Glucose control, cardiorespiratory fitness, and dietary composition in healthy men.

by

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Declaration

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Abstract

The prevalence and incidence of type 2 diabetes mellitus (T2DM) is worrisome and places a great burden on health care systems worldwide, while negatively affecting the quality of life of countless individuals. Continuous glucose monitoring (CGM) has not only improved the quality of diabetes care, but has also identified differences in the glucose profiles of individuals with otherwise seemingly healthy glucose control. The question arises whether CGM and the measurement of glycaemic variability would be more sensitive in identifying individuals who are at risk for imminent insulin resistance and subsequent T2DM.

It may have significant clinical value if it can be determined if free-living glycaemic variability is closer related to lifestyle factors, such as diet and exercise, than the traditional measures of glucose control. Hence, this study investigated glucose control and glycaemic variability during free-living conditions in apparently healthy men in relation to cardiorespiratory fitness (CRF) and dietary glycaemic load (GL).

Twenty-seven apparently healthy men of varying CRF levels and aged between 30 – 47 years, were included in the study. The participants underwent at least 7 days of CGM, while logging all food and drink. A modified treadmill Vam-éval ramp test was completed to measure peak aerobic exercise capacity. Each participant had blood samples drawn for the measurement of HbA_{1c} and completed a 2-h 75 g oral glucose tolerance test (OGTT) during which [glucose] and [insulin] was measured every 30 minutes. Inferential statistical analysis was done using Excel, while Cohen's effect sizes were calculated to describe the magnitude of differences between sub-groups.

CFR was best correlated to insulin sensitivity (HOMA-IR: r = -0.74; Matsuda index: r = 0.72), while dietary GL correlated most strongly with MAGE (r = 0.45). A K-means cluster analysis of the dietary macronutrient composition of the participants revealed three groups: high carbohydrate-low fat, low carbohydrate-high fat, and high-carbohydrate-high fat. The high carbohydrate-low fat cluster had the highest glycaemic variability, while the insulin levels were highest in the high carbohydrate-high fat cluster. No differences between clusters were found in traditional glucose control measures. The variation in the OGTT [insulin] was significantly more than the variation in [glucose]. Glycaemic variability indices did not identify more at-risk individuals than were identified with the traditional measures of glucose control.

This study could not vouch for early health risk detection among healthy, non-diabetic individuals using CGM. The results, however, showed that dietary macronutrient composition elicited larger differences in glycaemic variability, than in glucose control. Overall, it was evident that increases in insulin secretion occurs before there are any sustained increases in glucose levels.

This study provides evidence for the importance of both CRF and diet in the maintenance of metabolic health, as well as the importance of measuring insulin concentrations. Healthy, glucose tolerant individuals are not necessarily protected against hyperinsulinaemia after a high-carbohydrate meal. It is proposed that attention should shift from the measurement of glucose to the measurement of insulin for early risk detection - at least until future longitudinal studies are able to link risk to different glycaemic variability profiles.

Abstrak

Die voorkoms en groeikoers in die voorkoms van tipe 2 diabetes mellitus (T2DM) is kommerwekkend, plaas groot druk op gesondheidsorgstelsels wêreldwyd, en verlaag die lewenskwaliteit van ontelbare individue. Deurlopende glukose monitering (DGM) het die kwaliteit van diabetesbehandeling baie verbeter en het verskille geïdentifiseer in die glukose profiele van gesonde individue wat andersins blyk om gesonde glukose beheer te hê. Die vraag ontstaan of DGM en die meet van glisemiese variasie meer sensitief is om individue te identifiseer met verhoogde risiko vir toekomstige insulienweerstandigheid en gevolglike T2DM.

Indien vrylewende glisemiese variasie nader verwant aan leefstyl faktore (dieet en oefening) is as tradisionele maatstawwe van glukose beheer, kan dit betekenisvolle kliniese waarde inhou. Die doel van hierdie studie was om glukose beheer and glisemiese variasie gedurende vrylewende omstandighede in oënskynlik-gesonde mans, te ondersoek, asook die verband van hierdie parameters met kardiorespiratoriese fiksheid (KRF) en glisemiese lading (GL).

Sewe en twintig oënskynlik-gesonde mans, met verskillende vlakke van KRF en tussen 30 – 47 jaar oud, was by die studie ingesluit. Elke deelnemer het ten minste 7-dae se DGM deurgaan, waartydens hy dagboek gehou het van alle kos- en drank-inname. ‘n Gemodifiseerde trapmeul Vam-éval toets is voltooi om piek aerobiese oefenkapasiteit te bepaal. Elke deelnemer het bloed laat trek vir die meet van HbA_{1c} en het ook ‘n 75 g, 2-uur modelinge glukose toleransie toets afgelê waartydens [glukose] en [insulien] elke 30 minute gemeet is. Afleidende statistiese analise is met behulp van Excel gedoen, terwyl Cohen se effekgroottes bereken is om die grootte van verskille tussen sub-groepe te beskryf.

KRF het die beste met insuliensensitiwiteit gekorreleer (HOMA-IR: r = -0.74; Matsuda indeks: r = 0.72), terwyl GL die sterkste met MAGE gekorreleer het (r = 0.45). ‘n K-gemiddelde bondel analiese is op die makronutriënt samestelling van die deelnemers se diëte gedoen om drie groepe te identifiseer: hoë koolhidraat-lae vet, lae koolhidraat-hoë vet, en hoë koolhidraat-hoë vet. Die hoë koolhidraat-lae vet groep het die hoogste gliseiese variasie getoon, terwyl die insulienvlakke van die hoë koolhidraat- hoë vet groep die hoogste was. Geen verskille in tradisionele glukose beheer is tussen die groepe gevind nie. Die variasie in die [insulien] van die glukose toleransie toets was betekenisvol meer as die variasie in [glukose]. Die glisemiese variasie maatstawwe het nie meer individue met verhoogde risiko geïndentifiseer as die tradisionele maatstawwe van glukose beheer nie.

Hierdie studie kon nie ‘n saak uitmaak vir die vroeë risiko bepaling in gesonde, nie-diabetiese individue met behulp van DGM nie. Die resultate wys wel dat die makronutriënt samestelling van ‘n dieet gepaardgaan met groter verskille in glisemiese variasie, as glukose beheer. Oor die algemeen was dit duidelik dat verhogings in insulienvlakke voor volgehoue verhogings in glukosevlakke voorkom.

Hierdie studie wys op die belangrikheid van KRF vir dieet vir die handhawing van metaboliese gesondheid. Gesonde, glukose tolerante individue is nie noodwendig beskerm teen hoë vlakke van insulien ná ‘n hoë-koolhidraat maaltyd nie. Dit word voorgestel dat die aandag verskuif word vanaf die meet van glukose na insulien vir vroeë risiko-identifisering – ten minste totdat longitudinale studies risiko kan verbind met verskillende glisemiese variasie profiele.

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List of abbreviations

ACSM	American College of Sports Medicine
ADA	American Diabetes Association
AUC	area under the curve
BMI	body mass index
BF%	body fat percentage
CHD	coronary heart disease
CHO	carbohydrate
CIMO	carbohydrate-insulin model of obesity
CGM	continuous glucose monitor(ing)
CRF	cardiorespiratory fitness
CV%	coefficient of variation percentage
CVD	cardiovascular disease
FPG	fasting plasma glucose
GI	glycaemic index
GL	glycaemic load
GLUT4	glucose transporter type 4
h	hour
HbA_{1c}	glycated haemoglobin
HDL-C	high-density lipoprotein cholesterol
HIIT	high intensity interval training
HOMA-IR	homeostasis model assessment of insulin resistance
HR	heart rate
ISSN	International Society of Sports Nutrition
LDL-C	low-density lipoprotein cholesterol
MAGE	mean amplitude of glycaemic excursions (intra-day glycaemic variability)
MODD	mean of daily differences (inter-day glycaemic variability)
MRC	Medical Research Council
OGTT	oral glucose tolerance test
RER	respiratory exchange rate
RPE	rating of perceived exertion
SD	standard deviation
SEMDSA	Society for Endocrinology, Metabolism and Diabetes of South Africa

SWD	smallest worthwhile difference
T2DM	Type 2 Diabetes Mellitus
VO_2	oxygen consumption
vs.	versus
WAT	white adipose tissue
WHO	World Health Organisation
8-iso-PGF2α	8-iso prostaglandin F2α
[ ]	concentration

Glossary

General	
Postprandial	The period after food was consumed during which absorption of nutrients takes place.
Fasted / fasting	The post-absorptive state during which stored nutrients have to be utilised to provide for the body's energy needs.
Glycaemic variability	
CGM CV%	Coefficient of variation of the glucose values over the entire glucose monitoring period.
CGM SD	Standard deviation from the mean glucose value of the entire glucose monitoring period.
MAGE	Mean amplitude of glycaemic excursions. Also described as intra-day glycaemic variability.
MODD	Mean of daily differences. Also described as inter-day glycaemic variability.
Dietary intake variation	
Day-to-day individual variation	The mean and SD of both the SD and CV% of each participant's daily intake of each dietary constituent (energy, glycaemic load, carbohydrates, fat, and protein).
Day-to-day group variation	The mean and SD of the daily averages of the dietary constituents (energy, glycaemic load, carbohydrates, fat, and protein) of the entire participant group. (See table below.)

	Day 1	**Day 2**	**Day 3**		**SD**	**CV%**
Participant 1	Individual value for day 1	Individual value for day 2	Individual value for day 3	→	Day-to-day variation (SD) for participant 1	Day-to-day variation (CV%) for participant 1
Participant 2	Individual value for day 1	Individual value for day 2	Individual value for day 3	→	Day-to-day variation (SD) for participant 2	Day-to-day variation (CV%) for participant 2
Participant 3	Individual value for day 1	Individual value for day 2	Individual value for day 3	→	Day-to-day variation (SD) for participant 3	Day-to-day variation (CV%) for participant 3
	↓	↓	↓		**Average day-to-day individual variation (SD)**	**Average day-to-day individual variation (CV%)**
SD	SD for the group's dietary intake of day 1	SD for the group's dietary intake of day 2	SD for the group's dietary intake of day 3	**Average day-to-day group variation (SD)**		
CV%	CV% for the group's dietary intake of day 1	CV% for the group's dietary intake of day 2	CV% for the group's dietary intake of day 3	**Average day-to-day group variation (CV%)**		

Chapter 1 Problem statement

1.1 Background

Research suggest that the degree of blood glucose control is very much dependent on the intra- and inter-day variability in glucose levels, as well as the magnitude of glucose excursions in response to foods containing carbohydrate. For instance, Kohnert *et al.* (2012) are of the opinion that the effects of postprandial hyperglycaemia and large fluctuations in daily blood glucose concentrations are more damaging to one's health than chronically elevated daily glucose levels, while Hirsch (2005) stated that these parameters are more closely related to the risk for diabetic complications than fasted blood glucose levels. Evidence (Brownlee & Hirsch, 2006; Monnier *et al.*, 2006) also points to the harmful effects of glycaemic variability and postprandial hyperglycaemia in apparently healthy individuals.

Brownlee and Hirsch (2006) demonstrated that high postprandial glucose excursions are positively correlated to the production of free radicals and subsequent atherosclerotic pathogenesis in diabetic, as well as healthy individuals. Monnier *et al.* (2006) assessed the increased oxidative stress in type 2 diabetic patients and reported that acute glucose fluctuations are strongly correlated with free 8-iso prostaglandin F2α (8-iso PGF2α) (a reliable marker of oxidative stress), and is independent of mean 24-hour glucose levels, fasting plasma glucose values and HbA_{1c}. Similar observations were made by other researchers who also attributed the production of oxidative stress to glucose excursions and linking it to a heightened risk for vascular injury and subsequent cardiovascular disease (CVD) (Ceriello *et al.*, 2008; Quagliaro *et al.*, 2003). Furthermore, Esposito and colleagues (2002) found that acute hyperglycaemia in healthy individuals and those with impaired glucose tolerance caused an increase in the plasma concentrations of inflammatory cytokines. Inflammation has been widely linked to the pathogenesis of insulin resistance and type 2 diabetes mellitus (T2DM) (Akash *et al.*, 2018; Chen *et al.*, 2015; Moller, 2000) and to the increased risk for endothelial dysfunction in healthy individuals (Esposito *et al.*, 2002). It is alarming that the incidence of impaired glucose tolerance has risen among non-diabetic individuals and this reportedly triples their risk for future cardiovascular events or disease (Standl *et al.*, 2011).

The advancement in technology in recent years has made the application of continuous glucose monitoring (CGM) in clinical and research settings much easier. This has led to a shift in focus from measuring static parameters of glucose control to the continuous and dynamic measurement of

glycaemic variability (i.e. frequency and magnitude of 24-hour glucose oscillations) in the management of metabolic diseases, such as diabetes mellitus. However, it seems that CGM has a wider application than the management of already diagnosed diabetics, namely, it could serve as an early warning for individuals who may be at risk for future metabolic disease.

Supporting this rationality is a study by Thomas and colleagues (2016), for example, where CGM was used to determine the glucose profile of 10 sub-elite athletes over six days. They observed that, among these apparently healthy athletes, four individuals presented with a degree of glycaemic variability that could potentially indicate a pre-diabetic state as per their postprandial glucose excursions (i.e. > 7.8 $mmol \cdot L^{-1}$). The authors specifically commented that the athletes demonstrated differences in their tolerance of carbohydrates (regarding their abilities to maintain healthy postprandial glucose levels). Similar observations were made by Zeevi *et al.* (2015) on more than 800 healthy individuals. They found that an individual's glucose response to specific meals is not only dependent on the glycaemic load of the meal and insulin sensitivity of the individual, but also on lifestyle factors (e.g. diet and physical exercise), genetics and features of the gut microbiome. Although the researchers established that the amount of carbohydrate in a given meal is the primary determinant of the extent of the postprandial rise in blood glucose, the same meals had varying effects on the postprandial glucose response of the participants. The authors commented that individuals' tolerance to carbohydrate may vary considerably and suggested that sensitivity to carbohydrate is specific to each person.

In addition to diet, physical exercise is also regarded as an important aid in glycaemic control. It is, however, not clear what the exact mechanisms are whereby physical exercise exerts beneficial effects on glycaemic control (Roberts *et al.*, 2013), but the general consensus is that it lowers postprandial glucose (Cassidy *et al.*, 2017), insulin sensitivity (Gill, 2007; Roberts *et al.*, 2013) and HbA_{1c} (Solomon *et al.*, 2015; Winding *et al.*, 2018). Although acute exercise has been shown to improve postprandial glucose (Borror *et al.*, 2018; Shambrook *et al.*, 2018) and insulin resistance (Short *et al*, 2013), cardiovascular fitness has also been found to be inversely related to insulin resistance (Chen *et al.*, 2008) and positively related to glucose tolerance (Solomon *et al.*, 2015). In addition to the direct beneficial effects on glucose control, exercise is also implicated as a mediator in the development of chronic disease through its anti-inflammatory effects (Mathur & Pedersen, 2008). Although it could be asserted that exercise exerts protective effects towards one's health, the findings of Thomas *et al.* (2017) allude that certain individuals may actually be predisposed to insulin

resistance, irrespective of high physical fitness levels. In light of the findings of Zeevi *et al.* (2015) this predisposition may be related to carbohydrate intolerance.

In order to address questions relating to glucose control, it has to be established which markers are most appropriate for diagnoses of impaired glycaemic control, as well as to evaluate the effectiveness of interventions, such as diet or physical exercise. It seems, however, that there is not yet consensus in the literature as to which measure is most sensitive to detect changes in glucose control, or impaired glucose control. The diabetes and pre-diabetes diagnostic criteria of the American Diabetes Association (2017) (ADA) are based on either: i) glucose level after an 8-h fasted period; ii) 2-h post a 75 g glucose challenge; or iii) the level of HbA_{1c}. Numerous authors suggested, however, that the utilisation of fasting blood glucose levels and/or HbA_{1c} may not be the best methods for assessing glucose control, or the associated micro- and macrovascular risk in diabetic patients (Gorst *et al.*, 2015). It also needs to be considered that fasting blood glucose can fall within healthy ranges, while postprandial glucose tolerance is impaired (Bartoli *et al.*, 2011; Nathan *et al.*, 2007). HbA_{1c} is closely related to the average concentrations of blood glucose of the preceding two- to three-month period, but does not necessarily account for the degree of glycaemic variability (Brownlee & Hirsch, 2006; Cohen & Smith, 2008). Measuring postprandial glucose control rather than, or in addition to fasting blood glucose levels and HbA_{1c}, may provide valuable and additional information on an individual's degree of carbohydrate tolerance, and thus glucose control. The oral glucose tolerance test (OGTT) is more sensitive and specific than elevated fasting blood glucose, or HbA_{1c}, in identifying individuals with impaired glucose tolerance and those at higher risk of developing T2DM (Iskandar *et al.*, 2019; Kim *et al.*, 2016). Nonetheless, the 2-h [glucose] of the OGTT, on its own, is likely only 90% accurate in the diagnosis of diabetes mellitus (Kim *et al*, 2017).

Taking all of the above into account, as well as the convenience of CGM, it should be determined whether traditional measures of glucose control, including the OGTT, are sufficiently sensitive to identify individuals who may present with early signs of insulin resistance or pre-diabetes. Furthermore, the evidence that postprandial hyperglycaemia is associated with higher levels of oxidative stress and inflammation, even in non-diabetics, warrants the investigation of glycaemic variability in apparently healthy individuals.

1.2 Rationale for the study

With the prevalence and incidence of T2DM rising to epidemical numbers, it is crucial to establish what predisposes individuals to develop insulin resistance, as the latter is key in the pathogenesis of pre-diabetes and eventually, overt T2DM and metabolic disease. Since it is in every sense better to prevent than to treat, this study is important to determine whether the traditional markers of glucose control and insulin sensitivity, or alternatively, measures of glycaemic variability, are most sensitive in the detection of at risk individuals. Therefore, the potential exists that CGM may have broader applicability in the fight against metabolic disease, and specifically, T2DM.

The OGTT is widely used as a diagnostic test to determine whether an individual has impaired glucose tolerance. It has been suggested to be a more sensitive and specific marker of glycaemic control than HbA_1c or levels of fasting glucose. The question remains, though, what causes the transition from healthy glucose tolerance to impaired glucose tolerance? Is the data that can be obtained from an OGTT sufficient to determine whether an individual's glucose control is adequate, or are there other factors, especially lifestyle factors, that could contribute to varying degrees of glycaemic variability in individuals, even though they present with healthy glucose tolerance? If it is established that overall, intra-, and inter-day glycaemic variability, measured using CGM, show differences between individuals who otherwise present with healthy glucose control, the continuous measurement of blood glucose levels may become a useful diagnostic tool for earlier detection of declines in glucose control.

1.3 Purpose of the Study

Good evidence exists to support the link between exaggerated postprandial deviations in blood glucose levels and the risk for future cardiometabolic disease, even in apparently healthy persons and, in the absence of atypical traditional markers of glycaemic control. Therefore, the purpose of this study is to investigate glucose control and glycaemic variability during free-living conditions in apparently healthy men and in relation to cardiorespiratory fitness and dietary glycaemic load.

1.4 Aims

1.4.1 Primary Aims

- To explore the relationships between cardiorespiratory fitness (CRF) and glycaemic variability, the traditional markers of glucose control (HbA_{1c}, OGTT, and fasting plasma glucose) and insulin sensitivity.

Hypothesis 1: Cardiorespiratory fitness will be most strongly correlated with insulin sensitivity, followed by glycaemic variability and HbA_{1c}.

- To determine the relationships between dietary glycaemic load and glycaemic variability, glycaemic control, and insulin sensitivity.
  Hypothesis 2: Glycaemic load will be most strongly correlated to glycaemic variability, followed by insulin sensitivity (negative correlation) and glucose control (negative correlation).

1.4.2 Secondary Aims

- To investigate the effects of dietary intake with different macronutrient ratios on glycaemic variability, glucose control and insulin sensitivity.
  Hypothesis 3: Low carbohydrate-high fat food consumption will be associated with least glycaemic variability, healthy glucose control and high insulin sensitivity.
- To investigate the relationship between glycaemic variability and markers of glucose control.
  Hypothesis 4: There will be weak correlations between glycaemic variability and traditional glucose control measures.
- To determine the relationships between the parameters of glycaemic variability and insulin sensitivity.
  Hypothesis 5: There will be an inverse relationship between insulin sensitivity and glycaemic variability.

1.5 Objectives

1. Determine the CRF of participants via a maximal treadmill test.
2. Calculate the dietary glycaemic load of the participants over a 14-day period.
3. Perform a K-means cluster analysis using the macronutrient intake of the participants to group them according to dietary macronutrient composition.
4. Quantify the intra- and inter-day glycaemic variability of the participants over a 14-day period via CGM.
5. Test and quantify the traditional glycaemic control markers (HbA_1c, fasting glucose, 2-h glucose, and glucose and insulin area under the curve [AUC] of the OGTT) of the participants.
6. Measure the insulin resistance of the participants.
7. Measure body fat percentage (BF%), waist and hip circumferences, and height and weight of the participants.

1.6 Overview of chapters

The second chapter reviews the literature on glucose control, as well as the importance and measurement thereof. Factors influencing glucose control are also discussed, as well as the deterioration of glucose control and subsequent health effects. Studies reviewed include those that have identified increased glycaemic variability and/or postprandial glucose excursions in apparently healthy individuals.

Chapter three describes the study design, measures and methods used for data collection and analysis.

The results of the study are encapsulated in the fourth chapter. This includes the characteristics and CRF of the participants, as well as their glucose control and insulin sensitivity, and glycaemic variability.

Chapter five discusses the findings and limitations of the study.

Chapter six comprises the study conclusion, practical implications, and recommendations for future research.

Chapter 2 Literature review

2.1 Introduction

High postprandial glucose excursions have been positively correlated to the increased production of free radicals (Monnier *et al.*, 2006) and pro-inflammatory cytokines (Esposito *et al*., 2002) which is linked to a heightened risk for vascular injury and subsequent cardiovascular disease (CVD) (Ceriello *et al.*, 2008; Quagliaro *et al.*, 2003). It has also been linked to the pathogenesis of insulin resistance and type 2 diabetes (T2DM) (Akash *et al*., 2018; Chen *et al*., 2015; Moller, 2000). It is alarming that the incidence of impaired glucose tolerance has risen among non-diabetic individuals and this reportedly triples their risk for future cardiovascular events or disease (Standl *et al*., 2011).

Traditionally, fasting plasma glucose (FPG), glycated haemoglobin (HbA_{1c}) and the 2-h [glucose] after an oral glucose tolerance test (OGTT) are used as indicators of glucose control. Specified levels in the blood serve as the diagnostic criteria for T2DM and pre-diabetes (ADA, 2017); nevertheless, these cut-off values are not universally agreed on. Furthermore, these traditional measures do not reflect the degree of glucose fluctuations during the day (MacLeod *et al.*, 2013; Roberts *et al.*, 2013) as they only provide a snapshot of an individual's state of glucose control. The quantification of glucose control is complex, as it involves many aspects of bodily function and physiological conditions wherein glucose homeostasis needs to be maintained. Thus, it is very unlikely that a single measure can account for all aspects of glucose control (McDonnell *et al.*, 2005). It has been proposed that glycaemic variability is more sensitive to assess glucose control (Mikus *et al.*, 2012a; Mikus *et al.*, 2012b) and its parameters are better able to distinguish between healthy individuals and those with impaired glucose tolerance or T2DM (Acciaroli *et al.*, 2018; Longato *et al.*, 2018). Supporting this rationality are studies that revealed pre-diabetic glucose levels among apparently healthy sub-elite athletes (Thomas *et al.*, 2016). Furthermore, there is evidence that an individual's glucose response to specific meals is not only dependent on the glycaemic load of the meal, or the insulin sensitivity of the individual (Zeevi *et al.*, 2015).

The consensus is that regular exercise improves postprandial glucose (Cassidy *et al.*, 2017), insulin sensitivity (Gill, 2007; Roberts *et al*., 2013) and HbA_{1c} (Solomon *et al.*, 2015; Winding *et al.*, 2018). Notably, acute exercise has also been shown to improve postprandial glucose (e.g. exercising after a meal) (Borror *et al.*, 2018; Shambrook *et al.*, 2018) and insulin resistance, which is improved for several hours after a bout of exercise (Short *et al.*, 2013).

2.2 Control of blood glucose levels

The principle of homeostasis (i.e., the maintenance of a constant internal environment) is just as applicable to blood glucose levels as to all other physiological systems in the human body (Widmaier *et al.*, 2014). Control of blood glucose levels involves hormones and signalling pathways to maintain glucose levels within a certain range during two different conditions, namely the fasted (3 $mmol \cdot L^{-1}$ – 5.7 $mmol \cdot L^{-1}$) and the postprandial state (< 5.7 $mmol \cdot L^{-1}$ – 7.8 $mmol \cdot L^{-1}$). After the ingestion and metabolism of food, the fasted state resembles the homeostatic condition whereby stored energy is relied upon. Alternatively, the postprandial state resembles the homeostatic condition which includes immediate energy utilisation or the storage thereof (Widmaier *et al.*, 2014). Blood glucose regulating hormones and signalling pathways that take effect during the fasted state are responsible for the maintenance of constant blood glucose levels. Although other hormones, such as catecholamines, growth hormone and cortisol, influence blood glucose levels under certain conditions (Gerich, 2000; Lecavalier *et al.*, 1989), the principle hormones responsible for the control of blood glucose levels are the two pancreatic hormones, namely: insulin and glucagon (Widmaier *et al.*, 2014). During the postprandial state, raised blood glucose levels are counteracted by the disposal of glucose from the circulation into the systemic cells (ranging from the skeletal muscles to the brain and red blood cells) (Widmaier *et al.*, 2014).

An individual's postprandial blood glucose response is mostly related to the intake of food, in conjunction with the secretion of insulin and the sensitivity of the body's tissues to insulin (Blaak *et al.*, 2012). In healthy individuals, insulin is produced and secreted by the pancreatic islet beta-cells (Corkey, 2012; White & Copps, 2016) mainly in response to a rise in blood glucose levels (Meglasson & Matschinsky, 1986) and results in a subsequent lowering in glucose levels (Liang, 1994). An insulin response is also elicited by incretin hormones that are secreted into the circulation by the enteroendocrine cells of the stomach and intestines in response to the ingestion of food, especially carbohydrates (Drucker, 2007; Greiner & Bäckhed, 2011). The hormone, insulin, primarily facilitates the uptake of glucose from the blood into the systemic cells (Saltiel & Kahn, 2001).

After the consumption of food, pure sugars in their simplest forms (monosaccharides) are directly absorbed by the intestinal cells (Boron & Boulpaep, 2009). Monosaccharides that are bound in more complex forms, for example polysaccharides (e.g. starches comprised of long glucose polymer

chains), or sucrose (glucose and fructose) are digested into their monosaccharide constituents and then absorbed. Due to starches and carbohydrates consisting mostly of glucose subunits, the resultant product of digestion is mostly glucose (Widmaier *et al*., 2014). Galactose (forms lactose when bound to glucose) and fructose (the sugar naturally found in fruits; the other constituent of sucrose besides glucose) (Widmaier *et al.*, 2014) are ingested in smaller amounts. However, when the diet consists of excessive amounts of added sugar or high-fructose corn syrup, fructose is ingested in larger amounts (Fung, 2018). Many factors influence the rate of gastric emptying, the rate at which nutrients are absorbed, and subsequently the rate and extent at which blood glucose levels rise after ingestion of carbohydrate-containing foods (Russell *et al.*, 2016). Sugars and processed carbohydrates are absorbed faster and subsequently cause higher glucose excursions than complex and fibre-rich starches (Jenkins *et al.*, 1987).

Skeletal muscles are the main sites of postprandial glucose disposal (Defronzo, 2009), accounting for up to 75% of glucose uptake from the circulation via the action of insulin (Saltiel & Kahn, 2001). Insulin allows glucose to be transported from the blood into muscle cells via the activation of signalling pathways to facilitate translocation of GLUT4 transporters (glucose transporter type 4) to the plasma membranes of the cells (Petersen & Shulman, 2018; Samuel & Shulman, 2016). By allowing glucose transport into the muscle cells (otherwise termed glucose disposal), insulin promotes glucose utilisation (Petersen & Shulman, 2018). About 25% of the glucose that enters the cell is broken down via glycolysis and used for immediate energy production. The rate-limiting enzyme of glycolysis, hexokinase II, is upregulated by insulin for this purpose (Petersen & Shulman, 2018). The remaining 75% of the glucose is stored via glycogen synthesis, which is also promoted and enabled by insulin (Petersen & Shulman, 2018). Stored glycogen is broken down to glucose-6-phosphate and then directly used for energy production. Thus, all the glucose that is taken up by skeletal muscles is for the sole purpose of its own energy production (Petersen & Shulman, 2018).

White adipose tissue is another site of glucose disposal. This tissue only accounts for 5 – 10% of total glucose uptake, and is also dependent on the actions of insulin, via similar pathways as those in skeletal muscle cells (Samuel & Shulman, 2016).

The liver is responsible for about 25% of the postprandial uptake of glucose (MacLeod *et al.*, 2013), but is not dependent on insulin action for glucose transportation from the circulation (Gerich, 1993; Petersen & Shulman, 2018; Widmaier *et al*., 2014). The transition between the catabolic and anabolic processes of the liver is, however, largely regulated by insulin (Samuel & Shulman, 2016). Hepatic

glucose production is downregulated through the inhibition of the enzyme, glycogen phosphorylase (involved in glycogenolysis, the breakdown of stored muscle and liver glycogen to produce glucose) by insulin (Samuel & Shulman, 2016) and also through the inhibition of gluconeogenesis (hepatic glucose production from non-glycogen substrates such as lactate, glycerol and amino acids) (Gerich, 2000; Saltiel & Kahn 2001). Simultaneously, insulin stimulates glycogen synthesis (Petersen & Shulman, 2018) by activating the enzyme glycogen synthase (Samuel & Shulman, 2016), which results in an increased rate of glycogen synthesis (Caputo *et al.*, 2017). Excess glucose that could not be stored as glycogen, is utilised by de novo lipogenesis (Caputo *et al*., 2017).

Although insulin promotes the hepatic synthesis of macromolecules (Petersen & Shulman, 2018), the anabolic processes of the liver are, however, not solely dependent on insulin. The rate of gluconeogenesis and lipogenesis are also raised by an increased flux of substrates (Samuel & Shulman, 2016). Importantly, the liver is the only organ that can metabolise the monosaccharide, fructose. High levels of fructose in the liver also enhance hepatic glycogen production and de novo lipogenesis (Samuel & Shulman, 2016).

The liver is the main contributor to the maintenance of fasting blood glucose levels. It supplies about 80% of the glucose in post-absorptive conditions (Gerich, 2000), while insulin levels are at basal concentrations and glucagon levels are high (Breckenridge *et al.*, 2007). Glucagon is secreted by the pancreatic alpha cells when blood glucose levels are low in order to stimulate glycogenolysis and gluconeogenesis in the liver (Widmaier *et al*., 2014). The presence of the enzyme, glucose-6-phosphatase, allows the liver to produce glucose from glucose-6-phosphate (the product of glycogenolysis), which is then released into the circulation (Gerich, 1993; Widmaier *et* al., 2014).

Although skeletal muscle is another storage site for glycogen, this glycogen does not generate glucose for release into the bloodstream (Gerich, 1993). Rather, glucose-6-phosphate is directly broken down and used for energy by the skeletal muscle itself, without converting back to pure glucose (Widmaier *et al*., 2014). Skeletal muscles do, however, release lactate or alanine, which are alternative products of glycogen breakdown (Gerich, 1993) during anaerobic glycolysis (Petersen & Shulman, 2018). These three-carbon units are released into the circulation and mostly taken up and used by the liver as substrates for gluconeogenesis (Petersen & Shulman, 2018; Widmaier *et al*., 2014). During fasted or post-absorptive conditions, as well as during physical exercise, the liver therefore stands central to the regulation of blood glucose levels by producing and secreting glucose to prevent drops in blood glucose levels (Samuel & Shulman, 2016).

During fasting conditions, the control of blood glucose levels revolves mostly around the supply of glucose to the blood from the tissues in order for blood glucose levels to remain constant and not drop to hypoglycaemic levels. The liver is responsible for most of the glucose that is needed to maintain euglycaemia during fasted conditions of up to 12 – 14 hours (Gerich, 1993). As the period of a fast continues for longer, the glycogenolysis of other tissue, such as skeletal muscle, increases. Only after 48 hours of fasting does gluconeogenesis become the predominant blood glucose supply process and by 60 hours of fasting, gluconeogenesis is the only process whereby the liver provides glucose (Gerich, 1993). Ketone bodies also start increasing after two days of fasting and become a major source of energy as the fasted condition continues (Cahill, 2006; Newman & Verdin, 2014).

2.2.1 Typical fluctuations in blood glucose concentrations

Tight control of blood glucose levels is imperative in the maintenance of health (Blaak *et al.*, 2012; Fowler, 2011) since deleterious health consequences have long been attributed to frequent episodes of hypo- and hyperglycaemia (Gerich, 1993). Interestingly, oscillating glucose levels are more detrimental to the maintenance of health than consistent elevated fasting or daily mean levels of glucose (Ceriello *et al.*, 2008; Ceriello *et al.*, 2012; Ceriello & Kilpatrick, 2013). This finding will be discussed in more depth in section 2.5.1.

The typical range within which blood glucose fluctuations should and may occur is difficult to define, as various minimum and maximum concentrations are proposed by various authors. A typical healthy range has also not been defined by organisations, such as the American Diabetes Association (ADA) and the World Health Organization (WHO). For example, suggested ideal or healthy ranges vary from: > 2.2 $mmol \cdot L^{-1}$ to < 10.0 $mmol \cdot L^{-1}$ (Ludwig, 2002); 4.0 $mmol \cdot L^{-1}$ to 9.0 $mmol \cdot L^{-1}$ (Gerich, 2000); 4.4 $mmol \cdot L^{-1}$ to 6.6 $mmol \cdot L^{-1}$ (Ramasarma & Rafi, 2016); and 4.0 $mmol \cdot L^{-1}$ to 6.0 $mmol \cdot L^{-1}$ (Thomas *et al.*, 2016). Hypo- and hyperglycaemia are also terms that are widely used, without always being defined in absolute terms.

It is generally agreed that blood glucose levels should remain relatively stable, that fluctuations should be rare after an overnight fast, and that an average concentration of 5.0 $mmol \cdot L^{-1}$ in this state is deemed “healthy” (Gerich, 2000). According to two research groups, it was suggested that fasting blood glucose should never drop below 2.8 $mmol \cdot L^{-1}$ (Consoli *et al.*, 1987) or 3.0 $mmol \cdot L^{-1}$ (Gerich, 1993), however, there is no consensus on what is considered hypoglycaemia. In research and clinical settings, blood glucose concentrations below concentrations of 3.0 $mmol \cdot L^{-1}$ to 3.9 $mmol \cdot L^{-1}$ have

been labelled either 'hypoglycaemic', or the lower threshold for "healthy" fasting glucose. In other words, there is no consensus on what the minimum "healthy" glucose concentration is.

Hypoglycaemia is one of the major concerns for type 1 diabetes (T1DM) patients, as it occurs most frequently in this group (Joy *et al.*, 2010) compared to healthy individuals or type 2 diabetics (Lessan *et al*, 2015), unless the latter patients receive intensive drug treatment (SEMDSA, 2017). Severe and prolonged hypoglycaemia should not occur in healthy individuals; it is usually prevented by the actions of the physiological control systems and feedback loops that maintain glycaemic homeostasis.

Nirantharakumar *et al.* (2012) determined the prevalence of hypoglycaemia among non-diabetic in-patients from the University Hospital Birmingham using different cut-off glucose concentrations, i.e., 3.3 mmol·L^{-1}; 3.0 mmol·L^{-1}; 2.7 mmol·L^{-1}; 2.5 mmol·L^{-1}; and 2.2 mmol·L^{-1}. They estimated that 13 patients per 10 000 hospital admissions younger than 65 years would be considered hypoglycaemic with a 2.7 mmol·L^{-1} cut-off value, and 50 per 10 000 hospital admissions with a 3.3 mmol·L^{-1} cut-off value. Additionally, they discovered that all hypoglycaemic events were accompanied by co-morbidities namely: sepsis, kidney disease and alcohol-dependence. As a final comment, the researchers concluded that hypoglycaemia, defined as blood glucose levels lower than 2.7 mmol·L^{-1}, was very rare in non-diabetics outside of critical care. According to Gerich (1993), the liver can maintain blood glucose levels up to 60 hours of fasting (Gerich, 1993) and prevent significant drops in blood glucose during exercise. Remarkably, Stewart & Fleming (1973) reported no hypoglycaemic symptoms in an overweight, 27-year old male patient during a supervised therapeutic fast of 382 days.

A similar issue exists for the definition of hyperglycaemia. It is more difficult to define, with an absolute threshold, what is deemed "healthy", or what is associated with elevated risk. Once again, many different glucose concentrations have been used to classify postprandial hyperglycaemia. The ADA (2017) has set the upper threshold for ideal fasting glucose at 5.7 mmol·L^{-1}, while 7.8 mmol·L^{-1} is set as the pre-diabetes cut-off value after a 2-h OGTT. The ADA (2017) also contends that the peak glucose value in healthy individuals after a meal very rarely exceeds 7.8 mmol·L^{-1}. Consequently, a number of researchers consider this value (7.8 mmol·L^{-1}) the maximum postprandial glucose concentration in healthy individuals (Acciaroli *et al.,* 2018; Borg *et al.,* 2010; Madhu *et al.*, 2013; Rodriquez-Segale *et al.*, 2018), although others define hyperglycaemia at a higher limit of 10.0 mmol·L^{-1} (Cassidy *et al.,* 2017; MacLeod *et al.,* 2013). As will be discussed in section 2.5.1, the upper threshold is a critical parameter since a higher "glucose spike" after a meal is associated with more serious health risks.

Insulin reacts to the ingestion of food and the subsequent rising levels of blood glucose to stunt the postprandial surge in glucose and to lower the levels in the circulation. The secretion of insulin should match the relative rate of entry of absorbed glucose into the bloodstream. As glucose levels begin to drop due to the actions of insulin, the blood levels of insulin also decrease, following the same pattern as glucose (assuming healthy insulin sensitivity). Blood glucose levels are thus regulated via a typical negative feedback loop (Widmaier *et al*., 2014).

