will give us clues to the various factors that contribute to the development of these mental health problems.

WHAT IS DNA ANALYSIS OR GENETIC RESEARCH?

Genes are part of genetic material, also called DNA or RNA. Genes can be found by looking at a small blood sample and can be found in every cell our bodies. Our genes determine what we look like and sometimes what kind of diseases we may be at risk in getting. Worldwide, researchers who do genetic research are continuously discovering new information that may be of great benefit to future generations and to people who suffer from different types of diseases or conditions. Our study will therefore assist in bringing about this new information to show how changes in our genes may affect our health and ability cope.

WHAT ARE THE RISKS TO ME IF I AGREE TO JOIN THIS STUDY?

- You may experience minor pain or bruising at the site where blood is taken. Occasionally, some people experience fleeing dizziness or feel faint when their blood is drawn.
- The samples collected will only be for this study and will not be shared with anyone.

WHAT ARE THE BENEFITS TO ME IF I DECIDE TO JOIN THIS STUDY

Your personal results will be made known to you **only if they indicate** that you may:-

- Have a definite risk for developing a particular disorder.

- Have a condition or predisposition to developing a condition that is treatable or avoidable e.g. by a lifestyle modification.

There are no direct benefits to you taking part in this study. However, the findings may benefit future patients with stress-related mental health problems. This new information will provide us with a better understanding of the development of stress-related mental health problems and may result in the development of ways to lower the risk for these disorders, as well as helping us find new treatments.

WHAT ARE THE COSTS TO ME?

As explained earlier you will not be paid to take part in this study although you will be given R80 at each of the interviews to compensate for time spent completing the questionnaire and providing samples. You will also be given money to cover travel costs for each of the visits and we will provide you with a snack every time you come for the interviews.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study, contact:

Principal Investigator:

Principal Investigator
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For questions about your rights as the research participant, contact:

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GENETIC STUDIES SIGNATURE PAGE (adult)

I declare that: -

• I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable. I have had a chance to ask questions and all my questions have been adequately answered.

• I understand that taking part in this study is voluntary and I have not been pressurized to take part.

I **AGREE** to take part in a genetic research study entitled

_____
Participant Signature/Initial

OR

I **DO NOT AGREE** to take part in a genetic research study

_____
Participant Signature/Initial

_____	_____	_____
Participant's Name	Signature	Date
_____	_____	_____
Name of Study Staff Conducting Consent Discussion	Signature	Date
_____	_____	_____
Witness Name	Signature	Date

Addendum A3
HIV Informed Consent (for use by RICE study)

A STUDY ON WOMEN'S HEALTH AND WELL-BEING
HIV INFORMED CONSENT
ADULT WOMEN

INTRODUCTION

You have decided to participate in the Women's Health and Well Being Study. As part of the study procedures, you will be asked to do an HIV Test. Like all the other tests that will be done in this study, there will be no names on any of the specimens, only a special study number.

You are free to ask questions about this study at any time and, if you agree, you will be asked to sign this consent form. You will get a copy to keep. Your participation is entirely voluntary and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. Remember you are also free to withdraw from the study at any point, even if you do agree to take part initially.

This research study has been approved by the Medical Research Council of South Africa (MRC) Ethics Committee.

WHAT ARE MY RIGHTS?

You have the following rights:

1. Not to be tested for the AIDS virus without your free and informed consent.
2. To be given all relevant information on the harms, risks and benefits of taking, or not taking, the HIV test.
3. To receive pre-test counselling which is private and confidential, and which will inform you more about the test and its implications before you give consent.
5. To have your test result treated confidentially
6. To receive post-test counselling

IS THE TEST ALWAYS CORRECT? CAN THERE BE MISTAKES?

Even though the tests are very accurate, if your test result shows that you may be infected with the AIDS virus, we will have this confirmed by doing some additional tests.

Sometimes, a false positive result may occur in a small number of cases. A false positive means that the test shows positive when the person is not infected with the virus, by doing further tests we can see if the test is really a positive. The clinic staff and the laboratories follow a strict procedure to prevent these potential mistakes. In order to minimize false positive results, two different tests are performed.

WHAT DOES IT MEAN IF THE TEST IS NEGATIVE?

If your test result is negative, it means that you are not currently infected, but it does not mean that you may not become infected in the future.

WHAT DOES IT MEAN IF THE TEST IS POSITIVE?

If your test result is positive, it means that you may be infected with the AIDS. However, to be sure, we will do an additional two tests to confirm the result. You will be called back to the research clinic and our research nurse will discuss the information with you so that you can understand clearly what the test result means. You will be given your CD4 and viral load and referred to your local clinic for further HIV management.

WHAT ABOUT TESTING AT HOME

When you struggle to attend the clinic, we will try and visit you at home to hear how we can assist you to come to the clinic. If you unable to attend the clinic we will ask you if we can do a finger prick HIV test at your home. We will follow all the confidential procedures as explained above.

WHAT ARE THE COSTS TO ME?

There is no direct cost to you for having an HIV test done.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study, contact:

Principal Investigator
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For questions about your rights as the research participant, contact:

Chairperson: The MRC Research Ethics Committee
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HIV TESTING SIGNATURE PAGE(Adult)

I declare that: -

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable. I have had a chance to ask questions and all my questions have been adequately answered.

- I understand that taking part in this study is voluntary and I have not been pressurised to take part.

I DO AGREEE to have a HIV test

_____
Participant (Signature/Initial)

OR

I DO NOT AGREE to have a HIV test.

_____
Participant (Signature/Initial)

_____	_____	_____
Participant Name	Signature	Date
_____	_____	_____
Name of Study Staff Conducting Consent Discussion	Signature	Date
_____	_____	_____
Witness Name	Signature	Date

Addendum A4

IC for use at rape service clinics

A STUDY ON WOMEN'S HEALTH AND WELL-BEING

INFORMED CONSENT

RECRUITED AT _____

INTRODUCTION

Hello, welcome to our clinic. You are being invited to take part in the women's health and well-being study. It is a study that the Medical Research Council is conducting with women in Durban on women's health and wellbeing. We want to learn more about women's health so we can help the Government in better planning of their services so that they can meet women's health needs.

We also want to have a better understanding about women's physical and mental health problems, how long these problems last, what causes them, and what social factors and life experiences affect them, whether the problems persist and if the problems require treatment. We are also very interested to know how experiences of violence affect women's health. In addition, we want to get a better understanding on how women heal after being hurt.

This is an information and consent and form and we are inviting women between the ages of 18 – 40 years who attend Thuthuzela Care Centre/Rape Centres after they have been sexual assaulted. One of the primary reasons for doing this research is to understand the health problems that women who have been raped experience. Your participation in our research will not influence the health care that you receive at the Thuthuzela Care Centre/Rape Centres or your case. We will not ask you questions about the rape experience or the circumstances of the incident. We will ask about other sexual violence experiences. We understand that these questions might bother you and we will stop and can always ask these same questions at a later interview. However, we will also be prepared to help you if you need to speak to someone.

This study will be done over a period of three years and we will speak to women on repeat occasions. This means that you will be invited to come to the research clinic and have interviews done every 3 months. At each study visit we will ask you some interview questions, we will also ask you to give us permission to do some blood tests and tests on your urine as well as to provide a swab. We will explain more about this later. This means we will have to remain in contact so that we can invite you to return for the follow-up interviews over the three years.

