THE EFFECT OF AN EXERCISE INTERVENTION ON INSULIN SENSITIVITY, INSULIN SECRETION AND INSULIN CLEARANCE IN BLACK OBESE SOUTH AFRICAN WOMEN

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(DSMMEL001)

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“It seems impossible until it is done”

-Nelson Mandela

TABLE OF CONTENTS

AUTHOR'S DECLARATION

I, Melony Fortuin-de Smidt, hereby acknowledge that I know the meaning of plagiarism and I declare that all the work in this thesis, submitted for the degree of Doctor in Philosophy, save for that which is properly acknowledged, is entirely my own work.

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Date: 03 February 2020

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LIST OF ABBREVIATIONS

AIRg	Acute insulin response to glucose
BMI	Body mass index
CLp	Peripheral insulin clearance
DI	Disposition index
DXA	Dual-energy X-ray absorptiometry
EMCL	Extramyocellular lipid
FSIGT	Frequently sampled intravenous glucose tolerance test – insulin modified
HIE	Hepatic insulin extraction
HIEG	Hyperinsulinemic euglycemia clamp
HOMA2 IR%	Homeostatic model 2 of insulin resistance
HOMA2 B%	Homeostatic model 2 of Beta cell function
IMCL	Intramyocellular lipid
ISR	Insulin secretion rate
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
OGTT	Oral glucose tolerance test
SAT	Subcutaneous adipose tissue
S_I	Insulin sensitivity
South Africa	SA
TGs	Triglycerides
T2D	Type 2 Diabetes
VAT	Visceral adipose tissue
VO_{2peak}	Maximal oxygen uptake

SCIENTIFIC OUTPUTS FROM THIS THESIS

Papers

1. Goedecke JH, Mendham AE, Clamp L, Nono Nankam PA, Fortuin-de Smidt MC, Phiri L, et al. An Exercise Intervention to Unravel the Mechanisms Underlying Insulin Resistance in a Cohort of Black South African Women: Protocol for a Randomized Controlled Trial and Baseline Characteristics of Participants. *JMIR Res Protoc*. 2018 Apr 18;7(4):e75.

2. Fortuin-de Smidt MC, Mendham AE, Hauksson J, Hakim O, Stefanovski D, Louise Clamp L, Phiri L, Swart J, Goff LM, Micklesfield LK, Kahn SE, Olsson T, Goedecke JH. Effect of exercise training on hyperinsulinemia, insulin sensitivity and ectopic fat in obese black South African women: A randomized controlled trial. *European Journal of Endocrinology* 2020 183(1):51-61.

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1. Fortuin-de Smidt M, Alhamud A, Saleh MG, Mendham A, Hauksson J and Julia Goedecke J. The effect of exercise training on ectopic hepatic, pancreas and skeletal muscle lipid content in black obese South African women. **52nd Society for Endocrinology, Metabolism and Diabetes of South Africa Congress, Johannesburg, South Africa** *(Poster Presentation – 5-7 May 2017)*

2. Fortuin-de Smidt M, Mendham A, Alhamud A, Hauksson J, Olsson T and Goedecke J. A 12-week exercise intervention improves insulin sensitivity in sedentary black obese South African women unrelated to changes in ectopic fat accumulation. **18th International Congress of Endocrinology, Cape Town South Africa** *(Oral Presentation – 1-4 Dec 2018)*

3. Fortuin-de Smidt MC, Mendham AE, Hauksson J, Hakim O, Stefanovski D, Louise Clamp L, Phiri L, Swart J, Goff LM, Micklesfield LK, Kahn SE, Olsson T, Goedecke JH. Exercise training improves whole body insulin sensitivity and disposition index but does not alter ectopic fat content in obese black South African women. **Berzelius Symposium: Obesity and Type 2 Diabetes, Umea, Sweden** *(Poster Presentation, 11-12 Sep 2019)*

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Introduction: Black African populations present with low insulin sensitivity (S_I) and hyperinsulinemia, the latter due to high insulin secretion and reduced clearance. In addition, they exhibit lower levels of central and ectopic fat, compared to their white counterparts, contradicting the known correlates of S_I typically reported in white populations. Moreover, in black African women hyperinsulinemia is in excess of the level needed to compensate for low S_I, with a corresponding high disposition index (DI), a marker of insulin response that accounts for the level of S_I. Further, obese, black African women have a high risk for type 2 diabetes (T2D), but the correlates of hyperinsulinemia have not been fully elucidated, especially the role of ectopic fat and body fat distribution. Exercise training is beneficial to improve S_I and DI, however, whether these effects are mediated by changes in ectopic fat in skeletal muscle, liver and pancreatic depots is unknown. Accordingly, exercise training can be used as a model to assess the correlates of hyperinsulinemia and S_I in cohorts at high risk for developing T2D, such as obese black African women. This thesis therefore aims to describe the correlates of hyperinsulinemia and S_I and to evaluate the effect of exercise training on these components with emphasis on the role of body fat distribution and ectopic fat in mediating these changes.