Depending on the content and volume of the meal that was consumed, blood glucose typically reaches peak levels between 30 and 90 minutes and should return to basal levels between 120 to 180 minutes after a meal (ADA, 2001; Brand-Miller *et al.*, 2009; Gerich, 1993). It is suggested that postprandial glucose levels should not rise by more than 3.0 $mmol \cdot L^{-1}$ above fasting or basal levels (Tabák *et al.*, 2012) and should, ideally, only drop to the pre-prandial, fasting or basal concentrations (Jenkins *et al*., 1987; Ludwig, 2002). The latter is more likely to occur when glucose absorption from the intestines and the subsequent rise in blood levels happen over a prolonged period. In contrast, faster absorption rates cause higher postprandial blood glucose peak concentrations, as well as a larger area under the curve (AUC) of the total postprandial glucose response (Jenkins *et al*., 1987). Thus, the higher and quicker blood glucose rises after ingestion of carbohydrates or glucose, the more likely blood glucose levels are to "spike" and "over-shoot" typical postprandial glucose concentrations, as well as temporarily drop below basal levels in response to the secretion and action of insulin. Even though the latter situation could be seen as a typical response and usually occurs in the absence of hypoglycaemic symptoms (Gerich, 1993), it is an unfavourable reaction (Jenkins *et al*., 1987). The latter view is substantiated by evidence from more recent studies that are discussed in the next section.

2.3 Development of insulin resistance

The development of insulin resistance is the main driver for the increasing prevalence of elevated fasting glucose concentrations and postprandial hyperglycaemic episodes in an individual (Kahn, 2003; Templeman *et al.*, 2017). Thus, the deterioration of glucose control and rising circadian glucose levels could be described as the symptomatic manifestation of the development of, and progression towards T2DM in the presence of insulin resistance.

Petersen & Shulman (2018) defined insulin resistance as the state during which "higher circulating insulin levels are necessary to achieve the integrated glucose-lowering response", thus describing a

condition where the target tissues of insulin are less sensitive to the effects thereof, than what is typically expected. Hyperinsulinaemia is one of the major contributing causative factors leading to insulin resistance (Rizza *et al.*, 1985; Schofield & Sutherland, 2012). The development of insulin resistance due to hyperinsulinaemia necessitates for even more insulin to be secreted. Initially, glucose tolerance remains within healthy limits through compensatory increases in the secretion of insulin (Corkey *et al.*, 2012). However, persistent hyperglycaemia and hyperinsulinaemia exacerbate each other and systematically deteriorate towards the development of overt disease states. Pre-diabetes develops when blood glucose levels rise above healthy concentrations due to the pancreatic beta-cells being unable to secrete enough insulin (Tabák *et al.*, 2012). Further deterioration of beta-cell function and glucose control eventually leads to T2DM (Defronzo, 2009).

The precise mechanisms behind the development of insulin resistance is still an area of extensive research. Nevertheless, growing evidence suggests that the most likely explanation for deteriorating insulin sensitivity of the body's tissues is as a result of the body's reaction to over-nutrition (Caputo *et al.*, 2017), which can at least partly be attributed to improper nutrition (Ludwig & Ebbeling, 2018). Caputo *et al.* (2017) argues that over-nutrition has the ability to stimulate the vicious cycle of hyperinsulinaemia-insulin resistance.

The liver and skeletal muscles are the organs most involved in the development of insulin resistance and the consequential metabolic effects thereof (DeFronzo, 2009). White adipose tissue (WAT) insulin resistance has more recently been appreciated as an important role player in the pathophysiology of whole-body insulin resistance and related low-grade chronic inflammation (Caputo *et al.*, 2017; Petersen & Schulman, 2018; Shimobayashi *et al.*, 2018). Although glucose control processes are indeed integrated, the consequences of insulin resistance are tissue-specific (Petersen & Schulman, 2018).

Insulin resistance of the skeletal muscles leads to a decrease in insulin-dependent glucose uptake from the circulation (DeFronzo, 2009; Tabák *et al.*, 2012). As skeletal muscles are a large depot for postprandial glucose disposal, their insulin sensitivity is therefore important for the maintenance of healthy glucose tolerance. Accordingly, whole-body glucose turnover is largely dependent on skeletal muscle insulin sensitivity (Petersen & Schulman, 2018). Hyperinsulinaemia influences the insulin receptors of the skeletal muscle cells by altering the number of receptors, lowering their affinity for insulin and impairing signal transduction (Schofield & Sutherland, 2012). Hence, with the development of insulin resistance, GLUT4 translocation to the plasma membrane of muscle cells

is impaired so that glucose uptake decreases, and total body glucose uptake lowers as a result (Tabák *et al.*, 2012). When insulin resistance is not yet too severe, however, compensatory hyperinsulinaemia helps to preserve glucose transport so that healthy glucose tolerance levels are initially maintained (Peterson & Schulman, 2018). Impaired glucose tolerance develops when beta-cell dysfunction becomes evident, in addition to worsening insulin resistance (Schofield & Sutherland, 2012; Tabák *et al.*, 2012). Consequently, postprandial glucose levels elevate as a result (Nathan *et al.*, 2007). The development and progression of T2DM is especially related to postprandial hyperglycaemia (DiNicolantonio *et al.*, 2015), which adds to the vicious cycle.

The liver plays a very crucial and central role in the metabolic homeostasis of blood glucose levels and the progressing nature of insulin resistance towards T2DM (Caputo *et al*., 2017). When insulin action becomes less potent to suppress hepatic glucose production, hepatic insulin resistance ensues. Otherwise stated, the postprandial switch from net hepatic glucose production to net glucose uptake is less pronounced so that gluconeogenic rates in insulin-resistant livers are increased (Petersen & Schulman, 2018). Insulin-resistant skeletal muscle and WAT are largely involved in the development and progression of hepatic insulin resistance.

Insulin-resistant extrahepatic tissues cause an increased flux of substrates (i.e., glucose, glycerol, and free fatty acids) to the liver (Caputo *et al.*, 2017; Samuel & Shulman, 2016) and the uptake of these substrates by the liver is independent of insulin (Peterson & Schulman, 2018). This increased flux of substrates results in a series of effects. Firstly, an increased substrate flux to the liver results in upregulation of gluconeogenesis and lipogenesis (Samuel & Shulman, 2016). Increased hepatic fat production, or de novo lipogenesis, together with increases in free fatty acid delivery to the liver, contribute to high liver fat content and hepatic insulin resistance (Corkey *et al.*, 2012; Samuel & Shulman, 2016; Schofield & Sutherland, 2012), hepatosteatosis (Caputo *et al.*, 2017) and over time, non-alcoholic fatty liver disease (Samuel & Shulman, 2016). De novo lipogenesis is especially upregulated by excessive glucose and fructose delivery to the liver (Caputo *et al.*, 2017; Samuel & Shulman, 2016; Sevastianova *et al.*, 2012). Insulin potentiates de novo lipogenesis (Samuel & Shulman, 2016) which is, paradoxically, not diminished in the case of hepatic insulin resistance (Caputo *et al.*, 2017; Petersen & Schulman, 2018). The only present explanation of this paradoxical phenomenon, is that the increase in both de novo lipogenesis and liver fat content are due to the increased substrate delivery to the liver (which strongly associates with hepatic insulin resistance) in the case of chronic over-nutrition and extrahepatic insulin resistance (Caputo *et al.*, 2017; Petersen & Schulman, 2018). Over time, increases in liver fat content gives rise to the onset of chronic liver

inflammation, which is associated with tissue remodelling and fibrosis present in non-alcoholic steatohepatitis (Caputo *et al.*, 2017).

Insulin resistance of the liver also has extrahepatic effects, namely increases in circulatory levels of glucose and lipids. In fact, a marker of an insulin-resistant liver is high fasting plasma glucose levels (Nathan *et al.*, 2007; Tabák *et al.*, 2012). Insulin also regulates glycogen synthase (Petersen & Schulman, 2018), so that a decline in glycogen synthesis and storage by an insulin-resistant liver results in more glucose release into the circulation (Artese *et al.*, 2019). The concomitant diminished suppression of hepatic glucose production and the subsequent release of glucose into the circulation contributes to postprandial hyperglycaemia, in addition to fasted hyperglycaemia (Tabák *et al.*, 2012).

The major consequence of WAT insulin resistance is the failure to adequately suppress lipolysis (Artese *et al.*, 2019; Petersen & Schulman, 2018). The result is an increased release of free fatty acids into the circulation and subsequently the increased flux of free fatty acids to the liver (Caputo *et al.*, 2017). With adipocyte hypertrophy, there is a decrease in insulin-dependent glucose uptake, accumulation of oxidative stress products and development of endoplasmic reticulum dysfunction. Remodelling of WAT takes place secondary to chronic over-nutrition and obesity (Caputo *et al.*, 2017). Furthermore, insulin resistance of WAT is strongly associated with the increased expression and presence of pro-inflammatory macrophages and cytokines in the adipose tissue and subsequent chronic, low-grade inflammation (Caputo *et al.*, 2017; Shimobayashi *et al.*, 2018). This increase in systemic inflammation that is being ascribed to originating from insulin-resistant adipose tissue, has been proposed to contribute to the decline in whole-body insulin sensitivity (Caputo *et al.*, 2017; Schofield & Sutherland, 2012) and a major contributing factor to the metabolic syndrome (Caputo *et al.*, 2017).

2.4 Factors influencing glucose control

Poorly controlled blood glucose is a hallmark of type 1 and 2 diabetes mellitus (Lakhtakia, 2013). In addition to declining whole-body insulin sensitivity, which is the major factor contributing to worsening of glucose control, there are other independent factors that also influences the insulin response and insulin sensitivity of the body's tissues, namely: diet, gut microbiota, exercise, and cardiorespiratory fitness.

2.4.1 Dietary characteristics

One of the main factors that influences the postprandial blood glucose (and insulin) response is meal composition.

Carbohydrates cause the most marked increase in blood glucose levels, although the resultant postprandial glucose responses are not the same for all carbohydrate-containing foods. For this reason, Jenkins *et al.* (1987) first described the concept of the glycaemic index (GI) as a measure whereby carbohydrate foods are characterised according to its effect on blood glucose levels (Vrolix & Mensink, 2010). Although the GI of carbohydrates correlates ($r = 0.730$; $p < 0.001$) with the peak in blood glucose after ingestion (Brand-Miller *et al.*, 2009), the postprandial glucose AUC, however, does not purely depend on the type and characteristics of the ingested carbohydrate; it also depends on the amount of carbohydrates consumed. Therefore, it was suggested that the glycaemic load (GI × amount of carbohydrates) should be calculated (Willett *et al.*, 2002; Russell *et al.*, 2016), as glycaemic load is the best predictor ($r = 0.930$; $p = 0.022$) of postprandial glucose responses (Wolever & Bolognesi, 1996).

Refined carbohydrates, sugary foods and potatoes are typical high GI foods, while whole-wheat, high dietary fibre foods are usually of lower GI (Willet *et al.*, 2002). As was already mentioned, the speed at which carbohydrates are digested (influenced by the GI of the carbohydrates) and subsequently absorbed from the intestines into the blood, is a large determinant of the extent to which blood glucose rises (Jenkins *et al*., 1987). The rate of absorption also influences the total AUC of the glucose response (Jenkins *et al*., 1987). It is therefore not surprising that the consumption of habitual diets with a low glycaemic load associates (although weakly) ($\beta = 0.003$; $p = 0.034$) with lower levels of HbA_{1c} in T2DM Latino adults (Wang *et al.*, 2015).

The importance of postprandial blood glucose responses, even in healthy individuals, is underwritten by the "Carbohydrate-Insulin Model of Obesity" (CIMO) (Ludwig & Ebbeling, 2018). This model proposes that chronic over-nutrition leads to the development of insulin resistance (Caputo *et al*., 2017). According to the CIMO, the relative hypoglycaemia that is extremely prevalent after the ingestion of high GI meals further stimulates hunger and successive food intake (Ludwig & Ebbeling 2018; Ludwig 2002). The incremental rise in blood glucose in response to high GI carbohydrate foods stimulates a greater secretion of insulin and suppression of glucagon compared to the response following a low GI meal. This elicits a more exaggerated postprandial anabolic response with the effects of the high insulin-glucagon ratio persisting after GI nutrient absorption has ceased. The result

is a drop in blood glucose levels into the range of relative hypoglycaemia (below typical fasting levels). This hypoglycaemia triggers persistent hunger and food intake (Campfield *et al.*, 1996; Friedman & Granneman, 1983), and reportedly, with a preference for more high GI or sugary foods (Rodin *et al.*, 1985). Thus, a cycle of increased (high GI) food intake, hyperglycaemia and hyperinsulinaemia, followed by hypoglycaemia ensues (Ludwig, 2002). In other words, it could be suggested that excessive intakes of high GI foods can lead to higher glycaemic variability.

The results of a study by Ebbeling *et al.* (2020) support the CIMO. Ebbeling *et al.* (2020) found that energy expenditure increases with low- compared to high-carbohydrate diets. After at least 10% body weight loss during participation in the Framingham State Food Study (Shimy *et al.*, 2020), 164 participants were randomised to consume one of three diets with either 60%, 40% or 20% of energy from carbohydrates and 20%, 40% or 60% of energy from fats. All three diets comprised 20% of energy from proteins and continued for 20 weeks. Energy intake was controlled individually to ensure weight maintenance. This study revealed that energy requirements decreased as the percentage of carbohydrates increased. These findings support the rationality that greater amounts of ingested carbohydrates hinder the utilisation of energy, by promoting energy storage. The mechanism for the latter relates to the higher levels of insulin that accompany consumption of carbohydrates. Therefore, insulin is also referred to as the "fat-storing" hormone (Fung, 2018).

In theory, GI and/or glycaemic load should be ideal to establish which carbohydrate elicit more favourable postprandial glucose responses. However, there are additional meal components that influence, or effectively change, the GI and postprandial glucose response of mixed meals. For example, adding protein or fats to a meal usually (Venn & Green, 2007), but not always (Wolever & Bolognesi, 1996) lowers the effective GI of the meal. Fats tend to slow gastric emptying and subsequently slow the rate of absorption of ingested food, thus contributing to a lower postprandial glucose peak. The addition of protein to a meal also has the potential to lower the postprandial glucose response, although this is achieved through an increase of insulin secretion (Jenkins *et al.*, 1981; Linn *et al.*, 2000; Linn *et al.*, 1996). Certain essential and non-essential amino acids, such as arginine, leucine, isoleucine, alanine and phenylalanine, act as glucose-independent stimuli for insulin secretion (Russell *et al.*, 2016). Interestingly, there is evidence that vinegar added to a high GI meal may also lower the postprandial glucose response (Russell *et al.*, 2016).

As shown by Vrolix & Mensink (2010), GI cannot be used to accurately predict the incremental blood glucose AUC of a healthy individual, as significant variation exists between individuals in response

to the same GI carbohydrates. Otherwise stated, the postprandial glucose of a healthy individual is not fully accounted for by the GI and glycaemic load of the food that was ingested. This notion is supported by Hopper *et al.* (2013), who found that high glycaemic load meals correlated significantly with blood glucose concentrations at two hours after an OGTT in young adults, however, this relationship weakened with medium and low glycaemic load meals. Their data also revealed that postprandial glucose levels varied significantly among participants, as it is also dependent on the degree of insulin sensitivity of individuals.

To fully evaluate the effect of diet on glucose control, it is necessary to look at the long-term effects of diets or dietary constituents as well, and not only the immediate postprandial effect of certain foods.

Seidelmann *et al.* (2018) conducted a prospective cohort study over 25 years, which they included in a subsequent meta-analysis to investigate the association between carbohydrate intake and all-cause mortality in adults of all ages and from various countries. The meta-analysis revealed high (> 70% of energy) and low (< 40%) intakes of carbohydrates were associated with increased mortality (hazard ratios of 1.20 and 1.23, respectively), while a diet comprising of ~ 50% energy from carbohydrates, was associated with the lowest mortality. The authors noted that it is important to consider either which macronutrients replace carbohydrates when lowering carbohydrate intake, or which macronutrients are replaced by carbohydrate foods when increasing carbohydrate intake, as well as the sources of these foods. Although associations between carbohydrate intake and mortality were found, it cannot be said with certainty what percentage of carbohydrate intake holds the highest mortality risk. Results from studies included in the meta-analysis were not consistent and there were also significant trends of increased risk with other lifestyle factors, such as smoking and low physical activity. The inclusion of the number of different types of carbohydrates in a specific diet was also not accounted for in the meta-analysis. If it is true that individuals react differently to identical foods, it cannot be expected that large cohort studies on total carbohydrate intake can provide conclusive evidence that are generalizable.

It is suggested that lower GI diets decrease the risk for the development of T2DM (Willet *et al.*, 2002) and diets high in sugar are implicated in the aetiology of coronary heart disease, insulin resistance and subsequent impaired glucose tolerance (DiNicolantonio *et al.*, 2016). A meta-analysis by Ajala *et al.* (2013) reviewed numerous randomised controlled trials on the effects of dietary interventions lasting at least six months in adults older than 18 years. They found that the best diets for improving cardiovascular risk factors and glucose control, as measured by HbA_{1c}, were the low-carbohydrate

(20 g – 120 g carbohydrates per day, or 13% - 45% of energy intake from carbohydrates), low GI, Mediterranean and high-protein diets. This study's main finding was that modifying the macronutrient composition of various diets was effective in improving glucose control in people diagnosed with T2DM. Ajala *et al.* (2013) concluded that all diets where the intake of carbohydrates or glycaemic load are limited, had beneficial effects on glucose control, weight management and CVD risk in T2DM patients. Similarly, and in another systematic review and meta-analysis, Meng *et al.* (2017) reported that HbA_{1c} levels are positively affected by low-carbohydrate diets in comparison to normal- or high-carbohydrate diets in T2DM patients.

Tay *et al.* (2018) conducted a randomised controlled trial over a period of two years in type 2 diabetics. Both dietary intervention groups participated in supervised exercise sessions. They found that an energy-restricted, low-carbohydrate (14% of energy), high unsaturated fat (58% of energy) diet was equally effective to lower weight and HbA_{1c}, than an isocaloric high-carbohydrate (53% of energy), low-fat (< 30% of energy) diet. However, the low-carbohydrate diet was superior in sustaining greater reductions in diabetic medications. The blood lipoprotein profile and diurnal glucose stability (glycaemic variability) were also more favourably affected by the low-carbohydrate diet.

The diets and metabolic disease risk of more than 13 000 Koreans were analysed by Lee *et al.* (2018). Significant associations were found between the intake of carbohydrates, higher levels of triglycerides and lower levels of high-density lipoprotein cholesterol (HDL-cholesterol). This finding is consistent with many other studies (Dashti *et al.*, 2006; Hussain *et al.*, 2012; Meng *et al.*, 2017; Santos *et al.*, 2012; Tay *et al.*, 2014). Furthermore. elevated blood triglycerides, together with low [HDL-C] are associated with insulin resistance and a higher risk for CVD (Da Luz *et al.*, 2008; Ferrannini *et al.*, 2007; Ormazabal *et al.*, 2018).

The beneficial effects of lowering dietary carbohydrates are not limited to T2DM patients. Stentz *et al.* (2016) studied obese men and women with pre-diabetes, who were randomised into two groups. One group followed a high-protein diet (30% protein, 30% fat, 40% carbohydrate) and the other a high-carbohydrate diet (15% protein, 30% fat, 55% carbohydrate). All the participants (n = 12) on the high-protein diet experienced remission of their pre-diabetes, while two-thirds (8 of the 12 participants) in the high-carbohydrate group remained pre-diabetic. With the amount of fat controlled, the results of this study suggest that replacing carbohydrates for protein improves glucose

tolerance as measured by the OGTT. In fact, the high-protein group also presented with a large improvement in insulin sensitivity.

2.4.2 Carbohydrate sensitivity and the gut microbiome

Two independent studies conducted by Zeevi *et al*. (2015) and Thomas *et al*. (2016) (both discussed in more detail under "Continuous glucose monitoring and glycaemic variability") mentioned that it could be deducted from their results that the response to carbohydrate foods is an individualised phenomenon, with factors besides insulin sensitivity playing a role. Zeevi *et al*. (2015) specifically commented that individuals with similar glucose tolerance may have varying sensitivity to carbohydrates and subsequent diverse glucose responses to mixed meals. This perspective is supported by emerging evidence that the gut microbiota plays a definitive role in the blood glucose response to ingested food.

There is evidence that links obesity and diabetes mellitus, as well as low-grade inflammation to particularities of the gut microbiome (Cox *et al.*, 2015; Festi *et al.*, 2014; Greiner & Bäckhed, 2011; Van Olden *et al.*, 2015). The intestinal bacteria are highly involved in a number of physiological processes and regulatory systems (Van Olden *et al.*, 2015), among which are their involvement in the extraction of energy from ingested foods (Cox *et al.*, 2015; Zhang *et al.*, 2013). There is also a definitive role of the gut microbiota in glucose tolerance as seen in a study done by Suez *et al.* (2014). They found that the consumption of non-nutritive artificial sweeteners induced a worsening of glucose tolerance in relation to alterations of the gut microbiota. It seems that the composition and diversity of the gut microbiota, which is influenced by diet (Festi *et al*., 2014), genetic make-up and immune status (Greiner & Bäckhed, 2011), directly affect metabolic processing, energy balance and, ultimately, metabolic health (Van Olden *et al*., 2015). Numerous studies (reviewed by Cox *et al.* (2015) and Gentile and Weir (2018)) found that the intestinal microbiota is altered when habitual diet is changed, whether it be changes in macronutrient composition or a pronounced shift between the intake of plant or animal products. Thus, it seems that levels of low-grade inflammation, insulin sensitivity and postprandial metabolism of glucose and lipids are significantly affected by changes in the gut microbiome (Van Olden *et al.*, 2015).

The concentration of insulin after 30 minutes into a standard OGTT is used to distinguish between people who have a high or low insulin response to carbohydrates (Ludwig & Ebbeling, 2018). After ingestion of glucose (or any meal), the initial signal for insulin secretion by the pancreatic beta-cells

is mediated by the incretin hormones (Drucker, 2007). In other words, insulin secretion has been signalled even before blood glucose concentration increases significantly. This first-phase insulin secretion is signalled rapidly and is generally used to determine beta-cell function. It can also be determined by the amount of insulin that is secreted in the first 10 minutes of a hyperglycaemic clamp (Stumvoll *et al.*, 2000). The incretin hormones, therefore, play a crucial role in controlling the excursion in blood glucose in response to the consumption of glucose in any form (Drucker, 2007).

It can be argued that the incretin effect, considered together with the role of the gut microbiome in glucose tolerance, provides evidence that the extent of postprandial glucose excursions is not solely determined by insulin sensitivity. If healthy individuals, with the same insulin sensitivity, have differing incretin effects in response to carbohydrate-rich foods (Blaak *et al.*, 2012), it could explain the phenomenon of glucose tolerant individuals with varying responses to identical meals, as described by Zeevi *et al.* (2015). Although more evidence is needed, it could provide a preliminary explanation as to why free-living postprandial glucose excursions cannot be adequately predicted by the results of an OGTT. Furthermore, although not yet described, this explanation could also provide differential definitions for insulin sensitivity and carbohydrate sensitivity.

2.4.3 The role of exercise and cardiorespiratory fitness in glucose control

Exercise is widely considered important in the prevention and treatment of T2DM. Different types of physical activity and exercise have beneficial effects on both insulin resistance and glucose control (Roberts *et al.*, 2013). Lower levels of cardiorespiratory fitness (CRF) are also associated with an increase in the number of cardiometabolic disease risk factors (Grundy *et al.*, 2012). It is generally accepted that exercise is needed and beneficial for glucose control, but research is ongoing to determine how, when and what exercise should be implemented to be most effective in the improvement of glucose control. What type of exercise should be emphasised? At what intensity should one exercise, for how long and how frequently? What time of day is most effective to exercise? In an attempt to bring light to these questions, researchers have measured different aspects of glucose control, in combination with different exercise strategies.

HbA_{1c} has consistently been found to decrease with long-term exercise and improvements in CRF. Meta-analyses have been done that showed HbA_{1c} improves when T2DM patients participate in various types of exercise interventions that lasted at least six weeks to several months. One such meta-analysis (Snowling & Hopkins, 2006) summarized the effects of exercise interventions that lasted for at least 12 weeks, on glucose control and insulin sensitivity. They found that small

improvements in HbA_{1c} are elicited by aerobic, resistance and combination exercise. Slightly larger, (i.e. small to moderate improvements) were evident for fasting plasma glucose, 2-h [glucose] and insulin sensitivity. Resistance and all modes of aerobic exercise seemed to effectively improve all health outcomes, although there is a possible small benefit to combining aerobic and resistance exercise. Another meta-analysis and systematic review was done by Grace *et al.* (2017) which confirmed that aerobic exercise interventions of at least six weeks are effective in reducing HbA_{1c} and insulin resistance in T2DM patients. These improvements were concomitant with increases in CRF. In a cross-sectional study of obese individuals with and without T2DM (HbA_{1c} < 9%), Moxley *et al.* (2018) reported that cardiorespiratory fitness was significantly related to HbA_{1c}. Additionally, HbA_{1c} explained 19% of the variance in peak oxygen uptake (VO_{2peak}) and this relationship was independent of insulin sensitivity.

Liu *et al.* (2019) did a meta-analysis to investigate the effect of ~ three months of high-intensity interval training (HIIT) (at least three sessions per week) in T2DM individuals. In general, HIIT interventions improved HbA_{1c}, fasting insulin and glucose, as well as VO_{2peak}. HIIT was superior to moderate-intensity continuous aerobic exercise when comparing the improvements in HbA_{1c} and CRF. However, the two training interventions were equally effective in lowering fasting insulin and glucose.

It is understandable that higher intensities of exercise may bring about additional glucose control benefits, since glucose utilisation (and thus the rate of glucose uptake from the blood) by working skeletal muscles is greatly increased during exercise. Sylow *et al.* (2017) confirmed that the uptake of glucose by working skeletal muscles increases as the duration and intensity of exercise increases, thus explaining the superior results obtained with high intensity exercise.

Hopper *et al.* (2013) reported that the 2-h OGTT glucose concentration of young adults correlated significantly with their self-reported fitness levels ($r = 0.485$; $p = 0.028$). These results may also mean that a person's level of physical activity (i.e. low to high), in addition to objectively determined aerobic fitness, are positively related to glucose tolerance. These findings are in agreement with the results of the meta-analysis by Snowling and Hopkins (2006), as well as Solomon *et al.* (2015). In the latter cross-sectional study that included healthy and T2DM participants, there was a moderately strong association between CRF and glucose tolerance ($r = -0.325$; $p < 0.0001$). They also reported associations between CRF and HbA_{1c} ($r = -0.333$; $p < 0.001$), fasting plasma glucose ($r = -0.336$; $p < 0.001$), and insulin sensitivity ($r = 0.734$; $p < 0.001$).

In a cross-sectional study on T2DM patients, Jelleyman *et al.* (2017) found that physical activity (of any duration or intensity; as measured by accelerometers over seven days) was not related to fasting glucose. However, longer durations and intensity of physical activity was associated with lower 2-h glucose and improvements in insulin sensitivity.

In a study similar to that of Jelleyman *et al*. (2017), Sardinha *et al.* (2017) used accelerometers to investigate the role sedentary time and breaks in sedentary time in glucose control in 66 adults with T2DM. Breaks in sedentary time were defined as a period of more than one minute of physical activity. Increased sedentary time was associated with detrimental changes in all glucose control measures (HbA_{1c}, fasting- and 2-h glucose) and insulin resistance (HOMA-IR and Matsuda index), while higher number and duration of breaks in sedentary time were associated with improved glucose control measures and insulin resistance. When adjusted for time spent in moderate to vigorous physical activity, total sedentary time was associated with increases in fasting glucose ($\beta = 0.32$; $p = 0.037$) and breaks in sedentary time were associated with decreases in HOMA-IR ($\beta = -0.28$; $p = 0.047$) and fasting glucose ($\beta = -0.25$; $p = 0.046$). When adjusted for CRF, total sedentary time remained associated with increases in HbA_{1c} ($\beta = 0.25$; $p = 0.044$) and breaks in sedentary time remained associated with HOMA-IR ($\beta = -0.25$; $p = 0.036$), the Matsuda index ($\beta = 0.26$; $p = 0.036$), and fasting glucose ($\beta = -0.22$; $p = 0.038$).

Duvivier *et al.* (2017) also reported that limiting sedentary time leads to lower 24-h glucose AUC and improves insulin sensitivity in T2DM patients. They also speculated that insulin sensitivity may actually be more improved by the reduction in sedentary time through low intensity physical activity, rather than incorporating an acute exercise session during the day but not minimizing sedentary time.

Continuous glucose monitoring has enabled researchers to gain more insight on the role of exercise in glucose control. MacLeod *et al.* (2013) conducted a meta-analysis on the role of short-term (< 2 weeks) exercise interventions on the glucose levels of T2DM participants. The eight studies that were included in the analysis mostly involved a single exercise session, mostly cycling exercise and post-CGM was started either directly after the bout of exercise or measured during the day on which exercise was performed. The analysis revealed that acute exercise had no effect on fasting glucose levels or time spent at hypoglycaemic glucose concentrations. However, average 24-hour glucose and time spent above glucose concentrations of 10.0 $mmol \cdot L^{-1}$ were both lower (average reductions of 0.8 $mmol \cdot L^{-1}$ and 129 minutes per day, respectively) after a bout of exercise.

Borror *et al.* (2018) did a systematic review assessing the effect of different types of postprandial exercise on glucose levels in T2DM participants. Only randomised controlled trials were included and only if exercise was performed within three hours of a standardised meal. Study outcomes focussed on postprandial glucose AUC and 24-h glucose measures. The authors concluded that T2DM patients should ideally undertake moderate intensity aerobic exercise one hour after a meal. This type and timing of exercise was found to be safe and the most effective to minimise postprandial glucose levels. They also pointed out that not enough studies have been done on HIIT that was properly matched to moderate intensity continuous aerobic training. Studies on resistance exercise were also too few to make definitive conclusions, although it may be effective. Exercise is not only important for already diagnosed diabetic patients. Shambrook *et al*. (2018) reported positive and immediate effects on the glucose responses of inactive men after exercising 30 minutes after a meal and at any intensity.

An important observation by Moore *et al.* (2020) was that the acute benefits of exercise on glucose responses seems to be independent of CRF. They found that postprandial stair climbing was equally effective in lowering postprandial glucose excursions in non-diabetic individuals and irrespective of fitness level.

In summary, it seems that insulin sensitivity is most consistently improved by any form, intensity and duration of physical activity or exercise, in both diabetic and otherwise healthy individuals (Duvivier *et al.*, 2017; Grace *et al.*, 2017; Jelleyman *et al.*, 2017; Sardinha *et al.*, Snowling & Hopkins, 2006). Changes in insulin sensitivity in response to exercise training also seems to correlate with an individual's aerobic fitness (maximal oxygen uptake, VO_{2max}) (Cancino-Ramírez *et al.*, 2018; Solomon *et al.*, 2015).

Furthermore, acute exercise is a moderating factor in the immediate postprandial glucose response through the facilitation of enhanced glucose disposal (Ryder *et al.*, 2001; Stanford & Goodyear, 2014). Even in healthy individuals, habitual physical activity needs to be sustained to experience the postprandial glucose lowering benefits of exercise (Mikus *et al.*,2012a; Moore *et al*, 2020). Improvements in CRF do, however, also seem to be independently related to improvements in glucose control and insulin sensitivity (Liu *et al.*, 2018; Moxley *et al.*, 2018; Sui *et al.*, 2012).

2.4.4 Other factors influencing glucose control

Glucose control and glucose tolerance have also been related to other factors. For instance, obesity (as defined by BMI and waist circumference), together with a family history of T2DM, are strong predictors of declining glucose tolerance in non-diabetic individuals (Walker *et al.*, 2005). Brunner *et al.* (2006) also found that impaired glucose tolerance is associated with the degree of obesity, higher systolic blood pressure, and poorer lung function. The comorbidities of declining glucose control could possibly be described as mutual effects that occur due to the same root cause (i.e., hyperinsulinaemia), rather than necessarily being contributing factors (Crofts *et al.*, 2015). For example, does insulin resistance cause or precede obesity, and vice versa, or are insulin resistance and obesity both caused by hyperinsulinaemia?

Advancing age is widely associated with insulin resistance and declining glucose control (Brunner *et al.*, 2006; Ko *et al.*, 2006; Shimokata *et al.*, 1991, Zeevi, *et al.*, 2015), but the risks can be mitigated when individuals stay healthy and physically active (Rosenthal *et al.*, 1982). Age could thus be considered an overlapping or mediating factor in the development of insulin resistance which leads to a decline in glucose control and ultimately disease (Facchini *et al.*, 2001).

Although the influences of aging, obesity, a family history of T2DM, hypertension and poor lung function on glucose control are acknowledged, they will not be discussed in depth as these factors are beyond the scope of this thesis.

2.5 Consequences of insulin resistance

Primary hyperinsulinaemia that occurs secondary to postprandial hyperglycaemia can, over time, lead to chronic over-nutrition and the subsequent development of insulin resistance (Ludwig & Ebbeling, 2018). Type 2 Diabetes Mellitus has long been associated with increased morbidity and mortality, mainly due to CVD (McGurnaghan *et al.*, 2019), however, being pre-diabetic or having impaired glucose tolerance already increases one's risk for CVD (Ceriello & Motz, 2004; Haffner *et al.*, 1990). Clear associations exist between high levels of fasting insulin in pre-diabetic individuals and atherogenic CVD risk factors (Haffner *et al.*, 1990) and BMI (Hopper *et al.*, 2012; Odegaard & Chawla, 2013). Likewise, hyperinsulinaemia and insulin resistance stand central in the pathophysiology of metabolic diseases (Schofield & Sutherland, 2012; Tabák *et al.*, 2012).

The presence and degree of insulin resistance is a good predictor of age-related diseases, including hypertension, coronary artery disease, stroke, certain cancers and T2DM. In fact, it was proposed that the absence of insulin resistance completely negates the risk for age-related disease events (Facchini *et al.*, 2001).

Insulin resistance is a central characteristic of the metabolic syndrome (DeBoer, 2019). The metabolic syndrome is a clustering of risk factors that are strongly predictive of future CVD (DeBoer, 2019; McCracken *et al*., 2018), as it accelerates the atherogenic process due to it being a pro-inflammatory, pro-atherogenic and pro-thrombotic state (McCracken *et al.*, 2018). Metabolic syndrome also increases the risk for the development of T2DM (DeBoer, 2019; Esser *et al.*, 2014; Nolan & Prentki, 2019). Insulin resistance is not necessarily the principle causative factor leading to the development of other risk factors that collectively define the metabolic syndrome. Rather, insulin resistance is another consequence of a common underlying cause (hyperinsulinaemia) that also leads to the collective manifestation of central obesity, hypertension, elevated fasted levels of triglycerides, decreased HDL-C and increased fasting glucose (Nolan & Prentki, 2019; Reaven, 1988). Nolan and Prentki (2019) identified hyperinsulinaemia, in combination with chronic over-nutrition, as the common precursor of the clustering of symptoms seen in the metabolic syndrome. Inflammation plays a large pathological role in the development of insulin resistance and is independently associated with CVD risk associated with the metabolic syndrome (Esser *et al.*, 2014).

Chronic, low-grade systemic inflammation is well recognised as the key pathological factor of most, if not all chronic diseases, atherosclerosis, and CVD. Previously, high serum cholesterol levels were incorrectly labelled as the cause of atherosclerosis and subsequent disease (Tsoupras *et al.*, 2018). However, today it is recognised that atherosclerosis, which leads to CVD, is a chronic inflammatory disease (Taleb, 2016; Wu *et al.*, 2017).

Hypertrophied, insulin-resistant adipose tissue is believed to be the major origin of low-grade systemic inflammation (Asghar & Sheikh, 2017; McCracken *et al.*, 2018; Shimobayashi, *et al.*, 2018). Hyperinsulinaemia contributes to an increase in low-grade systemic inflammation, together with numerous other related factors. These factors include hyperglycaemia, excessive nutrient entry into cells (Nolan & Prentki, 2019) and increases in levels of oxidative stress (Tsoupras *et al.*, 2018). Oxidative stress occurs due to an overload of the electron transport chain, subsequent mitochondrial dysfunction (Nolan & Prentki, 2019) and accumulation of advanced glycation end products (Wu *et al.*, 2017) via increased glucose flux into glucose-metabolising tissues.

Sustained inflammation also contributes to endothelial dysfunction, which is strongly associated with atherosclerosis and CVD (Tsoupras *et al.*, 2018). Chronic oxidative stress is also a strong predictor of atherosclerosis (Wu *et al.*, 2017). Hyperglycaemia and oxidative stress are strongly implicated in the oxidation of low-density lipoprotein cholesterol (LDL-C) molecules (Wu *et al.*, 2017), which plays a significant role in endothelial dysfunction and atherosclerosis (Taleb *et al.*, 2016; Wu *et al.*, 2017). Moreover, insulin resistance and hyperinsulinaemia are also strongly implicated in the development of endothelial dysfunction, atherosclerosis, and CVD (Ormazabal *et al*., 2018).

If insulin resistance persists and progresses without intervention (i.e. lifestyle modification), metabolic syndrome, pre-diabetes and T2DM are the eventual fate (Esser *et al.*, 2014; Tabák *et al.*, 2012). With the amount of diabetic complications that occur due to the resultant hyperglycaemia (Fowler, 2011), the hallmark of diabetes, in addition to an even greater risk for CVD, the development of this overt disease state is, of course, not ideal.

2.5.1 Consequences of hyperglycaemia

In addition to the indirect effects of postprandial hyperglycaemia, namely the development of insulin resistance, obesity and associated conditions, hyperglycaemia itself also has direct negative health effects. Higher than healthy fasting glucose levels, postprandial glucose and HbA_{1c} are independent risk factors and predictors of vascular disease and mortality (Tabák *et al.*, 2012).

Several epidemiological studies highlight the importance of well-controlled glucose levels. The Whitehall study (Fuller *et al.*, 1983) and the UK Diabetes prospective study (Baldeweg & Yudkin, 1999) involved a large number of participants (18 403 men and over 5 000 men and women with T2DM, respectively) to assess the outcomes of long-term glucose levels. From the Whitehall Study it was evident that individuals in the highest percentiles of blood glucose ($\geq$ 5.4 $mmol \cdot L^{-1}$) 2-h after a 50 g glucose tolerance test had significantly higher rates of mortality due to stroke and coronary heart disease (Brunner *et al*., 2006; Fuller *et al*., 1983). The UK Diabetes Prospective Study found that fewer diabetic complications and risk of complications were attributable to lower levels of fasting plasma glucose (Colagiuri *et al.*, 2002) and reductions in HbA_{1c} levels (Manley, 2003).