IS MY PARTICIPATION VOLUNTARY IN THIS STUDY

This form gives you information about the study and what will be expected of you. Once you have read and understood and agree to participate, you will then be asked to sign your name on this form. You will be given a copy of this form to keep. It is important that you know that your participating is voluntary and you are free to withdraw from the study at any point, even after you have agreed to take part.

WHAT HAPPENS IF I DECIDE TO PARTICPATE IN THE STUDY?

If you agree to participate in the study then we will start with your baseline visit today. You will see and talk to both of us (nurse and research assistant) today. We will start by explaining in more detail about the procedures that will take place today and we will answer questions before you sign the informed consent for us to continue. The nurse will see you first and will do health assessments including the understanding of how you cope, taking of your blood pressure, your pulse, your weight and provide advice on healthy living. You will then be seen by the other researcher and she will ask

questions about yourself, your relationships with husbands and boyfriends and other health questions sexual and reproductive health matters, as well as questions about experiences of violence. There will be some questions about things which are often thought of as secrets. Let us know if you feel uncomfortable about these questions. At the end the nurse will take blood, urine and hair samples and will give you the answers to the tests as soon as we get them and will discuss your health with you. Everything that you tell the interviewer will be kept secret and you have the right not to answer any questions that you do not wish to answer. The answers to the questions will be entered on a computer and we expect the interviews with the two of us to take about 2 hours. We will also refer you to the health clinics if we think it is necessary.

WHAT ARE THE PROCEDURES IN THIS STUDY

If you are interested in being part of this study, the following will take place:

The Research assistant start by using a biometric system to log you on the study data entry system. A biometric system allows researchers to use your finger print to identify every time you return to the clinic. Your fingerprint will be linked to a number and this number will be used to store the information and this will ensure information remains safe and confidential. The reason why we need to identify fingerprints each time you visit the clinic is because the study is over a long period of time and patients' information can get mixed up so by entering your details with your study number it prevents your information getting mixed up with another participant's information. We will use another system to check if you are with another study. The will be the same procedure using your fingerprint to ccheck on the computer if you have already been with another study. ***We will ask you to give us permission to use the biometric system in a separate informed consent that you will sign***.

A study nurse will do a mental health assessment as well as a screen for high blood pressure as we explained earlier.

The research assistant will then do an interview asking the questions as we explained earlier and she will enter this on the computer as she speaks with you.

Hair sample- We will ask you to allow us to collect about 6-8 strands of hair between 1 ½ -6 cm long. We will take it from the back of the head, close to the scalp and we will do it at each visit. We aware that this might upset your hairstyle and we have a person here that will ensure that your hair is not disarranged. She will make sure that your hair looks exactly the same again after we taken the hair sample. The hair samples will be sent to a special laboratory in Germany where they will be examined and will provide us information about levels of stress we experience. The reason why it must be done in Germany is because we in South Africa do not have the machines and the equipment to examine it here. Because our hair grows slowly it will allow us to examine the levels of stress over the previous 2 months. Finding the levels of stress is very useful as it will assist us in coping better with stressful events in our lives. We will provide you with the test result as soon as we get it. We will be very careful about how we take the hair. In order to take a hair sample the following will be important:

- Your hair must be longer than 1 ½ cm
- You must not have any signs of hair loss or baldness
- You do not have had a severe physical disease in the last 5 years
- You have not been taking anti-depressants in the last 6 months

We will then collect some samples to screen your health as part of the study to answer the questions we have about women's health at each time when you are interviewed. The following are tests and samples that we plan to collect at the screening visit as well as all study visits thereafter:

- **Urine test**- we will require a sample of your urine to test for pregnancy. We will give you the result as soon as it has been tested in the lab here at the clinic. This test will be done every time you visit.
- **Swab from your vulva** – this is to test for infections passed through sex. This test is done by taking a smear from vagina. This is similar to a pap smear test but we do not use instruments and the nurse will assist you and show you how to do it. You will do it in private. The results will be given and discussed with you as soon as it comes back from the laboratory. This test will be done every time you visit.
- **Blood tests-** Blood will be collected from you for various tests. This will be done by the nurse and we will collect about 4-5 teaspoons of blood at each study visit.

We will collect the blood for the following:

- **To test for diabetes, cholesterol levels, kidney and heart diseases in blood.** Women who experience stress and mental problems may be more likely to develop heart cardiovascular disease risk factors such as diabetes, high cholesterol and related kidney problems. We would like to test for diabetes and cholesterol (fat) levels in blood as well as any related kidney problems. We would also like to test for other indicators (signs) of heart disease in the blood. This is a very common illness and it is often hidden. We will also test for fat in your blood which can contribute to heart disease. We will also give you the test results once we receive it from the laboratory. This test only be done on 3 of the visits.
- **To test for infections that passed through sex, including herpes infection and HIV**. We will inform you of your STI test results and whether any treatment is needed. If treatment is needed we will assist you in receiving treatment.
    - **HIV test:** you will receive counselling before the HIV test and also after the test. You will be told the HIV result as soon as it is available. The nurse will speak to you about the meaning of the results, how you feel, and ways to prevent HIV and other sexually transmitted infections. Sometimes the HIV test is not clear and in this case we will do an additional test until we confirm your results for sure. We will discuss the repeat test with you and what this means. The result will be kept secret. The information will be kept by the Medical Research Council and the people who see it will not know your name. The information will not be given to anyone else who may come to learn that we have it. ***We will ask you to give us permission to do the HIV test at each study visit or home visit in a separate informed consent.*** This test will be done at every visit or during a home visit when you unable to attend the clinic. The test done at the home visit will be a finger prick only
- We will not repeat the HIV test if you are found to be HIV positive. We will do other tests if you are HIV positive. We will do a CD4 test and a test for your viral load. By measuring the CD4 cells in your body we can determine the progression of the HIV. CD4 cells are cells that give us information on how well our immune system is working and they tell us when a person should start with anti-retroviral therapy (HIV treatment). We will also measure the viral load as this tells us how well the treatment is working. We will measure these every six months during the research, and we will give you the outcome of the test result and help you to understand what it means. We will also refer you to your local health care clinic to get the necessary treatment. This test will be done at each visit depending on if you are HIV positive.

- **P24 Antigen Test**: If you seroconvert during the study- this means you become HIV positive during the period of this study then we will do a special test called a p24 antigen test which will tell us information on how long ago the infection happened. This May mean that we have to prick you again as we only know that your HIOV status has changed after testing the blood.
- **Herpes test:** We will also test the blood for herpes (1 teaspoon), which is a virus that can cause you to have painful ulcers around your vagina, although in many people who are infected there is little pain. This is an important virus as people who have it are at greater risk of catching HIV if they have sex with a person who is HIV positive. We want to test to see if you have this. We will give you the test result as soon as we get it back from the laboratory and we will help you to understand the result. This test will be done every time you visit.

- **Genetic research** - We will take a blood sample (about 2 teaspoons) to examine the genes in your blood.
  What is Genetic research? Genetic material, also called DNA or RNA, is usually obtained from a small blood sample. Genes are found in every cell in the human body. Our genes determine what we look like, such as the colour of our eyes or how tall we will grow. Sometimes, genes also give us information about what kind of diseases we may be susceptible to, such as heart disease or diabetes. Worldwide, researchers in the field of genetics are continuously discovering new information that may be of great benefit to future generations and also that may benefit people today, who suffer from particular diseases or conditions. Some factors in our environment can bring about changes in our genes and we are particularly interested to know about changes in the genes that will affect our ability to cope***. We will ask you to give us permission to take a sample of blood for genetic testing at each study visit in a separate informed consent.*** This test will not be done on every visit.