Methods: Firstly**,** a cross-sectional analysis of 45 obese (BMI 30-40 kg/m^2) black South African women (age 20-35 years) without T2D was conducted. Thereafter the women were block randomized into an exercise training (n=23) or no exercise (control, n=22) group. The exercise training group participated in a 12-week combined aerobic and resistance training programme (40-60 min session, 4 days/week) supervised by a

biokineticist. Pre and post-intervention testing included assessment of acute insulin response to glucose (AIRg), S_I, DI (AIRg x S_I), insulin secretion rate (ISR), hepatic insulin extraction (HIE) and peripheral insulin clearance (CLp) (frequently sampled intravenous glucose tolerance test); body fat mass and regional adiposity (dual-energy X-ray absorptiometry); hepatic, pancreatic and skeletal muscle fat and abdominal subcutaneous (aSAT) and visceral adipose tissue (VAT) (magnetic resonance imaging); intramyocellular (IMCL) and extramyocellular fat content (EMCL) (magnetic resonance spectroscopy).

Results: The baseline results showed that a high DI was associated with low VAT (r-0.565, p<0.001), pancreatic fat, soleus IMCL and EMCL with VAT explaining most of the variance in DI (32%). S_I was inversely associated with VAT (rho -0.417, p=0.007) and AIRg was inversely and HIE was positively associated with VAT-aSAT ratio (rho -0.345, p=0.029 and rho 0.510, p=0.011, respectively). DI was positively associated with CLp (rho 0.528, p=0.006), while its components (S_I and AIRg) were not. Results from the intervention showed that exercise training increased DI (median (interquartile range): 6.1 (3.6-7.1) to 6.5 (5.6-9.2) $x10^3$ arbitrary units, p=0.028), S_I (2.0 (1.2-2.8) to 2.2 (1.5-3.7) $(mU/l)^{-1}min^{-1}$, p=0.005) and VO_{2peak} (mean ± standard deviation: 24.9±2.42 to 27.6±3.39 ml/kg/min, p<0.001), with no changes in control group. Exercise training decreased body weight (84.1±8.7 to 83.3±.9.7 kg, p=0.038) and gynoid fat mass (18.5±1.7 to 18.2±1.6%, p<0.001). AIRg, ISR, HIE, CLp, aSAT, VAT and ectopic fat were unchanged after exercise training. However, the control group increased body weight and aSAT. The increase in S_I and DI were not associated with changes in body composition, body fat distribution or ectopic fat.

Conclusion: Novel results from our cross-sectional analysis showed that, in obese black South African women, DI was positively associated with peripheral insulin

clearance, probably due to higher S_I of peripheral tissue. Moreover, the most important correlate of a high DI was low VAT independent of ectopic fat accumulation in other sites. Further, we showed that low AIRg and high HIE correlated with a high VAT-aSAT ratio, while low S_I was associated with high VAT. These associations require further exploration to determine direction of causality. Findings from our exercise intervention study extend on previous research by showing that moderate-to-high intensity combined aerobic and resistance exercise training increased S_I and improved cardiovascular fitness, but insulin secretion, hepatic insulin clearance, ectopic and central fat depots did not change. Our results suggest that hyperinsulinemia may not occur solely as a compensatory mechanism for low S_I and that ectopic and central fat might not be the primary correlates of insulin resistance in this cohort. Rather, intrinsic factors within muscle and adipose tissue may be putative mediators for observed improvements in the metabolic outcomes but will require further elucidation. Further research is required to confirm the causal role of VAT on low DI and to determine whether a long-term exercise training program and/or a low carbohydrate/glycemic index diet will reduce AIRg in those with hyperinsulinemia.