Overall, the UK Diabetes Prospective Study supports the notion that the lowering of blood glucose in T1DM and T2DM patients reduces the incidence of microvascular complications, together with decreasing morbidity and mortality, which coincides with chronic conditions related to diabetes

(Baldeweg & Yudkin, 1999). Cavalot *et al.* (2011) investigated the role of glycaemic control parameters (fasting blood glucose, HbA_{1c}, and blood [glucose] 2-h after meals) in all-cause mortality and cardiovascular events and concluded that in addition to utilising HbA_{1c}, postprandial glucose levels also associate strongly with mortality and cardiovascular events. Another observational study in more than 15 000 non-diabetic individuals also reported that the 2-h postprandial [glucose] adds to the ability to predict risk of death due to cardiovascular disease (Lin *et al.*, 2009).

Ceriello and Motz (2004) proposed that to prevent the development of T2DM and CVD, postprandial hyperglycaemia should be controlled in order to minimise the production of oxidative stress and the subsequent inflammation. The latter is considered to be involved in the root causes of beta-cell dysfunction and the pathogenesis of insulin resistance, T2DM (Akash *et al.*, 2018), atherogenesis (Wu *et al.*, 2017) and CVD (via endothelial dysfunction) (Förstermann *et al.*, 2017). Furthermore, hyperglycaemia also provides favourable carcinogenic conditions (Chang & Yang, 2016), implicating hyperglycaemia in almost all non-communicable diseases.

A key observation from the literature is that persistent glucose oscillations have more detrimental health effects than chronically elevated, but stable glucose concentrations. Ceriello *et al.* (2008) explored the effects of acute increases in blood glucose in comparison to constantly high glucose levels over a period of 24 hours in T2DM patients and healthy controls. The average glucose concentrations were the same for the chronically elevated hyperglycaemia conditions (10.0 $mmol \cdot L^{-1}$ and 15.0 $mmol \cdot L^{-1}$) and oscillation glucose (glucose was kept high every six hours at 15.0 $mmol \cdot L^{-1}$ and normalised for the next six hours). Importantly, the glucose oscillations caused the highest increases in oxidative stress and endothelial dysfunction markers in both groups.

Monnier *et al.* (2006) studied the association between 24-h urinary excretion rates of free 8-iso prostaglandin F2α (8-iso-PGF2α), a well-recognised marker of oxidative stress, in T2DM patients and healthy controls and their two-day CGM-derived mean amplitude of glucose excursions (MAGE) (the arithmetic mean of glucose excursions) (Service *et al.*, 1970). MAGE is a measure of intra-day glycaemic variability (Monnier *et al.*, 2008). The T2DM patients exhibited significantly higher glucose fluctuations and had a higher production of the oxidative stress marker compared to the healthy controls. MAGE was linearly related to 8-iso-PGF2α, with no threshold value. Furthermore, 8-iso-PGF2α was not related to measures of long-term glucose control, including HbA_{1c}, fasting glucose and mean daily glucose.

Further insight into glycaemic variability is provided by studies from Ceriello *et al.* (2012) and Ceriello *et al.* (2013). In both studies, individuals' recovery from hypoglycaemia was investigated. After hypoglycaemia was induced with the use of a clamp, participants (T1DM patients and healthy controls) were infused with glucose, either to reach and maintain euglycaemia, or hyperglycaemia for two hours, before attaining and maintaining ideal glucose levels for six hours. Collectively they found that the negative effects, namely increased oxidative stress and inflammation, which are resultant from hypoglycaemia, are worsened when a period of hyperglycaemia follows a hypoglycaemic event. The effects were seen in both the diabetic, as well as the healthy control participants.

2.6 Measures of glucose control

Glucose control is traditionally assessed using either one or a combination of the following: fasting plasma glucose, HbA_{1c} and the glucose level 2-h after a 75 g oral glucose load (ADA, 2017). All these measures are routinely used to diagnose individuals with either pre-diabetes or overt diabetes mellitus, according to certain cut-off criteria for each. None of these tests can, however, be considered the gold standard of glucose control which embodies all facets of glucose homeostasis.

The concept of glycaemic variability as an alternate measure of glucose control, emerged with the development and use of CGM in clinical and research settings. Glycaemic variability is a broad term used to describe inter- and intra-day fluctuations in blood glucose levels (Monnier *et al.*, 2008; Suh & Kim, 2015). Glycaemic variability describes more specifically postprandial glucose, as well as the differences between peaks and nadirs in glucose levels, compared to the traditional measures of glucose control.

It has been suggested that HbA_{1c} is the best indicator of long-term glucose control, whereas the measurement of fasting or 2-h OGTT [glucose] is very limited to a specific time-point. Especially in glucose tolerant individuals, fasting or 2-h [glucose] can vary when measurements are repeated and thus a single measure may not adequately represent the true, or average values (Kim *et al.*, 2016).

HbA_{1c} is influenced by chronic fasting, as well as postprandial glucose (Roberts *et al.*, 2013), giving an indication of the average glucose levels over a period of 2 - 3 months preceding the measurement (ADA, 2001). Concordantly, HbA_{1c} is closely related to mean levels of glucose ($r = 0.51$; $p < 0.001$) as measured with CGM (Kohnert *et al.*, 2007). The degree to which postprandial glucose excursions

contribute to an individual's HbA_{1c}, depends on their diabetic status; the relative contribution of fasting glucose increases as HbA_{1c} levels rise (Monnier *et al.*, 2003). For individuals at the upper level of healthy HbA_{1c} values, abnormal fasting blood glucose levels are very rare; rather, the increase in 2-h OGTT glucose levels seem to be responsible for most of the rise in HbA_{1c} levels, at least initially (Woerle *et al.*, 2004). Individuals who present with similar degrees of glycaemic variability may have very different percentages of HbA_{1c}, and vice versa (McDonnell *et al.*, 2005). Especially among individuals without significant decrements in their overall glucose control, HbA_{1c} does not adequately account for their glycaemic variability (Suh & Kim, 2015) and is much less indicative of short-term glycaemic variability (Kohnert *et al.*, 2007; Roberts *et al.*, 2013; Wang *et al.*, 2012). Shah *et al.* (2019) also showed that HbA_{1c} cannot predict the variability in glucose levels in a sample of healthy persons. Hanefeld *et al.*, (2014) also proposed that glucose control abnormalities may present before a significant rise in HbA_{1c}.

Thus, the greatest limitation in HbA_{1c} measures is its inability to provide information on short-term fluctuations, i.e. its magnitude and frequency (ADA, 2001). Nevertheless, HbA_{1c} is the most useful measure to assess overall, long-term glycaemia in T2DM patients as it correlates well with long-term complications. It has, however, little meaning for T1DM patients (ADA, 2001) or apparently healthy individuals that may be at risk for future T2DM due to large daily glucose fluctuations, but in the absence of high mean levels of blood glucose.

The OGTT is the only diagnostic test that specifically identifies the presence of impaired glucose tolerance (Iskandar *et al.*, 2019). The glucose values at 2-h correlates positively ($r = 0.55$; $p < 0.001$) with HbA_{1c} levels (Woerle *et al.*, 2004), but there is not perfect concordance between the two measures (Iskandar *et al.*, 2019). Postprandial glucose also does not necessarily correlate with fasting glucose levels, especially in non-diabetic individuals. Only fasting glucose values above a certain threshold (5.5 $mmol \cdot L^{-1}$ to 6.0 $mmol \cdot L^{-1}$ (Schrot, 2004; Turner, 1998)) are associated with risk, while there is no such threshold for postprandial glucose (Blaak *et al.*, 2012; Levitan *et al.*, 2004). This may suggest that postprandial glucose is an earlier indicator of disease risk than fasting glucose. Besides, it is well-documented that people with healthy fasting glucose concentrations may have impaired glucose tolerance (Bartoli *et al.*, 2011; Woerle *et al.*, 2004).

A high fasting blood glucose level is recognised as a risk factor for CVD (Colagiuri *et al.*, 2002), is used as a criterion for the diagnosis of diabetes and pre-diabetes (ADA, 2017) and is useful to identify individuals who are at increased risk for T2DM (Tirosh *et al.*, 2005). Nevertheless, assessing

impaired glucose control with an isolated measure of fasting blood glucose will not identify individuals who currently present as healthy, but could be at risk due to long-term elevated postprandial blood glucose levels. Fasting blood glucose does not provide adequate information about metabolic glucose homeostasis and persistently high fasting glucose also occurs too late in the development of T2DM to be utilised as an early risk detector (Bartoli *et al.*, 2011).

Furthermore, the OGTT glucose value at 2-h is probably most relevant to T2DM patients, as this is typically when their glucose levels peak after a meal (ADA, 2001). Healthy individuals, however, reach peak glucose levels earlier, i.e. on average after 60-minutes (ADA, 2001). Measuring 2-h [glucose] in healthy individuals may therefore not provide meaningful information when considering the clinical importance (as has been discussed) of the actual peak of the postprandial glucose response.

Overall, it seems that the measurement of fasting blood glucose alone may be the least specific indicator of overall glucose control and it could be deduced that fasting blood glucose is the least sensitive in the detection of a deterioration in overall glucose control, at least in the initial phase. Concordantly, studies related to glucose control and/or diabetic status, mostly utilises either the standard 75 g OGTT (Acciaroli *et al.*,2018; Hanefeld *et al.*, 2014; Wang *et al.*, 2012), or HbA_{1c} (Shah *et al.*, 2019; Zeevi *et al.*, 2015) to classify participants as healthy, pre-diabetic or diabetic.

2.6.1 Continuous glucose monitoring and glycaemic variability

In diabetic patients, glycaemic variability, in addition to HbA_{1c}, is a known risk factor for microvascular complications (Hirsch, 2005) and should be considered a future routine parameter when aiming to optimise glucose control (Suh & Kim, 2015). An exercise intervention study on T2DM patients by Karstoft *et al.* (2013) serves as an example of how glycaemic variability measures are more sensitive to changes in glucose control than HbA_{1c} and parameters derived from the OGTT. After four months of a continuous- or interval walking exercise intervention in T2DM patients, no significant changes in fasting glucose, HbA_{1c}, OGTT 2-h glucose, maximum glucose or AUC were found. However, significant changes in the average and maximum glucose (as measured by CGM) before and after the interval walking intervention were observed. The authors noted, though, that the dissimilar changes in the CGM and OGTT parameters could be the result of a slight delay in the post-OGTT testing compared with the CGM following the exercise training period. Therefore, the acute effects that exercise may have had on glucose control cannot be ignored.

The studies by Borg *et al.* (2010), Hanefeld *et al.* (2014), and Rodriquez-Segale *et al.* (2018) add further evidence of the disconnect between CGM measures and HbA_{1c}. They highlighted that it cannot be assumed that a person within the healthy ranges of HbA_{1c} will have a completely ideal glucose profile, or that they are necessarily safe from potentially harmful glucose excursions. Additionally, Madhu *et al.* (2013) and Wang *et al.* (2012) provide reasons why the OGTT may also not be adequate to predict the risk for future development of T2DM or CVD. If ideal glucose tolerance was the sole factor that predisposed individuals to the harmful effects of postprandial glucose excursions or glycaemic variability, the OGTT should have been fully predictive of an individual's CGM profile. This is not the case. Bartoli *et al.* (2011) commented that the OGTT is unable to predict future risk in individuals who currently present with normal glucose tolerance.

The two studies by Ceriello and colleagues (2012; 2013) provide valuable insight why increased glycaemic variability, specifically, can be regarded an independent risk factor for diabetic complications (Hirsch, 2005). These researchers demonstrated that the oxidative stress and inflammation that are caused by hyperglycaemic excursions are greater when a hyperglycaemic excursion is followed by hypoglycaemia, compared to normoglycaemia. Furthermore, it also explains why it is potentially important to assess glycaemic variability in apparently healthy individuals. A person with large glycaemic variability may present with the same daily mean glucose value as another individual with a more stable glucose profile. This is because excessive acute hyperglycaemia can be "cancelled out", in terms of mean values, by equally excessive nadirs; an important consideration when measuring average glucose or HbA_{1c}.

With the utilisation of CGM in research settings, it was found that study participants with normal glucose tolerance present with episodes of postprandial hyperglycaemia in the pre-diabetic range. Thomas *et al.* (2016) recruited 10 sub-elite athletes to investigate their ability to maintain optimal levels of blood glucose concentrations together with their typical heightened energy expenditure and dietary intake. The study is limited in terms of sample size, as well as the lack of tests to assess glucose tolerance or insulin sensitivity, however, the individuals were all healthy and had good levels of fitness (determined by resting heart rate being below 60 beats per minute and 6 or more hours of training per week). The CGM blood glucose profiles of the athletes over a period of 6 days, however, showed large interpersonal variation. Even when the postprandial period of two hours was removed, four of the athletes spent over ~ 70% of the time above glucose levels of 6.0 $mmol \cdot L^{-1}$, which is in the range of fasting pre-diabetes concentrations (ADA, 2017). In fact, three athletes presented with

pre-diabetic fasting glucose concentrations. Although the participants' diets were not controlled, the authors commented on the large difference in sensitivity to carbohydrate foods that was observed between the athletes.

Zeevi *et al.* (2015) developed an algorithm to predict postprandial glucose responses in non-diabetics. They integrated numerous factors, namely the gut microbiome, physical activity, habitual diet, anthropometrics (height, body mass, waist, and hip circumferences) and blood parameters (HbA_{1c}, total cholesterol and HDL-C). The individuals' blood glucose responses to identical meals (all containing 50 g carbohydrates) could not be predicted by either HbA_{1c} or the glycaemic load of the meals. All the aforementioned factors played a role in the significant inter-individual differences in glucose responses to the identical meals. They also found that it was not the same meal that caused the highest glucose response in different individuals. The data therefore suggest that people have individualised sensitivity to carbohydrates. The effect of adding protein or fats to a meal also differed between individuals. In addition to the observed variation in postprandial glucose responses, the data from individual CGM profiles showed that glucose excursions reached levels well above 7.8 $mmol \cdot L^{-1}$ on several occasions, and some excursions reached concentrations up to 10.0 $mmol \cdot L^{-1}$. The latter approaches the top range of pre-diabetic post-challenge values and is similar to the glucose concentrations that were used in hyperglycaemia experimental studies, for instance Ceriello and colleagues (2008). A glucose concentration of 10.0 $mmol \cdot L^{-1}$ has previously been used as the targeted upper limit in clinical settings for diabetic patients on intensive therapy (Heine *et al.*, 2004; Monnier *et al.*, 2008).

A few researchers aimed to assess whether CGM could be used to differentiate between seemingly healthy individuals with adequate glucose control and those who show signs of derangements. For instance, Borg *et al.* (2010) and Rodriquez-Segale *et al.* (2018) used CGM to investigate the degree of glucose control in persons with ideal HbA_{1c} values. Both studies revealed that, in free-living conditions, a high percentage (73% and 93%, respectively) of healthy subjects exceeded the OGTT pre-diabetes glucose threshold of 7.8 $mmol \cdot L^{-1}$. Neither study performed OGTTs, therefore, although the participants were healthy according to HbA_{1c}, glucose tolerance was not measured directly. Diet was recorded in both studies, but not statistically controlled for. Thus, deductions regarding the role of diet and glucose tolerance on the prevalence of free-living hyperglycaemia cannot be made from either of these studies.

Madhu *et al.* (2013) questioned whether CGM is more sensitive than the OGTT for early detection of T2DM. Participants included individuals ranging from healthy to those diagnosed with T2DM. The CGM profiles of the group with healthy glucose tolerance showed many excursions that exceeded the pre-diabetic cut-off value. The degree of glucose control impairment, as assessed by CGM-derived mean glucose, standard deviation (SD), inter-quartile range (IQR), coefficient of variation (CV%), minimum and maximum values and range of glucose, were in line with the classification of the two groups. Individuals with healthy glucose tolerance and pre-diabetes could be identified as higher-risk, based on derangements of their 24-h CGM glucose profiles. Although Wang *et al.* (2012) used different CGM-derived glycaemic variability indices, namely MAGE, largest amplitude of glycaemic excursions (LAGE), mean of daily differences (MODD) and postprandial glucose excursion (PPGE), in addition to mean blood glucose and SD of mean blood glucose, their findings were similar to that of Madhu *et al.* (2013). Furthermore, they identified 22% of individuals with healthy glucose tolerance (n = 53) who had excursions exceeding concentrations as high as 11.1 mmol·L^{-1}. In the latter two studies, the participants' meals were controlled during the CGM period, making the findings even more important. The findings of both studies are in agreement with the suggestion that individuals with similar glucose tolerance may have variable sensitivity to carbohydrates and subsequent different glucose responses to mixed meals (Zeevi *et al.,* 2015), possibly irrespective of insulin sensitivity.

The relevance of the 7.8 mmol·L^{-1} cut-off value for peak postprandial glucose, additionally to its use as the target for the 2-h OGTT value is motivated by the ADA (2001) as follow: "In nondiabetic individuals, plasma glucose concentrations peak ~60 minutes after the start of a meal, rarely exceeds 7.8 mmol·L^{-1} and return to pre-prandial levels within 2–3 hours." This threshold, however, should not be suggested as an absolute value distinguishing between "safe" and "risky" postprandial glucose. Rather it can be regarded as a descriptive parameter to quantify relative hyperglycaemia exposure, since the risk associated with postprandial glucose excursions do not have a threshold value. Researchers agree that risk and deleterious effects increase linearly from healthy blood glucose ranges (Brunner *et al.,* 2006; Ceriello & Genovese, 2016; Levitan *et al.,* 2004; Monnier *et al.,* 2006).

CGM and glycaemic variability offers a more comprehensive description of all aspects involved in glucose control compared to any of the other measures that are routinely used. The use of fasting glucose measurements, on its own, to screen for impairments in blood glucose levels is not adequate. There is strong evidence that points to postprandial hyperglycaemia being an independent risk factor, as well as the parameter that associates most strongly with CVD and chronic disease risk (Blaak *et*

al., 2012; Ceriello & Genovese, 2016). Furthermore, irrespective of diabetic status, it is clear that postprandial hyperglycaemia has numerous deleterious health effects.

Considering the evidence in favour of the independent importance of postprandial hyperglycaemia and glucose fluctuations over HbA_{1c} and fasting blood glucose levels, CGM-derived measures of glucose control could perhaps become the preferred method to detect at-risk individuals. Additionally, especially considering the findings of Zeevi *et al.* (2015), who demonstrated the effects of specific dietary constituents on the postprandial glucose response of different individuals, CGM could possibly highlight potential risk associated with lifestyle, rather than only assessing general glucose tolerance with the standard OGTT. The isolated assessment of postprandial glucose with an OGTT will not necessarily provide any information on whether an individual could be at risk of future development of insulin resistance, metabolic syndrome or T2DM, because this test does not necessarily give an accurate indication of the free-living postprandial glucose responses of the said individual.

It can be argued that CGM profiles are not yet adequately described in healthy populations (Acciaroli *et al.,* 2018; Shah *et al.,* 2019). This is especially regarding risk that may be associated with CGM profiles and glycaemic variability among healthy individuals. To my knowledge, there are also not yet any large prospective studies that investigated the long-term effects related to different CGM profiles. Why do once-healthy individuals develop insulin resistance and declining glucose tolerance? Differences in the CGM profiles of healthy individuals would not have been evident if glucose tolerance were the only factor influencing free-living glucose control. Lifestyle factors, such as dietary characteristics and physical fitness, in relation to CGM profiles should be investigated further. T2DM is after all described, even by the general public, as a lifestyle disease.

2.7 Conclusion

From the literature, it seems that the amount and quality of dietary carbohydrates are the strongest predictor of the postprandial glucose response. Regular exercise and CRF are also important in the regulation of postprandial rises in blood glucose and for the maintenance of peripheral insulin sensitivity. The postprandial glucose response has, however, been identified as an independent individual characteristic among apparently healthy individuals and studies (Thomas *et al.,* 2016; Zeevi *et al.,* 2015) utilising CGM have observed that apparently healthy individuals may present with increased free-living glycaemic variability. High postprandial glucose and subsequent relative

hypersecretion of insulin are arguably the primary risk factors for the development and progression of obesity, insulin resistance and subsequent related diseases. The questions that remain are: i) whether certain individuals are more likely to develop insulin resistance-related diseases?; ii) whether CGM can be used for earlier detection of those at risk ?; and finally, iii) how important is dietary composition and CRF, in relation to each other, and for the maintenance of healthy glucose control and insulin sensitivity?

Chapter 3 Methodology

3.1 Study Design

3.1.1 Participants

The study was cross-sectional in design and convenience sampling was used to recruit volunteers to participate in the study. The participants included staff of Stellenbosch University; thus, institutional permission was sought. Posters (Appendix C) were posted in buildings on campus. Invitations to participate in the study were spread via word of mouth and adverts posted on the Sport Physiology Laboratory website and Twitter account, as well as the Department of Sport Science's Facebook page.

The required sample size was calculated with G*Power 3 (Faul *et al.*, 2009) and based on the results of (Moxley *et al.*, 2018). It was calculated that 28 participants would be sufficient to detect a statistically significant difference in insulin sensitivity (with a power of 0.90 and 5% level of significance) in men with low to high levels of fitness. It was therefore decided to recruit a minimum of 30 volunteers to make provision for potential dropouts.

Participants were included if:

- they were male;
- they were 30 to 50 years old;
- they had a body mass index (BMI) between 20 and 35 kg/m^2;
- they were not on a calorie- or a specific carbohydrate-restrictive diet, or had been in the 3-month period prior to the study;
- they did not make any significant changes to their diet or dietary patterns in the 3-months prior to their inclusion into the study.

Participants were excluded if:

- they reported acute illness, chronic disease or any other medical problems during the health screening session (Appendix A) that deemed them not suitable to partake in a maximal exercise test;
- they had any diagnosed metabolic condition or disease (e.g. type 1 or type 2 diabetes mellitus, [diabetes is a broad term which covers both 'mellitus' and 'insipidus' – for the purpose of this study, the term 'diabetes' will refer only to diabetes mellitus] hypo- or hyperthyroidism), or were using any chronic medication for any of these conditions;

- they had a musculoskeletal injury which would have constrained their performance during the maximal exercise test;
- they reported any skin conditions or hypersensitivity that would have caused skin irritation while wearing the glucose monitoring sensor;
- they were taking aspirin, as the salicylic acid could cause inaccurate continuous glucose monitoring (CGM) readings (https://www.myfreestyle.com/freestyle-libre-pro-cgm-system).

Figure 3.1 shows the inclusion of volunteers into the study, reasons for exclusion, and reasons for unsuccessful completion of study participation.

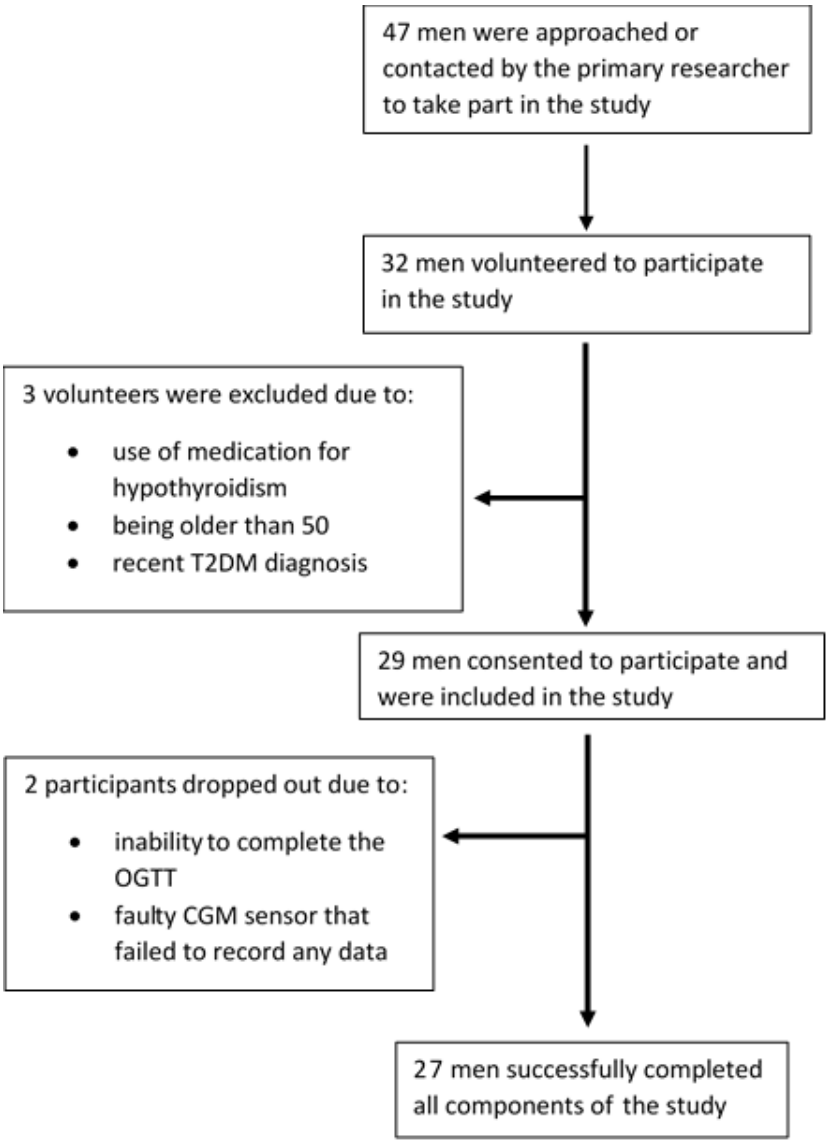

Figure 3.1 Volunteers and subsequent participants included in the study.

3.1.2 Procedures

On their first visit to the Sport Physiology Laboratory, volunteers who showed interest in the study underwent a screening session to determine if they qualified according to the stipulated inclusion and exclusion criteria. The screening included the completion of a health screening form (Appendix A). After an explanation of the details and practical implications of the study, and the volunteer agreed to participate in the study, they were asked to sign the informed consent form (Appendix B).

After being included in the study, the rest of the session comprised of the following:

1. Body composition and anthropometrical measurements.
2. The participant was fitted with a Freestyle Libre Pro continuous glucose monitoring sensor (Abbott Diabetes Care Inc.) which they were asked to wear for two weeks to assess glycaemic variability. They were also given an explanation on how to accurately log all food and drink intake while wearing the glucose monitor.
3. Participants completed a running test on the treadmill to measure their maximal aerobic exercise capacity.
4. An appointment was made for a visit to the nearest pathology laboratory for the collection of a fasted blood sample for the measurement of fasting plasma glucose, insulin, and glycated haemoglobin (HbA_{1c}), and an oral glucose tolerance test (OGTT) and.
5. The researcher made appointments with each participant to collect their glucose monitoring sensor from a location of their choice when their monitoring period was completed.

3.2 Tests, Measures and Equipment

3.2.1 Health Screening Questionnaire (Appendix A)

A modified version of the American College of Sports Medicine (ACSM) Medical Screening Questionnaire (Riebe *et al.*, 2016) was used to identify any medical conditions that may have warranted the participant's exclusion from the study. The ACSM questionnaire is in the public domain and was specifically developed for research laboratories such as the Sport Physiology Laboratory in the Department of Sport Science.

3.2.2 Anthropometric measurements

While participants were barefoot and wearing minimal clothing, their body mass and stature were determined. Stature is defined as the perpendicular distance between the transverse planes of the

vertex and the inferior aspects of the feet. Stature was measured with a sliding stadiometer (Seca, Hamburg, Germany) according to the stretch stature method. The participants were asked to stand barefoot on the scale and with their heels together. The heels, buttocks and upper part of the back touched the scale. The individual's head was placed in the Frankfort position (Kent, 2006). This is when the orbital (lower edge of the eye socket) and the tragion (the notch superior to the tragus of the ear) are horizontally aligned. The participant was then asked to take a deep breath and the researcher then carefully moved the headboard down the vertex and placed it firmly down on the head of the participant, compressing the hair as much as possible. The measurement was taken to the nearest 0.5 cm.

Body mass was measured using a calibrated electronic scale (UWE BW-150, 1997 model Brisbane, Australia) and recorded to the nearest 0.1 kg.

Waist and hip circumferences were also measured. Waist circumference was taken as the smallest circumference between the last rib and the superior iliac crest while the participant was breathing quietly. While the participant was standing with feet together, hip circumference was measured as the largest perimeter around the buttocks. Both circumferences were measured to the nearest 0.5 cm.

3.2.3 Body composition

An ImpediMed® SFB7-08L050042 multi frequency body composition analysis device (ImpediMed® Limited, Brisbane, Australia) was used to determine the participants' body fat percentages and fat-free masses. Participants were instructed to avoid all forms of exercise and the consumption of alcohol 12 hours prior to the measurement, as well as asked to avoid eating two hours before the measurements were recorded. They were also required to empty their bladders prior to lying down for their measurements and to remove all jewellery and socks. Participants lay on a plinth in a supine position for 10 minutes before the measurement was taken. At the time when the measurement was recorded, they were asked to extend their arms by their sides, while resting their hands with their palms down and their legs slightly apart. After the anatomical placement areas were cleaned with an alcohol swab and allowed to dry, the electrodes were placed on the right hand and right foot, at least 5 cm apart. Hand placements were on the wrist between the radial styloid process and ulnar styloid process and on the dorsal surface of the hand, 1 cm proximal to the knuckle of the middle finger. Foot placements were located in the ankle joint region between the two malleoli and on the dorsal surface of the foot, 1 cm proximal to the metatarsal phalangeal joint of the second toe.

3.2.4 Continuous glucose monitoring (CGM)

Participants were fitted with a FreeStyle Libre Pro glucose monitoring sensor (Abbott Diabetes Care Inc.) in the Sport Physiology Laboratory. The monitor contains a small sensor that is inserted through the skin by a small needle. This sensor stays in place and automatically measures the of the adipose tissue interstitial fluid glucose concentration every minute, for up to two weeks. According to the manufacturer the Freestyle Libre Pro is, on average, within 12.3% of the Yellow Springs Instrument analyser reference standard. This was confirmed by Bailey *et al.* (2015) in which the FreeStyle Libre sensor's glucose results were highly correlated with capillary blood glucose readings ($r = 0.95$). The accuracy remained throughout the 14 days of wear and readings were not affected by BMI, age, clinical site, or HbA_{1C}. The manufacturers do, however, warn that caution should be exercised when identifying hypoglycaemia or when interpreting sensor glucose readings under 3.3 $mmol \cdot L^{-1}$, as there is a slight possibility of false low values. Bailey *et al*. (2015) reported that 40% of the low values (< 3.3 $mmol \cdot L^{-1}$) in their study were in actual fact above 4.5 $mmol \cdot L^{-1}$.

The sensors were applied to the participants' triceps area, as instructed by the manufacturers, and participants were requested to take utmost care that it remained fixed until it was removed 2 weeks later. Non-allergenic waterproof plasters were applied over the sensors to prevent them from dislodging. Participants were also supplied with extra plasters. They were allowed to apply any tape that they were comfortable with in order to protect their sensors. Participants were blinded to their live glucose data as the sensor stored all the readings until it was extracted by the researcher using the digital reader, which remained in the laboratory. Sensors that were dislodged within the first week (< 7 days) were replaced with a new sensor, so that a minimum of 7 days were recorded for each participant.

The data obtained from the glucose monitors was used to determine the following parameters:

- Mean: the average of all the glucose values ($mmol \cdot L^{-1}$) over the total glucose monitoring period, calculated as the sum of all the glucose values, divided by the total number of values (Hill *et al.*, 2011).
- Standard deviation (SD) ($mmol \cdot L^{-1}$) around a mean glucose value.
- Coefficient of variation (CV%): SD normalised by the mean and expressed as percentage (Valletta *et al.*, 2014).
- MAGE (mean amplitude of glycaemic excursions): the mean value of the average height of glucose excursions or deviations greater than 1 SD (Hill *et al.*, 2011).

- MODD (mean of daily differences): the average of the differences between glucose values on the same time of each monitoring day (Hill *et al.*, 2011).
- Average time per day (minutes) above 6 mmol·L^{-1} (Thomas *et al.* 2016; Ceriello *et al.* 2008) and 7.8 mmol·L^{-1} (Shah *et al.*, 2019; Rodriguez-Segade *et al.*; Borg *et al.*, 2010) and below 3 mmol·L^{-1} (Danne *et al.*, 2017).

MAGE (mmol·L^{-1}) is typically used to assess intra-day variability, whereas MODD (mmol·L^{-1}) depicts the inter-day variability for each individual (Monnier *et al.*, 2008; Rodbard, 2009). A higher MAGE value therefore means larger intra-day variability, while a higher MODD value depicts greater day-to-day variation. Values below a MODD of 1 represent similarity in glucose patterns of each day analysed (McDonnell *et al.*, 2005).

CV% was used to assess over-all glycaemic variability. According to Acciaroli *et al.* (2018) it is a powerful statistical measure for this purpose. The higher the coefficient of variation, the greater the level of dispersion around the mean glucose value.

MAGE, MODD, mean glucose and SD around the mean [glucose] were calculated using the EasyGV© software developed by Nathan R. Hill (Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital and Tse Medical Academy, Harris Manchester College, University of Oxford, Oxford, United Kingdom) (https://www.phc.ox.ac.uk/research/technology-outputs/easygv).

3.2.5 Blood measurements

Participants went to the nearest pathology laboratory in the morning and after an overnight fast (> 8 hours). Blood plasma samples were taken for the following tests:

Glycated haemoglobin (HbA_{1c})

HbA_{1c} is a single measure that reflects the average plasma [glucose] of the prior two- to three months (WHO, 2006). 6.5% (48 mmol·mol^{-1}) is considered the cut-off diagnostic value for diabetes mellitus (WHO, 2019; SEMDSA, 2017), while 5.7% is considered the lower threshold for pre-diabetes (ADA, 2017).

Fasting plasma glucose (FPG)

FPG ≥ 5.6 mmol·L^{-1} is considered a pre-diabetic value (ADA, 2017; SEMDSA, 2017).

Fasting insulin

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the fasted concentrations of glucose and insulin: HOMA-IR = (fasting insulin [$\mu U \cdot L^{-1}$] × fasting glucose [$mmol \cdot L^{-1}$])/22.5. A HOMA-IR score > 1.7 – 2.0 was considered higher than a healthy value (Gayoso-Diz *et al.*, 2013).

Oral Glucose Tolerance Test (OGTT)

The standard procedure of the OGTT was followed. The test requires participants to drink a syrup solution containing 75 g of glucose. Blood samples were collected before consumption of the glucose solution (0 min), as well as at 30 min, 60 min, 90 min and 120 min post-consumption. Blood samples were analysed at all time points to obtain the plasma glucose and insulin concentrations.

Healthy glucose tolerance is defined as a glucose concentration < 7.8 $mmol \cdot L^{-1}$ after two hours of consuming the glucose solution (ADA, 2017).

Whole-body insulin sensitivity index was calculated using the area under the glucose and insulin curves, as described by Matsuda & DeFronzo (1999) and using the following formula:

$$Matsuda\ index = \frac{10000}{\sqrt{g_0 \times i_0 \times \frac{(g_0 \cdot 15 + g_{30} \cdot 30 + g_{60} \cdot 30 + g_{90} \cdot 30 + g_{120} \cdot 15)}{120} \times \frac{i_0 \cdot 15 + i_{30} \cdot 30 + i_{60} \cdot 30 + i_{90} \cdot 30 + i_{120} \cdot 15}{120}}}$$

g_{xtime} – glucose concentration after *x* minutes ($mg \cdot dL^{-1}$)
i_{xtime} – insulin concentration after *x* minutes ($mIU \cdot L^{-1}$)

Participants were classified as insulin-resistant if they had values < 4.3 (Gutch *et al*, 2015).

3.2.6 Vam-éval (VO_{2peak} running exercise test)

A modified treadmill Vam-éval (Cazorla & Léger, 1993) ramp test was completed by the participants to measure their peak aerobic exercise capacity as an indication of their cardiorespiratory fitness (CRF). A Saturn h/p/cosmos treadmill (Nussdorf-Traunstein, Germany) with specialised computer software (Cosmed Quark CPET, Rome, Italy) was used. This software integrates the breath-by-breath gas sampling and heart rate recordings (COSMED wireless HR monitor, Italy) to continuously record exercise intensity and selected cardiorespiratory parameters throughout the test. The gas analysers were calibrated to 16% O_2, 4% CO_2 and balance N_2 calibration gases, while the turbine flow meter was calibrated with a 3 L calibration syringe before each test.

Before initiation of each test, participants were fitted with an adjustable safety harness while standing stationary on the treadmill. They then warmed up for 5 min at a speed of 8 $km \cdot h^{-1}$ after which they were allowed to drink water. The test began at a speed of either 9 or 11 $km \cdot h^{-1}$, depending on the participant's subjective assessment of general fitness status. Three of the participants started the test at 11 $km \cdot h^{-1}$, whereas the remaining 24 participants started at 9 $km \cdot h^{-1}$. The speed of the treadmill increased by 0.5 $km \cdot h^{-1}$ after each minute. The test was terminated once the participant indicated he was unable to continue.

According to the 10th edition of ACSM's Guidelines for Exercise Testing and Prescription (Riebe *et al.*, 2016), respiratory exchange rate (RER) is considered the most accurate non-invasive indicator of a maximal exercise effort. However, not all participants were able to reach a RER of ≥ 1.10 indicative of a maximal test. Peak VO_{2peak} was therefore reported instead of VO_{2max}.

Nonetheless, in accordance with the ASCM's guidelines (Riebe *et al.*, 2018), the following criteria were considered indicators of a maximal test:

(i) a plateau in VO_2 (or failure to increase VO_2 by 150 $mL \cdot min^{-1}$) with increased workload;
(ii) failure of heart rate to increase with increases in workload;
(iii) a rating of perceived exertion (RPE) at peak exercise > 17 on the 6–20 scale (Borg, 1982) (Appendix D);
(iv) a peak RER ≥ 1.10.

Pre-exercise test requirements:

VO_{2peak} testing was conducted in the Sport Physiology Laboratory in the Department of Sport Science, Stellenbosch University. The ambient temperature in the laboratory was kept between 20°C and 24°C for all testing. To standardise the participant's metabolic state during the testing sessions they:

- did not eat for at least two hours prior to testing;
- avoided alcohol ingestion at least 12 hours before testing;
- avoided caffeine-containing drinks at least 4 hours before testing;
- avoided vigorous activities: RPE above 12 on the Borg scale (Borg, 1982) or any unaccustomed exercise at least 24 hours before testing.