- **Storage of left-over specimens –** If you agree to participate in the study and agree to have the above blood specimens taken we will ask you to allow us to store the leftover samples such as leftover blood. These leftover samples might be useful for future related studies and we will use it to look for additional evidence of disease progression. For example, we may further look at your genes. We will store this in a safe place called a repository. Nobody will have access to this leftover blood sample other than the study researchers and it will not be used unless the blood can be used in a study approved by the Ethics committee. We plan to keep this leftover blood sample for 10 years and it will be destroyed after 10 years if it is not used. There are no names on any of the specimens, only a special study number. The people who run the repository and the scientists who later use the specimens will not know your name or any other information about you that might identify you. You may decide that you do not want your leftover blood samples stored for future research studies. You can still participate in this study even if you make this decision, any leftover specimens from you will be destroyed at the end of the study. People always have the right to stop participating in research. If you decide that you do not want researchers to be able to use the leftover specimens in the repository, you can contact the clinic staff. They will tell the repository that the specimens with your study code number should not be studied, and these specimens will be destroyed. There are no direct benefits to you from storing your leftover specimens. You may be helping people in the future from the results of studies using the stored specimens. All these specimens are being collected as part of the RICE study in which you are participating. We are not asking you to give any additional specimens for storage, so there is no additional risk associated with collection. The specimens are stored only by code number (not your name) so there is no risk of loss of privacy.

Will you get told your results?

You will not normally get the result of the genetic testing as these tests are experimental. Also, the blood samples from all participants will be de-identified and analysed together and not individually. While the genetic test will not benefit you directly, the research many benefit to people who have experienced a traumatic event in the future. In addition, the tests done on the samples of blood stored may not be ready for many years. Your personal results will be made known to you only under special circumstances and **only if they indicate** that you may:

- have a definite risk for developing a particular disorder
- have a condition or predisposition to developing a condition that is treatable or avoidable, e.g. by a lifestyle modification
- need genetic counseling.

When this happen you will be supported by the staff at the genetic clinic who are experts in helping people understand these diseases. It is however very unlikely that this will happen as such diseases are rare.

The same apply for results from tests done on the stored blood samples. If the researcher's findings could provide important information for your medical care, then they will contact the research staff at your site with the results, and the staff at your clinic can link the code with your name and notify you of the results. If you would like to be contacted with this sort of information, you must notify the study staff of any changes in your address or phone number.

We will ask you to give us permission to store the blood at each study visit in a separate informed consent.

WHAT ARE THE BENEFITS TO ME IF I PARTICIPATE IN THIS STUDY

The main benefit will be in gaining information about your physical and mental health. If we detect that you have any mental or physical health problems, with your permission, we will refer you to your local health care facility for treatment. It is very beneficial for you to learn your HIV status, if you are negative you can make decisions to protect yourself to make sure you remain negative. If you are positive it is important to know so you can get on treatment as soon as you need it and can look after yourself. If you have sexually transmitted infections, we will find this out on the day of your visit and we will treat you for this infection, it is important to know so these can be treated as otherwise you may develop other health problems or become infertile. It is also very useful to know early if you are pregnant so you can receive early care.

Benefits for society

It is very important for South Africa women that we understand their health better and can plan health services that can properly meet their physical and mental health needs. In order to do this effectively it is necessary to have information about what places at women at risk of health problems and how great this risk is. By participating in this study, you will be playing an important role in helping us build this knowledge.

WHAT ARE THE RISKS?

There are no major medical risks involved with participating in this study. Some of the questions may make you feel sad, especially when we ask about experiences of violence, but the study staff will give you support if this happens. There should not be a risk that your private information becomes known to anyone because the study staff will use a special project ID number to identify you and the information you provide. All the staff involved in this study have been given special training on the importance of confidentiality. Some of the women in the study have been invited for the interviews because they experienced trauma and violence in their lives and some of them have

not. Only the interviewer will know whether you have been invited because of violence in your life, no one else will know this.

WHAT ARE THE COSTS TO ME?

There is no cost to you for participating in this study. Y You will be given R80 at the baseline visit and at every visit thereafter you will receive an increment of R20_ to compensate for time spent completing the questionnaire and giving the samples. You will also be given money to cover travel costs for each of the visits and we will provide you with a snack every time you come for the interviews.

WHAT IF I CHANGE MY MIND AFTER JOINING THE STUDY?

At any stage you may change your mind and no longer participate. You can then stop participating and will not be punished in any way for this.

INSURANCE FOR THE STUDY PROVIDED BY THE MEDICAL RESEARCH COUNCIL

If you fall ill, suffer any side effects or if you are injured in any study related manner, contact the researchers immediately at the numbers provided below. The MRC, as sponsors of this study has taken out the necessary insurance to cover you as a research participant.

WHO CAN I ASK IF THERE ARE ANY PROBLEMS WITH THE STUDY?

Principal Investigator
Dr Naeemah Abrahams
Deputy Director, Gender & Health Research Unit, Medical Research Council
Cape Town Office
TEL: 021 9380823
CELL 082 461 7542
Email: naeemah.abrahams@mrc.ac.za

Project Coordinator
Alesha Sewnath
Gender & Health Research Unit, Medical Research Council
Gender & Health Research Unit, Medical Research Council
RICE study- RK Khan Hospital, Chatsworth, Durban
TEL: 031 242 3721
Email: alesha.sewnath@mrc.ac.za
Study Office: Tel: 031 2423720

For questions about your rights as the research participant, contact:

Chairperson: The MRC Research Ethics Committee
Prof. Moodley
Medical Research Council
P.O.Box 19070
Tygerberg
7505
Tel. 021-9380867
Email: adri.labuschagne@mrc.ac.za

MAIN STUDY SIGNATURE PAGE

I declare that: -

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable. I have had a chance to ask questions and all my questions have been adequately answered.

- I understand that taking part in this study is voluntary and I have not been pressurised to take part.

I **DO AGREE** to join the study.

_____
Participant's Signature /Initial

OR

I **DO NOT AGREE** to join the study.

_____
Participant's Signature /Initial

_____	_____	_____
Participant's Signature	Signature	Date
_____	_____	_____
Name of Study Staff Conducting Consent Discussion	Signature	Date
_____	_____	_____
Witness Name	Signature	Date

STORAGE OF LEFT OVER BLOOD SPECIMEN SIGNATURE PAGE

I declare that: -

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable. I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is voluntary and I have not been pressurized to take part.

I **DO AGREE** for the leftover blood specimens to be stored and used for future tests as discussed in this consent form**.**

_____
Participant Signature/Initial

OR

I **DO NOT AGREE** to have any of my leftover blood specimens to be stored in the repository for use in studies in future.

_____
Participant Signature/Initial

_____	_____	_____
Participant's Name	Signature	Date
_____	_____	_____
Name of Study Staff Conducting Consent Discussion	Signature	Date
_____	_____	_____
Witness Name	Signature	Date

HAIR COLLECTION SIGNATURE PAGE

I declare that: -

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable. I have had a chance to ask questions and all my questions have been adequately answered.

- I understand that taking part in this study is voluntary and I have not been pressurized to take part.

I **DO AGREE** to the collection of my hair sample as discussed in this consent form.

_____
Participant's Signature/Initial

OR

I **DO NOT AGREE** to give a hair sample for analysis in this study.