CHAPTER 1: LITERATURE REVIEW

1.1 EPIDEMIOLOGY OF TYPE 2 DIABETES AND OBESITY – THE SOUTH AFRICAN CONTEXT

The prevalence of type 2 diabetes (T2D) is increasing worldwide with trends in Africa surpassing other world regions (1). The global prevalence of T2D in adults has increased from 4.7% in 1980 to 8.5% in 2014 (2). Furthermore, the International Diabetes Federation (IDF) predicts that the number of people living with diabetes aged 20-79 years will increase from 463 million in 2019 to 700 million in 2045 (1). Globally, sub-Saharan Africa has the highest projected rate of increase in T2D over the next 26 years, increasing by 143% from 19 million in 2019 to 47 million by 2045 (1). In addition, T2D is a significant contributor to morbidity and mortality worldwide with T2D associated morbidity and mortality rates more prevalent in populations of African descent compared to white populations (3,4). The increased prevalence of T2D coincided with an increase in the global prevalence of overweight and obesity of 27.5% for adults between 1980 and 2013 (5). In Africa, mean BMI increased in both men and women between 1980 and 2014, however the increase was greater in women (6). Regional differences were also noted with Northern and Southern African women having a mean BMI higher than the global average (6). The link between BMI and prevalence of T2D is evident with a strong positive association found in both sexes in 1980 and 2014 (correlation coefficient ~0.9) (6).

The IDF estimated that in 2019 approximately 4.6 million people were living with diabetes in South Africa (SA), with a further 2.4 million people undiagnosed (1). Furthermore, black African urban populations seemed to be more affected as the

diabetes prevalence increased from 8% in 1990 to 12.2% in 2009 (7). Concomitantly, the obesity epidemic has been increasing in SA (8) and excess weight was shown to contribute to 86.9% of T2D cases (9). Moreover, BMI and hyperglycemia are the second and third most important contributors to mortality and morbidity in SA, after unsafe sex (6). Notably, women are disproportionately affected, with an overweight and obesity prevalence of 68% compared to 31% in men in 2016 (10), with SA women having the highest prevalence of obesity in Sub-Saharan Africa (5). Furthermore, within SA, black SA women (40.9%) have a higher prevalence of obesity, compared to white (30.6%) SA women (10). Clearly, black SA women are disproportionately affected by the obesity epidemic, and the increase in T2D prevalence in this population is concerning. Understanding the correlates of insulin sensitivity (S_I), insulin secretion and clearance, the key components in the pathogenesis of T2D, in obese, black SA women are therefore of interest.

1.2 Physiology of insulin function, secretion and clearance

Glucose is an essential energy source to human cells, but is toxic to tissue that is exposed to prolonged elevated plasma levels. Insulin, plays a key role in the regulation of plasma glucose levels within a narrow range by ensuring the uptake and safe storage of glucose within muscle (11). Processes involved in the regulation of plasma insulin levels such as the secretion, action and clearance of insulin are therefore integral in the maintaince of glucose homeostasis.

1.2.1 INSULIN FUNCTION

Although insulin exerts its effect on many body tissues (12), this review will focus on the effects on muscle, liver and adipose tissue. Essentially, within the muscle, insulin promotes the uptake of plasma glucose and the net synthesis of glycogen. In the liver, insulin suppresses endogenous glucose production (EGP) and stimulates glycogen synthesis, while increasing lipogenesis. In the adipose tissue, insulin inhibits lipolysis and favours the uptake of plasma free fatty acids and the synthesis of triglycerides (TGs) (13). The cellular pathways that culminate in these insulin effects are initiated when insulin binds to its receptor. Although the downstream insulin effects may differ depending on the tissue, the initial insulin signalling pathway are similar between tissues. Firstly, autophosporylation of the insulin receptor β-subunit leads to tyrosine phosphorylation of the insulin receptor substrate molecule (IRS-1 or IRS-2). This sets off a cascade of phosphorylation events that involves activation of key cellular molecules such as phospatidyl-inositol-3-kinase (PI3K), protein kinase 1 (PDK1), Akt1/2 and protein kinase C (PKC), amongst others. Akt activates AS160 and promotes the translocation of glucose transporter containing vesicles to the cell membrane. Akt also stimulates glycogen synthase which induces glycogenesis (14). Suppression of EGP in the liver is mediated by Akt, which inactivates the forkhead transcription factor (FOXO1), an important regulator of gluconeogenic genes. Moreover, insulin activates the sterol-regulatory-element-binding protein-1c (SREBP1c), which promotes transcription of lipogenic genes and promotes *de novo* lipogenesis (DNL) (13). Further, the uptake of free fatty acids into the tissue is initiated by insulin stimulating the expression of lipoprotein lipase (LPL), an important enzyme involved in the the hydrolysis of plasma

TGs (15). Further, insulin inhibits hormone sensitive lipase (HSL), the rate-limiting enzyme involved in lipolysis (13) and thus promotes storage of TGs.