3.2.7 Logging of food and drink

Participants either used a link to a Google form (Appendix E) to log all their food and drink consumption, or they kept a written diary (Appendix F) during the glucose monitoring period. A thorough explanation as to how all dietary intake were to be logged was given individually to each participant. Participants were encouraged to continue with their usual dietary habits and to not make any changes to their habitual dietary routine or intake until they completed their participation in the study. An ISSN (International Society of Sports Nutrition) certified sport nutritionist analysed the dietary data, using the South African MRC FoodFinder® software, to calculate the macronutrient contents. Glycaemic load (GL) (i.e. glycaemic index multiplied by the amount [g] of carbohydrate) of the logged food consumption was calculated using this data. Glycaemic index (GI) values were obtained from the South African GI and GL Guide (Steenkamp & Delport, 2016). Because the addition of fat and protein influence the postprandial glucose and insulin responses, participants were also grouped according to their macronutrient intakes by means of a K-means cluster analysis.

3.3 Statistical Analyses

Data and statistical analysis were conducted using Microsoft® Excel® for Microsoft 365 MSO (16.0.12827.20438) 64-bit.

Descriptive statistics for body composition, CRF, blood parameters, insulin sensitivity and glycaemic variability are reported as mean ± SD. The Kolmogorov-Smirnov test confirmed that all the variables followed a Gaussian distribution.

Glycaemic variability was calculated from the CGM data using the EasyGV© software (section 3.2.4). Missing glucose readings were interpolated with a straight-line estimation using the same EasyGV© software. At least seven full days of glucose monitoring was needed for a participant's data to be included into the analysis.

T-tests for independent groups were performed for all the variables of interest (body composition, dietary intake, CRF, glucose control, insulin sensitivity or glycaemic variability) to determine whether there were statistically significant differences between participants with a full set of CGM data and those whose sensors dislodged or ceased working before 14 days of monitoring were recorded. There were no statistically significant differences in either the CGM data, nor the personal characteristics of the 18 men who had 12 or more valid CGM monitoring days and the 9 men who

had 7 – 11 days of CGM data ($0.16 < p < 0.98$) (see Appendix G for complete results). For this reason, the complete CGM data set of each participant was used for analysis, except for the day on which the person underwent the OGTT. The data on this particular day were excluded from the CGM analyses for all participants.

K-means cluster analysis (Likas *et al.*, 2003) was used (algorithm run by MATLAB® R2020a [9.8.0.1380330]) to identify three groups based on the macronutrient intake of the participants. Single-factor analyses of variance (ANOVA) and post hoc T-tests for independent groups (with Bonferroni's correction) were performed to confirm differences in percentage intakes of fat, carbohydrates, and protein. The same statistical analysis was done to test for differences in body composition, glucose control, insulin sensitivity, OGTT concentrations of glucose and insulin, and glycaemic variability indices between the three groups. Glucose and insulin AUC's for the OGTTs of each cluster were calculated according to the method of Matthews *et al.* (1990). Additionally, Cohen's effect sizes and 90% confidence intervals were calculated to describe the magnitude of the differences between the clusters for all these variables. The smallest worthwhile difference between clusters was calculated as 0.2 x the pooled standard deviation of the variable. Threshold values for Cohen's *d* were > 0.2 (small), > 0.6 (moderate), > 0.8 (large) and > 1.2 (very large) (Hopkins *et al.*, 2009).

Pearson's product-moment correlation coefficient (*r*) was used to describe the association between dietary glycaemic load, and CRF and glucose control, insulin sensitivity, glycaemic variability, and body composition measures. The effect sizes of the correlations were described as small (> 0.1), moderate (> 0.3), large (> 0.5), very large (> 0.7) (Hopkins *et al.*, 2009).

3.4 Ethical considerations

The study protocol was approved by the Health Research Ethics Committee (HREC) at Stellenbosch University (S19/10/262; Project ID: 12997). All testing and laboratory procedures were performed in accordance to the Declaration of Helsinki.

The participants were informed that their participation was completely voluntary and that they could withdraw at any time. Each individual signed an informed consent form before partaking in the study. There were no serious risks involved as all participants were healthy, nonetheless, dizziness, fainting

and discomfort during the maximal exercise test could have been experienced and participants were aware thereof.

All tests and procedures were thoroughly explained to the participants and full understanding thereof was confirmed before any test or measurement was initiated. This was to ensure minimal risk and to ensure accuracy and consistency of measurements.

Emergency personnel that were qualified in basic life support (BLS), able to perform cardiopulmonary resuscitation (CPR) and able to use an Automated External Defibrillator (AED) (which was in the Sport Physiology Laboratory) were on the premises during all laboratory testing sessions. No adverse events took place during any of the exercise tests.
Blood samples were all taken in pathology laboratories by qualified personnel who all followed the standard health and safety procedures.

Chapter 4 Results

4.1 Participants

Twenty-nine healthy men qualified to participate in the study and 27 complete data sets were included for analysis. Table 4.1 describes the physical characteristics of these participants. Nineteen men were aged 30 to 39 years and the remaining eight participants were aged between 40 and 47 years.

Table 4.1 Age and body composition characteristics of the participants (n = 27).

	Mean ± SD	Minimum - Maximum
Age (years)	36.85 ± 4.84	30 – 47
Body mass (kg)	87.87 ± 14.02	70.6 – 120.0
Body fat %	20.71 ± 6.17	5.9 – 30.5
Fat free mass (kg)	69.35 ± 9.73	54.0 – 87.57
Waist:Height	0.50 ± 0.06	0.42 – 0.67
Waist:Hip	0.86 ± 0.05	0.78 – 1.00

The body fat percentages of five (18.5%) participants were *very poor*, five (18.5%) were *poor*, six (22%) were *fair*, seven (26%) were *good*, two (7%) were *very lean* and two (7%) were *excellent* (Riebe *et al.*, 2016).

Ten (37%) participants had waist-to-hip circumferences ≥ 0.9 (WHO, 2008). These same participants also had unhealthy waist-to-height ratios of ≥ 0.5 (Ashwell & Gibson, 2016). Except for one of these participants, who had a *fair* body fat percentage, the remaining nine had *poor* or *very poor* body fat percentages. An additional three participants who had healthy waist-to-hip and waist-to-height ratios, had *poor* body fat percentages.

4.2 Cardiorespiratory fitness (CRF)

Only six (22%) participants were able to reach RER values ≥ 1.10. All participants indicated that they were exhausted and no longer able to continue when their tests were terminated. Four (15%) of the participants indicated that they were too exhausted to continue with the exercise test before any of the other three criteria for a maximal exercise test, as per the ACSM (Riebe *et al*., 2016), were reached. Two of the four criteria for a maximal test were met by five (18.5%) of the participants,

three criteria were met by 15 (55.6%) participants and four (15%) of the participants met all four criteria.

One participant (4%) had *very poor* CRF, two (7%) *poor*, eight participants' (30%) fitness levels were classified as *fair*, six (22%) had *good* CRF and 10 participants (37 %) had *excellent* CRF (Riebe *et al.,* 2016:126).

Table 4.2 Peak cardiorespiratory fitness characteristics of the participants (n = 27).

	Mean ± SD	**Minimum - Maximum**
VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$)	45.91 ± 7.40	30.4 – 58.7
VO_{2peak} ($ml \cdot kg^{-1}$)	3979 ± 585	2619 – 4941
HR_{peak} (bpm)	186 ± 9	173 – 205
RER_{peak}	1.06 ± 0.04	0.99 – 1.13
Peak running speed ($km \cdot h^{-1}$)	13 ± 2	10 – 17.5

bpm, beats per minute; HR, heart rate; RER, respiratory exchange rate.

Only one participant with *poor* or *fair* fitness had a "*good*" body fat percentage; the rest (n = 10) had either *fair, poor*, or *very poor* body fat percentage classifications. Likewise, only one participant with a *good* level of CRF, was in the "*poor*" category of body fat percentage, while the rest of the participants with *good* or *excellent* CRF (n = 15), had *fair* to *excellent* body fat percentages.

4.3 Continuous glucose monitoring (CGM) period

The participants were asked to wear their continuous glucose monitoring (CGM) sensors for a full two-week period. Unfortunately, the sensors in some participants detached from their arms or ceased recording data before the two weeks were completed. In six cases, the sensor came off either within two days of the monitoring period, or it failed to record more than a day or two; these were subsequently replaced with a new sensor. The first sensor's data were discarded. If the sensor failed or detached after seven days, it was not replaced. Therefore, the minimum number of days that constituted a complete data set was seven full days, which included five workdays and one two-day weekend for all the participants. Figure 4.1 illustrates the distribution of CGM monitoring days among the participants.

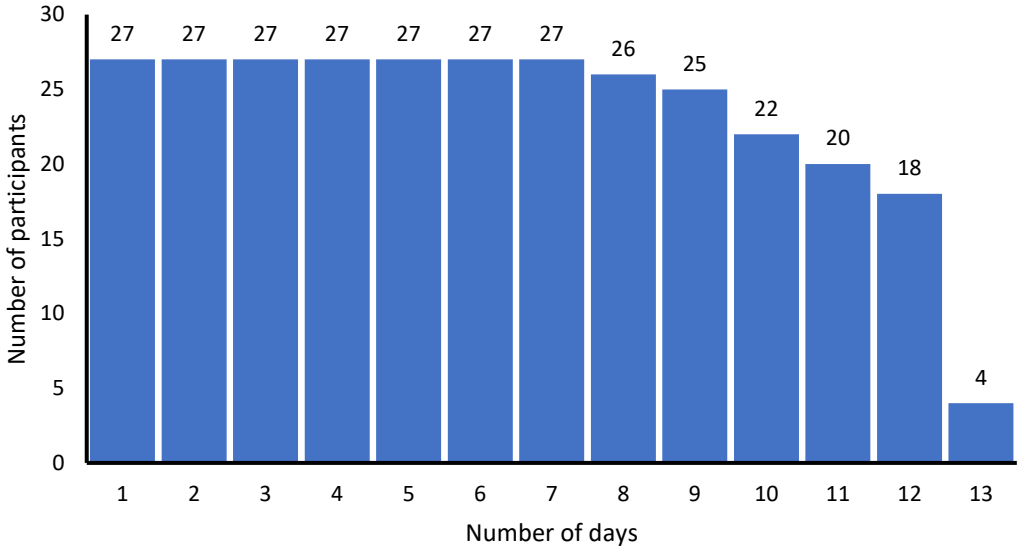

Figure 4.1 Number of glucose monitoring days for the group of participants.

There were no statistically significant differences in body composition, dietary intake, CRF, glucose control, insulin sensitivity or glycaemic variability between the participants with 12 or 13 days' CGM data and those with 7 – 11 days of CGM data. Results for this analysis are shown in Appendix G.

4.4 Dietary characteristics

Judging by the range in values, the dietary characteristics and eating patterns among participants varied considerably, especially regarding absolute carbohydrate and energy intake (Table 4.3). The average daily contribution of sugar to the energy intake of the participants was 9.8% (± SD 3.9%) (range: 2.8 – 17.5%). There was also large interpersonal and intrapersonal daily variation in energy intake as evidenced by Figures 4.2 – 4.4 and Table 4.4.

Table 4.3 Dietary characteristics of the participants during the continuous glucose monitoring period (n = 27).

	Mean ± SD	Minimum - Maximum
Average daily GL	149 ± 49	43 – 239
Average daily CHO intake (g)	267 ± 72	105 – 398
Average daily dietary fibre intake (g)	20.6 ± 6.3	10 – 33
Average daily sugar intake (g)	65.7 ± 28.3	22 – 130
Average daily energy intake (kJ)	11245 ± 1831	7976 – 15125
% dietary CHO intake	41 ± 7	24 – 54
% dietary protein intake	19 ± 4	13 – 27
% dietary fat intake	40 ± 5	32 – 53

GL, glycaemic load; CHO, carbohydrate. Sugar refers to all monosaccharides and disaccharides; CHO refers to polysaccharides.

For the whole group, the intake of protein as percentage of total energy intake was statistically significantly lower than the intake of carbohydrates ($p < 0.0001$; ES = 3.889; 90% CI: 19 - 26) and fat ($p < 0.0001$; ES = 4.786; 90% CI: 20 - 24). The relative intake of carbohydrates and fat did not differ significantly ($p = 0.82$; ES = 0.083; 90% CI: -3.4 – 4.4).

There were no significant differences in the average GL intake between monitoring days ($p = 0.988$; ES = 0.060; 90% CI: -270 – 270) (figure A1 in Appendix H).

The participants' daily average carbohydrate intake did not differ significantly between days ($p = 0.949$; ES = 0.057; 90% CI: -85 – 92) (Figure A2 in Appendix H)

There were no statistically significant differences in average fat intake between the CGM days ($p = 0.295$; ES = 0.020; 90% CI: -0.22 – 0.95) (Figure A3 in Appendix H).

There were no statistically significant differences in average protein intake across the monitoring days for the whole group ($p = 0.543$; ES = 0.006; 90% CI: -0.17 – 0.37) (Figure A4 in Appendix H).

Table 4.4 The average group day-to-day variation (mean ± SD) in dietary energy intake, glycaemic load, and macronutrient consumption.

	SD	CV%
Energy intake	3586 ± 1059 kJ	32.6 ± 5.9%
Glycaemic load	60.5 ± 25.4	41.8 ± 15.7%
Carbohydrate	121.4 ± 20.0 g	46.1 ± 7.4%
Fat	55.1 ± 13.3 g	45.4 ± 8.5%
Protein	42.5 ± 5.9 g	37.6 ± 5.7%

The day-to-day dietary composition of the participants' food varied more than the energy intake (Table 4.4). The group's day-to-day variability (CV%) was larger for carbohydrate intake than for fat (ES = 0.083; 90% CI: -4.4 – 5.7) and protein intake (ES = 1.290; 90% CI: 4.6 – 12). There was a large difference in fat intake variability compared with protein variability (CV%) between individuals (ES = 1.086; 90% CI: 2.8 – 13).

Tables showing the group's day-to-day macronutrient variability, average day-to-day individual (intrapersonal) variation in dietary components, and the differences in the intrapersonal variations of the participants' dietary macronutrient intakes are included in Appendix H.

4.4.1 Characteristics of the macronutrient clusters

Based on the proportions of individual dietary macronutrient intake, three clusters were identified (Figure 4.2). Cluster 1 (n = 9, 33.3%), in comparison to the other two clusters, could be described as the high carbohydrate-low fat (high CHO) group, whereas cluster 2 (n = 6, 22.2%) can be described as the low carbohydrate-high fat (low CHO-high fat) group and cluster 3 (n = 12, 44.4%) the high carbohydrate-high fat group (high CHO-high fat).

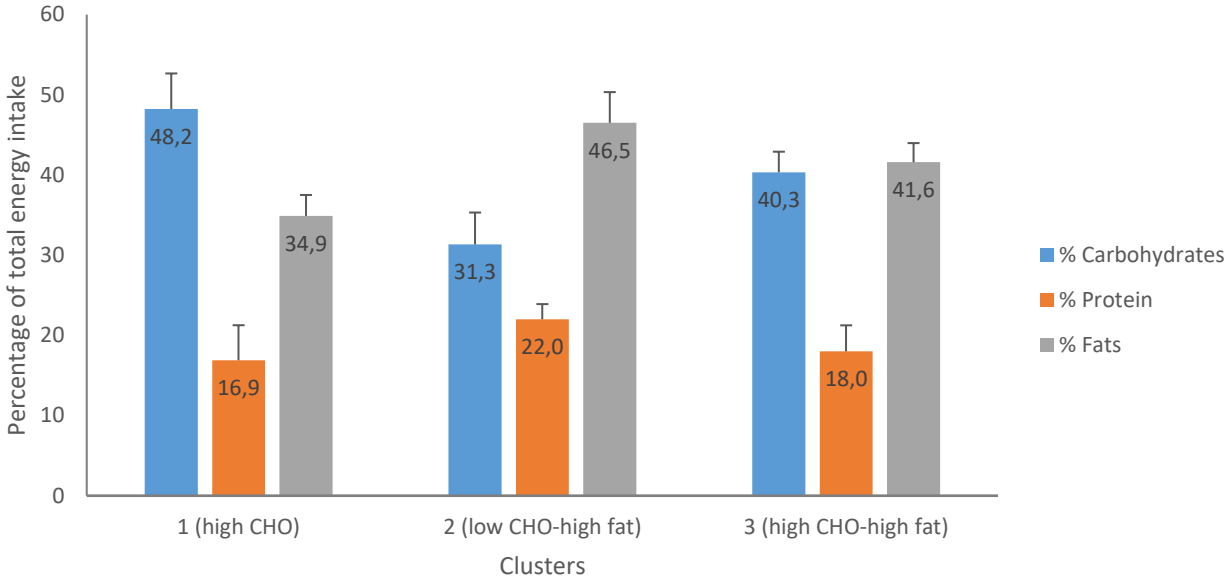

Figure 4.2 The percentage energy contribution from the macronutrients for participants in the three clusters.

The intake of fat and carbohydrates differed significantly between the three groups ($p < 0.01$). There was a large difference in protein intake between clusters 1 and 2 (16.9% vs 22%, $p = 0.019$; ES = 1.41; 90% CI: 1.7 – 8.5), and clusters 2 and 3 (22% vs 18 %, $p = 0.013$; ES = 1.38; 90% CI: 1.5 – 6.5), but only a small difference between clusters 1 and 3 (16.9% vs 18%, $p = 0.51$; ES = 0.30; 90% CI: -1.8 – 4.0).

The average glycaemic load was 185.7 (± SD 42.0) for cluster 1, 99.5 (± SD 28.3) for cluster 2 and 146.1 (± SD 40.3) for cluster 3 (p = 0.001). The differences in glycaemic load between clusters 1 and 2 ($p = 0.0007$; ES 2.301; 90% CI: 51 - 120), clusters 1 and 3 ($p = 0.041$; ES = 0.965; 90% CI: 8.3 – 71) and clusters 2 and 3 ($p = 0.023$; ES = 1.260; 90% CI: 14 – 79) were all large. The average daily energy intake of cluster 2 was 10 309 (± SD 1593 kJ), which was less than for cluster 1, which was 11 557 (± SD 1243 kJ) ($p = 0.112$; ES = 0.898; 90% CI: 13 – 21) and cluster 3, which was 11 478 (± SD 2243) ($p = 0.27$; ES = 0.567; 90% CI: -630 – 3000). There was no difference in energy intake between clusters 1 and 3 ($p = 0.925$; ES = 0.042; 90% CI: -1400 – 1500).

4.4.2 Differences between the body composition measures of the clusters

The average BF%, waist-to-hip and waist-to-height ratios, as measures of obesity, were highest for cluster 3, while BF% was lowest for cluster 2. Moderate differences in BF% were detected between cluster 2 and 1, and large differences between cluster 2 and 3. Waist-to-hip and waist-to-height ratios,

as indicators of central obesity, were both lowest for cluster 1, although the differences between cluster 1 and 2 was small to negligible.

Table 4.5 Body composition of individuals per cluster (means +- SD) and differences between the clusters. Difference values are ES (90% CI).

	Mean ± SD			ES (90% CI)		
	Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 1 vs 2**	**Cluster 1 vs 3**	**Cluster 2 vs 3**
Body mass (kg)	82.1 ± 6.84	88.8 ± 14.56	91.7 ± 17.05	0.645** (-16 – 3.1)	0.698** (-20 – 0.9)	0.178 (-17 – 11)
BF%	20.2 ± 3.53	16.6 ± 7.91	23.2 ± 6.03	0.641** (-1.6 – 8.9)	0.576* (-6.9 – 1.0)	0.984*** (-12 – -0.7)
FFM (kg)	65.5 ± 5.53	73.5 ± 10.37	70.2 ± 11.42	1.032*** (-15 – -0.77)	0.496* (-12 – 2.5)	0.301* (-6.4 – 13)
Waist:Hip	0.84 ± 0.045	0.87 ± 0.046	0.88 ± 0.061	0.071 (-0.046 – 0.039)	0.763** (-0.83 – 0.0)	0.680** (-0.088 – 0.011)
Waist:Height	0.47 ± 0.039	0.48 ± 0.040	0.53 ± 0.072	0.332* (-0.05 – 0.024)	1.033#*** (-0.11 – -0.02)	0.771** (-0.1 – 0.007)

#p = 0.03; all other p-values are > 0.05; *small difference, **moderate difference, ***large difference.

4.5 Glucose control

4.5.1 Whole group results

A summary of the laboratory blood tests for measures of glucose control and insulin resistance are presented in Table 4.6. All participants, except one (participant 01), presented with healthy fasting plasma glucose concentrations (< 5.6 mmol·L^{-1}). One of the participants with a healthy fasting glucose (5.1 mmol·L^{-1}) had a 2-hr post OGTT glucose level of 7.8 mmol·L^{-1}, which is the cut-off value for the classification of pre-diabetes. All the other participants had healthy 2-hr glucose concentrations.

Four participants had HbA_{1C} levels on or above the cut-off value for healthy individuals (pre-diabetic range: 5.7% – 6.1%). None of them, however, reached an HbA_{1C} level of 6.5% that is typically used for the diagnosis of diabetes (WHO, 2019; SEMDSA, 2017). The remaining 23 participants presented with healthy HbA_{1C} values.

Table 4.6 Traditional glucose control measures and insulin sensitivity indexes (n = 27).

	Mean ± SD	Minimum - Maximum	Healthy values
HbA_{1C} (%)	5.43 ± 0.27	5.1 – 6.1	< 5.7*
Fasting plasma glucose (mmol·L^{-1})	5.01 ± 0.32	4.3 – 5.7	< 5.6*$
2-h glucose (mmol·L^{-1})	4.63 ± 1.44	1.7 – 7.8	< 7.8*#
Maximum glucose (mmol·L^{-1})	7.48 ± 1.37	5.1 – 10.3	-
Fasting insulin (mIU·L^{-1})	5.95 ± 3.66	1.9 – 18.6	< 10.7**
HOMA-IR	1.31 ± 0.77	0.43 – 3.64	< 1.7-2.0***
Matsuda Index	9.36 ± 4.37	2.69 – 21.16	> 4.3****

HbA_{1c}, Glycated haemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; *healthy values as per the American Diabetes Association (2017); $SEMDSA (2017) #World Health Organization (2006); **(Ter Horst *et al.*, 2015); ***Gayoso-Giz *et al.* (2013); ****Gutch *et al.* (2015).

According to the Matsuda index, four of the participants were identified as insulin-resistant (Figure 4.3). Among these was the participant (03) with the high 2-h glucose value (7.8 mmol·L^{-1}) and one of the participants (07) with a HbA_{1C} value in the pre-diabetes range. These four individuals also had HOMA-IR values of 2.04 and higher. Another individual was identified as insulin-resistant according to the HOMA-IR (value of 2.09), but not by the Matsuda index (value of 5.07) (cut-off value of 4.3 as suggested by Gutch *et al.;* 2015). None of the participants had HOMA-IR values within the cut-off range (1.7 - 2.0).

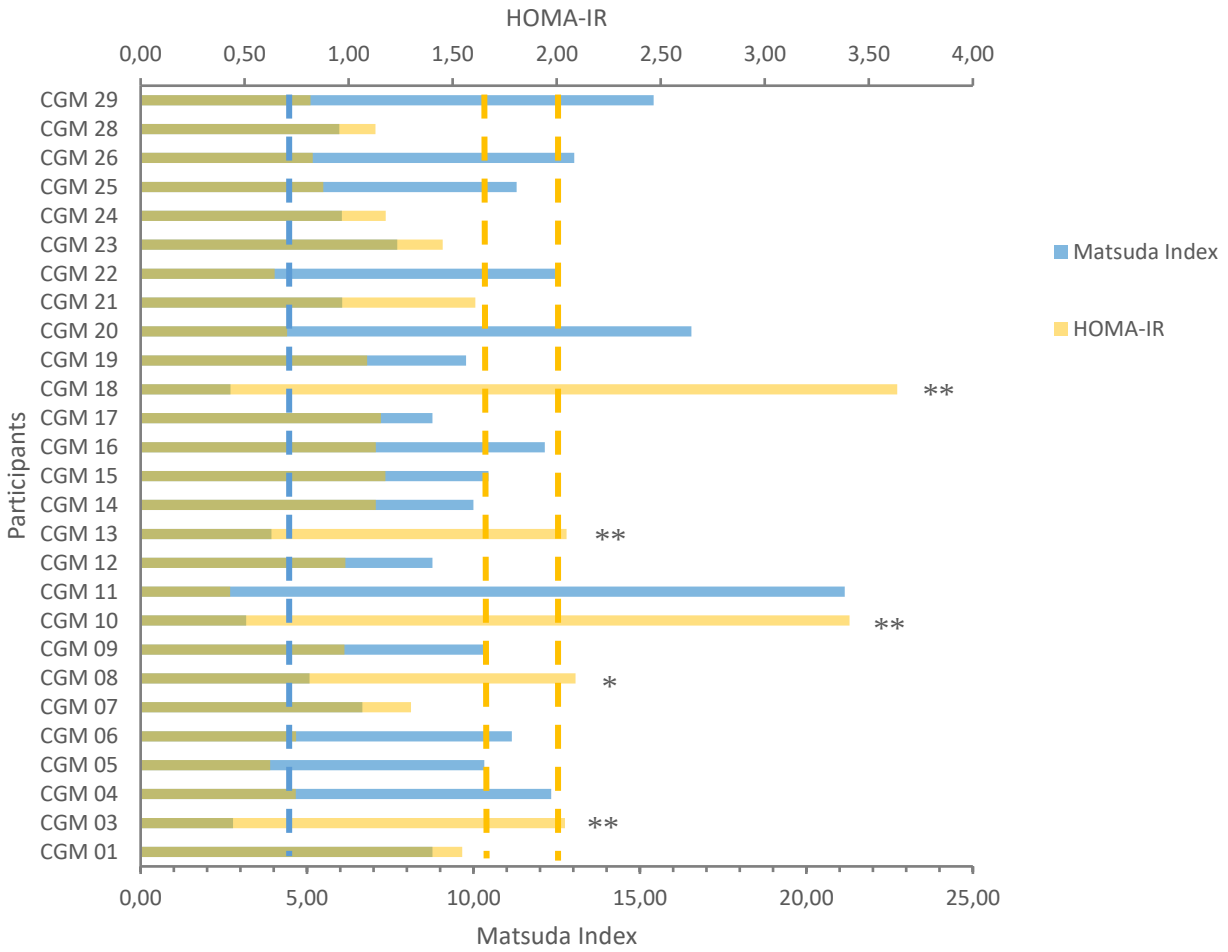

Figure 4.3 Individual insulin sensitivity/-resistance indices. The blue and yellow dashed lines represent the cut-off value for the Matsuda index and range for HOMA-IR, respectively. The overlapping of the bars presents as green. Matsuda index values to the right of the blue dashed line (4.3) represent the healthy values, while HOMA-IR values left of the range between the two yellow dashed lines (1.7 and 2.0), represent the healthy values. * Unhealthy HOMA-IR; ** unhealthy Matsuda and HOMA-IR indexes.

The maximum glucose concentration during the OGTT was measured after 30 minutes for 22 (81%) of the participants. Four participants (15%) reached maximum [glucose] after one hour and one participant after 90 min. The latter participant's maximum [glucose] was, however, a mere 5.1 $mmol \cdot L^{-1}$.

The participants' average maximum [glucose] during the OGTT was 7.5 (± SD 1.4) mmol·L^{-1} (range 5.1 – 10.3 mmol·L^{-1}). The maximum [glucose] of ten (37%) of the participants exceeded 7.8 mmol·L^{-1}. In 16 (59%) participants the 2-h post OGTT glucose level was lower than their individual fasting level. Eight (30%) participants' 2-h glucose levels were higher than their fasting levels, while three (11%) participants had identical fasting and 2-h glucose concentrations. The participant with the highest 2-h [glucose] also had the highest [insulin] at 90 and 120 min. In fact, his final insulin measurement (120 min) was the highest of all his values. This participant's Matsuda index was the second lowest. The participant with the lowest Matsuda index had the highest overall [insulin], namely 165.2 mIU·L^{-1} after 60 min. His insulin levels, however, returned to normal (i.e. below 40 mIU·L^{-1}) after 120 min, namely 28.9 mIU·L^{-1}.

The average ± SD [glucose] and [insulin] trend of the participants during the OGTT is illustrated in Figure 4.4. The average variability (CV%) in glucose values (21.5 ± 9.9%) was less than for insulin values (80.9 ± 27.5%) (p = 0.004).

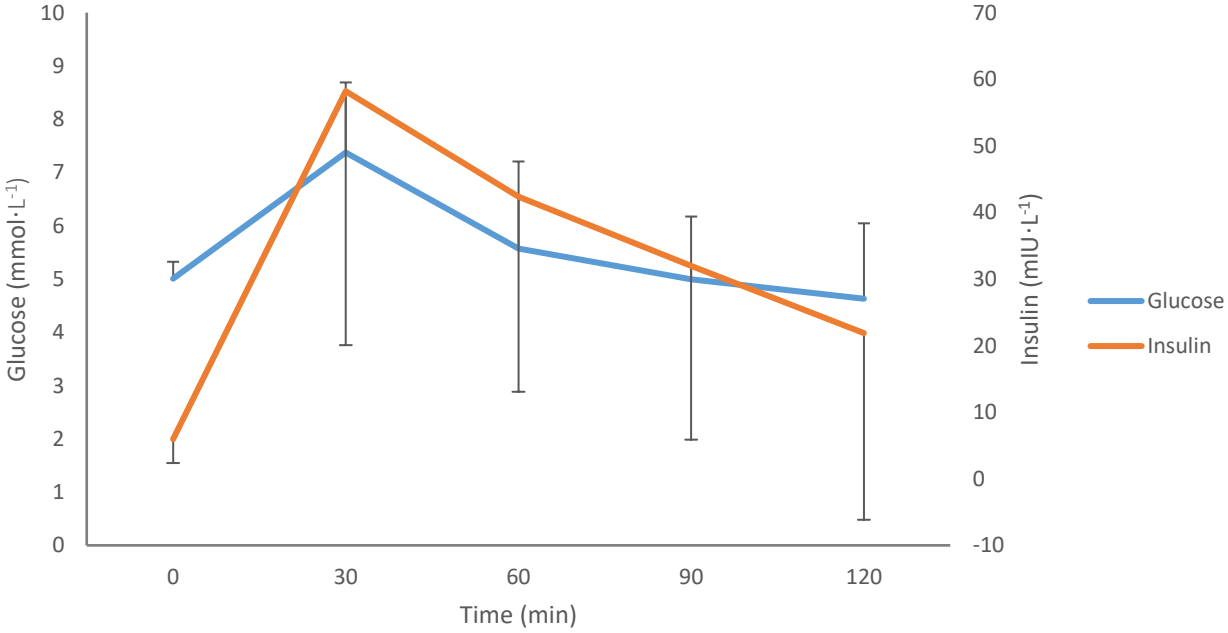

Figure 4.4 The average (± SD) of the [glucose] and [insulin] of the study sample during the OGTT.

4.5.2 Macronutrient clusters

4.5.2.1 Glucose control and insulin sensitivity

Table 4.7 illustrates that the differences between the clusters were more evident and consistent for insulin sensitivity / -resistance indexes than for glucose control measures.

Table 4.7 Glucose control and insulin sensitivity measures for the respective macronutrient clusters.

	Clusters (mean ± SD)			Cluster differences ES (90% CI)		
	1	**2**	**3**	**1 vs 2**	**1 vs 3**	**2 vs 3**
HbA_{1C} (%)	5.46 ± 0.34	5.42 ± 0.18	5.42 ± 0.27	0.133 (-0.23 –0.31)	0.129 (-0.19 –0.27)	0 (n.a.)
FPG ($mmol \cdot L^{-1}$)	4.87 ± 0.30	5.07 ± 0.22	5.08 ±0.36	0.731** (-0.46 –0.055)	0.637** (-0.48 –0.04)	0.051 (-0.3 –0.27)
2-h glucose ($mmol \cdot L^{-1}$)	4.49 ± 1.85	4.50 ± 1.21	4.80 ± 1.30	0.007 (-1.5 –1.5)	0.200* (-1.5 – 0.88)	0.235* (-1.4 –0.81)
HOMA-IR	1.09 ± 0.43	0.87 ± 0.19	1.7 ± 0.97	0.621** (-0.11 –0.55)	0.779** (-1.2 – -0.01)	1.028*** (-1.5 – -0.13)
Matsuda Index	10.03 ± 3.92	11.23 ± 2.24	7.9 ± 5.21	0.357* (-4.3 –1.9)	0.446* (-1.5 – 5.7)	0.734** (-0.62 –7.2)

All p-values > 0.05; *small difference, **moderate difference, ***large difference.

There were no significant differences in HbA_{1c} between the clusters ($p > 0.05$; ES < 0.2). The 2-h [glucose] between clusters 1 and 2 and FPG between clusters 2 and 3 did not differ either ($p > 0.05$; ES < 0.2). There was a small increase in 2-h [glucose] from clusters 1 to 3 ($p = 0.656$; ES = 0.200) and from cluster 2 to 3 ($p = 0.644$; 0.235). There was a moderate increase in FPG from cluster 1 to cluster 2 ($p = 0.189$; ES = 0.731) and from cluster 1 to cluster 3 ($p = 0.165$; ES = 0.637).

Insulin sensitivity (as determined by the Matsuda index) was highest in cluster 2 (compared to cluster 1: $p = 0.510$; ES = 0.357; compared to cluster 3: $p = 0.161$; ES = 0.734). Likewise, insulin resistance (HOMA-IR) was the highest in cluster 3 ($p = 0.093$; ES = 0.779, compared to cluster 1 and $p = 0.057$; ES = 1.028, compared to cluster 2).

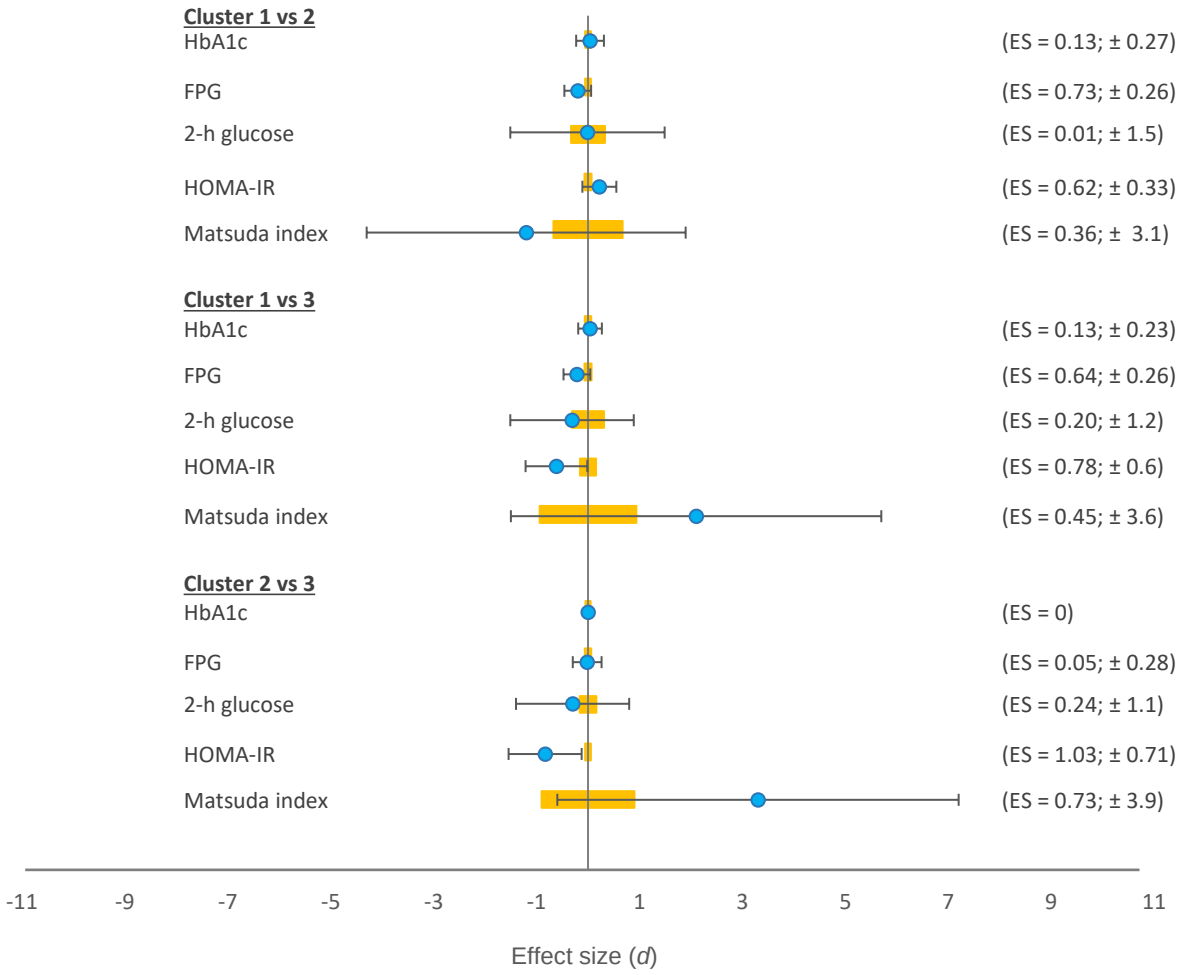

Figure 4.5 Differences in glucose control and insulin sensitivity between clusters 1 & 2, 1 & 3, and 2 & 3. The blue dots represent the mean difference in the respective measures between the clusters with error bars representing the 90% confidence intervals. The yellow bars show the smallest worthwhile difference.

4.5.2.2 Oral Glucose Tolerance test

There was a small difference in average OGTT [glucose] between cluster 3 and cluster 1 ($p = 0.054$; ES = 0.293) and cluster 2 ($p = 0.082$; ES = 0.241), but there was no difference in the average [glucose] of cluster 1 and 2 ($p = 0.708$; ES = 0.049).