_____
Participant's Signature/Initial

_____	_____	_____
Participant's Name	Signature	Date
_____	_____	_____
Name of Study Staff Conducting Consent Discussion	Signature	Date
_____	_____	_____
Witness Name	Signature	Date

Addendum A5
RICE main IC for family clinics

A STUDY ON WOMEN'S HEALTH AND WELL-BEING
INFORMED CONSENT
RECRUITED AT _____ FAMILY PLANNING CLINIC

RICE - WOMEN'S HEALTH AND WELLBEING STUDY

INTRODUCTION

Hello, welcome to our clinic. You are being invited to take part in the women's health and well-being study. It is a study that the Medical Research Council is conducting with women in Durban on women's health and wellbeing. We want to learn more about women's health so we can help the Government in better planning of their services so that they can meet women's health needs.

We also want to have a better understanding about women's physical and mental health problems, how long these problems last, what causes them, and what social factors and life experiences affect them, whether the problems persist and if the problems require treatment. We are also very interested to know how experiences of violence affect women's health. In addition we want to get a better understanding on how women heal after being hurt.

This is an information and consent and form and we are inviting women between the ages of 18 – 40 years who attend the (*name* _____) family planning clinic/who use contraceptives.

This study will be done over a period of three years and we will speak to women on repeat occasions. This means that you will be invited to come to the research clinic and have interviews done every 3 months. At each study visit we will ask you some interview questions, we will also ask you to give us permission to do some blood tests and tests on your urine as well as to provide a swab. We will explain more about this later. This means we will have to remain in contact so that we can invite you to return for the follow-up interviews over the three years.

IS MY PARTICIPATION VOLUNTARY IN THIS STUDY

This form gives you information about the study and what will be expected of you. Once you have read and understood and agree to participate, you will then be asked to sign your name on this form. You will be given a copy of this form to keep. It is important that you know that your participating is voluntary and you are free to withdraw from the study at any point, even after you have agreed to take part.

WHAT HAPPENS IF I DECIDE TO PARTICPATE IN THE STUDY?

If you agree to participate in the study then we will start with your baseline visit today. You will see and talk to both of us (nurse and research assistant) today. We will start by explaining in more detail about the procedures that will take place today and we will answer questions before you sign the informed consent for us to continue. The nurse will see you first and will do health assessments including the understanding of how you cope, taking of your blood pressure, your pulse, your weight and provide advice on healthy living. You will then be seen by the other researcher and she will ask questions about yourself, your relationships with husbands and boyfriends and other health questions sexual and reproductive health matters, as well as questions about experiences of violence. There will be some questions about things which are often thought of as secrets. Let us know if you feel uncomfortable about these questions. At the end the nurse will take blood, urine and hair samples and will give you the answers to the tests as soon as we get them and will discuss your health with you. Everything that you tell the interviewer will be kept secret and you have the right not to answer any questions that you do not wish to answer. The answers to the questions will be entered on a computer and we expect the interviews with the two of us to take about 2 hours. We will also refer you to the health clinics if we think it is necessary.

WHAT ARE THE PROCEDURES IN THIS STUDY

If you are interested in being part of this study, the following will take place:
The Research assistant start by using a biometric system to log you on the study data entry system. A biometric system allows researchers to use your finger print to identify every time you return to the clinic. Your fingerprint will be linked to a number and this number will be used to store the information and this will ensure her information remains safe and confidential. The reason why we need to identify your fingerprint each time you visit the clinic is the study is done over a long period of time, the patients' information can get mixed up so by entering your details with your study number it prevents your information getting mixed up with another participant's information. We will use another system to check if you are with another study. The will be the same procedure using your fingerprint to check on the computer if you have already been with another study. ***We will ask you to give us permission to use the biometric systems in a separate informed consent that you will sign***.

A study nurse will do a mental health assessment as well as a screen for high blood pressure as we explained earlier.

The research assistant will then do an interview asking the questions as we explained earlier and she will enter this on the computer as she speaks with you.

Hair sample- We will ask you to allow us to collect about 6-8 strands of hair between 1 ½ and 6 cm long. We will take it from the back of the head, close to the scalp and we will do it at each visit. We aware that this might upset your hairstyle and we have a person here that will ensure that your hair is not disarranged. She will make sure that your hair looks exactly the same again after we taken the hair sample. The hair samples will be sent to a special laboratory in Germany where they will be examined and will provide us information about levels of stress we experience. The reason why I it must be done in Germany is because we in South Africa do not have the machines and the equipment to examine it here. Because our hair grows slowly it will allow us to examine the levels of stress over the previous 2 months. Finding the levels of stress is very useful as it will assist us in coping better with stressful events in our lives. We will provide you with the test result as soon as we get it. We will be very careful about how we take the hair. In order to take a hair sample the following will be important:
Your hair must be longer than 1 ½ cm
You must not have any signs of hair loss or baldness
You do not have had a severe physical disease in the last 5 years
You have not been taking anti-depressants in the last 6 months

We will then collect some samples to screen your health as part of the study to answer the questions we have about women's health at each time when you are interviewed. The following are tests and samples that we plan to collect at the screening visit as well as all study visits thereafter:

- **Urine test-** we will require a sample of your urine to test for pregnancy. We will give you the result as soon as it has been tested in the lab here at the clinic. This test will be done every time you visits.

- **Swab from your vulva** – this is to test for infections passed through sex. This test is done by taking a smear from vagina. This is similar to a pap smear test but we do not use instruments and the nurse will assist you and show you how to do it. You will do it in private. The results will be given and discussed with you as soon as it comes back from the laboratory. This test will be done every time you visits.

- **Blood tests**: Blood will be collected from you for various tests. This will be done by the nurse and we will collect about 4-5 teaspoons of blood at each study visit.

We will collect the blood for the following:

- **To test for diabetes, cholesterol levels, kidney and heart diseases in blood.** Women who experience stress and mental problems may be more likely to develop heart cardiovascular disease risk factors such as diabetes, high cholesterol and related kidney problems. We would like to test for diabetes and cholesterol (fat) levels in blood as well as any related kidney problems. We would also like to test for other indicators (signs) of heart disease in the blood. This is a very common illness and it is often hidden. We will also test for fat in your blood which can contribute to heart disease. We will also give you the test results once we receive it from the laboratory. This test only be done on 3 of the visits
- **To test for infections that passed through sex, including herpes infection and HIV**. We will inform you of your STI test results and whether any treatment is needed. If treatment is needed we will assist you in receiving treatment.

- **HIV test:** you will receive counseling before the HIV test and also after the test. You will be told the HIV result as soon as it is available. The nurse will speak to you about the meaning of the results, how you feel, and ways to prevent HIV and other sexually transmitted infections. Sometimes the HIV test is not clear and in this case we will do an additional test until we confirm your results for sure. We will discuss the repeat test with you and what this means. The result will be kept secret. The information will be kept by the Medical Research Council and the people who see it will not know your name. The information will not be given to anyone else who may come to learn that we have it.
- ***We will ask you to give us permission to do the HIV test at each study visit or home visit in a separate informed consent.*** This test will be done at every visit or during a home visit when you unable to attend the clinic. The test done at the home visit will be a finger prick only
    - We will not repeat the HIV test if you found to be HIV positive. We will do other test if you are HIV positive. We will do a CD4 test and a test for your Viral load. By measuring the CD4 cells in your body we can determine the progression of the HIV. CD4 cells are cells that give us information on how well our immune system is working and they tell us when a person should start with anti-retroviral therapy (HIV treatment). We will also measure the viral load as this tells us how well the treatment is working. We will measure these every six months during the research, and we will give you the outcome of the test result and help you to understand what it means. We will also refer you to your local health care clinic to get the necessary treatment. This test will be done at each visit depending on if you are HIV positive.