1.2.2 INSULIN SECRETION

The pancreas is a retroperitoneal organ that has both exocrine and endocrine functions. The islets of Langerhans, dispersed amongst the exocrine cells, contribute ~4.5% to the overall pancreatic volume (16). Insulin is synthesized, processed, packaged and secreted from the β-cells, which is the most abundant cell within the islets (16). This thesis will now provide a summary of the processes involved in insulin secretion, which has been published before in a detailed review by Tokarz *et al*. (17).

The synthesis of insulin involves transcription and then translation into pre-proinsulin peptide by the ribosomes on the endoplasmic reticulum. The endoplasmic reticulum is also where the peptide is cleaved into pro-insulin and folded. After pro-insulin has been stabilized into a 3D-form it is transported to the Golgi-apparatus where it is packaged into secretory vesicles. Inside the vesicles, pro-insulin is cleaved into insulin and C-peptide. Insulin secretion occurs from two different pools. The first phase insulin secretion reflects readily available insulin already present in storage vesicles within the β-cells, whereas the second phase insulin secretion reflects newly synthesized insulin. Insulin secretion is triggered by various stimuli of which glucose is the main stimulus. The cascade of events starts with glucose entering the β-cells through glucose transporter (GLUT1) where it is immediately phosphorylated to glucose-6-phosphate by glucose kinase. This reaction produces ATP which gives rise to an increase in the ATP:ADP ratio within the cell and results in the closure of the K^+-ATP dependent channel. Depolarization follows and the Ca^{2+} gated channels open. An influx of Ca^{2+} into the cell triggers the release of insulin and C-peptide in equimolar quantities via

exocytosis. This K^+-ATP dependent pathway is not the sole pathway responsible for β-cell insulin secretion. Other factors that influence β-cell insulin secretion are free fatty acids, amino acids, neuronal factors (cholinergic stimulation increases insulin secretion and adrenergic stimulation via catecholamines reduces insulin secretion), hormonal factors (somatostatin inhibits insulin secretion, while incretins derived from the gut stimulate insulin secretion), and adipokines (leptin can increase insulin secretion) (17).

1.2.3 Insulin clearance

The liver is the main organ involved in insulin clearance and clears approximately 50-70% of insulin during first pass through the liver (18). Insulin is also cleared by peripheral tissue such as the kidneys, muscle and adipose tissue, amongst others. However, the kidneys are the most important site, clearing over 80% of extrasplanchnic insulin (19), followed by the muscle (20). After binding of insulin to its receptor, CEACAM1, a transmembrane protein, is activated by phosphorylation and subsequently initiates the internalization of the insulin-receptor complex by endocytosis (17). Inside the endosome the insulin degrading enzyme (IDE) degrades insulin bound to its receptor into smaller particles. Next, these smaller particles are delivered to lysosomes where further degradation occurs via proteolysis, after which the insulin receptor is recycled back to the cell membrane (17).

1.3 Overview of the pathogenesis of Type 2 Diabetes

Our understanding of the pathophysiology of T2D has certainly improved since the 19th century when Claude Bernhard identified the role of the liver in gluconeogenesis and Joseph von Mering and Oscar Minkowski first discovered the central role of the

pancreas in the development of diabetes (21). Further, the advancement of molecular science and the conduct of longitudinal studies in populations at risk aided our understanding of the causal factors involved in the development of T2D, which are multifactorial. T2D is characterized by high fasting glucose or impaired glucose tolerance (IGT) and involves two key factors, a reduced sensitivity of tissue to insulin (insulin resistance) and pancreatic β-cell dysfunction (22).