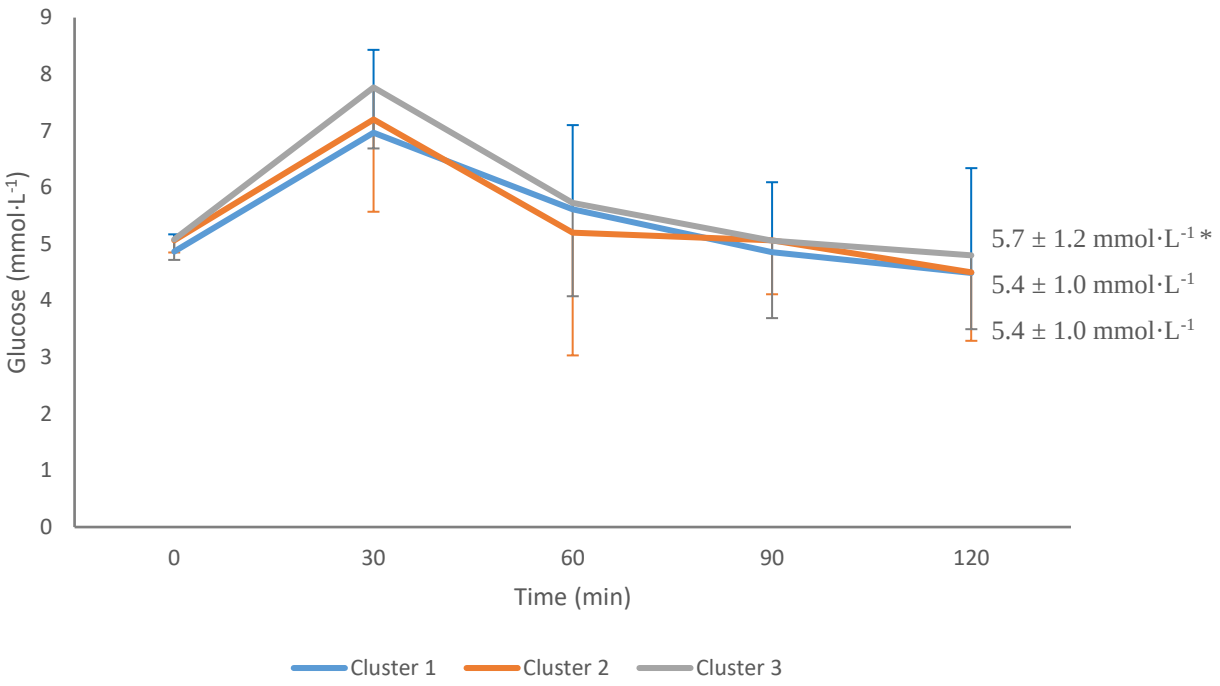

Figure 4.6 A comparison of the blood glucose responses during the 2-hr OGTT among clusters. Values are means ± SD. * Small difference between average [glucose] of cluster 1 and 3, and cluster 2 and 3.

The differences in the average [insulin] during the OGTT between the clusters were larger than the differences in the average [glucose]. There were large differences between the average OGTT [insulin] of cluster 2 compared to cluster 1 (p = 0.023; ES = 1.262) and cluster 3 (p = 0.013; ES = 1.503), while there was a small difference between the average [insulin] of clusters 1 and 3.

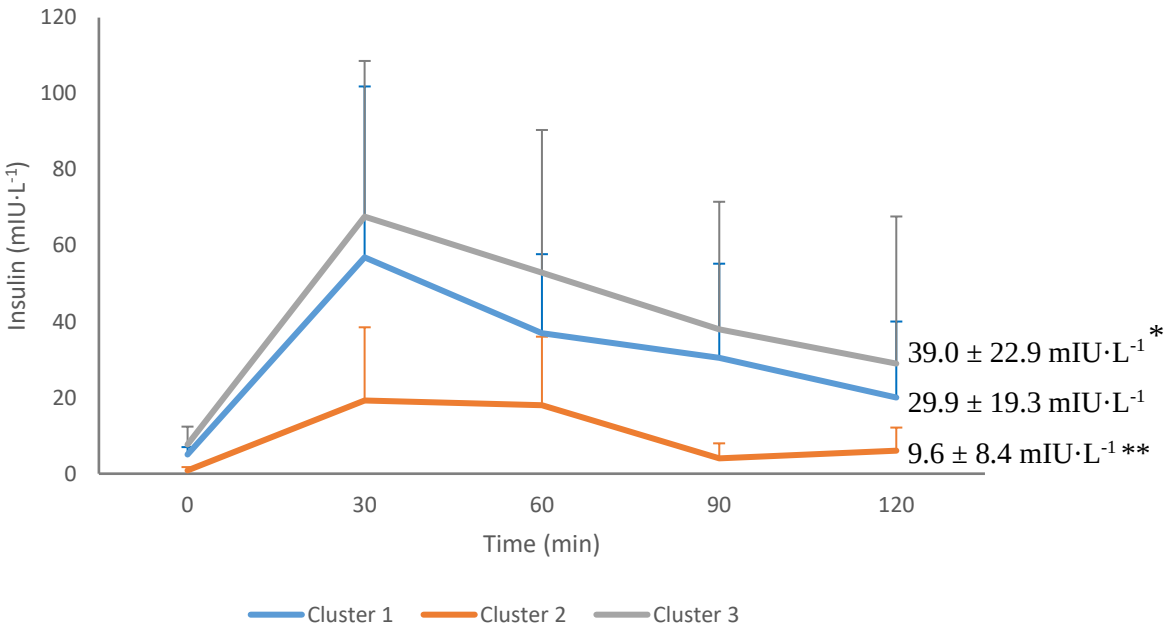

Figure 4.7 A comparison of serum insulin responses during the 2-hr OGTT among clusters. Values are means + SD. * The difference in average [insulin] between cluster 1 and 3 was small; ** the differences in the average [insulin] between cluster 1 and 2, and cluster 2 and 3 were large.

The differences in [insulin] between the clusters at individual time points during the OGTT were generally larger than the differences in [glucose] (See Tables A4 and A5 in Appendix H). This was evident in effect sizes, even though post hoc tests revealed no statistically significant differences ($p > 0.05$) between either [insulin] or [glucose] at any time point.

There was no meaningful difference in [glucose] between clusters 1 and 2 at 120 min, clusters 1 and 3 at 60 min, and clusters 2 and 3 at 0 min and 90 min ($p > 0.05$; ES < 0.02). Small differences were evident between clusters 1 and 2, at 30 min, 60 min and 90 min, between clusters 1 and 3 at 90 and 120 min, and between clusters 2 and 3 at 30 min, 60 min, and 90 min. A moderate difference was seen between clusters 1 and 2 for fasting [glucose], as well as between clusters 1 and 3 for fasting and 30 min [glucose].

There was a difference in [insulin] between all the clusters at all time points during the OGTT. The size of the difference in [insulin] during the OGTT between clusters 1 and 2 was moderate at fasting and 120 min, and small at all other time points; the [insulin] of cluster 1 were higher at all time points. A moderate difference was seen between clusters 1 and 3 for fasting and 60 min [insulin], with a small difference at all other time points; the average [insulin] of cluster 3 were higher. There was a large difference in fasting [insulin] between clusters 2 and 3. All other [insulin] differences between

cluster 2 and 3 were moderate. The [insulin] at all time points were the highest in cluster 3 and the lowest in cluster 2.

There was no meaningful difference in the [glucose] AUCs of clusters 1 and 2 (p = 0.987; ES = 0.009). There was a small increase in [insulin] AUC for cluster 1, compared to cluster 2 (p = 0.338; ES = 0.481). The [glucose] and [insulin] AUCs were larger for cluster 3 than cluster 1 (p = 0.492; ES = 0.309 and p = 0.417; ES = 0.365, respectively). The AUCs of both [glucose] and [insulin] of cluster 3 were larger than cluster 2 (p = 0.553; ES = 0.303 and p = 0.111; ES = 0.776, respectively).

4.6 Glycaemic variability

4.6.1 Whole group results

The means ± SD for the glycaemic variability parameters and time above and below set concentrations of the study sample are depicted in Table 4.8. None of the individuals presented with glucose concentrations at or above 10.0 mmol·L^{-1} at any time point during the period of CGM. 10.0 mmol·L^{-1} is frequently used as the value to describe hyperglycaemia (Danne *et al*., 2017; Paing *et al*., 2020).

Table 4.8 Glycaemic variability indices from continuous glucose monitoring data (n = 27).

	Mean ± SD	Minimum – Maximum	Healthy population reference values
Mean glucose (mmol·L^{-1})	4.6 ± 0.4	3.9 – 5.4	5.03 – 6.69*
SD (mmol·L^{-1})	0.79 ± 0.15	0.57 – 1.19	0.44 – 1.37*
CV%	17.32 ± 4.1	10.67 – 30.16	7.74 – 22.45*
MAGE (mmol·L^{-1}) (intra-day variability)	1.00 ± 0.20	0.72 – 1.54	0.8 – 3.02* 0.0 – 2.8**
MODD (mmol·L^{-1}) (inter-day variability)	0.70 ± 0.16	0.46 – 1.08	0.4 – 1.41* 0.0 – 3.5**

SD, Standard deviation from the average glucose concentration over the CGM monitoring period; CV%, coefficient of variation; MAGE, mean amplitude of glycaemic excursions; MODD, mean of daily differences. *Reference values according to Foreman *et al.* (2020) (2.5th – 97.5th percentile) and **Hill *et al*. (2011) (mean ± 2 SD).

The average (± SD) time per day that the participants spent above [glucose] of 6 mmol·L^{-1}, was 84 min (± 57) (range: 10 – 205). The average (± SD) time per day that the participants spent at and

above [glucose] of 7.8 mmol·L^{-1}, and at or below 3 mmol·L^{-1} was 4 min (± 5) (range: 0 – 15) and 22 min (± 44) (range: 0 – 188) , respectively.

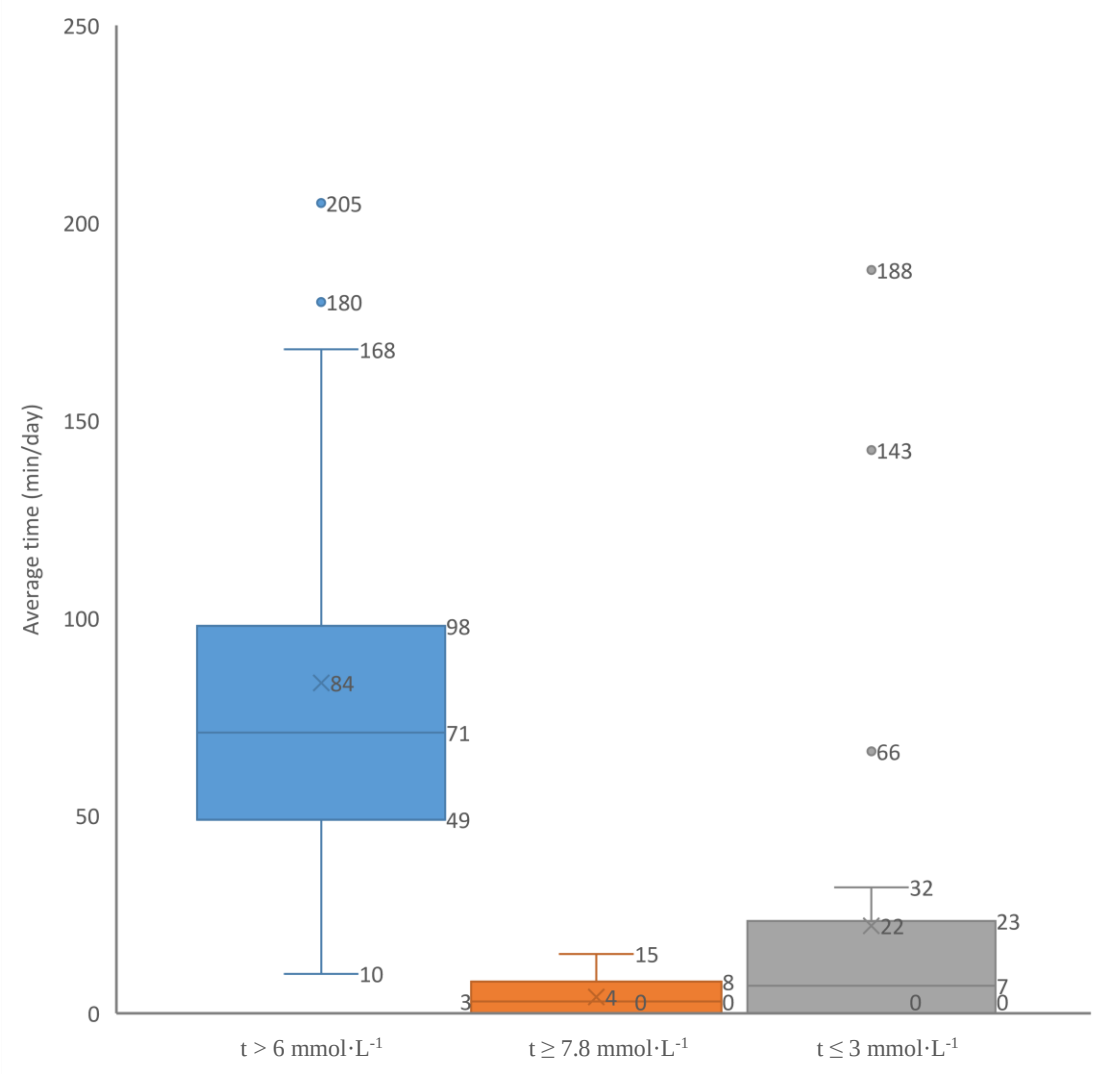

Figure 4.8 Average time per day spent at, above and below specified [glucose] thresholds.

Seventeen (63%) participants recorded glucose values ≥ 7.8 mmol·L^{-1} during the CGM period (Figure 4.9).

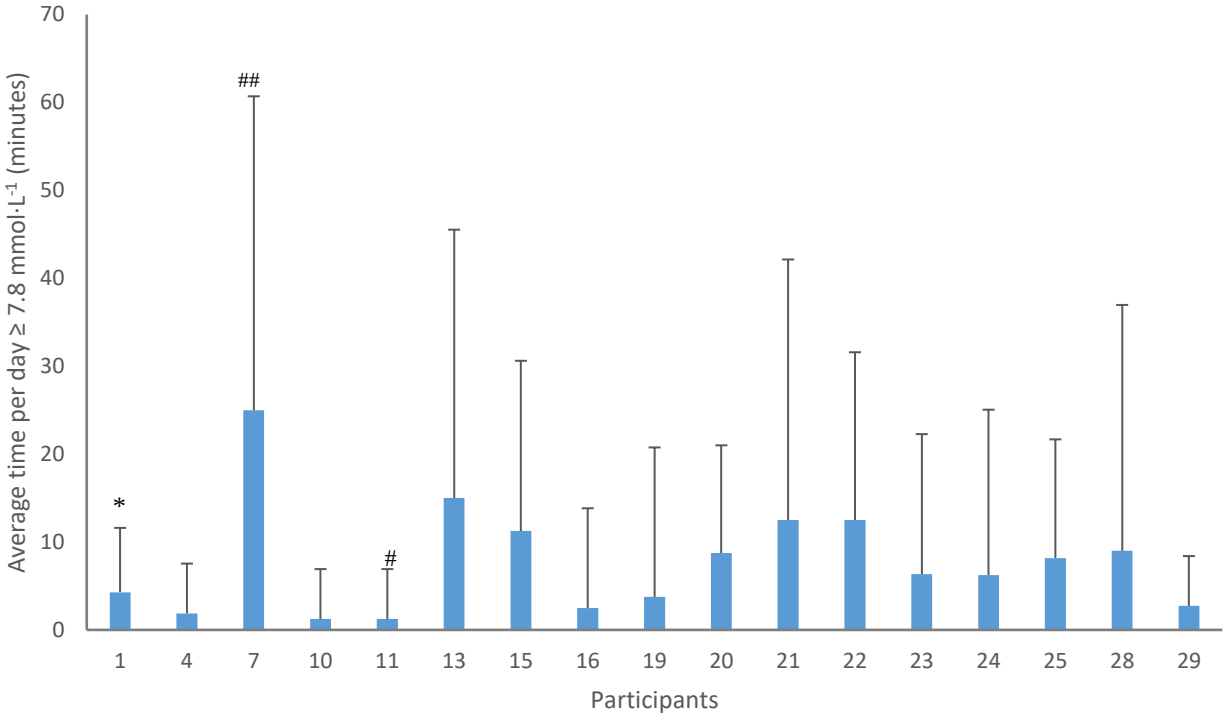

Figure 4.9 Individuals' average (+ SD) time per day with high (≥ 7.8 mmol·L^{-1}) glucose values. *Participant 1: least overall glycaemic variability, ##participant 7: most inter- and intra-day glycaemic variability and #participant 11: most overall glycaemic variability.

Glucose values below 3 mmol·L^{-1} were recorded on one or more occasions by 18 (67%) participants (Figure 4.10). Among these 18 participants, minimum values of 2.2 mmol·L^{-1} were recorded on at least one occasion for some individuals (n = 8), however, only two of these participants spent a significant amount of time at this minimum value (on average 58 and 101 min per day, respectively). Collectively, participants had more often low (≤ 3 mmol·L^{-1}) [glucose] (22 ± 44 minutes per day), than high values (≥ 7.8 mmol·L^{-1}) (4 ± 5 min on average per day) (p = 0.049).

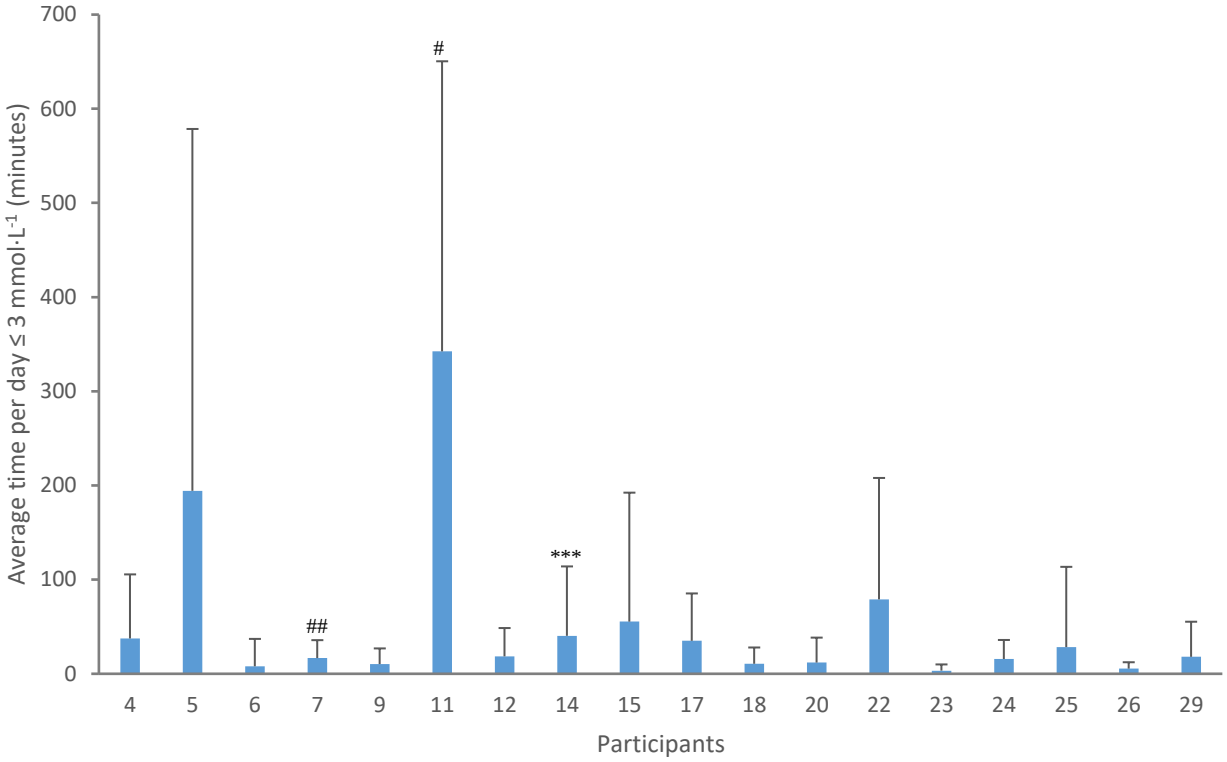

Figure 4.10 Individuals' average (+ SD) time per day with low (≤ 3 mmol·L^{-1}) glucose values. ##Participant 7: most inter- and intra-day glycaemic variability, **participant 11: most overall glycaemic variability and *** participant 14: least inter-day glycaemic variability.

Representative continuous glucose monitoring profiles

The highest inter- and intra-day glycaemic variability as per MODD and MAGE, respectively, was observed in the same individual (Figure 4.12 and Figure 4.13). His overall glycaemic variability (CV%) was the second highest among the study sample. This individual also spent most time per day above 6 mmol·L^{-1} and his average daily GL was the highest of all participants. His HOMA-IR and Matsuda indexes were 1.3 and 6.66, respectively, which does not classify him as being insulin-resistant. The individual with the highest CV% (Figure 4.11) had the second highest MAGE and MODD values. He was deemed the most insulin sensitive in the study sample, with an HOMA-IR of 0.43 and a Matsuda index of 21.16. The participants with the least inter- and intra-day and overall glycaemic variability were different for each of the parameters.

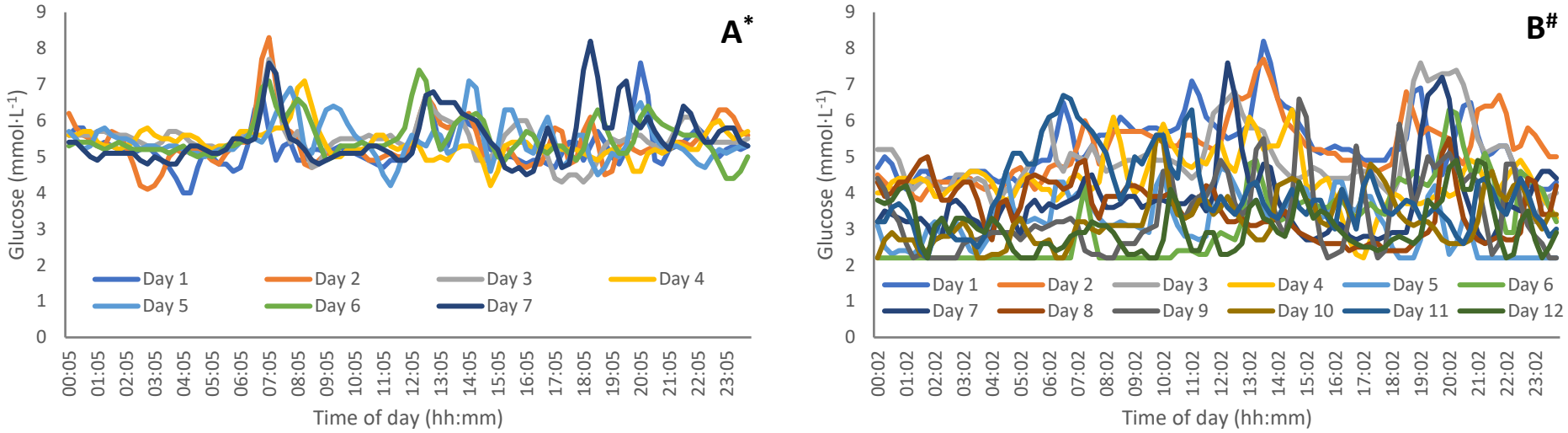

Figure 4.11 Individual CGM profiles of the participant with (A) the least (participant 01) and (B) most (participant 11) overall glycaemic variability (CV%).

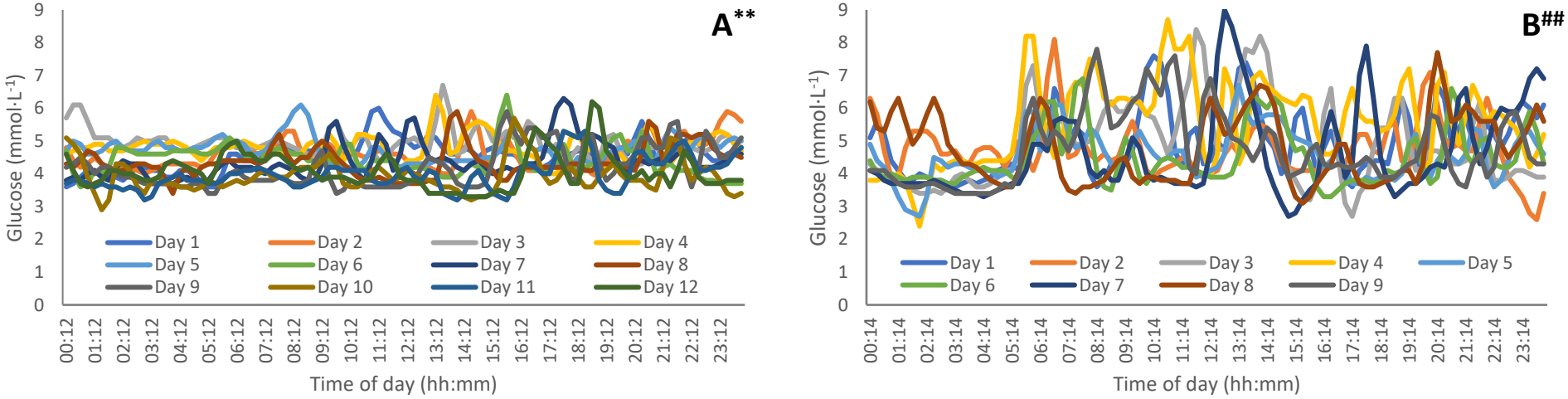

Figure 4.12 Individual CGM profiles showing the (A) least (participant 08) and (B) most (participant 07) intra-day glycaemic variability as per MAGE.

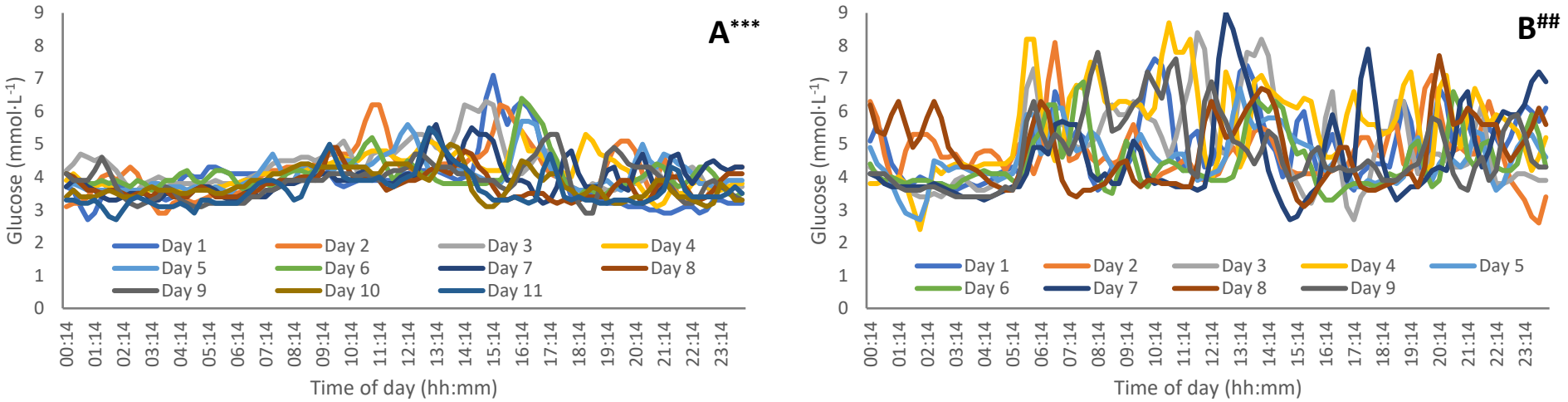

Figure 4.13 Individual CGM profiles showing the (A) least (participant 14) and (B) most (participant 07) inter-day glycaemic variability as per MODD.

4.6.2 Clusters

The glycaemic variability indices between the clusters are compared in Table 4.9 The glycaemic variability indices of the clusters and differences between the clusters. The highest average values for all the indices were in cluster 1. The lowest glycaemic variability is not attributed to a single cluster but are distributed between clusters 2 and 3. None of these differences was statistically significant. Small differences were evident between clusters 1 and 2 for CGM glucose SD (lower for cluster 2), between clusters 1 and 3 for MODD (lower for cluster 3) and time per day spent above 6 mmol·L^{-1} (lower for cluster 3). Differences were seen between clusters 2 and 3 in CGM glucose SD (lower for cluster 3), CV% (lower for cluster 3), MODD (lower for cluster 3) and time per day spent above 6 mmol·L^{-1} (lower for cluster 2). Moderate differences were evident between clusters 1 and 2 for MAGE (lower for cluster 2) and time per day spent above 6 mmol·L^{-1} (lower for cluster 2), and between cluster 1 and 3 for CGM glucose SD (lower for cluster 3) and MAGE (lower for cluster 3).

Table 4.9 The glycaemic variability indices of the clusters and differences between the clusters.

	Clusters (mean ± SD)			**Cluster differences ES (90% CI)**		
	1	**2**	**3**	**1 vs 2**	**1 vs 3**	**2 vs 3**
Mean glucose (mmol·L^{-1})	4.6 ± 0.35	4.6 ± 0.34	4.6 ± 0.47	0.150 (-27 – 37)	0.017 (-0.31 – 0.33)	0.104 (-0.42 – 0.33)
SD (mmol·L^{-1})	0.83 ± 0.14	0.81 ± 0.13	0.74 ± 0.16	0.199* (-0.15 – 0.1)	0.590* (-0.03 – 0.21)	0.415* (-0.07 – 0.2)
CV%	18.3 ± 3.5	17.8 ± 3.5	16.4 ± 4.8	0.151 (-2.7 – 3.8)	0.134 (-1.4 – 5.2)	0.313* (-2.5 – 5.3)
MAGE (intra-day **variability)**	1.10 ± 0.2	0.93 ± 0.2	0.95 ± 0.2	0.784** (-0.37 – 0.03)	0.749** (-0.003 – 0.3)	0.136 (-0.16 – 0.12)
MODD (inter-day variability)	0.73 ± 0.2	0.71 ± 0.1	0.66 ± 0.2	0.156 (-0.17 – 0.12)	0.414* (-0.06 – 0.2)	0.296* (-0.09 – 0.18)
t > 6 mmol·L^{-1} (min·day^{-1})	95 ± 58	61 ± 39	86 ± 64	0.666** (-14 – 82)	0.151 (-38 – 56)	0.431* (-75 – 25)

All p-values > 0.05; *small difference, **moderate difference.

4.7 Associations between cardiorespiratory fitness and glycaemic variability, glucose control and insulin sensitivity.

There was no clear association between CRF and the glycaemic variability indices – either small (CV%, t > 6 mmol·L^{-1}, mean glucose, SD) or trivial correlations (MODD and MAGE) were found. HbA_{1c} was moderately and negatively correlated with CFR, but fasting plasma glucose and 2-h glucose did not correlate with CRF. HOMA-IR and the Matsuda index were both very strongly associated with CRF. Higher CRF values were associated with lower insulin resistance (lower HOMA-IR), better insulin sensitivity (increased Matsuda index), lower OGTT insulin AUC (large correlation), and a higher OGTT glucose AUC (small correlation).

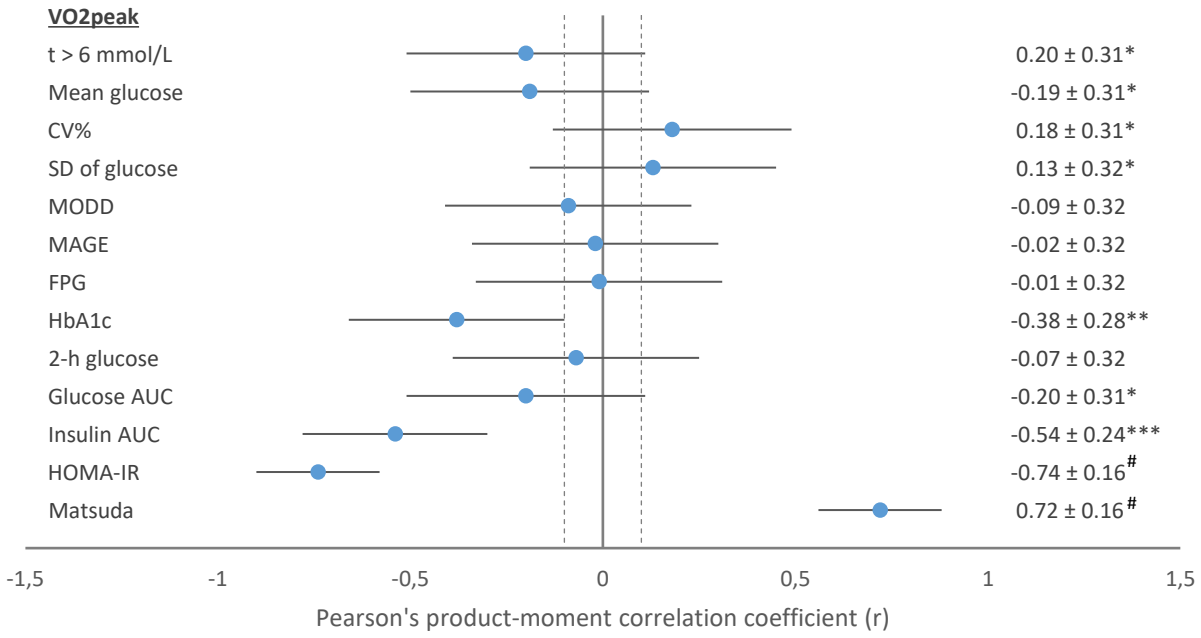

Figure 4.14 Correlations between CRF and glycaemic variability, glucose control and insulin sensitivity. Correlations are represented as r-values ± 90% confidence intervals. The vertical dashed lines represent the r-values of -0.1 and 0.1, respectively. Correlation coefficients and confidence intervals that cross both lines are deemed unclear. Correlation effect sizes: *small, **moderate, *** large, # very large.

4.8 Associations between average daily glycaemic load and glycaemic variability, glucose control, and insulin sensitivity.

Insulin sensitivity and/or -resistance did not correlate with habitual daily dietary glycaemic load. 2-h glucose showed the strongest (moderate negative) correlation with glycaemic load among all the glucose control measures. A high glycaemic load was associated with moderate increases in intra-

day (MAGE) and inter-day (MODD) glycaemic variability, as well as daily time spent above 6 mmol·L^{-1}. Overall, glycaemic variability (CV% and SD) and mean CGM glucose revealed only small correlations with dietary glycaemic load.

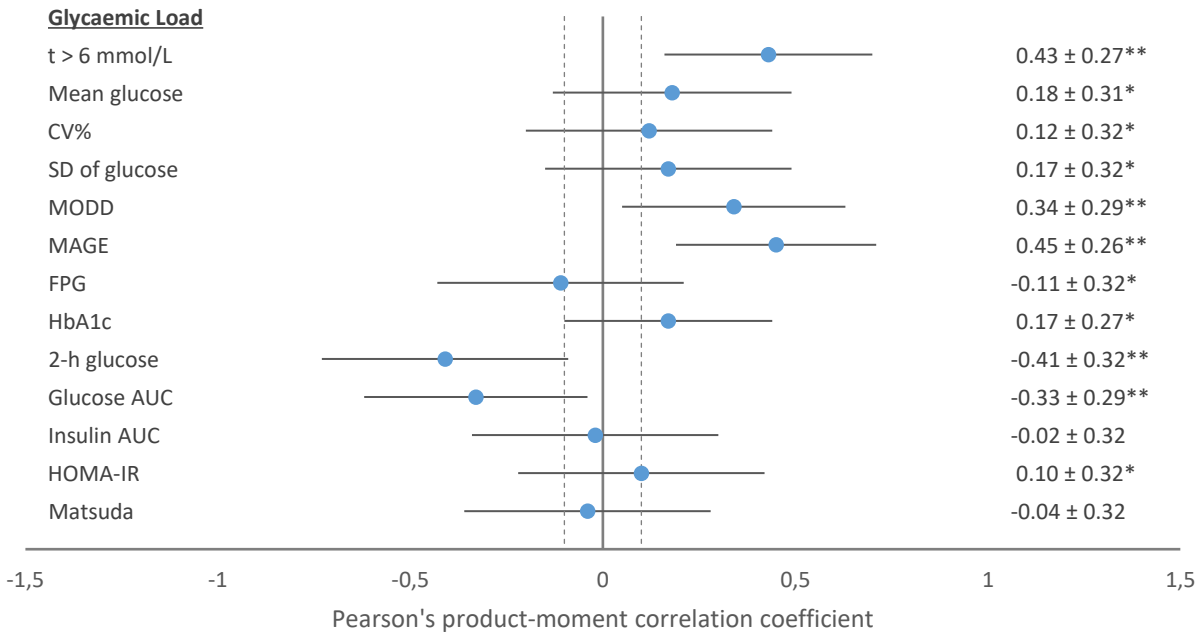

Figure 4.15 Correlations between glycaemic load and glycaemic variability, glucose control and insulin sensitivity. Correlations are represented as r-values ± 90% confidence intervals. The vertical dashed lines represent the r-values of -0.1 and 0.1, respectively. Correlation coefficients and confidence intervals that cross both lines are deemed unclear. Correlation effect sizes: *small, **moderate.

4.9 Associations between glycaemic variability and glucose control.

Higher HbA_{1c} values were related to moderate increases in both mean glucose and average daily time above 6 mmol·L^{-1}, as well as a small increase in MAGE. Increased fasting plasma glucose was associated with small increases in time per day spent above 6 mmol·L^{-1}, overall glycaemic variability (CV% and SD) and moderate increases in inter- and intra-day glycaemic variability (MODD and MAGE, respectively). 2-h OGTT [glucose] was slightly and negatively correlated with time per day spent above 6 mmol·L^{-1} and overall glycaemic variability (CV% and SD). 2-h OGTT [glucose] revealed moderate negative correlations with inter-day (MODD), and intra-day glycaemic variability (MAGE).