- **P24 Antigen Test**: If you seroconvert during the study- this means you become HIV positive during the period of this study then we will do a special test called a p24 antigen test which will tell us information on how long ago the infection happened. This May mean that we have to prick you again as we only know that your HIOV status has changed after testing the blood.

- **Herpes test:** We will also test the blood for herpes, which is a virus that can cause you to have painful ulcers around your vagina, although in many people who are infected there is little pain. This is an important virus as people who have it are at greater risk of catching HIV if they have sex with a person who is HIV positive. We want to test to see if you have this. We will give you the test result as soon as we get it back from the laboratory and we will help you to understand the result. This test will be done every time you visit.

- **Genetic research** - We will take a blood sample (about 2 teaspoons) to examine the genes in your blood.

What is Genetic research? Genetic material, also called DNA or RNA, is usually obtained from a small blood sample. Genes are found in every cell in the human body. Our genes determine what we look like, such as the colour of our eyes or how tall we will grow. Sometimes, genes also give us information about what kind of diseases we may be susceptible to, such as heart disease or diabetes. Worldwide, researchers in the field of genetics are continuously discovering new information that may be of great benefit to future generations and also that may benefit people today, who suffer from particular diseases or conditions. Some factors in our environment can bring about changes in our genes and we are particularly interested to know about changes in the genes that will affect our ability to cope. This test will not be done on every visit.

- **Storage of left over specimens –** If you agree to participate in the study and agree to have the above blood specimens taken we will ask you to allow us to store the leftover samples such as leftover blood. These leftover samples might be useful for future related studies and we will use it to look for additional evidence of disease progression. For example, we may further look at your genes - We will store this in a safe place called a repository. Nobody will have access to this leftover blood sample other than the study researchers and it will not be used unless the blood can be used in a study approved by the Ethics committee. We plan to keep this leftover blood sample for 10 years and it will be destroyed after 10 years if it is not used. There are no names on any of the specimens, only a special study number. The people who run the repository and the scientists who later use the specimens will not know your name or any other information about you that might identify you. You may decide that you do not want your leftover blood samples stored for future research studies. You can still participate in this study even if you make this decision, any leftover specimens from you will be destroyed at the end of the study. People always have the right to stop participating in research. If you decide that you do not want researchers to be able to use the leftover specimens in the repository, you can contact the clinic staff. They will tell the repository that the specimens with your study code number should not be studied, and these specimens will be destroyed. There are no direct benefits to you from storing your leftover specimens. You may be helping people in the future from the results of studies using the stored specimens. All these specimens are being collected as part of the RICE study in which you are participating. We are not asking you to give any additional specimens for storage, so there is no additional risk associated with collection. The specimens are stored only by code number (not your name) so there is no risk of loss of privacy.

Will you get told your results?

You will not normally get the result of the genetic testing as these test are experimental. Also the blood samples from all participants will be de-identified and analysed together and not individually. While the genetic test will not benefit you directly, the research many benefit to people who have experienced a traumatic event in the future. In addition, the tests done on the samples of blood stored may not be ready for many years. Your personal results will be made known to you only under special circumstances and **only if they indicate** that you may:

- have a definite risk for developing a particular disorder
- have a condition or predisposition to developing a condition that is treatable or avoidable, e.g. by a lifestyle modification
- need genetic counseling.

When this happens, you will be supported by the staff at the genetic clinic who are experts in helping people understand these diseases. It is however very unlikely that this will happen as such diseases are rare.

The same apply for results from tests done on the stored blood samples. If the researcher's findings could provide important information for your medical care, then they will contact the research staff at your site with the results, and the staff at your clinic can link the code with your name and notify you of the results. If you would like to be contacted with this sort of information, you must notify the study staff of any changes in your address or phone number.

We will ask you to give us permission to store the blood at each study visit in a separate informed consent.

WHAT ARE THE BENEFITS TO ME IF I PARTICIPATE IN THIS STUDY

The main benefit will be in gaining information about your physical and mental health. If we detect that you have any mental or physical health problems, with your permission, we will refer you to your local health care facility for treatment. It is very beneficial for you to learn your HIV status, if you are negative you can make decisions to protect yourself to make sure you remain negative. If you are positive it is important to know so you can get on treatment as soon as you need it and can look after yourself. If you have sexually transmitted infections, we will find this out on the day of your visit and we will treat you for this infection, it is important to know so these can be treated as otherwise you may develop other health problems or become infertile. It is also very useful to know early if you are pregnant so you can receive early care.

Benefits for society

It is very important for South Africa women that we understand their health better and can plan health services that can properly meet their physical and mental health needs. In order to do this effectively it is necessary to have information about what places at women at risk of health problems and how great this risk is. By participating in this study, you will be playing an important role in helping us build this knowledge.

WHAT ARE THE RISKS?

There are no major medical risks involved with participating in this study. Some of the questions may make you feel sad, especially when we ask about experiences of violence, but the study staff will give you support if this happens. There should not be a risk that your private information becomes known to anyone because the study staff will use a special project ID number to identify you and the information you provide. All the staff involved in this study have been given special training on the importance of confidentiality. Some of the women in the study have been invited for the interviews because they experienced trauma and violence in their lives and some of them have not. Only the interviewer will know whether you have been invited because of violence in your life, no one else will know this.

WHAT ARE THE COSTS TO ME?

There is no cost to you for participating in this study. You will be given R80 at the baseline visit and at every visit thereafter you will receive an increment of R20_at each of the study visits to compensate for time spent completing the questionnaire and giving the samples. You will also be given money to cover travel costs for each of the visits and we will provide you with a snack every time you come for the interviews.

WHAT IF I CHANGE MY MIND AFTER JOINING THE STUDY?

At any stage you may change your mind and no longer participate. You can then stop participating and will not be punished in any way for this.

INSURANCE FOR THE STUDY PROVIDED BY THE MEDICAL RESEARCH COUNCIL

If you fall ill, suffer any side effects or if you are injured in any study related manner, contact the researchers immediately at the numbers provided below. The MRC, as sponsors of this study has taken out the necessary insurance to cover you as a research participant.

WHO CAN I ASK IF THERE ARE ANY PROBLEMS WITH THE STUDY?

Principal Investigator
Dr Naeemah Abrahams
Deputy Director
Gender & Health Research Unit, Medical Research Council
Cape Town office: TEL: 021 9380823
FAX: 021 9380310
CELL 082 461 7542
Email: naeemah.abrahams@mrc.ac.za

Project Coordinator
Alesha Sewnath
Gender & Health Research Unit, Medical Research Council
TEL: 031 242 3721
Email: alesha.sewnath@mrc.ac.za
Study Office; Tel: 031 2423720

For questions about your rights as the research participant, contact:

Chairperson: The MRC Research Ethics Committee
Prof. Moodley
Medical Research Council
P.O.Box 19070, Tygerberg,
7505
Tel. 021-9380687
Email: adri.labuschagne@mrc.ac.za

MAIN STUDY SIGNATURE PAGE

I declare that: -

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable. I have had a chance to ask questions and all my questions have been adequately answered.

- I understand that taking part in this study is voluntary and I have not been pressurised to take part.

I **DO AGREE** to join the study.

_____
Participant's Signature /Initial

OR

I **DO NOT AGREE** to join the study.

_____
Participant's Signature /Initial

_____	_____	_____
Participant's Signature	Signature	Date
_____	_____	_____
Name of Study Staff Conducting Consent Discussion	Signature	Date
_____	_____	_____
Witness Name	Signature	Date

STORAGE OF LEFT OVER BLOOD SPECIMEN
SIGNATURE PAGE

I declare that: -

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable. I have had a chance to ask questions and all my questions have been adequately answered.

- I understand that taking part in this study is voluntary and I have not been pressurized to take part.

I **DO AGREE** for the leftover blood specimens to be stored and used for future tests as discussed in this consent form**.**

_____
Participant Signature/Initial

OR

I **DO NOT AGREE** to have any of my leftover blood specimens to be stored in the repository for use in studies in future.

_____
Participant Signature/Initial

_____	_____	_____
Participant's Name	Signature	Date
_____	_____	_____
Name of Study Staff Conducting Consent Discussion	Signature	Date
_____	_____	_____
Witness Name	Signature	Date

HAIR COLLECTION SIGNATURE PAGE

I declare that: -

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable. I have had a chance to ask questions and all my questions have been adequately answered.

- I understand that taking part in this study is voluntary and I have not been pressurized to take part.

I **DO AGREE** to the collection of my hair sample as discussed in this consent form.

_____
Participant's Signature/Initial

OR

I **DO NOT AGREE** to give a hair sample for analysis in this study.

_____
Participant's Signature/Initial

_____	_____	_____
Participant's Name	Signature	Date
_____	_____	_____
Name of Study Staff Conducting Consent Discussion	Signature	Date
_____	_____	_____
Witness Name	Signature	Date

Addendum B: SOCIO-DEMOGRAPHIC Questionnaire			
Q1READ: Let us start			**SKIP**
FUNDA: Asiqale			
101 **Q2**	**What is your date of birth?** Lunini usuku lwakho lokuzalwa?	Day [ ][ ] Month [ ][ ] Year [ ][ ][ ][ ]	
102 **Q3**	**What is your highest level of education?** Ugcine kuliphi ibanga esikoleni?	SUB A/GRADE1.....1 SUB B/GRADE 2.....2 STD 1/GRADE 3.....3 STD 2/GRADE 4.....4 STD 3/GRADE 5.....5 STD 4/GRADE 6.....6 STD 5/GRADE 7.....7 STD 6/GRADE 8.....8 STD 7/GRADE 9.....9 STD 8/GRADE 10.....10 STD 9/GRADE 11.....11 STD 10/GRADE 12.....12 INCOMPLETE DEGREE /QUALIFICATION.....13 COMPLETED DEGREE / QUALIFICATION.....14	
103 **Q4**	**What race group do you consider yourself?** Uyiluphi uhlanga?	BLACK/AFRICAN.....1 COLOURED.....2 WHITE.....3 ASIAN/INDIAN.....4 OTHER, PLEASE SPECIFY.....5	
104 **Q5**	**What is your home language (the one you grew up speaking with your family)?** Ukhuluma luphi ulimi ekhaya (owakhula ulukhulumai nomden)?	ENGLISH.....1 ZULU.....2 S.SOTHO.....3 XHOSA.....4 TSWANA.....5 SHANGAAN/TSONGA.....6 AFRIKAANS.....7 NDEBELE.....8 PEDI/N. SOTHO.....9 VENDA.....10 SWAZI.....11 OTHER, PLEASE SPECIFY.....12	
105 **Q6**	**Are you currently studying** Uyafunda njengamanje?	YES .....1 NO.....0	

106 **Q7**	**What is your main source of income?** **Multiple question maximum of two answers** Imuphi umthombo omkhulu othola ngawo imali?	SELF-EMPLOYED.....1 INCOME FROM FULL TIME EMPLOYMENT.....2 INCOME FROM PART TIME EMPLOYMENT.....3 CHILD SUPPORT GRANT..D.....4 SOCIAL GRANT FOR DISABILITY.....5 PARTNER SUPPORT.....6 FAMILY SUPPORT (PARENTS/GRANDPARENTS).....7 NO INCOME.....8 OTHER , PLEASE SPECIFY.....9 _____-	 SKIP 108 SKIP 108 SKIP 108 SKIP 108 SKIP 108
107 **Q8**	**How much do you earn per month, before tax, and including benefits?** Uhola malini ngenyanga, ingakakhishwa intela, sekuhlangene nenzuzo?	R1 – R500.....1 R501 – R1000.....2 R1 001 – R2000.....3 R2001 – R5000.....4 R5001 – R10 000.....5 R10 001 – R20 000.....6 R20 000 OR MORE.....7	
108 **Q9**	**Would you say that the people in your home often, sometimes, seldom or never go without food?** Ungathi ekhaya lakho abantu balala bengadlile izikhathi eziningi, ngezinye izikhathi, kancane noma akukaze kwenzeke?	OFTEN.....1 SOMETIMES.....2 SELDOM/HARDLY EVER.....3 NEVER 4	
109 **Q10**	**If you have an emergency and R200 was needed immediately, would you say it would be very easy, easy, quite difficult or very difficult to find the money?** Uma kunesimo esiphuthumayo, kudingeka uthole u-R200 ngokushesha, ungathi kungabalula kakhulu, kungabalula, kungabanzinyana, noma kungabanzima kakhulu ukuwuthola?	VERY DIFFICULT.....1 QUITE DIFFICULT.....2 EASY.....3 VERY EASY.....4	

Addendum C
Contact details and consent to be contacted

RECRUITMENT AND RETENTION INFORMATION FORM
THE WOMEN'S HEALTH AND WELL-BEING STUDY

1. Study ID _____

2. First name _____

3. Surname _____

4. Preferred name or names

a. _____
b. _____
5. Cell phone number _____

6. Home address _____

7. Is this your family home, if not what is the address_____

_____
8. Cell number of relative #1 _____

9. Cell number of relative # 2_____

10. Name and address of employer_____

11. Phone number of employer
_____

12. Cell phone number and name of friend _____

13. Which church are you a member of and where is
it_____

14. Are you a member of any choirs or clubs, if so which ones_____

CONSENT TO BE CONTACTED BY RESEARCHER
WOMEN'S HEALTH AND WELLBEING STUDY

The Gender and Health Research Unit at the Medical Research Council (MRC) is undertaking research with women using our services here in Durban on women's health and wellbeing. They want to learn more about women's health so we can help the Government in better planning of their services so that they can meet women's health needs.
They are planning to interview women using health facilities in Durban. They want to recruit a group of 2018 women in total and to interview them on different occasions over three years. This will enable them to measure and try to understand changes that occur in women's health and life circumstances over this time.
They have asked us here at the clinic to only tell you a little bit about their research and if you agree to speak to them they will provide much more information. We are not asking you to decide whether you want to take part in their study but we are asking that you agree to be contacted by the researcher. We would like for your permission for your personal details to be stored confidentially and given to the researcher at the Gender and Health Unit. If you agree she will contact you at some time in the week and we will explain to you all about the study and will only then ask you permission to participate in the study. When the researcher contacts you she will not reveal the reason for the call to anyone but to you.
We are not asking you to agree to participate in the research but only to agree to be contacted by another person. You will then be free to agree to participate in the study or to decline. If you feel that you do not want your contact details to be passed to the researcher then you must feel free to say so. The services you receive here at the clinic remains the same and will not be influenced by your decision.