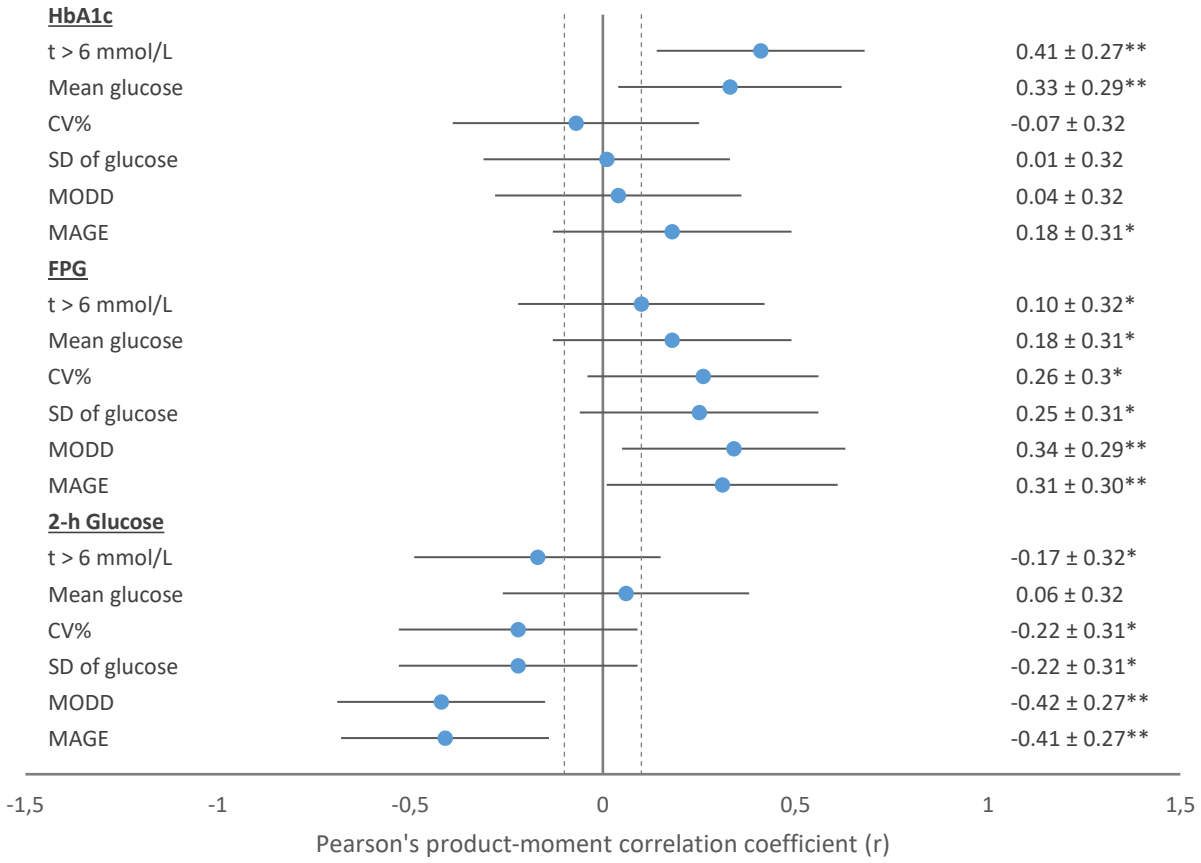

Figure 4.16 Correlations between glucose control measures and glycaemic variability indices.
Correlations are represented as r-values ± 90% confidence intervals. The vertical dashed lines represent the r-values of -0.1 and 0.1, respectively. Correlations between these two values and confidence intervals that cross both lines are deemed unclear. Correlation effect sizes: *small, **moderate.

4.10 Association between glycaemic variability and insulin sensitivity.

Small positive correlations were found between insulin resistance and daily time spent above [glucose] of 6 mmol·L^{-1}, as well as mean glucose. Insulin resistance were negatively correlated with overall glycaemic variability (CV% and SD) and intra-day glycaemic variability (MAGE).

Higher insulin sensitivity was moderately correlated with both CGM glucose CV% and SD, but less with MAGE and MODD. Mean glucose and average time per day spent above 6 mmol·L^{-1} both were negatively correlated with insulin sensitivity.

Figure 4.17 Correlations between insulin sensitivity and glycaemic variability (per person over monitoring period). Correlations are represented as r-values ± 90% confidence intervals. The vertical dashed lines represent the r-values of -0.1 and 0.1, respectively. Correlations between these two values and confidence intervals that cross both lines are deemed unclear. Correlation effect sizes: *small, ** moderate.

4.11 Association between insulin sensitivity and glucose control.

The strongest correlation was observed between the Matsuda index and HbA_{1c}. Insulin resistance (HOMA-IR) was positively associated with HbA_{1c} and 2-h glucose. FPG did not correlate with either insulin sensitivity or resistance.

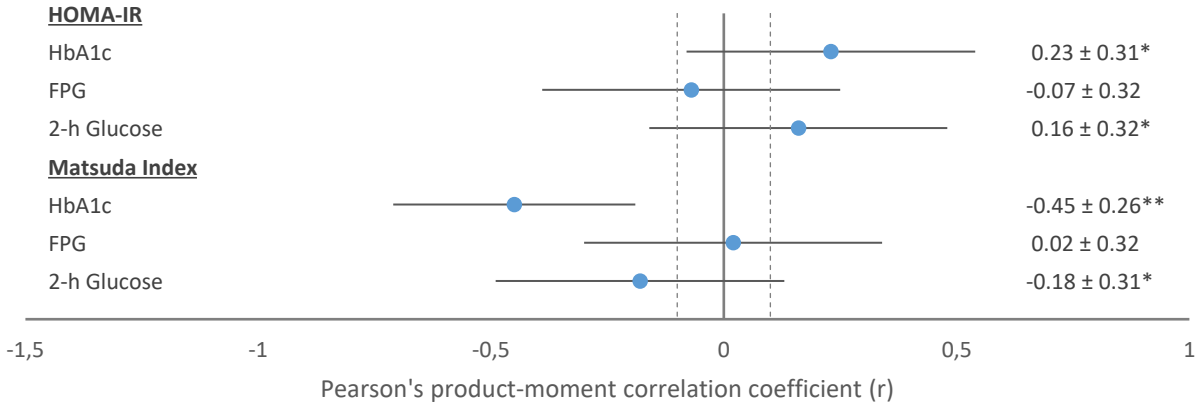

Figure 4.18 Correlations between insulin sensitivity indexes and glucose control measures.
Correlations are represented as r-values ± 90% confidence intervals. The vertical dashed lines represent the r-values of -0.1 and 0.1, respectively. Correlation coefficients and confidence intervals that cross both lines are deemed unclear. Correlation effect sizes: *small, **moderate.

4.11 Association between age and body composition, CRF, glucose control, insulin resistance, and glycaemic variability.

Advancing age was associated with small decreases in body mass and insulin sensitivity and a moderate decrease in fat free mass. Advancing age was also associated with small increases in body fat percentage, mean glucose, insulin AUC, MAGE and average time per day spent above 6 mmol·L^{-1}.

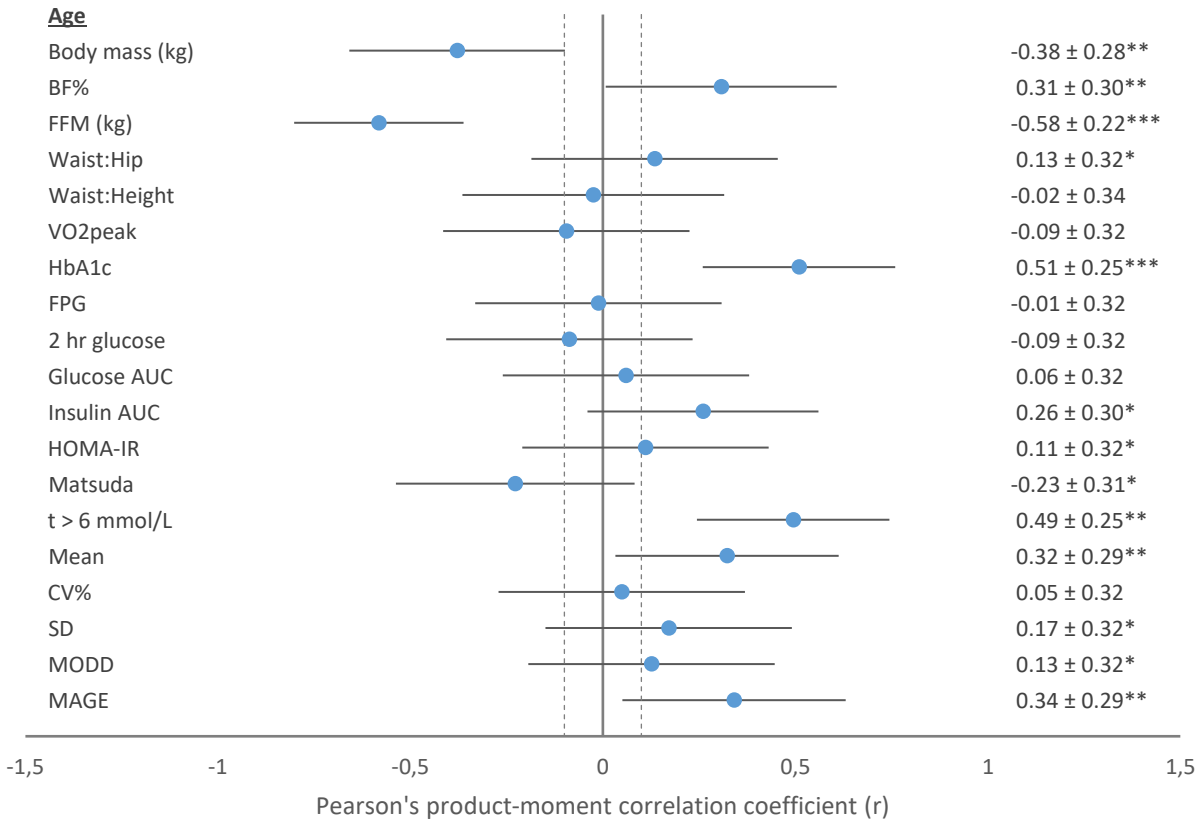

Figure 4.19 Correlations of body composition, CRF, glucose control, insulin sensitivity and glycaemic variability with age. Correlations are represented as r-values ± 90% confidence intervals. The vertical dashed lines represent the r-values of -0.1 and 0.1, respectively. Correlations between these two values and confidence intervals that cross both lines are deemed unclear. Correlation effect sizes: *small, **moderate, ***large.

4.12 Associations between body composition and glycaemic load, glycaemic variability, glucose control, and insulin sensitivity.

Overall CRF levels and insulin sensitivity / -resistance were most strongly associated with measures of body composition. The strongest correlation was observed between CRF and waist-to-height ratio correlated and the weakest with fat free mass.

HbA_{1c} was the only traditional glucose control measure that correlated meaningfully with body composition; however, all correlations were small. The association between HbA_{1c} and waist-to-hip ratio was the strongest.

The Matsuda index and HOMA-IR correlated the strongest, but only moderately with the waist-to-hip and waist-to-height ratios, with HOMA-IR having stronger associations with both ratios compared with the Matsuda index.

The body composition markers that correlated best with glycaemic variability, varied between all indices. MODD did not have any meaningful associations with the body composition measures. The strongest correlation was between fat free mass and the average time per day spent at [glucose] of 6 $mmol \cdot L^{-1}$. Increases in fat free mass was associated with a small to medium decrease in time spent above 6 $mmol \cdot L^{-1}$ [glucose].

Table 4.10 Associations between body composition measures with age, CRF, GL, glucose control, insulin sensitivity and glycaemic variability (Pearson's product-moment correlation coefficients).

	Body mass (kg)	***BF %***	***FFM (kg)***	***Waist:Hip***	***Waist:Height***
Age (years)	-0.38**	0.31*	-0.58***	0.13*	-0.03
VO2peak ($ml \cdot kg \cdot min^{-1}$)	-0.54***	-0.71#	-0.23*	-0.74#	-0.80#
Glycaemic load	0.11*	0.07	0.29*	-0.07	0.11*
HbA1c (%)	0.22*	0.42**	0.03	0.47**	0.33**
FPG ($mmol \cdot L^{-1}$)	0.03	-0.05	0.06	-0.07	-0.03
2-h glucose ($mmol \cdot L^{-1}$)	-0.04	-0.07	-0.01	0.13*	0.09
HOMA-IR	0.27*	0.48**	0.03	0.77#	0.79#
Matsuda Index	-0.16*	-0.52***	0.10*	-0.68***	-0.67***
CGM glucose CV %	-0.15*	-0.19*	-0.05	-0.29*	-0.26*
t > 6 $mmol \cdot L^{-1}$	-0.24*	0.37**	-0.45**	0.24*	0.09
Mean glucose ($mmol \cdot L^{-1}$)	-0.12*	0.37**	-0.32**	0.32**	0.15*
SD ($mmol \cdot L^{-1}$)	-0.24*	-0.09	-0.20*	-0.22*	-0.25*
MODD ($mmol \cdot L^{-1}$)	-0.06	0.17*	-0.12	0.00	-0.01
MAGE ($mmol \cdot L^{-1}$)	-0.21*	0.25*	-0.34**	-0.07	-0.14*

Correlation effect sizes: *small; **medium; ***large, #very large.

4.13 Summary of results

Table 4.11, Table 4.12, and Table 4.13 highlights the specific outcomes for which individual participants' recorded an atypical outcome measure. An outcome measure was regarded atypical if it surpassed the cut-off values as depicted in Table 4.8. The participants are listed according to their macronutrient clusters.

In total, 56 (14%) atypical markers were recorded among all the participants. The average atypical markers per participant is 1.0 in cluster 1, 0.83 in cluster 2, and 3.5 in cluster 3. Thirty-four (42% of the total possible body composition markers) of the atypical markers (60%) were related to body composition and of these, 24 were present in cluster 3. Only five of the 34 (15%) markers were concomitant with unhealthy glucose control measures, while 11 (32%) were concomitant with either elevated fasting insulin, or the presence of insulin resistance.

Only two participants had increased overall glycaemic variability, of which one participant did not present with any atypical body composition markers. Five participants had atypical body composition markers, but no other accompanying atypical markers. In all cases, low CRF was accompanied with at least one other atypical marker for glucose control, insulin resistance, or glycaemic variability, in combination with at least one unhealthy body composition marker.

Table 4.11 Summary of atypical measures for the participants in cluster 1.

		Participants in cluster 1 (high carbohydrate-low fat)								
	Atypical values	**4**	**7**	**9**	**13**	**16**	**19**	**20**	**22**	**24**
Age (years)		32	45	43	42	33	30	35	41	39
BF%	Poor & very poor		X							
Waist:Hip	≥ 0.9				X					X
Waist:Height	≥ 0.5				X					X
CRF	Poor & very poor		X							
HbA1c	≥ 5.7%									
FPG	≥ 5.6 mmol·L^{-1}									
2-h glucose	≥ 7.8 mmol·L^{-1}									
Fasting insulin	≥ 10.7 mlU·L^{-1}									
HOMA-IR	≥ 1.7 – 2.0				X					
Matsuda Index	≥ 4.3				X					
Mean glucose	≥ 6.7 mmol·L^{-1}									
SD	≥ 1.37 mmol·L^{-1}									
CV%	≥ 22.45 mmol·L^{-1}		X							
MAGE	≥ 2.80 mmol·L^{-1}									
MODD	≥ 1.41 mmol·L^{-1}									
Total unhealthy markers		**0**	**3**	**1**	**4**	**0**	**0**	**0**	**0**	**2**

Table 4.12 Summary of atypical measures for the participants in cluster 2.

		Participants in cluster 2 (low carbohydrate-high fat)					
	Atypical values	**5**	**6**	**12**	**15**	**25**	**29**
Age (years)		39	32	34	30	38	35
BF%	Poor & very poor					X	
Waist:Hip	≥ 0.9				X	X	
Waist:Height	≥ 0.5				X	X	
CRF	Poor & very poor						
HbA1c	≥ 5.7%						
FPG	≥ 5.6 mmol·L^{-1}						
2-h glucose	≥ 7.8 mmol·L^{-1}						
Fasting insulin	≥ 10.7 mlU·L^{-1}						
HOMA-IR	≥ 1.7 – 2.0						
Matsuda Index	≥ 4.3						
Mean glucose	≥ 6.7 mmol·L^{-1}						
SD	≥ 1.37 mmol·L^{-1}						
CV%	≥ 22.45 mmol·L^{-1}						
MAGE	≥ 2.80 mmol·L^{-1}						
MODD	≥ 1.41 mmol·L^{-1}						
Total		**0**	**0**	**0**	**2**	**3**	**0**

Table 4.13 Summary of atypical measures for the participants in cluster 3.

		Participants in cluster 3 (high carbohydrate-high fat)											
	Atypical values	**1**	**3**	**8**	**10**	**11**	**14**	**17**	**18**	**21**	**23**	**26**	**28**
Age (years)		36	36	34	40	36	32	30	36	47	34	44	42
BF%	Poor & very poor	X	X	X	X			X	X	X			X
Waist:Hip	≥ 0.9	X	X	X	X			X	X	X			X
Waist:Height	≥ 0.5	X	X	X	X			X	X	X			X
CRF	Poor & very poor			X	X				X				
HbA1c	≥ 5.7%	X							X	X			
FPG	≥ 5.6 mmol·L^{-1}	X											
2-h glucose	≥ 7.8 mmol·L^{-1}		X										
Fasting insulin	≥ 10.7 mlU·L^{-1}				X				X				
HOMA-IR	≥ 1.7 – 2.0		X	X	X				X				
Matsuda Index	≥ 4.3		X		X				X				
Mean glucose	≥ 6.7 mmol·L^{-1}												
SD	≥ 1.37 mmol·L^{-1}												
CV%	≥ 22.45 mmol·L^{-1}					X							
MAGE	≥ 2.80 mmol·L^{-1}												
MODD	≥ 1.41 mmol·L^{-1}												
Total		**5**	**6**	**5**	**7**	**1**	**0**	**3**	**8**	**4**	**0**	**0**	**3**

Chapter 5 Discussion

5.1 Introduction

The purpose of this study was to investigate the glucose control and free-living glycaemic variability of apparently healthy men in relation to cardiorespiratory fitness (CRF) and dietary intake.

Due to the prevalence and incidence of T2DM that is rising to epidemical numbers, it is crucial to establish what predisposes people to develop insulin resistance, as this condition is a key role-player in the pathogenesis of pre-diabetes and eventually, overt T2DM and metabolic disease. Since it is in every sense better to prevent than to treat, it is important to know whether the traditional markers of glucose control and insulin sensitivity, or alternatively, measures of glycaemic variability, reliably detect at risk individuals.

The OGTT is widely used as a diagnostic test to determine whether an individual has impaired glucose tolerance. It has been suggested to be a more sensitive and specific marker of glycaemic control than HbA_{1c}, or levels of fasting glucose. The question remains, though, what causes someone to progress from healthy glucose tolerance to impaired glucose tolerance? Is the information from an OGTT sufficient to determine whether an individual's glucose control is adequate, or are there other factors, especially lifestyle factors (specifically, CRF and diet macronutrient composition), that contribute to varying degrees of glycaemic variability in individuals, even though they present with normal glucose tolerance? If it is established that intra- and inter-day glycaemic variability, as measured with a CGM, show differences between individuals who are otherwise considered having normal glucose control, the continuous measurement of blood glucose levels may become a useful diagnostic tool.

5.2 Overview of findings

5.2.1 Primary aims

- To explore the relationships between cardiorespiratory fitness (CRF) and glycaemic variability, the traditional markers of glucose control (HbA_{1c}, OGTT, and fasting plasma glucose) and insulin sensitivity.
  Hypothesis 1: Cardiorespiratory fitness will be most strongly correlated with insulin sensitivity, followed by glycaemic variability and HbA_{1c}.

This hypothesis is partly accepted. CRF was most strongly associated with insulin sensitivity. It was negatively correlated to HOMA-IR (proxy indicator of insulin resistance) and positively to the Matsuda index (whole body insulin sensitivity). These associations of insulin sensitivity with CRF were stronger compared to the measures of glucose control and glycaemic variability. Among the glucose control and glycaemic variability measures, HbA_{1c} had the strongest (positive) correlation with CRF.

- To determine the relationships between dietary glycaemic load and glycaemic variability, glycaemic control, and insulin sensitivity.
  Hypothesis 2: Glycaemic load will be most strongly correlated to glycaemic variability, followed by insulin sensitivity (negative correlation) and glucose control (negative correlation).

  This hypothesis is partly accepted. Dietary glycaemic load was not associated with insulin sensitivity or –resistance but was strongly and positively correlated with MAGE (intra-day glycaemic variability) and time per day spent above 6.0 $mmol \cdot L^{-1}$. Weak correlations were found between dietary glycaemic load and glucose control.

5.2.3 Secondary aims

- To investigate the effects of dietary intake with different macronutrient ratios on glycaemic variability, glucose control and insulin sensitivity.
  Hypothesis 3: Low carbohydrate-high fat food consumption will be associated with least glycaemic variability, healthy glucose control and high insulin sensitivity.

  This hypothesis is accepted. The lowest glycaemic variability and best insulin sensitivity was observed for the low CHO-high fat cluster. Glycaemic variability was the highest in the high CHO-low fat cluster, whereas insulin sensitivity was the lowest in the high CHO-high fat cluster. Significant differences between the clusters in glucose control measures were not found.

- To investigate the relationship between glycaemic variability and markers of glucose control.

  Hypothesis 4: There will be weak correlations between glycaemic variability and traditional glucose control measures.

This hypothesis is accepted. Glycaemic variability did not correlate well with the traditional measures of glucose control.

- To determine the relationships between the parameters of glycaemic variability and insulin sensitivity.

  Hypothesis 5: There will be an inverse relationship between insulin sensitivity and glycaemic variability.

  This hypothesis is rejected. An increase in insulin resistance was associated with small decreases in glycaemic variability. Likewise, an increase in whole-body insulin sensitivity was associated with increases in glycaemic variability.

5.3 Purpose of the study

The purpose of this study was to explore the potential of CGM as an early detector of glycaemic/glucose control (and insulin resistance) and its relation to CRF and dietary intake.

Twenty-seven apparently healthy individuals participated in the study; 22 participants were classified as healthy according to their glucose control (as per the traditional measures and glycaemic variability). Four participants could be classified as potentially prediabetic according to the traditional measures of glucose control – three according to HbA_{1c} (of which one also had a FPG concentration equal to the pre-diabetic cut-off value) and one according to the OGTT 2-h [glucose].

Although the participant with the elevated FPG reported to be fasted when his bloods were drawn, one cannot dismiss the possibility that he was not completely fasted for at least 8 hours. The latter participant was also the person with the highest average CGM glucose value, which is in line with the high FPG measure. All the participants' glucose control measures were well below the diabetic values (ADA, 2017).

Only two participants were identified with glycaemic variability above the healthy range of CGM CV% values. One of these participants also had a pre-diabetic HbA_{1c} value, but the other participant was deemed healthy, according to the investigated measures of the current study.

From the above, it does not appear that CGM-derived glycaemic variability indices are more useful than the traditional glucose control measures in the identification of individuals with diminishing glucose control. Notably, however, the results of this study should be interpreted in light of the considerations and limitations regarding CGM and the measurement of glycaemic variability. These will be discussed next, before discussing the findings of the study from section 5.5 onwards.

5.4 Continuous glucose monitoring

5.4.1 Accuracy of glucose sensor readings

As per the manufacturer, the correlation between the readings of the FreeStyle Libre Pro sensor (the sensors that were used in this study) and capillary [glucose] is very high (r = 0.954; n = 8739). The mean absolute relative difference (MARD) (the average relative error of all sensor readings) of the sensor versus the finger-prick reference [glucose] is 11.1%.

Parkes *et al.* (2000) developed a consensus error grid with different zones (A to E) to distinguish between discordant results that will have clinical implications or not. Two methods or instruments that agree completely (e.g. exactly the same concentration), will provide a data point in the centre of zone A on a correlation line graph. Zone A represents the area on the graph where slight errors in measurement have no effect on clinical action. Zone B represents the degree of errors where slightly different clinical actions are required. In the comparison of the FreeStyle Libre Pro sensor with the YSI (Yellow Springs Instrument) analyser reference tests, 86.7% of the sensor's glucose values fell within zone A and 99.7% fell in zones A and B (Bailey *et al.*, 2015). These findings strongly support the manufacturer's claims regarding the sensor's accuracy.

Bailey *et al.* (2015) also showed that the sensors remain stable throughout the 14-day wear period after they were calibrated in the factory. Tsoukas *et al.* (2020) reported a MARD of 11.2% (compared to plasma [glucose]) for sensors that were 1 – 2 days old and 13 – 14 days old, and a MARD of 6.6% when the sensors were 5 – 7 days old. Dye *et al.* (2010) reported a concordance correlation of 0.8764 (Kendall's W, n = 15, p < 0.001) between glucose sensor values and capillary whole blood concentrations. Although the latter researchers used Glucoday® continuous ambulatory glucose monitoring devices, these sensors also measure interstitial [glucose].

It should also be considered that the CGM sensors measures interstitial glucose and not blood glucose. It has been reported that interstitial glucose yields up to 10% lower OGTT AUCs, compared to blood

glucose values (Hasson *et al.*, 2010). On the other hand, interstitial glucose assessment is considered a suitable replacement for the measurement of blood glucose (Rebrin & Steil, 2000). The main consideration when using a sensor to record OGTT responses, is the time lag in interstitial [glucose] that could range between 5 to 12 minutes (Rebrin & Steil, 2000) or even 15 minutes (Dye *et al.*, 2010). Considering the aims of the current study, the lag-time of the sensors was not a confounding factor.

Several authors reported the occurrence of low [glucose] (< 3.9 mmol·L^{-1}) in their data (Mazze *et al.*, 2008; Shah *et al.*, 2019; Thomas *et al.*, 2016). For instance, Mazze *et al.* (2008) found that participants with normal glucose tolerance spent an average of 3 ± 3% of the day (equating to ± 45 min per day) below 3.9 mmol·L^{-1} and an average of 1 ± 1% of the day (equating to ± 15 min per day) below 3.3 mmol·L^{-1}. These findings are comparable to those of the current study where the average time per day spent below 3.0 mmol·L^{-1} was 22 min). However, none of the participants in our study reported symptoms of hypoglycaemia. The manufacturer of the Freestyle Libre Pro glucose sensors reported that 40% of the values that they recorded below 3.3 mmol·L^{-1} were actually $\geq$ 4.4 mmol·L^{-1} according to standard laboratory analysis of blood glucose. Therefore, these hypoglycaemic glucose concentrations should be interpreted with caution.

5.4.2 Glucose profiles of the participants

It is a common finding that healthy individuals can present with glucose excursions of up to and above 10.0 mmol·L^{-1} (Borg *et al.*, 2010; Madhu *et al.*, 2013; Thomas *et al.*, 2016; Zeevi *et al.*, 2015). In a more recent study, Hall *et al.* (2018) investigated the CGM profiles of individuals with healthy glucose control according to the traditional measures. In agreement with previous studies, they identified normal glucose tolerant individuals with postprandial glucose excursions, as measured by CGM, in the pre-diabetic and diabetic ranges. They also confirmed the finding of Zeevi *et al.* (2015) that showed individuals react differently to the same meals when looking at the extent of the postprandial glucose excursions.

None of the participants in the current study reached the degree of glucose excursions that have been reported in the previous studies, although 17 (63%) participants did exceed 7.8 mmol·L^{-1} at least once during their monitoring period. No participant reached a 10.0 mmol·L^{-1} [glucose], which, according to experimental studies, is the extent of hyperglycaemia that causes inflammation (Esposito *et al.*, 2002), oxidative stress (Ceriello, *et al.*, 2008; Monnier *et al.*, 2006), and endothelial damage (Ceriello *et al.*, 2013). The clinical significance and possible long-term effects of the glycaemic variability and

glucose excursions in the profiles of the participants in the current study is therefore difficult to interpret. Possible reasons for the lower observed peak [glucose] in this study might be related to the small number of participants and the strict inclusion criteria, as well as the fact that the glucose monitors only record 15-min average [glucose]. Furthermore, the athletes in the study by Thomas *et al.* (2016) were consuming very large amounts of carbohydrates and sugar, while standard meals were given to the participants in the studies of Hall *et al.* (2018) and Zeevi *et al.* (2015). These standard meals were specifically planned to elicit high postprandial glucose responses. Also, as described in section 5.6, South Africans typically do not follow diets that are low in fat (Vorster *et al.*, 2013) and this was consistent with the current study sample. The addition of fat to a meal typically lowers the postprandial glucose response (Collier & O'Dea, 1983).

5.4.3 The glycaemic variability profiles of the participants

Foreman *et al.* (2020) considered 48 hours as the minimum time needed to reliably measure glycaemic variability. With the exception of two previous studies (Borg *et al.,* 2010; Riddlesworth *et al.,* 2018) where CGM was used for more than a week, most other studies are limited to 2 – 3 days of CGM data (Gude *et al.*, 2017; Hanefeld *et al* 2014; Hill *et al.,* 2011; Paing *et al.,* 2020; Shah *et al*., 2019). In this study, glycaemic variability was calculated from at least 7 full days' CGM recordings, thus exceeding most other studies. Hence, the findings of this study can be considered with confidence.

The participants in this study presented with varying degrees of glycaemic variability, however, none of the indices were higher (indicating large variability) than the normative values for healthy populations, as defined by Hill *et al.* (2011). The latter study included 70 non-diabetic individuals, however, some had fasting plasma [glucose] of < 6.7 mmol·L^{-1}, which is 1.1 mmol·L^{-1} above the 5.7 mmol·L^{-1} cut-off value for pre-diabetes (American Diabetes Association, 2017; SEMDSA, 2017). Tabák *et al*. (2012) noted that overt T2DM is only diagnosed after the disease state has already progressed to the point of beta-cell dysfunction. Thus, even though the study participants of Hill *et al.* (2011) excluded individuals with overt T2DM, they undoubtedly included individuals who were pre-diabetic, and thus not healthy. Therefore, the reported normative glycaemic variability values of Hill *et al*. (2011) cannot be considered true and valid normal ranges for healthy individuals.

Foreman *et al.* (2020) also aimed to determine reference values for CGM-derived glycaemic variability indices (mean glucose, SD, CV, MAGE, MODD) of healthy (n = 470), pre-diabetes (n = 184) and T2DM participants (n = 197). However, their reference values largely overlapped between

the different categories. In other words, the range of values (2.5^{th} – 97.5^{th} percentiles) that was suggested for heathy participants, overlapped between 86% to 99% with the prediabetic range and between 63.8% – 81.1% with the diabetic range. In the case of the 10^{th} – 90^{th} percentiles for healthy persons, 76.4% – 84.3% of the values overlapped with the pre-diabetic range, while 27.7% – 54.7% overlapped with the diabetic range.

In the absence of clear reference values, the interpretation of glycaemic variability indices is problematic and thus the biggest obstacle in its utilisation in clinical practice. Hence, despite the convenience of CGM sensors and the valuable data it records, it is currently not possible to definitively categorise individuals as healthy, or in varying degrees of disease states; at least not based on glycaemic variability indices alone. The findings of the current study are confirmation of this inference.

Some individuals presented with insulin resistance and postprandial hyperinsulinaemia, however, their CGM-derived indices of glycaemic variability were not atypical. Thus, it is plausible that the postprandial glycaemic excursions that is currently deemed healthy or without risk, is actually lower than the level of 7.8 $mmol \cdot L^{-1}$ that is presently accepted (American Diabetes Association, 2017; González-Rodríguez *et al.*, 2019). Neither Foreman *et al.* (2020) and Hill *et al.* (2011), who provided reference values for CGM-derived indices, nor studies that aimed to characterise typical ambulatory glucose profiles (Borg *et al.*, 2010; Nomura *et al.*, 2011; Mazze *et al.*, 2008) provide suitable information to assess potential future risk for the development of insulin resistance-related chronic disease states. In order to derive reliable reference values that can be used in clinical settings, the long-term monitoring of exclusively healthy individuals is needed.

5.4.5 Limitations and considerations of glycaemic variability measures

The current study was unable to conclude that glycaemic variability may be a better and earlier method than traditional measures of glucose control for the detection of individuals possibly at risk of deteriorating metabolic health. Nonetheless, the current limitations of the measures of glycaemic variability should be considered before summarily dismissing the potential importance of CGM in healthy individuals.

CV% has been suggested by multiple authors as the best measure of overall glycaemic variability (Acciaroli *et al.*, 2018; Danne *et al.*, 2017; Foreman *et al.*, 2020). Conversely, it is also frequently said that no single measure has yet been identified to optimally represent glycaemic variability and

no single measure is able to characterize all aspects of glucose control and metabolism (Monnier *et al*., 2008; Peyser *et al.*, 2018; Rodbard, 2009; Suh & Kim, 2015). Multiple measures and in combination are therefore used when assessing glycaemic variability. For instance, MODD is a measure of inter-day glycaemic variability, describing the average difference in daily glycaemic patterns (Hill *et al*., 2011). Overall stability of day-to-day glucose is thus assessed by this measure. A limitation, though, is that individuals without fixed daily mealtimes will have a larger MODD value compared to another individual with fixed eating patterns, even though their glucose control, average glucose, times-in-zone and glucose tolerance may be the same. Furthermore, there is no evidence in the literature that these two hypothetical individuals will be at different degrees of risk should MODD be the only difference between them.

MAGE is the most-used parameter to quantify average intra-day glycaemic variability (Hill *et al*., 2011) since it is related to the extent of postprandial glucose excursions (Suh & Kim, 2015). Unfortunately, none of the glycaemic variability measures give information regarding the frequency of glucose excursions. Equal MAGE values for two individuals can, for example, be originating from two different glucose profiles. For example, a single glycaemic excursion of 1.0 mmol·L^{-1} above SD will equate to an individual having a MAGE value of 1, while a second individual may experience four glucose excursions, e.g. one of 2.0 mmol·L^{-1} above his SD, another 1.2 mmol·L^{-1} above SD and two of 0.7 mmol·L^{-1} SD which will also result in a MAGE value of 1. The latter individual, however, clearly experience more severe glycaemic variability.

Time spent in the range of 3.9 mmol·L^{-1} to 7.8 mmol·L^{-1} (Shah *et al.*, 2019), or 10.0 mmol·L^{-1} is widely used (Paing *et al*., 2020; Rodbard, 2009; Vigersky & McMahon, 2019) and recommended when investigating glucose control (Danne *et al*., 2017). These limits may, however, create an inaccurate picture of the degree of glucose control, or glycaemic variability, in a healthy individual, especially with regards to the lower limit. As was reported by Rodbard (2020), time spent within a zone decreases when an individual's mean glucose is below 6.6 mmol·L^{-1}, increasing the time spent in hypoglycaemia([glucose] < 3.9 mmol·L^{-1}). Furthermore, [glucose] below 3.0 mmol·L^{-1} is widely considered a clinical hypoglycaemic concentration, especially in healthy persons (Danne *et al*., 2017; Shah *et al*., 2019;) and without any signs or symptoms that accompany hypoglycaemia (mainly involving the nervous system and brain dysfunction). Venous [glucose] as low as 2.75 mmol·L^{-1} may be deemed normal (Guerci *et al.*, 2013). This leaves the question how applicable the lower limit of 3.9 mmol·L^{-1} is to describe glucose control in non-diabetic populations. Perhaps this is the exact reason why numerous studies involving non-diabetic populations only consider hyperglycaemic

excursions, or time spent above 7.8 mmol·L^{-1} (Borg *et al.*, 2010; Gonzalez-Rodriguez *et al*., 2019; Márquez-Pardo *et al*, 2020).

It is uncertain whether it is the relative or the absolute height of a glucose excursion that holds most associated risk. If two hypothetical individuals namely, person X with a baseline [glucose] of 3.3 mmol·L^{-1} and person Y with a baseline [glucose] of 5.4 mmol·L^{-1} both present with excursions up to 7.7 mmol·L^{-1}, will the excursions be associated with the same risk? Uncertainty regarding this scenario questions the true risk associated with large glycaemic variability in healthy, non-diabetic populations. Considering that the normal postprandial increase in [glucose] is 3.0 mmol·L^{-1} (Tabák *et al.*, 2012), one wonders whether higher glycaemic variability, but lower average [glucose] (person X) , means higher risk for future development of insulin resistance or CVD. Alternatively, is person Y with lower overall glycaemic variability, but higher average [glucose] and similar average glycaemic excursions than person X at higher risk for future disease?

The design of the current study was not suitable to address these uncertainties and larger, longitudinal studies are needed for definitive answers. It has not yet been investigated what the potential long-term implications of varying degrees of glycaemic variability have in the development of insulin resistance. Nonetheless, an important finding of the current study was that hyperinsulinaemia can be present without any abnormalities in glucose control or glycaemic variability parameters.

5.5 Associations between cardiorespiratory fitness and measures of glucose control

The findings of this study suggest that CRF is most strongly related to insulin sensitivity in comparison to glucose control and glycaemic variability. This finding adds to the existing evidence that insulin sensitivity improves with high levels of CRF (Carcino-Ramirez *et al.*, 2018; Grace *et al.*, 2017; Solomon *et al.*, 2015). Although maximal aerobic capacity (up to ~ 40% of VO_{2max}) (Bouchard *et al.*, 1998; Bouchard *et al.*, 1986) and the potential to improve maximal aerobic capacity with exercise training is. to a certain degree, genetically determined (Keller *et al.*, 2011; Timmons *et al.*, 2010), different types of training is well known to improve maximal aerobic capacity (Lundby *et al.*, 2017; Roca *et al.*, 1992; Vollaard *et al.*, 2009). Thus, it remains a possibility that the correlation between insulin sensitivity and CRF is not due to CRF level *per se*. Rather, it could be due to the effects of the regular and increasing levels of physical exercise training that are needed to elicit notable changes in CRF. In other words, improved insulin sensitivity that is associated with high

CRF could, in fact, be due to the cumulative effects of acute exercise that have been shown previously to improve insulin sensitivity, even in young and healthy individuals (Blaak *et al*., 2012; Duvivier *et al.*, 2017; Short *et al.*, 2013).

Following insulin sensitivity and -resistance, HbA_{1c} was the next strongest (negative) associated with CRF. Neither FPG, 2-h [glucose], nor glycaemic variability indices revealed meaningful associations with CRF. Insulin sensitivity had, in turn, the strongest association with HbA_{1c} among all the glucose control and glycaemic variability measures. A small association was found between insulin sensitivity and overall glycaemic variability. From this study it thus seems that overall glucose levels may decrease with increased CRF, but it does not seem that glycaemic variability or fluctuations and overall postprandial glucose excursions are related to CRF.

It must be mentioned that there is no consensus as to what constitutes a true maximal aerobic capacity test (Riebe *et al.*, 2016). Researchers apply any single, or any combination of five criteria to declare a test maximal, however, the implementation of these criteria to assess whether a test was maximal has been found not viable (Poole *et al.*, 2008). All participants in the current study reached at least one criterion indicating a maximal test, but not all attained definitive plateaus in VO_2. For these reasons, VO_{2peak} was reported instead of VO_{2max} and it is thus possible that the participants' true cardiorespiratory fitness was underestimated. It is also possible that another parameter of physical exercise, such as frequency, duration or intensity may in fact correlate better with glucose control, rather than aerobic fitness (CRF) level.