Thank you
I _____ (your name) understand the paragraphs above and hereby grant consent for a researcher from the Gender & Health Research Unit to contact me.
_____ (your signature)
Contact Details
My telephone numbers: _____ (home)
_____ (cell)
_____ (work)
_____ (Other)
A close friend or family's phone number:
Name _____: Number _____
Address (optional): _____
_____
If you have questions contact: Naeemah Abrahams
Gender and Health Research Group, Medical Research Council
Tel: 021 9380445 Fax: 121 9380310 Email: nabraham@mrc.ac.za

TECHNISCHE UNIVERSITÄT DRESDEN
Faculty of Science. Department of Psychology, Chair of Biopsychology

Instructions for Hair Sampling

Preparation

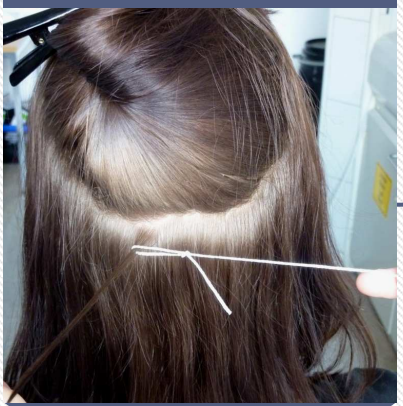
Required materials:

- ✓ Aluminum foil
- ✓ Scissors
- ✓ Paper clip
- ✓ Hairgrip (Supplied)
- ✓ Comb (Supplied)
- ✓ Twine loop (see instructions)

Step 1

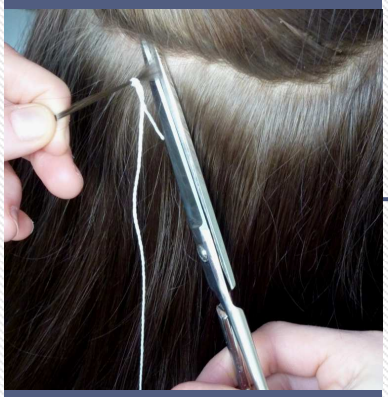
- Separate the hair strand at the back of the head using the hairgrip
- Separate **2-3 hair strands (overall diameter similar to half the diameter of a pencil)** approx. 2 cm above the scalp and comb the strands

Step 2



- Thread the combed strands **through the loop** and **tighten** the loop

Step 3

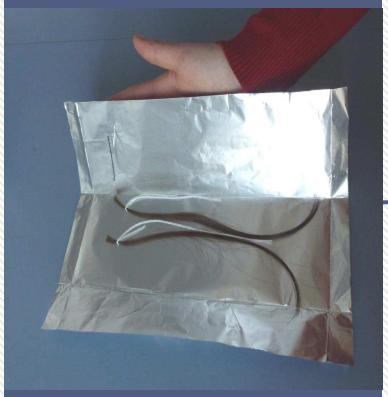


- Cut the hair **as close as possible to the scalp**

Step 4

- Place the strands onto the **aluminium foil**
- Mark the scalp-near end of the strands with a permanent marker on the aluminium foil

Step 5



- Fold the aluminium foil
- If the sample is longer than the aluminium foil, the hair strands can be cut at the distal end

Step 6

- Fix the folded aluminium foil at the scalp-near end with a paper clip
- **Do not fold** the sample
- Place the aluminium foil with the hair protocol (including participant ID) into a transparent folder
- Store the sample in a dry and dark place

Addendum D2

Standard Operational Procedures: HAIR SAMPLING

For this study we will be adhering to the procedural protocol of the **University of Dresden: Faculty of Science, Department of Psychology, Chair of Biopsychology**. (See included in this document)

Requirements

Study personnel tasked to perform hair sampling procedures should be adequately trained to perform the scientific procedure of obtaining hair samples.

In addition to the given Protocol, please note the following applicable to this study:

- Ensure that the participant has given informed consent to the procedure
- Ensure that the participant fully understands the procedure and the risks thereof, and that the participant has been given sufficient opportunity to ask questions and discuss the procedure if needed.
- Ensure that the participant is comfortable and in the most appropriate position to perform the procedure.
- Ensure that all equipment and supplies for the procedure is ready and at hand.
- Put on gloves
- Perform procedure as explained in Protocol. In addition, the instructional video can be viewed.
    - Hair samples obtained should be at least 3cm long, but can be longer.
- Prepare the twine slip-knots before the procedure commences. This is done as follows:

To make the slip knot, wind the yarn into a circle, with the end of the yarn on top.

Pass your fingers through the loop.

Grasp the yarn end which is still attached to the ball.

Pull the yarn through the loop.

Pull on the loop to tighten up the knot.

- Prepare the aluminium foil pieces before the procedure commences. This is done as follows:
    - From roll of aluminium foil provided, cut rectangular pieces of approximately 10cm x 20cm
    - When inserting the hair sample, fold the aluminium foil as explained in the Protocol document
- Inform participant when procedure is completed and ensure comfort of participant.
- After samples have been folded in the aluminium foil, label the sample by writing the patient number on the foil with a black marker
- Complete the hair sample information sheet and insert it with the foil into a plastic bag
- Seal and secure the bag
- Pack and store the samples as prescribed in the Protocol Document.
- Ensure correct instructions for safe shipping is followed when sent for analysis.

Addendum E

MATERIAL TRANSFER AGREEMENT

Between

STELLENBOSCH UNIVERSITY ("SU")

Physical Address	R.W. Wilcocks Building 2037, Victoria Street, Stellenbosch, 7600, South Africa
Postal Address	Private Bag X1, Matieland, Stellenbosch, 7602, South Africa
Telefax Number	+27 (0)21 808 4537
Telephone Number	+27 (0)21 808 2187
Contact Person: **Contract** related matters	Legal Advisor: Mr Mark Mulder mmulder@sun.ac.za
Contact Person: **Project and financial** related matters Email Address	Prof Soraya Seedat sseedat@sun.ac.za
Signature who warrants that s/he is duly authorised to sign	
Name	Prof NC Gey Van Pittius
Position	Deputy Dean, Research: Faculty of Medicine & Healthcare
Date	17/07/2014

and

[TU Dresden] ("Recipient")

Physical Address	Lehrstuhl Biopsychologie TU Dresden Andreas-Schubert-Bau Zellescher Weg 19, Raum 220 D-01062 Dresden, Germany
Postal Address	As above
Telefax Number	+49-351-4633-7274
Telephone Number	+49-351-4633-9660
Contact Person	Prof. Dr. Clemens Kirschbaum
Email Address	clemens.kirschbaum@tu-dresden.de
Signature who warrants that s/he is duly authorised to sign	
Name	Dr Clemens Kirschbaum
Position	Dean, Faculty of Science TU Dresden
Date	May 28, 2014

Recipient Scientist

Facility/Laboratory Address	Lehrstuhl Biopsychologie TU Dresden Andreas-Schubert-Bau Zellescher Weg 19, Raum 220 D-01062 Dresden, Germany
Telephone Number	+49-351-4633-7274
Email Address	clemens.kirschbaum@tu-dresden.de
Signature	

Name	Dr Clemens Kirschbaum
Position	Head of Biopsychology Lab, TU Dresden
Date	May 28, 2014

Research Project title: Hormones in hair as possible predictive biomarkers of posttraumatic stress disorder in persons who have been raped	**Contract number: S003749**
Research Period: 2014-2017	**SU Ethical Clearance number:**

Material type:
Human tissue or blood samples [**x**]; Cell components [ ]; Plants or organisms [ ]; Animals [ ]; Genetically Modified Organisms [ ]
Bioprospecting: [YES / **NO**]
Use of indigenous biological resources: [YES / **NO**]

1 The Recipient acknowledges that the Material is confidential and proprietary to SU. The transferred Materials shall be used solely for non-commercial research purposes to carry out the Research Project (clause 15) only at the Recipient's facility/laboratory under the direction of the Recipient's Scientist.

2 Legal title to the Material will remain with SU. Nothing in this Agreement: (i) grants the Recipient any rights over the Material (other than as specifically granted by this Agreement) or under any patent, plant breeders' right or other intellectual property right, nor any right to use, or (ii) permits the use of any products or processes containing, using, or directly derived from the Material, for profit-making or commercial purposes.

3 The Recipient will not make use of, or permit anyone else to make use of, the Material or a product directly derived from the Material for commercial purposes or for any other purposes other than for the Research Project without SU's prior written consent. In such event, it agrees to negotiate in good faith with SU for the grant of an appropriate licence or the conclusion of a revenue sharing agreement, if justified. SU will have no obligation to grant a licence.

4 It is expressly acknowledged and agreed by the Recipient that the Materials may not be used for work on humans, in clinical trials or for diagnostic purposes involving human subjects and that the Materials must at all times be used in accordance with all applicable laws and regulations.

5 The Recipient agrees not to transfer, transmit or in any other way provide access to the Materials, to any third party without the written consent of SU. Such consent will not be unreasonably withheld if the third party is an academic research institution as long as such third party signs an equivalent Material Transfer Agreement with SU. Upon request by SU, the Recipient will return such of the Material as may be required to the SU or dispose of such Material as directed by SU and Recipient shall certify such disposal upon request.

6 Upon request the Recipient will notify SU of the results of the Research Project and provide SU with samples of such results developed through the use of the Material. Neither party may register or apply for the registration of any intellectual property right with respect to the results of the Research Project (including without limitation under patent or plant breeders' right) without the prior written consent of the other party. The Recipient grants SU a fully paid-up, non-exclusive right to use any results developed through the use of the Material transferred under this Agreement for its own internal, non-profit academic research and teaching purposes.

7 Material delivered pursuant to this agreement by SU to the Recipient is understood to be experimental in nature and may have hazardous properties. SU makes no representations and provides no warranties of any kind, either expressed or implied by law, with respect to the Material or any information associated to it. In particular, SU hereby excludes and disclaims any express or implied warranty of reasonable quality, merchantability or fitness for a particular purpose, that the Material or any information associated with it, is free from defects (latent or otherwise), or that use of the Material will not infringe any intellectual property right or other third party rights.

8 In no event will SU or its personnel be liable to the Recipient for any direct or indirect losses or damages whatsoever, arising in connection with this agreement, save to the extent that the limitation of liability contained herein is not permitted by applicable law.

9 Risk of loss or damage to the Material will pass to the Recipient upon delivery to the transport carrier. The use of the Material will be at the sole and exclusive risk of the Recipient. The Recipient hereby indemnifies and agrees to hold SU harmless against any and all losses that may arise in connection with the Materials, including any loss or damage to the Material in transit.

10 The Recipient agrees to treat all information pertaining to the Material as confidential and proprietary to SU, including the properties, characteristics, content and composition thereof and the potential uses and methods of use thereof and will not disclose any information pertaining to the Material to any third party without the written consent of the SU including pursuant to clause 11. The Recipient will also ensure that the Recipient's Scientist and all other persons allowed to access the Material comply with this clause.

11 SU recognises the desire of the Recipient to publish details of academic research in scientific journals or theses and SU agrees that the Recipient will be free to publish results of the Research Project, providing that SU is provided with a copy of any such manuscript or abstract at least thirty (30) days prior to submitting such publication to the scientific journal or to examiners, to give SU the opportunity of requesting the removal of any proprietary confidential information pertaining to the Material. Recipient must comply with SU's removal requirement to the reasonable satisfaction of SU prior to submitting such publication. Manuscripts should be sent to SU's technology transfer company, InnovUS Pty Ltd, or its Research Contracts Office, Division for Research Development, for review of such proprietary information content. SU agrees to maintain such results, copy of any such manuscript or abstract in confidence and not to engage in any written dissemination or in any dissemination by other methods of results obtained by Recipient from use of the Materials for the purpose described in Clause 6.

However, this clause 11 shall not preclude either party's attribution of authorship in, and distribution of academic literature reporting the results of research conducted with the Materials, where applicable.

12 The Recipient agrees to provide appropriate acknowledgement of the source of the Materials in all publications. Each party agrees not to use or refer to this Agreement in any promotional activity, or use the names or marks of the other without express written permission.

13 General

13.1 This Agreement shall come into force on the date on which it is signed by both parties and shall remain in force for the duration of the Research Period, or as long as the Recipient has possession of the Materials if longer.

13.2 Either the Recipient or SU may terminate this Agreement forthwith by thirty (30) days prior notice of termination in writing:

13.2.1 If either party commits a material breach of this Agreement, which in the case of a breach capable of remedy is not remedied within thirty (30) days of the receipt by the party in default of notice identifying the breach and requiring its remedy, or.

13.2.2 Termination without cause

Upon termination of this Agreement, Recipient's rights to use the Material will cease and Recipient will discontinue all use of the Material, but all other terms hereunder will continue unaffected.

13.3 Neither party shall assign or transfer any interest in this Agreement without prior written approval of the other party.

13.4 No amendment, consent or waiver of terms of this Agreement shall bind either party unless in writing and signed by all parties. Any such amendment, consent, or waiver shall be effective only in the specific instance and for the specific purpose given.

13.5 This Agreement embodies the entire agreement between the parties hereto and no provision of this Agreement may be changed except by the mutual written consent of the parties hereto.

13.6 This Agreement shall be governed by the South African Law and the South African Courts shall have exclusive jurisdiction to deal with any dispute which may arise out of or in connection with this Agreement.

13.7 The use of South African biological resources is governed by the National Environmental Management Biodiversity Act, Act 10 of 2004 (NEMBA) and its associated subordinate legislation. To engage in any bioprospecting activity using biological material with South African origin a bioprospecting permit must be obtained from the Department of Environmental Affairs:

The Director General
Department of Environmental Affairs
Private Bag x447
PRETORIA
0001

Enquiries:
The Director: Resource Use
Mr. Muleso Kharika
Tel: +27 12 310 3578 / 3451
Fax: +27 12 320 4087 / 7026
Email: Jkharika@environment.gov.za
www.environment.gov.za

14 The Material

This agreement concerns the following types and quantities of material to be provided to the Recipient:

Type of Material	Quantity	Place of Origin	Already Identified Potential Uses
Hair (taken from the scalp) from individuals who have been raped and non-rape exposed controls	Samples from 2100 participants	KwaZulu Natal, South Africa	-

Material includes all progeny generated from the Material supplied and that part of all derivatives and the derivative's progeny which contains any of the Material supplied or its progeny.

15 The Research Project

The manner in which and the extent to which the Material may be used by the Recipient are as follows:

The hair samples will be used to measure cortisol and other steroid hormones (e.g. DHEA, corticosterone) using a gold-standard liquid chromatography-mass spectrometry approach that allows for measurement at high sensitivity and specificity.

~ END ~