5.6 Dietary characteristics of the participants

All the participants were encouraged to follow their habitual diets and eating patterns. None of the participants followed any restrictive diets and a wide range of foods were consumed. If any, one would classify their diets as high carbohydrate-high fat, with the percentage of energy intake from carbohydrates and fats being 41 ± 7% and 40 ± 5%, respectively. The current dietary guidelines for South Africans stipulate that the intake of carbohydrates should constitute 50% of total energy intake, with less than 10% of total energy intake from added sugars (Vorster *et al*., 2013). South Africans, however, typically eat foods with less than 50% of energy from carbohydrates and they also tend to consume more sugars than 10% of daily energy. Their intake of dietary fibre is also low (< 20 g per day) (Vorster *et al*., 2013). The tendency for lower carbohydrate intake was consistent with the average dietary intake of the participants. The average intake of sugars of the participants met the

recommended amount, while their fibre intake bordered on low (average of 20.6 ± 6.3 g per day). The participants' total intake of fats was, on average, 10% higher than is recommended by current national and international dietary guidelines (WHO, 2018; Vorster *et al.*, 2013), however, no individual participant's diet could be outrightly called a low-carbohydrate, high-fat diet. The latter is defined as 15 – 20% of energy from carbohydrates and 60 – 65% energy from fat (Burke *et al.*, 2018).

Daily caloric intake varied considerably among participants, as well as between days for the same individual. Nevertheless, the average daily energy intake for each participant remained fairly consistent. Unusually high or low energy intake on a particular day was typically followed by a reverse pattern on the next day.

The average day-to-day variations in daily macronutrient and glycaemic load consumption for each individual, as well as for the group were calculated. The average individual day-to-day (intrapersonal) variation in glycaemic load was larger than the group's average day-to-day variation. The large intrapersonal day-to-day variability in glycaemic load would have negatively influenced the strength of the correlations between glycaemic load and glycaemic variability, glucose control and insulin sensitivity measures. Thus, with less variance and a larger sample size, the relationships between the outcome variables might be stronger.

The group's average day-to-day variation in dietary macronutrient intake revealed that there was also considerable variation in protein consumption and even more for fat intake. This finding warranted further investigation into the dietary composition of the participants, other than the glycaemic load alone. These results are discussed in section 5.8.1.

5.7 Associations between glycaemic load and measures relating to glucose control

To my knowledge, this is the first study to specifically investigate the associations of glycaemic variability indices with dietary glycaemic load. The rationale for this analysis was based on the known relation between carbohydrate ingestion and postprandial glucose excursions.

It was found that a higher daily dietary glycaemic load was associated with increases in time per day spent above 6.0 $mmol \cdot L^{-1}$, MAGE and MODD. Of all the variables, MAGE was the variable most strongly associated with dietary glycaemic load. This was an expected finding. The observation that

the height of postprandial glucose excursions is determined by the type of carbohydrates consumed, led to the development of GI values for carbohydrates (Jenkins *et al.*, 1981). However, it is also known that postprandial glucose is independently affected by the amount of CHO ingested. Thus, the concept of glycaemic load was introduced which takes both the type and amount of carbohydrate consumed into consideration (Foster-Powell *et al.*, 2002). It is thus intuitive that the glycaemic load of an individual's diet should, at least in part, affect the magnitude of the spikes in postprandial glucose values.

Dietary glycaemic load was minimally correlated to HbA_{1c}, suggesting that this measure of glucose control is unable to predict the effect of a healthy individual's diet on glucose control. This finding is partly supported by the results on the differences between the macronutrient clusters of participants, as discussed in section 5.8.1. Briefly, it was discovered that there were large differences in OGTT insulin levels and glycaemic variability indices between the clusters, whereas there were almost no differences in traditional glucose control measures.

5.8 The characteristics of the participant clusters according to dietary macronutrient intake

To gain further insight into the role of diet on glycaemic variability, glucose control and insulin sensitivity, the participants in the current study were grouped into three clusters according to their reported macronutrient intake. The cluster analysis was performed to include the collective inputs of all the macronutrients, as the postprandial insulin response is not solely dependent on carbohydrates. Protein, for example, can cause postprandial insulin secretion that is disproportionate to the incremental glucose AUC (Wolever *et al.*, 2016). Fats do not independently stimulate secretion of insulin, but the co-ingestion of fats with other foods has an influence on the postprandial glucose and insulin levels (Nuttall & Gannon, 1991). For instance, Collier & O'Dea, (1983) observed that the consumption fat together with carbohydrates lowers the postprandial glucose response, although it does not necessarily influence insulin secretion. This finding is confirmed by the MAGE results of the current study, namely that the high CHO-low fat cluster had a higher average MAGE than the high CHO-high fat cluster which had higher insulin values. When considering the role of the incretin hormones on insulin secretion, together with the role of the gut microbiome on the postprandial glucose and insulin responses, the combined effects of all the macronutrients cannot be ignored by grouping participants only according to carbohydrate intake.

The macronutrient percentages of participants in the high CHO was the closest to current dietary recommendations. Current recommendations stipulate that energy intake should be 50% from carbohydrates and less than 30% from fats. In the case of the high CHO, their energy from carbohydrates was 48.2% (± 4.4%) and the energy from fats was 34.9% (± 2.6%). The low CHO-high fat was the closest to a low carbohydrate-high fat diet, although their intake of carbohydrates (31.3 ± 4.0% of energy intake) was too high and their fat intake (46.5 ± 3.8%) was too low (Burke *et al*., 2018). The high CHO-high fat cluster's diet was characterised by almost equal contributions to the daily energy intake by carbohydrates and fats (40.3 ± 2.6% and 41.6 ± 2.4% from carbohydrates and fats, respectively). Interestingly, the latter macronutrient breakdown was recently described as a moderate-carbohydrate diet (Ebbeling *et al.*, 2020).

5.8.1 Differences between the dietary macronutrient clusters

The first secondary aim of this study was to investigate the differences between habitual dietary macronutrient compositions on glycaemic variability, glucose control, and insulin sensitivity. This analysis clearly showed that hyperinsulinaemia is prevalent before any meaningful changes in [glucose] during the OGTT, average CGM [glucose], or other traditional glucose control measures become evident.

The differences between clusters were most pronounced for insulin sensitivity and the [insulin] of the OGTTs. The high CHO-high fat cluster had the highest insulin response during the OGTT, the low CHO-high fat cluster had the lowest insulin response during the OGTT, and the intermediate insulin response came from the high-CHO cluster. Similar distinctions were not reflected in the OGTT [glucose], fasting plasma glucose, 2-h glucose, HbA_{1c} levels, or average CGM [glucose].

Regarding glycaemic variability, the most pronounced differences were observed in MAGE (intra-day variability). MAGE was highest for the high CHO-high fat cluster, with no significant difference between the low CHO-high fat and high CHO-high fat clusters. Furthermore, the time per day spent above [glucose] of 6.0 $mmol \cdot L^{-1}$ increased between clusters as the percentage of dietary carbohydrates increased. This finding thus supports the correlations between glycaemic load, MAGE and time per day spent above 6.0 $mmol \cdot L^{-1}$ (section 5.7). Carbohydrates are the principle dietary component that causes a rise in blood glucose levels (Ludwig, 2002). Hence the more carbohydrates consumed throughout the day, the higher the expected postprandial glucose excursions, as well as time spent above fasting [glucose] levels. This deduction explains why dietary glycaemic load is considered the best predictor of postprandial glucose (Ludwig & Ebbeling, 2018).

Although the glucose variability measures cannot be directly related to insulin levels, as insulin cannot be measured continuously with glucose, an interesting observation was nonetheless made. The five-point OGTTs revealed higher average insulin levels and insulin AUC for the high CHO-high fat cluster (moderate intake of carbohydrates), than the high CHO cluster (highest intake of carbohydrates), which had the highest MAGE values and daily time spent above 6.0 $mmol \cdot L^{-1}$. These results suggest that a diet consisting of equally high amounts of carbohydrates and fat might be the strongest elicitor of hyperinsulinaemia; but that the presence of hyperinsulinaemia does not necessarily coincide with the degree of free-living glycaemic variability or any other measures of blood glucose levels.

The role of a high carbohydrate-high fat diet in the development of hyperinsulinaemia and/or insulin resistance, as suggested by the findings of the current study, can be considered by looking at the metabolic effects of these macronutrients. The foods that we consume have specific hormonal responses that determine the way in the body uses the ingested calories. The most important hormone is insulin, which is secreted directly in response to a rise in blood glucose and subsequently puts the body in a state of anabolism during which circulatory metabolic fuels are decreased. The latter is achieved because insulin facilitates glucose uptake into the systemic cells, while also suppressing adipose tissue to release fatty acids. Combined, these actions promote the production and storage of glycogen and fat (Ludwig & Ebbeling, 2018). The addition of fats to a carbohydrate meal might lower the postprandial glucose to a degree, but not necessarily the insulin response (Collier & O'Dea, 1983). This is explained by the fact that the postprandial insulin response is not only dependent on the stimulus from elevated blood glucose levels, but also by incretin hormones that are secreted into the circulation by the enteroendocrine cells of the stomach and intestines in response to the ingestion of food, especially carbohydrates (Greiner & Bäckhed, 2011 & Drucker, 2007).

The CIMO (Ludwig & Ebbeling, 2018) proposes that consistently high intakes of carbohydrates cause obesity due to the high postprandial levels of insulin and its subsequent effects. High circulating concentrations of insulin promote the storage of carbohydrates and fats and these are consequently not readily available for energy production. It also stimulates feelings of hunger and hence, more energy intake. Hyperinsulinaemia, in response to high carbohydrate intake (as in the high CHO and high CHO-high fat clusters), raises one's appetite due to the suppression of the breakdown and utilisation of stored glycogen and fats for energy production. Hence, hyperinsulinaemia is the underlying cause of overnutrition and obesity and not the consequence of overnutrition and obesity.

It can also be argued that high intakes of fat, together with high intakes of carbohydrates, can be expected to have more detrimental effects than a high carbohydrate-low fat diet. Even though post prandial glucose may not be higher in response to a high carbohydrate-high fat diet, the amount of carbohydrates in the latter diet is still enough to keep the body from utilising fats (stored and consumed). The high intake of fats, together with a high intake of carbohydrates, potentially place the body in prolonged periods of energy storage, due to high insulin secretion, similar to a high carbohydrate-low fat diet. Ingesting equally high amounts of carbohydrates and fats probably exceeds the body's need for energy, which inevitably are then stored, or utilised for immediate energy purposes and the remainder is then stored. It is possible that overnutrition manifests earlier when excessive amounts of fat are consumed with equally high amounts of carbohydrates. In line with the CIMO, Caputo *et al.* (2017) and Peterson & Shulman (2018) also concluded that chronic overnutrition leads to the development of insulin resistance.

The high CHO-high fat cluster's diet is comparable to the Western diet, which is typically a high-sugar (or high simple carbohydrates), high-fat diet (Gentile & Weir, 2018; Mente *et al.*, 2009). Chronic consumption of this type of diet is highly associated with disease states, such as coronary heart disease (Mente *et al.*, 2009), CVD (Ramji & Davies, 2015), obesity and T2DM (Mbanya *et al.*, 2010; Verma & Hussain, 2017). The findings of the current study revealed that the highest levels of insulin were present among participants whose diets were high in fat, as well as carbohydrates, and thus support the association between a typical "Western diet" and chronic disease.

5.10 Cardiorespiratory fitness, diet, and the maintenance of health

The findings of the study suggest that CRF is more related to better insulin sensitivity, than it is to lower average glucose levels (mean CGM glucose and HbA_{1c}) or postprandial glucose excursions (MAGE, 2-h [glucose] and OGTT glucose AUC). Therefore, even though there is value in studies that investigate the effects of exercise on various aspects of glucose control, the findings of studies that focus solely on glucose control may possibly underestimate the beneficial health effects of exercise regarding the promotion of metabolic health.

The current study revealed very strong associations between CRF and insulin sensitivity, whereas average dietary glycaemic load did not correlate with insulin sensitivity. Based on the strong positive correlation between CRF and insulin sensitivity, the importance of CRF as a possible and important protective factor against the diminishing of insulin sensitivity cannot be disregarded. Furthermore, it

must be considered that CRF was directly and objectively measured, while glycaemic load is an average value that was dependent on the collection of many estimated values. The strength of the association between glycaemic load and insulin sensitivity in the results of this study may be lower than the true association.

Considering the studies that have found a positive effect of acute exercise on the insulin sensitivity of healthy individuals as discussed in section 2.4.3, it is also plausible that increases in CRF may be linked to a larger effect on insulin sensitivity over a shorter period of time, compared to that of dietary glycaemic load. This does, however, not disregard the importance of dietary intake for the prevention of insulin resistance.

Glycaemic load was more strongly associated to MAGE than CRF. Neither CRF, nor daily glycaemic load revealed any noteworthy associations with overall glycaemic variability.

The findings highlighted above reiterate the view of Malhotra *et al.* (2015) that "you cannot outrun a bad diet". On the other hand, insulin responses to the OGTT and insulin sensitivity might be more important for health preservation, as both showed stronger associations with CRF than with dietary glycaemic load. This by no means suggests that exercise (as a mediator of CRF) will fully protect individuals from becoming hyperinsulinaemic, or insulin-resistant while they are eating a diet of ultra-processed and/or junk foods and diets which are high in sugar. No author argues against these types of diets being a sure promoter of health decline (Fernström *et al.*, 2019; Herforth *et al.*, 2019; World Health Organization, 2018). If anything, these findings demonstrate why a 'good/healthy' diet (which restricts carbohydrates, thus preventing high secretion of insulin), combined with physical exercise promotes long-term health better than either diet or exercise alone. This assertion is supported by Hopper *et al.* (2013) who suggested that the postprandial glucose response to a meal is dependent on an individual's insulin sensitivity, together with the glycaemic load of the meal.

The question regarding the importance of diet versus exercise for the maintenance of health touches on the topic of "the fat-but-fit paradox", as discussed by Ortega *et al*. (2018). Findings from many observational studies led to the formulation of the phrase "fat-but-fit" which suggests that CRF may be more important for the maintenance of health compared with an ideal body weight (Ortega *et al*, 2018). Although the relation between body composition and diet, CRF and glucose control in the current study was not a key consideration, it did provide evidence that hyperinsulinaemia can be present in healthy weight individuals; reiterating that one is not necessarily healthy when at "healthy"

weight. Furthermore, together with insulin sensitivity that was best correlated with CRF, these findings add to the proposition of the fit but fat paradox. This does not, however, contradict the evidence on the importance of diet, irrespective of CRF. The current study's finding that different dietary macronutrient compositions is associated with significant differences in OGTT insulin levels, support the importance of dietary intake. Interestingly, evidence also exists that weight loss is not necessarily needed to reap the benefits of either a low-carbohydrate diet (Francois *et al.*, 2017), or exercise interventions (Hamer & O'Donovan, 2010).

Central to the notion that both diet and exercise are important for the maintenance of health, is the review by Francois *et al.* (2017). In their review the authors discuss the benefits of combining dietary carbohydrate restriction with HIIT in the treatment of T2DM patients. It is very reasonable to expect added benefits when HIIT is added to a carbohydrate-restricted diet or vice versa. A combination of exercise with a diet that does not stimulate hypersecretion of insulin would promote metabolic health to a larger extent, than either intervention independently.

Metabolic health is defined in terms of the presence of the metabolic syndrome criteria (Ortega *et al.*, 2016). The manifestation of the metabolic syndrome marks the presence of insulin resistance (Kelly *et al.*, 2014; Nolan & Prentki, 2019; Reaven, 1988). The observation that some overweight individuals may be metabolically healthy (related to the fat-but-fit paradox) (Ortega *et al.*, 2016), while "healthy" weight individuals present with hyperinsulinaemia or insulin resistance, adds to the existing evidence in favour of the measurement of insulin levels for the early detection of individuals at risk for future metabolic disease. Identifying the cause allows for earlier intervention and prevention, rather than waiting for symptoms (the metabolic syndrome) to arise before attempting to intervene. Thus, being overweight is not necessarily synonymous to being metabolically unhealthy.

5.11 Effects of hyperinsulinaemia

As was apparent from the differences between the dietary clusters, changes in insulin levels occur before any changes in glucose control. This is not a surprising finding, as the literature widely supports the notion that hyperinsulinaemia is at the root of the development of non-communicable, lifestyle diseases (Crofts *et al.*, 2015).

The sooner any disease risk is identified, the sooner lifestyle changes can be made in order to slow down, stop, and reverse developing conditions. Earlier lifestyle interventions in at-risk individuals

will buy more time, leaving more room for the preferred medicine, namely, preventative lifestyle changes, to become effective. The identification of hyperinsulinaemia should thus be the goal for early identification of disease risk.

As was already indicated by Rizza *et al.* (1985), insulin resistance is caused by hyperinsulinaemia in a feed-forward control process. Thus, it can be argued that once insulin resistance develops, the postprandial glucose response, that is low enough to prevent harmful metabolic effects downstream, is significantly less than what is required in a healthy insulin sensitive individual. Thus, as insulin resistance progresses, the postprandial glucose response that could be deemed healthy, is theoretically reduced, which explains why the development of insulin resistance and T2DM is typically described to be progressive (Taylor, 2013). In other words, the same specific postprandial glucose response in a glucose tolerant, insulin sensitive individual will not be as damaging as it is in an individual who is already insulin-resistant. This is, however, speculative, and possibly an oversimplified line of reasoning. Either way, future well-designed longitudinal studies will have to be conducted to test this theory. A similar speculation was made by Francois *et al.* (2018). They mentioned the degree of insulin resistance of an individual may be the determining factor as to how drastically dietary carbohydrates should be restricted to elicit health improvements.

One can further argue that measuring glucose control as the primary marker in the screening of an individual's risk for T2DM and subsequent risk for CVD (Ceriello, 2005; Tabák *et al.*, 2012) is not optimal, as deteriorations in glucose control indicate that an individual has already progressed to a disease state (Tabák *et al.*, 2012; Wilcox, 2005). It is well-described in the literature that hyperinsulinaemia and/or insulin resistance is present before glucose tolerance declines and pre-diabetes starts to develop (DeFronzo & Ferrannini, 1991). Impaired glucose tolerance develops due to beta-cell dysfunction, secondary to hyperinsulinaemia and subsequent insulin resistance (DeFronzo, 2009; Tabák *et al.*, 2012; Wilcox, 2005). Elevated glucose levels, or abnormal measures of glucose control, are thus symptoms that develop due to a longer-standing root cause, i.e., hyperinsulinaemia (Crofts *et al.*, 2015). Addressing metabolic health only once glucose levels are atypical is an unnecessarily late reaction. Instead, measuring insulin levels presents health care workers with the opportunity to identify individuals at risk much earlier (Crofts *et al.*, 2015). The definition and manifestation of the metabolic syndrome supports this notion. Elevated fasting glucose is merely one of the symptoms of the metabolic syndrome (Huang, 2009), whereas hyperinsulinaemia has been identified as the unifying cause of all the major symptoms (Kelly *et al.*, 2014; Nolan & Prentki, 2019; Reaven, 1988), i.e., hypertriglyceridaemia (Raygor *et al.*, 2019), hypertension (Wang

et al., 2017), and central obesity (Shimobayashi *et al.*, 2018; Templeman *et al.*, 2017) that collectively define a state of being at an increased risk for the development of T2DM and CVD (Huang, 2009; McCracken *et al.*, 2018).

Considering that hyperinsulinaemia is directly implicated in the mechanisms of certain cancers, atherosclerosis, endothelial dysfunction, gestational- and T2DM, hypertriglyceridaemia, non-alcoholic fatty liver disease, obesity, dementia and neuropathies (Crofts *et al.*, 2015), it makes sense that early detection and subsequent lifestyle interventions (i.e., alterations in diet and the addition of physical exercise) will go a long way in the prevention of the metabolic syndrome and other eventual chronic disease states. Prevention is always preferred above treatment. If waiting for the manifestation of diminished glucose control or other symptoms of the metabolic syndrome before intervening, it would mean treatment, not prevention.

There was no clear relation between free-living glycaemic variability and hyperinsulinaemia in the current study. Glycaemic variability also did not correlate strongly with glucose tolerance or insulin sensitivity. Whether amplified glycaemic variability within the typical ranges of healthy individuals, in the absence of hyperinsulinaemia or insulin resistance, is a possible risk factor for deteriorating metabolic health in the future, cannot be deduced from the findings of this cross-sectional study. Future longitudinal studies would be needed to investigate this possibility.

5.12 Associations between glycaemic variability and traditional measures of glucose control

A secondary aim of this study was to investigate the associations between the traditional measures of glucose control and glycaemic variability. These associations are important when considering whether CGM would suffice as an early detection method of declining glucose control. The findings of this study, are however, inconclusive, as no meaningful associations were found between traditional glucose control measures and glycaemic variability.

Foreman and colleagues (2020) investigated similar relationships in 851 individuals (men and women). of which 470 had healthy glucose metabolism, 184 were pre-diabetic and 197 participants were T2DM patients. They reported the strongest correlation between mean glucose and fasting plasma glucose, and secondly between mean glucose and HbA_{1c} in glucose tolerant individuals, whereas the current study found fasting plasma glucose to be better correlated to MAGE and MODD,

rather than mean glucose. Similar to the current study, Foreman *et al.* (2020) found a strong correlation between mean CGM [glucose] and HbA_{1c} in participants with pre-diabetes or diabetes, with no notable association with overall glycaemic variability. This confirms the views about HbA_{1c}, namely that it is unable to reflect individual differences in glycaemic variability (MacLeod *et al*., 2013; Roberts *et al*., 2013).

Foreman *et al.* (2020) reported larger positive correlations between glycaemic variability and traditional glucose control measures for their T2DM group, compared to the group of healthy participants. This finding, together with the generally weak associations found in the current study, suggest that the sensitivity of the traditional measures of glucose control may be diminished in healthy individuals, compared to individuals with T2DM.

The large difference in the sample size of the current study and that of Foreman *et al.* (2020) means that comparisons should be made with caution. The small numbers of the current study, as well as the narrow age range of the participants, affects the strength of the correlations negatively. Nonetheless, the findings should stimulate interest in further and larger-scaled investigations. Thus far, however, it is questionable whether the traditional measures of glucose control adequately quantify the degree of glycaemic variability as measured by CGM in healthy individuals.

5.12.1 Glucose tolerance and its association with free-living glycaemic variability

Interesting findings regarding the associations between 2-h [glucose], the glycaemic variability indices, and glycaemic load, deserve specific attention. As the name implies, the OGTT is designed to quantify individuals' glucose tolerance. The 2-h OGTT [glucose], specifically, is used to differentiate between individuals with normal or impaired glucose tolerance. Glucose tolerance is dependent on the complex interplay of insulin secretion and clearance and the actions of insulin, namely, promoting glucose disposal from the bloodstream and the inhibition of glucose production endogenously (Bergman, 1989). It also predicts the degree of postprandial glucose excursions that can be expected during free-living conditions. However, it is not glucose tolerance *per se* that is important, but the severity and frequency of glucose excursions during free-living conditions (Kohnert *et al.*, 2012; Monnier *et al.*, 2008). It is expected that an individual with impaired glucose tolerance will experience higher postprandial glucose excursions, compared to an individual with normal glucose tolerance. Better glucose tolerance should theoretically "protect" an individual against excessive postprandial glucose spikes (Hanefeld *et al.* 2014). Thus, it was expected that individuals with better glucose tolerance will present with lower glycaemic variability. The relevance

of the 2-h [glucose] to quantify glucose tolerance in healthy, non-diabetic individuals, is, however, questionable.

Hanefeld *et al.* (2014) investigated the CGM profiles of individuals with normal and impaired glucose tolerance according to their OGTT results. They found that both overall and intraday glycaemic variation (SD of CGM glucose and MAGE, respectively), were significantly higher in the glucose intolerant participants, compared with the glucose tolerant individuals. They also found that the 2-h [glucose] of the OGTT is closer related to MAGE in glucose intolerant individuals than healthy, glucose tolerant individuals. Therefore, they proposed that the 2-h [glucose] is more representative of the expected postprandial glucose responses during free-living conditions in those who are already glucose intolerant in comparison with healthy individuals with healthy glucose tolerance. Madhu *et al.* (2013) investigated the 24-h CGM glycaemic profiles of normal glucose tolerant individuals and T2DM patients, who were categorised based on their OGTT results. They concluded that the OGTT results overestimated the control of glucose in comparison with the 24-h glucose profiles. In other words, the glucose excursions that typically occur during free-living conditions were higher than the participants' 2-h [glucose]. The findings of Madhu *et al.* (2013) help to explain the findings of the current study regarding the associations that were found with 2-h [glucose].

The 2-h [glucose] of the participants in the current study was not related to their free-living glucose excursions, i.e. higher 2-h [glucose] did not coincide with higher MAGE. In fact, the contrary was closer to the truth. Lower 2-h [glucose] was moderately associated with higher MAGE values, as well as other glycaemic variability indices. This finding is somewhat in agreement with Brand-Miller *et al.* (2009) who demonstrated peaks in postprandial [glucose] after as little as 30 min. Furthermore, the ADA (2001) suggested that the 1-h OGTT glucose value may be a better measure of glucose tolerance in non-diabetic individuals, than the 2-h value. This is exactly what Foreman *et al.* (2020) reported, namely, overall glycaemic variability correlated best with the 1-h OGTT glucose value, as opposed to the [glucose] at other time points during the OGTT. The 1-h [glucose] was the peak [glucose] during the OGTT. Foreman *et al.* (2020) subsequently suggested that more attention be given to the peak [glucose] during a 2-h OGTT, instead of only considering whether the 2-h value is above or below a threshold value.

It would make sense that the 2-h [glucose] will only be representative of peak postprandial [glucose] once considerable insulin resistance or impaired glucose tolerance has developed, as explained by Matsuda & DeFronzo (1999). Furthermore, since hyperinsulinaemia is present before insulin

resistance, which again is present before impaired glucose tolerance develops (Crofts *et al.*, 2015; Tabák *et al.*, 2012), the OGTT and especially the 2-h [glucose] is probably largely incapable of early risk detection, or of providing any information, really, for normal glucose tolerant, but possibly insulin-resistant, individuals. It can thus be deduced that the 2-h [glucose] from the OGTT cannot be used as an interchangeable term for postprandial glucose in normal glucose tolerant individuals and is in line with the negative correlation that was observed between 2-h [glucose] and MAGE in the current study.

Although from a different perspective, the current study's results support the suggestion that the 2-h [glucose] value cannot be used to quantify a healthy individual's glucose tolerance. It was observed that an individual can present with significant insulin resistance and postprandial hyperglycaemia without an atypical 2-h [glucose] (i.e. exceeding 7.8 $mmol \cdot L^{-1}$). The latter is used as the cut-off to differentiate between healthy, glucose tolerant individuals and impaired glucose tolerance or prediabetic individuals (ADA, 2017; SEMDSA, 2017). There were four individuals in the current study that were insulin-resistant and ten individuals (37% of the participants) whose maximum [glucose] during the OGTT (after 30 – 60 min) exceeded 7.8 $mmol \cdot L^{-1}$, even though their 2-h value was well below this cut-off value.

The mismatch between 2-h [glucose] and MAGE is also a possible corroboration of the mechanism behind the proposed carbohydrate-insulin model of obesity (CIMO) of Ludwig & Ebbeling (2018). The negative correlation between 2-h [glucose] and MAGE in the current study could be understood in light of the reactive response to high postprandial glucose excursions. Ludwig and Ebbeling (2018) explained that higher peak [glucose] in response to carbohydrate intake causes greater insulin secretion, which is followed by a rapid drop in blood [glucose] and potentially to levels lower than fasting [glucose]. The 2-h [glucose] in healthy individuals may thus be more characteristic, or descriptive, of the recovery from hyperglycaemia, rather than it being reflective of the postprandial glucose excursion. At the very least, it seems that the 2-h [glucose] does not reliably quantify or describe glucose tolerance in non-diabetic individuals.

Further evidence of the above argument is that there were no meaningful associations between the 2-h [glucose] and insulin sensitivity, body composition or age in the current study. Decreases in glucose tolerance occur secondary to the development of insulin resistance (Crofts *et al.*, 2015; Tabák *et al.*, 2012). Decreased glucose tolerance and increased insulin resistance is subsequently well-known to be associated with advancing age, as well as obesity (Brunner *et al.*, 2006; Ko *et al.*, 2006;

Shimokata *et al.*, 1991; Walker *et al.*, 2005). There is thus no evidence in this study that the 2-h [glucose] is indicative of the presence or absence of health risk related to glucose metabolism in apparently healthy individuals.

5.13 Association between glycaemic variability and insulin sensitivity

With insulin being largely responsible for the drop in postprandial [glucose] (Gerich, 2000) and worsening glucose control being a symptom of insulin resistance (Hall *et al.*, 2018), it was expected that glycaemic variability would be correlated with insulin resistance. In contrast, higher glycaemic variability was associated with better insulin sensitivity, as measured by both the Matsuda index and the HOMA-IR. This finding could first and foremost corroborate the model developed by Zeevi *et al.* (2015) which they used to predict the postprandial glucose responses of healthy individuals based on specific diets. An important finding by this study group, which was confirmed by Hall *et al.* (2018), was that healthy individuals do not experience the same postprandial glucose responses to the same foods. A possible explanation for this observation relates to the gut microbiome, which is likely an important role player in the absorption and metabolism of ingested foods (Suez *et al.*, 2014; Zeevi *et al.*, 2015).

Insulin sensitivity of the glucose-accepting tissues may thus not be the determining factor in the magnitude of the postprandial glucose excursions that can be expected. In fact, better insulin sensitivity, theoretically, only enhances the efficiency of glucose disposal. The incretin hormones that elicit the first-phase secretion of insulin may have an influence on the degree that glucose spikes directly after the ingestion of carbohydrates (Drucker, 2007). Alternatively, the height of the immediate postprandial [glucose] is dependent on how much glucose enters the blood stream and at what rate. Only after blood glucose levels have started to rise, is the pancreas stimulated to secrete insulin.

According to the CMIO (Ludwig & Ebbeling, 2018), higher postprandial glucose stimulates higher levels of insulin to be secreted. The more insulin enters the blood stream, and the more insulin sensitive the peripheral tissues are, the faster blood glucose levels will drop. If the rate of glucose disposal is high, the mechanisms responsible for homeostasis of blood glucose are challenged and cannot transition from the postprandial state to the postabsorptive state fast enough (Ludwig, 2002). The resultant high postprandial glucose spike (stimulating high levels of insulin to be secreted which leads to fast glucose disposal in an insulin sensitive individual) is thus an over compensatory drop in

blood [glucose] to hypoglycaemic concentrations. A nadir in the blood glucose profile has thus arisen following the excessive peak. Hence, it can theoretically be understood why, in healthy glucose tolerance individuals, greater insulin sensitivity was associated with higher degrees of glycaemic variability (within the healthy ranges).

Of note, the description of the physiological process above will not be applicable to individuals with overt insulin resistance, or after glucose tolerance has developed. In this case, insulin resistance will cause glucose disposal to occur at slower rates, which will subsequently cause higher elevations in postprandial [glucose].

Regardless of the explanation behind the association between glycaemic variability and insulin sensitivity in healthy, normal glucose tolerant participants, the so-to-speak "inability" of insulin sensitivity to protect healthy individuals against postprandial [glucose] spikes, emphasizes the importance of diet composition in the protection against less than optimal high levels of postprandial glucose and insulin.

5.14 Summary of main findings

The main finding of this study is that CGM and glycaemic variability indices were not able to detect health risk in individuals earlier than traditional glucose control, but that changes in insulin secretion (development of hyperinsulinaemia) occur before any abnormalities in glucose metabolism can be identified. The variability in OGTT insulin concentrations was more than the variability in the glucose concentrations of the OGTT, suggesting postprandial insulin levels are a better predictor of glucose control than postprandial glucose.

CRF was very strongly correlated with insulin sensitivity, more so than dietary glycaemic load. This advocates for the importance of exercise and high levels of aerobic fitness for the maintenance of healthy insulin sensitivity and the prevention of deterioration in metabolic health.

Notable differences in insulin resistance and insulin profiles after an OGTT were found between groups of participants who habitually consumed diets of varying macronutrient compositions. These differences in insulin resistance and insulin profiles were larger than differences in the OGTT glucose levels, HbA_{1c} (no difference between macronutrient clusters) and glycaemic variability indices. The largest difference between the three dietary groups, in terms of glycaemic variability indices, was the

difference in MAGE. Interestingly, the highest insulin levels and highest insulin resistance was found in the group of participants who habitually consumed foods with equally high percentages of carbohydrates and fats, while MAGE was more dependent on the percentage carbohydrates consumed per day. In other words, the results suggest that a diet which is high in carbohydrates and fats is more likely to be detrimental by eliciting hyperinsulinaemia, than a diet that is high in carbohydrates, but moderately low in total fat intake. It can thus not be concluded that postprandial glucose excursions are the largest determining factor in the development of hyperinsulinaemia and insulin resistance. It seems, from the results of this study, that a diet high in fats and carbohydrates will most likely lead to the development of hyperinsulinaemia and insulin resistance. The participants whose diets were high in fat and moderately low in carbohydrates had the lowest insulin levels, insulin resistance, and MAGE.

Insulin sensitivity was not a strong predictor of glycaemic variability and average postprandial glucose excursions within healthy ranges in non-diabetic men and during free-living conditions. This finding emphasizes the importance of diet to minimize high postprandial levels of glucose and insulin, even in healthy, insulin sensitive individuals.

This study was the first, to my knowledge, that investigated glucose control and free-living glycaemic variability of apparently healthy men in relation to cardiorespiratory fitness and dietary intake. To my knowledge it is also the first study that compared glycaemic variability, glucose tolerance and insulin sensitivity of healthy individuals with habitual diets of different macronutrient compositions. The findings of this study provide specific evidence of the presence of hyperinsulinaemia among individuals with healthy glucose control and glycaemic variability indices.

5.15 Study limitations

The sample size of the study was small, which minimizes the external validity of the study. Furthermore, a non-probability sampling method was used to recruit participants. However, the risk for selection bias was mitigated in that volunteers were enrolled consecutively into the study, in other words as they announced their interest to participate, provided they met the stipulated inclusion and exclusion criteria.

It is possible that the CGM devices that were used might have underestimated the degree of glycaemic variability by being unable to record absolute maximum glucose values. This is due to the sensors

measuring glucose concentrations every minute, but only recording the average concentration of every 15-minute period. As briefly discussed under section 5.4.1, interstitial glucose measurements are accurate in comparison to blood or plasma glucose concentrations, but it is possible that the degree of postprandial glucose may be under-reported by glucose sensors. The latter consideration is likely to have a small but added effect on the measurement of postprandial glucose excursions, compared with the possible smoothing effect of the 15-minute average values.

No standardised meals were provided at any time during the study; participants continued with their habitual diets. This could be seen as both a limitation and a strength. The free-living CGM made it impossible to compare the glycaemic variability of the individuals independent of diet. At the same time, however, the findings are arguably more applicable to everyday life, as the results of the study are able to address the applicability of the traditional glucose control tests to free-living conditions of healthy men.

The dietary analysis, specifically the calculation of glycaemic load, must be interpreted with caution. The average glycaemic load for each participant was calculated by only considering the carbohydrate constituents of each meal that was consumed. It is known that other individual meal components (such as the addition of fat, protein or vinegar, cooking methods,) have a marked effect on the overall GI of a meal (Russell *et al.*, 2016) and the subsequent glycaemic load. It is unfortunately not possible to account for all the possible factors to accurately calculate the glycaemic load of each meal. Other meal components or ingredients, cooking methods, food temperature, etc., were thus ignored.

Accurate cut-off values for a healthy HOMA-IR is difficult to determine as the variability in the threshold value that determines insulin resistance is large and is dependent on individual characteristics, such as ethnicity (Gayoso-Diz *et al.*, 2013; Wallace, Levy & Matthews, 2004). Individuals in the current study were from different ethnic backgrounds and may thus have been wrongly categorised as either insulin-resistant or non-insulin-resistant.

Chapter 6 Conclusions

6.1 Overview of findings

The findings of this study permit the flowing conclusions:

Hypothesis 1 is partly accepted.
Cardiorespiratory fitness had the strongest associations with the markers of insulin sensitivity, followed by HbA_{1c}, mean glucose and overall glycaemic variability.

Hypothesis 2 is partly accepted.
Dietary glycaemic load was most strongly associated with intraday glycaemic variability (MAGE), followed by time per day spent above 6 $mmol \cdot L^{-1}$, and inter-day glycaemic variability (MODD). No meaningful associations were found between dietary glycaemic load and insulin sensitivity, or glucose control.

Hypothesis 3 is accepted.
The low carbohydrate-high fat dietary group presented with the lowest indices of glycaemic variability and best insulin sensitivity. MAGE was highest for the high carbohydrate-low fat group, while insulin levels during the OGTT were the highest for the high CHO-high fat cluster. Differences in glucose control between the clusters were negligible or small.

Hypothesis 4 is accepted.
The strongest correlations among the glycaemic variability indices and traditional glucose control measures were between 2-h [glucose], MAGE and MODD. Both correlations were moderately negative. Moderate positive correlations were found between FPG, and MAGE and MODD. HbA_{1c} was not predictive of glycaemic variability.

Hypothesis 5 is rejected.
Better insulin sensitivity was associated with higher overall glycaemic variability, but lower mean [glucose]. Insulin sensitivity does not seem to protect against free-living postprandial hyperglycaemia in healthy, glucose tolerant individuals.

6.2 Practical implications

Instead of looking into the utilisation of CGM and measurement of glycaemic variability for health risk detection, the measurement of insulin levels should enjoy more attention. Collectively, the results suggest that insulin, instead of glucose, should be measured to screen and determine risk in individuals. Insulin levels are a better and earlier predictor of future metabolic abnormalities and the development of glucose intolerance or diminished glucose control than any measures of blood glucose, whether it is the traditional measures of glucose control or CGM-derived indices.

The findings of the current study support the need for both exercise (and increases in cardiovascular fitness) and diet for the long-term maintenance of metabolic health. More specifically, a diet that prevents hyperinsulinaemia (i.e. low in carbohydrates and high in healthy fats) and exercise to promote insulin sensitivity are very important factors to prevent the development of insulin resistance and subsequent cardiometabolic disease.

6.3 Recommendations for future research

It would be valuable to repeat the study on a larger group of individuals and over a broader age range in order to test the findings of the current study. This would help to gain further insight into the relationships of cardiorespiratory fitness and diet with glycaemic variability, glucose control, and insulin resistance. A larger study sample could also provide more insight into the prevalence of increased free-living glycaemic variability in individuals with healthy HbA_{1c} and fasting glucose levels and healthy glucose tolerance.

Conducting a similar study, but with the inclusion of some standard meals will allow the determination of individuals' responses to carbohydrate foods. This may help to make more sense of the glycaemic variability data and the relations thereof to glucose control and insulin sensitivity.

Due to the cross-sectional design of the current study, it could not be elucidated whether increased glycaemic variability (within the normal ranges of healthy individuals), in the absence of hyperinsulinaemia or insulin resistance, is a possible risk factor for future manifestation of deteriorating metabolic health. This study will have to be repeated on a larger sample and including individuals with higher glycaemic excursions and with the addition of a longitudinal component, to properly investigate the effect varying degrees of glycaemic variability on long-term health.

Available glycaemic variability reference values have, been derived from large cohorts that were grouped according to the traditional measures of glucose control. Reference values are needed that are based on the risk associated with various degrees of glycaemic variability.

6.4 Conclusion

This is the first study, to my knowledge, to specifically investigate the associations of glycaemic variability indices with dietary glycaemic load. Glycaemic variability better reflects the impact of dietary intake on glucose levels, compared to the traditional measures of glucose control.

Glycaemic variability indices were not able to identify atypical glucose metabolism independently from the traditional measures of glucose control. The variability in insulin levels during the OGTT was higher than the variability in the glucose levels during the OGTT and is additive support that changes in insulin secretion are evident before glucose levels become abnormal.

Furthermore, this study provides evidence to support the importance for both exercise and a healthy diet for the maintenance of metabolic health. More specifically, the current study found that cardiorespiratory was strongly associated to insulin sensitivity, while dietary glycaemic load and macronutrient composition as important considerations in the prevention of hypersecretion of insulin and subsequent development of insulin resistance. The results of this study suggest a diet that is equally high in carbohydrates and fat, is most likely to illicit hyperinsulinaemia and thus, over time, insulin resistance.

The results of this study, combined with the extensive literature on the detrimental effects of hyperinsulinaemia and insulin resistance, provide motivation to highly recommend shifting the focus from various means of measuring glucose control to the measurement of fasting and postprandial insulin levels. More attention should also be given to the definition of “healthy” insulin levels that would aid in the prevention of declining health.

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Appendix A:

Modified ACSM Medical Screening Questionnaire taken from *ACSM's guidelines for exercise testing and prescription* 9th ed. (Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins Health, pp.25.)

Health Status Questionnaire

On this questionnaire, a number of questions regarding your physical health are to be answered. Please answer every question as accurately as possible so that a correct assessment can be made. Please mark the space to the left of the question to answer "yes". Leave blank if your answer is "no". Please ask if you have any questions. Your response will be treated in a confidential manner.

Name:_____ Date:_____

Medical Screening – ACSM Medical Screening Questionnaire

- Do you have any personal history of heart disease?
- Do you suffer from chronic migraines?
- Do you suffer from any anxiety disorders?
- Do you have any personal history of metabolic disease (thyroid, renal, liver)?
- Do you know of any traumatic brain injury you have obtained? Are you using medication to treat a previous incident?
- Have you suffered from a severe head injury/concussion within 6 months of this study?
- Have you had diabetes for less than 15 years?
- Have you had diabetes for 15 years or more?
- Have you experienced pain or discomfort in your chest apparently due to blood flow deficiency?
- Any unaccustomed shortness of breath (perhaps during light exercise)?
- Have you had any problems with dizziness or fainting?
- Do you have difficulty breathing while standing or sudden breathing problems at night?
- Do you suffer from ankle oedema (swelling of the ankles)?
- Have you experienced a rapid throbbing or fluttering of the heart?
- Have you experienced severe pain in leg muscles during walking?
- Do you have a known heart murmur?
- Do you have any family history of cardiac or pulmonary disease prior to age 55?
- Have you been assessed as hypertensive on at least 2 occasions?
- Has your serum cholesterol been measured at greater than 5.4mmol/l?
- Are you a cigarette smoker?
- Would you characterize your lifestyle as "sedentary"?

Medical History

__ Are you currently being treated for high blood pressure?
If you know your average blood pressure, please enter: _____/_____
Please Check All That Apply.

_ has doctor ever found an abnormal ECG?
_ Abnormal Chest X-Ray?
_ Rheumatic Fever?
_ Low Blood Pressure?
_ Asthma?
_ Bronchitis?
__ Emphysema?
__ Other Lung Problems?

_ Limited Range of Motion?
_ Recently Broken Bones?
_ Arthritis?
_ Bursitis?
_ Swollen or Painful Joints?
_ Foot Problems?
_ Knee Problems?
__ Back Problems?
__ Shoulder Problems?

_ Stroke?
_ Epilepsy or Seizures?
_ chronic Headaches or Migraines?
_ Persistent Fatigue?
_ Stomach Problems?
_ Hernia?
__ Anemia?

_ Has a doctor imposed any activity restrictions? If so, please describe:

_____

_____

_____

Family History

Have your mother, father, or siblings suffered from (please select all that apply):

___ Heart attack or surgery prior to age 55
___ Stroke prior to age 50
___ Congenital heart disease or left ventricular hypertrophy
___ High cholesterol
___ Diabetes
___ Obesity
___ Hypertension
___ Osteoporosis
___ Asthma
___ Leukemia or cancer prior to age 60

Medications

Please Select Any Medications You Are Currently Using

_ Diuretics
_ Beta Blockers
_ Vasodilators
_ Alpha Blockers
_ Calcium Channel Blockers
_ Other Cardiovascular
_ NSAIDS/Anti-inflammatories (Motrin, Advil)
_ Cholesterol
_ Diabetes/Insulin
_ Other Drugs (record below).

Please list the specific medications that you currently take:

_____

_____

Emergency Contacts

Please list your general practitioner and person to be contacted in case of emergency

Doctor:_____ Phone:_____

Contact:_____ Phone:_____

Activities and Goals

On average, how many times do you exercise per week? _____

On average, how long do you exercise? _____ minutes

On a scale from 1 to 10, how intense is your typical workout (circle one):

Very Easy **1 2 3 4 5 6 7 8 9 10** Very Intense

For each activity that you participate in, indicate your typical exercise time in minutes per session:

Running/Jogging:	_____	Weight Training:	_____	Skiing/Boarding:	_____
Walking:	_____	Aerobics Classes:	_____	Yoga/Martial Arts:	_____
Stair Climbing:	_____	Swimming:	_____	Other:	_____
Bicycle/Spinning	_____	Racquet Sports:	_____		

Lifestyle

Are you a cigarette smoker? _____ If so, how many per day? _____

Previously a cigarette smoker? _____ If so, when did you quit? _____

How many years have you smoked or did you smoke before quitting? _____

Do you/did you smoke: cigarettes? cigars? pipe?

Please rate your daily stress levels (select one):

Low Moderate High: I enjoy the challenge High: sometimes difficult to handle High: often difficult to handle

Do you drink alcoholic beverages? _____

How many units of alcohol do you consume per week: _____ (see Alcohol Units Calculator below)

Alcohol Units Calculator

Type of Drink	Units
1 glass of wine	1
1 pub measure of spirits (Gin, Vodka etc.)	1
1 can of beer	1.5
1 bottle of strong lager	2.5
1 can of strong lager	4
1 bottle of wine	7
1 litre bottle of wine	10
1 bottle of fortified wine (port, sherry etc.)	14
1 bottle of spirits	30

Dietary Habits: Please select all that apply

___ I seldom consume red or high fat meats
___ I pursue a low-fat diet
___ I eat at least 5 servings of fruits/vegetables per day
___ I almost always eat a full, healthy breakfast
___ My diet includes many high-fiber foods
___ I rarely eat sugar of high-fat dessert

Other

Please indicate any other medical conditions or activity restrictions that you may have. It is important that this information be as accurate and complete as possible.

_____

_____

_____

Is any of this information critical to understanding your readiness for exercise? Are there any other restrictions on activity that we should know about?

_____

_____

Thank you for taking the time to complete this questionnaire!

Appendix B

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF RESEARCH PROJECT:	
The role of cardiorespiratory fitness on measures of glucose control and glycaemic variability in healthy men aged 30 - 50 years.	
DETAILS OF PRINCIPAL INVESTIGATOR (PI):	
Title, first name, surname: Ms Jonine Möller	**Ethics reference number:** S19/10/262
Full postal address: Department Sport Science, Stellenbosch University, Private Bag X1, Matieland, Stellenbosch, 7601	**PI Contact number:** 0727833633

We would like to invite you to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff any questions about any part of this project that you do not fully understand. It is very important that you are completely satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary,** and you are free to decline to participate. In other words, you may choose to take part, or you may choose not to take part. Nothing bad will come of it if you say no: it will not affect you negatively in any way whatsoever. Refusal to participate will involve no penalty or loss of benefits or reduction in the level of care to which you are otherwise entitled to. You are also free to withdraw from the study at any point, even if you do agree to take part initially.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University.** The study will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, the South African Guidelines for Good Clinical Practice (2006), the Medical Research Council (MRC) Ethical Guidelines for Research (2002), and the Department of Health Ethics in Health Research: Principles, Processes and Studies (2015).

What is this research study all about?

The study will be conducted in the Sport Physiology Laboratory in the Department of Sport Science. We will invite 30 men to participate in the study and recruitment will continue until we achieve this number.

The aim of the study is to assess fluctuations in glucose during your everyday life in comparison to the usual diagnostic tests which are used to determine glucose control. We also want to determine to what degree fitness and dietary influence these measures.

If you agree to take part in the study and the screening procedure (which will involve the filling in of a questionnaire about your health status) indicate that you are eligible (if medical clearance needs to be obtained before you may partake in the study; the doctor's consultation will be paid for by the study's insurance), you will continue with the rest of the session in the laboratory, which could last an hour. Your height, weight, waist and hip circumferences and % body fat will be measured, after which you will be fitted with a continuous glucose monitor which you will wear continuously for two weeks. The application and wearing of the monitor are completely painless. It will be applied to the back of your upper arm. The glucose monitor will measure your glucose levels every minute for as long as it stays on your arm until the two weeks are over. You won't be aware of the measurements. You may sleep, shower, swim and exercise with the sensor on. You will not be able to see your live glucose readings, but you will receive a full report after you have completed the study. While wearing the glucose monitor, you will be asked to log all food and drink and keep a diary of the exercise and physical activity that you do. It will be thoroughly explained to you how to go about doing this. Google forms will be used for the purpose of loggings, but you may keep a paper-based diary if you prefer. You will then perform a maximal exercise test on the treadmill, to end off

the session with. We will fit you with a heart rate monitor around your chest and a face mask to measure your respiratory gas exchange. You will be required to run on the treadmill while the speed is increased by 0.5 km/h after every minute. You will be asked to run until you are exhausted. The data that we gather from this test will allow us to determine your maximal aerobic capacity.

We will also make an appointment for a morning on which you will be accompanied to the nearest pathology laboratory. You will need to fast overnight. A fasting blood sample will be drawn to measure glycated haemoglobin (HbA_{1c}), glucose and insulin. You will then drink a solution of 75 g of glucose after which blood samples will be taken every 30 min, again for glucose and insulin, until 120 min have passed. You will be allowed to take your laptop to work or a book to read while you sit and wait.

Why do we invite you to participate?

You are invited to take part in this study because you have indicated your interest in the research project by responding voluntarily to the invitation asking for participants and you meet the inclusion criteria for the study. The inclusion criteria stipulate that you are a healthy male, not restricting dietary carbohydrates, didn't change your dietary habits in the last 6 months and are between 30 and 50 years of age.

What will your responsibilities be?

We ask that you complete all questionnaires as honestly as possible and that you follow the instructions of the researchers before, during and after the testing sessions as best you can. You will also be asked to give your best efforts during the exercise test. In case of any deviations from the instructions (see below), we ask that you inform the researchers.

Prior to the testing session, we ask that you do the following:
1. do not eat for the 2 hours prior to testing;
2. avoid caffeine-containing drinks and alcohol ingestion at least 12 hours before testing;
3. avoid vigorous activities or any unaccustomed exercise at least 24 hours before testing.

You are also required to report your daily food intake honestly and as accurately as possible. It is also very important for you to make sure the glucose sensor does not come off your arm. It cannot be reapplied once it has been put on your arm. You will be given plasters to help to keep it in place.

Will you benefit from taking part in this research?

The continuous glucose monitoring data together with your meal-loggings could provide very important and useful insight into your glucose responses to certain foods and meals. You will receive a personal report on all your test results and these findings will be discussed with you in person.

Are there any risks involved in your taking part in this research?

There will be no serious risks involved in the study; nonetheless, you may experience dizziness, physical discomfort, muscle fatigue and in rare instances, fainting, during the maximal aerobic capacity exercise test. In the case that you experience any of these symptoms the exercise test will be stopped immediately, and the researchers will take the necessary steps to make you comfortable. Biokineticists are on standby in the Department of Sport Science who are trained in first aid and willing to assist. Should any serious emergency arise, you will be stabilized and transported to the emergency room of Stellenbosch Medi-Clinic.

Although scarce, some people may experience a hyposensitive skin reaction (such as an allergic reaction, itching, pain, redness and burning) to the adhesive of the glucose monitoring sensors. Other side effects may also develop, such as sleep disturbances, muscle soreness or subcutaneous haemorrhage. You may choose to withdraw from the study should any side effect be experienced. You will also be referred to a doctor, if necessary. All medical costs will be covered by the study insurance.

Even though it is unlikely, what will happen if you get injured somehow because you took part in this research study?

Stellenbosch University will provide comprehensive no-fault insurance and will pay for any medical costs that came about because participants took part in the research. The participant will not need to prove that the researchers were at fault.

Will you be paid to take part in this study and are there any costs involved?

For your time invested in the laboratory tests, you will receive R21.00/hour commensurate with unskilled labour rates in accordance with NHREC (2012) guidelines for "Payment of trial participants in South

Africa". In addition, you will receive R100 for each visit to the laboratory to cover travel expenses if you do not reside in Stellenbosch.

Protection of participant information, confidentiality and identity

Upon induction into the study you will receive a coded identification which will be used on all your documents and computer files related to the project. All the hard copies of your documents will be locked in the office of the supervisor, Prof Terblanche. Only the student investigator (Ms Möller), the laboratory manager (Ms Engelbrecht) and the supervisor (Prof Terblanche) will have access to the original records, the data and the locked cabinet where hard copies of documents will be stored. A sign in and out method will be implemented to ensure the safety of the documents.

All computer files (i.e. Excel spreadsheets) and data which are collected via instruments in the Sport Physiology Laboratory (i.e. the data during the exercise tests) will be saved in a dedicated password protected folder on the laboratory computer and the password will only be available to the student investigator. This computer is also password protected and only the laboratory manager, Ms Engelbrecht, has access to the computer. Data files that are saved on computers for access by the research team will be de-identified. In other words, your data will be stored using your project identification number and not your name or other identifiable information.

It is likely that the results from this study will be presented at national and international conferences and published in international peer-reviewed academic journals. Only group data and related statistics will be reported. You will therefore not be personally identifiable in these presentations and publications.

Is there anything else that you should know or do?

If you have any questions or concerns about this study, please feel free to contact Jonine Möller [072783 3633; 18266231@sun.ac.za] and/or the supervisor Prof Elmarie Terblanche [082 707 6501; et2@sun.ac.za].

You may phone the Health Research Ethics Committee at 021 938 9677/9819 if there still is something that the researchers have not explained to you, or if you have a complaint.

You will receive a copy of this information and consent form for you to keep safe.

Declaration by participant

By signing below, I ……..……. agree to take part in a research study entitled *"The role of cardiorespiratory fitness on measures of glucose control and glycaemic variability in healthy men aged 30 - 50 years."*

I declare that:

- I have read this information and consent form, or it was read to me, and it is written in a language in which I am fluent and with which I am comfortable.
- I have had a chance to ask questions and I am satisfied that all my questions have been answered.
- I understand that taking part in this study is **voluntary,** and I have not been pressurised to take part.
- I may choose to leave the study at any time and nothing bad will come of it – I will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan that we have agreed on.

Signed at (*place*) .....…….. on (*date*) ……...…….. 20….

....
Signature of participant **Signature of witness**

Declaration by investigator

I *(name)* ……..…… declare that:

- I explained the information in this document in a simple and clear manner to ……..
- I encouraged him/her to ask questions and took enough time to answer them.
- I am satisfied that he/she completely understands all aspects of the research, as discussed above.
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.)*

Signed at (*place*) .....…….. on (*date*) ……...…….. 20…..

....
Signature of investigator **Signature of witness**

Appendix C

Sportwetenskap
Sport Science
STELLENBOSCH

Sport Physiology Laboratory

SEE EXACTLY WHAT HAPPENS TO YOUR GLUCOSE IN RESPONSE TO YOUR LIFESTYLE

STUDY PARTICIPANTS NEEDED...

...to do one fitness test, do one blood test and to wear a continuous glucose monitor for 2 weeks while continuing with every-day life

Are you:

30 - 50 years old;
fit / unfit (doesn't matter);
Healthy ?
Sorry! Men only

CONTACT Jonine for more info:
0727833633
18266231@sun.ac.za

Appendix D

Rating	Descriptor
6	No exertion at all
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard (heavy)
16	
17	Very Hard
18	
19	Extremely hard

Appendix E

Food & Drink intake

- Please log EVERYTHING that you eat or drink (water and non-caloric drinks excluded).
- Provide a description of the quantity, for example, 1 apple, 100 g chicken, 1 tsp honey. If you don't have a kitchen scale please provide any description that will help us determine the quantity, for example, a palmful, heaped tablespoon, the size of a deck of cards, etc.
- Please be as precise as possible - too much information is better than too little.
- Remember to include sauces/dressings and the oil or butter used for frying or milk in coffee and tea, for example.
- If making a dish, list the total ingredients of the recipe the first time you eat of the dish; thereafter you can just log portion size with a shorter description of the meal.
- Please try to avoid eating foods that you do not know the ingredients of for as long as you are wearing your glucose monitor.
- Food labels may by photographed and uploaded if/when applicable.
- If logging several meals at once, please clearly mark the time of intake of each item in the description.

* Required

1. Name & Surname: *

2. Date? *

Example: January 7, 2019

3. Time?

Example: 8:30 AM

4. What did you eat / drink and how much of it? *

5. Food label / Ingredients list / Nutritional information / Recipe (Optional):

Files submitted:

Appendix F

Food & Drink Intake

Please log EVERYTHING that you eat or drink (water and non-caloric drinks excluded).

Provide a description of the quantity, for example, 1 apple, 100 g chicken, 1 tsp honey. If you don't have a kitchen scale please provide any description that will help us determine the quantity, for example, a palmful, heaped tablespoon, a the size of a deck of cards, etc.

Please be as precise as possible - too much information is better than too little.

Remember to include sauces/dressings and the oil or butter used for frying or milk in coffee and tea, for example.

If making a dish, list the total ingredients of the recipe the first time you eat of the dish; thereafter you can just log portion size with a shorter description of the meal.

Please try to avoid eating foods that you do not know the ingredients of for as long as you are wearing your glucose monitor.

Food labels may by photographed and attached if/when applicable.

Date

Time	**Meal description (with ingredients & quantity)**

Drinks

Appendix G

	7 – 11 days CGM	12 & 13 days CGM	
	Mean ± SD	Mean ± SD	*p; ES*
n	9	18	
Age (years)	36 ± 5	37 ± 5	0.64; 0.20
Body mass (kg)	88.3 ± 15.9	87.7 ± 13.5	0.91; 0.04
Body fat %	22.9 ± 5.4	19.6 ± 6.4	0.19; 0.54
Fat free mass (kg)	67.7 ± 10.7	70.2 ± 9.4	0.55; 0.25
Waist:Height	0.49 ± 0.04	0.50 ± 0.07	0.73; 0.16
Waist:Hip	0.85 ± 0.04	0.86 ± 0.06	0.73; 0.18
VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$)	45.3 ± 6.9	46.23 ± 7.8	0.75; 0.21
Average daily GL	158.8 ± 55.8	144.0 ± 46.5	0.47; 0.30
Average daily CHO intake (g)	276.8 ± 71.6	262.33 ± 74.4	0.63; 0.20
Average daily energy intake (kJ)	11641 ± 1822	11046 ± 1855	0.44; 0.32
% dietary CHO intake	42.1 ± 8.3	40.4 ± 6.74	0.57; 0.23
% dietary protein intake	16.7 ± 3.2	19.4 ± 3.9	0.08; 0.73
% dietary fat intake	41.2 ± 5.9	40.1 ± 5.0	0.59; 0.21
HbA_{1C} (%)	5.47 ± 0.5	5.41 ± 0.2	0.62; 0.18
Fasting plasma glucose ($mmol \cdot L^{-1}$)	5.04 ± 0.5	4.99 ± 0.2	0.68; 0.15
2-hour glucose ($mmol \cdot L^{-1}$)	4.08 ± 1.2	4.91 ± 1.5	0.16; 0.59
HOMA-IR	1.11 ± 0.3	1.42 ± 0.9	0.35; 0.41
Matsuda Index	9.52 ± 3.0	9.29 ± 5.0	0.90; 0.05
Mean glucose ($mmol \cdot L^{-1}$)	4.7 ± 0.4	4.6 ± 0.4	0.37; 0.25
SD ($mmol \cdot L^{-1}$)	0.79 ± 0.16	0.79 ± 0.15	0.98; 0.00
CV%	17.06 ± 3.96	17.46 ± 4.28	0.82; 0.10
MAGE (intraday variability)	1.05 ± 0.23	0.97 ± 0.18	0.32; 0.41
MODD (inter-day variability)	0.72 ± 0.18	0.69 ± 0.15	0.70; 0.19

Appendix H

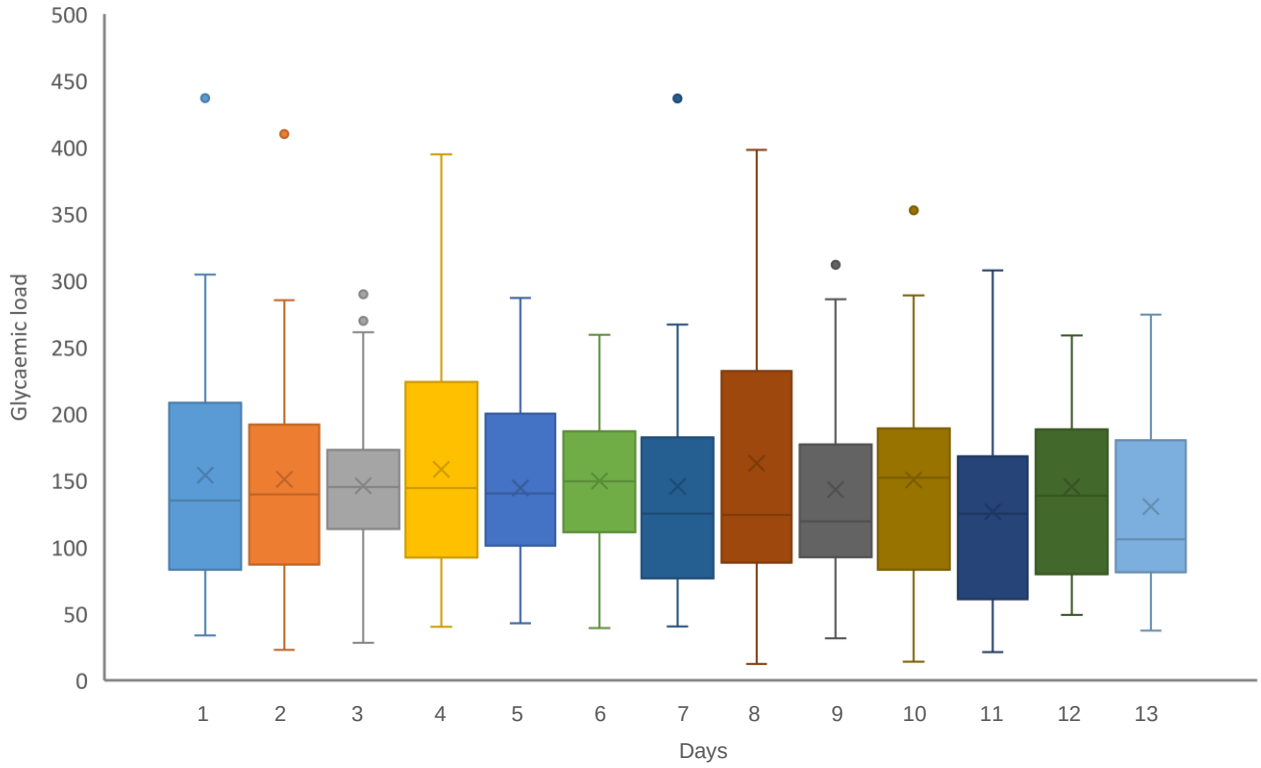

Figure A1. The daily dietary GL of the participants during the CGM period. The horizontal line in the box represents the median value, while the X marks the mean GL value.

The outlying GL values (defined as 1.5 times the interquartile range larger than the third quartile) were from different individuals each day.

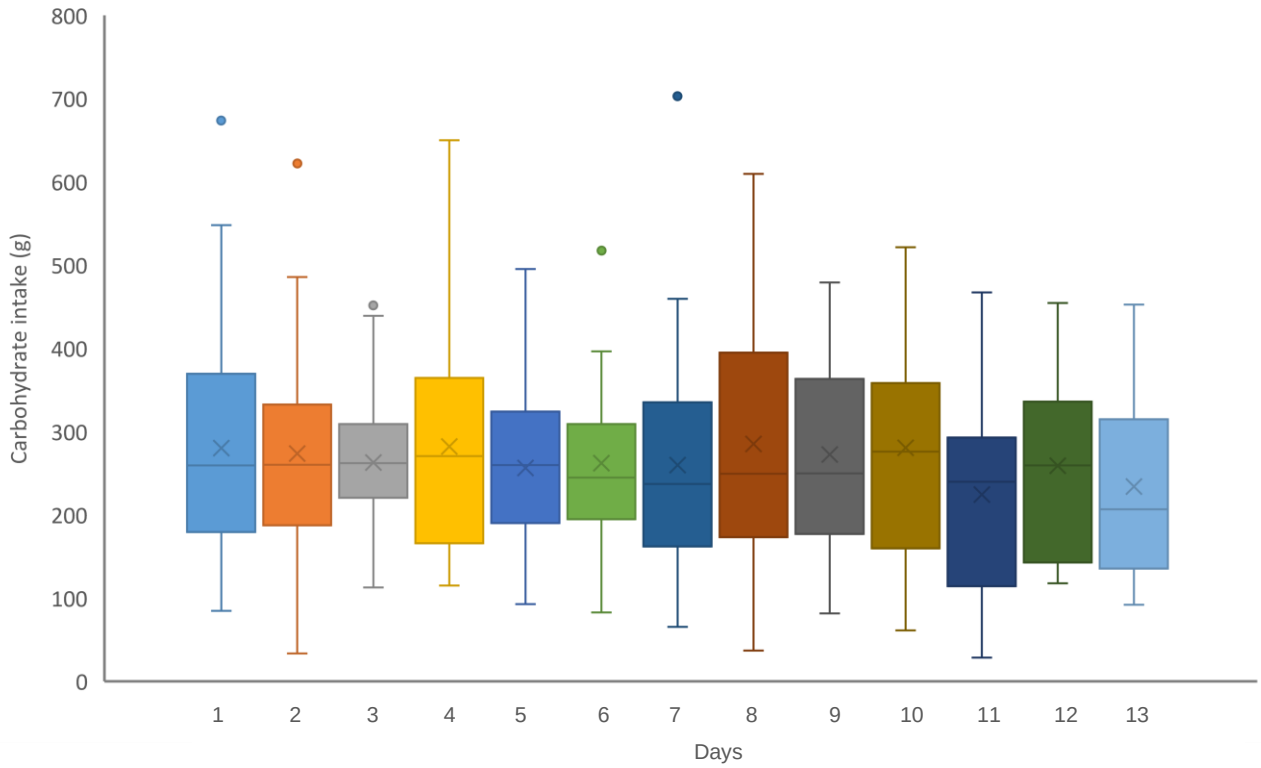

Figure A2. Daily dietary carbohydrate intake of the participants during the CGM period. The horizontal line in the box represents the median value, while the X marks the mean carbohydrate intake value.

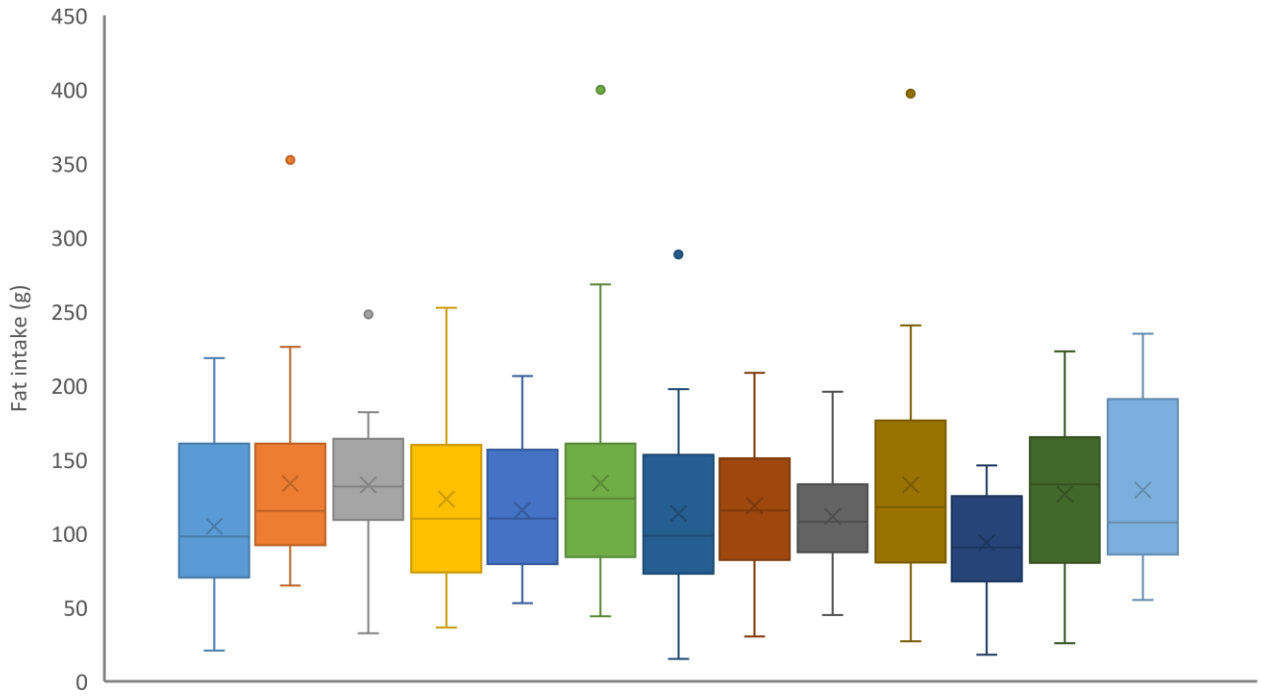

Figure A3. Daily dietary fat intake of the participants during the CGM period. The horizontal line in the box represents the median value, while the X marks the mean fat intake value.

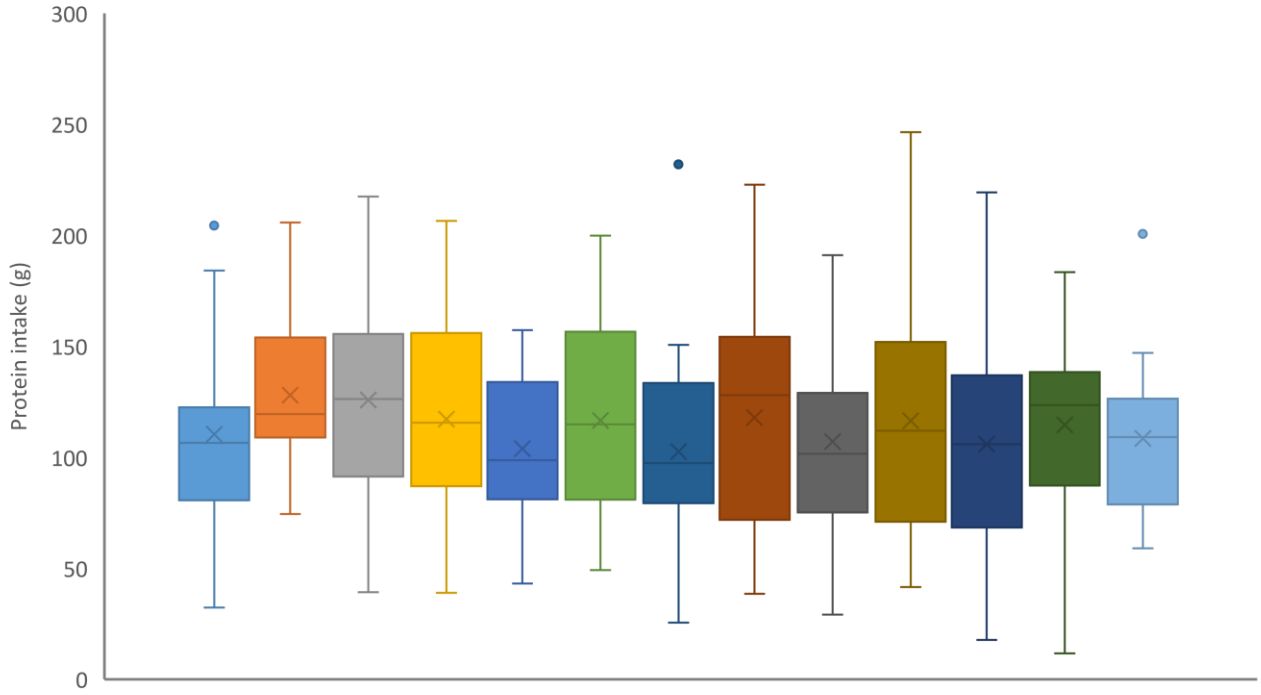

Figure A4. Daily dietary protein intake of the participants during the CGM period. The horizontal line in the box represents the median value, while the X marks the mean protein intake value.

Table A1. Summary of the magnitude of differences (ES ± CI 90%) in the group's day-to-day macronutrient variability.

	SD (ES; 90% CI)	**CV% (ES; 90% CI)**
Carbohydrate vs. fat	3.901; 50 – 83*	0.083; -4.4 – 5.7
Carbohydrate vs. protein	5.342; 63 – 94*	1.290; 4.6 – 12*
Fat vs. protein	1.227; 5.1 – 20*	1.086; 2.8 – 13*

*$p < 0.05$.

The average day-to-day individual (intrapersonal) variation in dietary glycaemic load was more than the average day-to-day individual (intrapersonal) variation of the macronutrient intakes (Table). The average day-to-day individual (intrapersonal) variation in glycaemic load was also more than the group's average day-to-day variation in glycaemic load.

Table A2. The average day-to-day individual (intrapersonal) variation (mean ± SD) in dietary energy, glycaemic load, and macronutrient intakes.

	SD	CV%
Energy intake	4020 ± 501 kJ	36.1 ± 5.2%
Glycaemic load	78.5 ±13.9 g	53.6 ± 8.8%
Carbohydrates	95.5 ± 38.6 g	36.6 ± 14.7%
Fat	52.3 ± 17.4 g	43.5 ± 12.2%
Protein	38.8 ± 10.2 g	35.0 ± 9.8%

The average day-to-day individual (intrapersonal) variability (CV%) in carbohydrate intake was smaller than for fat intake (ES = 0.510; 90% CI: -11 – -2.4), but not for protein intake (ES = 0.130; 90% CI: -2.3 – 5.6). The day-to-day individual (intrapersonal) variability (CV%) in fat consumption was larger than the variability of protein consumption (ES = 0.769; 90% CI: 5.3 – 12).

Table A3. The differences in the intrapersonal variations of the participants' dietary macronutrient intakes.

	SD (ES; 90% CI)	CV% (ES; 90% CI)
Carbohydrate vs. fat	1.443; 1.443*	-0.510; -11 – -2.4*
Carbohydrate vs. protein	2.009; 45 – 68*	0.130; -2.3 – 5.6
Fats vs. proteins	0.947; 7.4 – 20*	0.769; 5.3 – 12*

*$p < 0.05$.

Table A4. [Glucose] and comparisons between the clusters of the individual time points.

	Clusters (mean ± SD)			Cluster differences (ES (90% CI))		
	1	2	3	1 - 2	1 - 3	2 - 3
0 min	4.9 ± 0.3	5.1 ± 0.2	5.1 ± 0.4	0.731** (-0.06 –0.46)	0.637** (-0.04 –0.48)	0.051 (-0.27 –0.3)
30 min	7.0 ± 1.5	7.2 ± 1.6	7.8 ± 1.1	0.153 (-1.2 –1.7)	0.637** (-4.0 –5.6)	0.444* (-0.55 – - 1.7)
60 min	5.6 ± 1.5	5.2 ± 2.2	5.7 ± 1.6	0.231* (-2.1 –1.3)	0.072 (-1.1 –1.3)	0.288* (-1.1 –2.1)
90 min	4.9 ± 1.2	5.1 ± 1.0	5.1 ± 1.4	0.186 (-0.85 –1.3)	0.154 (-0.8 –1.2)	0.007 (-1.1 –1.1)
120 min	4.5 ± 1.9	4.5 ± 1.2	4.8 ± 1.3	0.007 (-1.5 –1.5)	0.120 (-0.88 –1.5)	0.235* (-0.81 –1.4)

All $p > 0.05$; *small difference, **moderate difference.

Table A5. [Insulin] means ± SD and comparisons between the clusters of the individual time points.

	Clusters (Mean ± SD)			Cluster differences (ES; 90% CI)		
	1	**2**	**3**	**1 vs 2**	**1 vs 3**	**2 vs 3**
0 min	5.0 ± 2.0	3.9 ± 0.9	7.7 ± 4.7	0.725** (-0.27 –0.34)	0.692** (-0.27 –5.5)	0.978*** (0.37 – 7.2)
30 min	56.9 ± 45.0	41.3 ± 19.3	67.6 ± 41.0	0.420* (-50 – 19)	0.251* (-22 – 43)	0.740** (-4.8 – 57)
60 min	37.0 ± 20.8	29.4 ± 18.0	52.9 ± 37.5	0.382* (-26 –11)	0.505* (-8.1 –40)	0.719** (-5 – 52)
90 min	30.5 ± 24.8	22.3 ± 4.0	38.0 ± 33.6	0.417* (-26 –10)	0.248* (-16 – 31)	0.561* (-8.7 – 40)
120 min	20.0 ± 20.0	10.5 ± 6.1	29.0 ± 38.7	0.592* (-25 – 5.5)	0.278* (-16 – 33)	0.573* (-9.7 – 47)

All $p > 0.05$; *small difference, **moderate difference, ***large difference