Insulin resistance occurs when a normal amount of insulin produces a subnormal biological response (23). However, the effects can differ by organ. Insulin resistance in the muscle is characterized by the reduced uptake of glucose and glycogenesis (24). Hepatic insulin resistance involves reduced suppression of EGP, however the effect of insulin on DNL remains intact (25). Adipose tissue insulin resistance is defined by reduced suppression of lipolysis by insulin, resulting in elevated levels of plasma free fatty acids, which may be redistributed to ectopic sites (26). Considerable strides have been made to unravel the molecular mechanisms involved in reduced insulin action with defects both at the receptor level and in the insulin signalling pathway being implicated (13). An increase in BMI parallels the presence of insulin resistance and different theories (lipotoxicicity, inflammation, adipokines) have emerged to explain excess accumulation of fat as a cause of insulin resistance (27–29). However, these mechanisms may not act in isolation. Therefore a paradigm that considers the interconnectedness of these various theories, together with intrinsic cellular mechanisms such as mitochondrial dysfunction, endoplasmic reticulum and oxidative stress (30) may all contribute towards the underlying pathogenesis of insulin resistance. That being said, such a paradigm is complex and to investigate all these components at once is not always feasible. Nevertheless, the adipose tissue expandibility hypothesis suggests a finite ability of adipocytes to store fat (31). This may explain insulin

resistance in the midst of obesity by taking the differential association between regional fat depots and S_I into account while providing a reason for ectopic fat accumulation and its role in hepatic and peripheral insulin resistance (32).

The ability of the pancreatic islet β-cells to secrete insulin is critical in delaying the onset of T2D. Even when insulin resistance is present, the pancreatic β-cells and the liver regulate insulin secretion and clearance, respectively, to ensure higher plasma levels of insulin to maintain normoglycemia (33). Notably, a failure to ensure an adequate insulin response for the level of S_I, will result in deterioration of glucose tolerance and progression towards T2D (22). Indeed, longitudinal studies in Pima Indians, whom have a high risk for T2D (34), have illuminated the key role of insulin secretory dysfunction, together with low S_I in the progression to T2D (35,36). Subsequently, another study in Pima Indians evaluated whether the contribution of insulin response and S_I to the progression of T2D differed depending on the severity of IGT (36). They demonstrated that during the progression from normal glucose tolerance to IGT and from IGT to T2D, both a low insulin response and low S_I were additive predictors in the progression of worsening glucose tolerance. Further, the role of insulin response in the progression of T2D was found to be similar across different ethnicities (African Americans, Hispanics and non-Hispanic Whites) (37). This longitudinal study reported that while a reduction was found in S_I, regardless of the glucose tolerant status at follow-up, the direction and magnitude of change in the insulin response determined the glucose tolerance status after a follow-up of 5.2 years (37). Further, the insulin response is dependent on both insulin secretion and clearance. Based on a longitudinal study in healthy African American offsprings of T2D patients, it appears that the deterioration in insulin secretion contributes more to the reduced insulin response and subsequently increased risk for T2D compared to the reduction in hepatic insulin clearance (38). While the course of

T2D may be similar across ethnicities, the underlying mechanisms of low S_I and the ability to increase the acute insulin response may still differ amongst ethnic groups.

1.4 ETHNIC DIFFERENCES IN INSULIN SENSITIVITY

Lower S_I is frequently found in black Africans compared to white populations. Indeed, South African ethnic-comparison studies (Table 1.1) showed that obese pre-menopausal black women had lower S_I compared to white women without T2D, measured using a frequently sampled intravenous glucose tolerance test (FSIGT) (39), and a hyperinsulinemic-euglycemic clamp (HIEG) (40). This finding was also apparent in normal-weight women (39,41). Nevertheless, two South African studies using the HIEG (42) and the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) index (43) found no differences in S_I between white and black women that were also obese, pre-menopausal and without T2D. Whether differences in quantifying S_I (fasting vs stimulated or steady state vs dynamic conditions) or whether adjusting for the fat mass index (42,43) or not (39,40) may account for these discrepant findings are not clear.

Further afield, ethnic-comparison studies conducted in the United States of America (USA) and the United Kingdom (UK) included participants over a wider age range (children to post-menopausal women), and level of glucose tolerance (Table 1.2). Amongst those without T2D, a lower S_I was found in African American adults compared to their age and BMI matched white counterparts. This finding was apparent regardless of BMI (44–47) or age (45,48–50). However, amongst those with T2D, no significant ethnic differences in S_I have been reported (51–53). In children, the influence of

lifestyle/environmental factors on S_I are minimized and therefore observed differences could be attributed to genetics or epigenetics. Notably, some studies showed that black African American children had lower S_I compared to white children even prior to the onset of obesity (45,54–56) (Table 1.3), while other studies in both children and adults found no ethnic difference in S_I (42,43,49,53,57–62). Again, methodological differences in quantifying S_I may contribute towards these heterogenous findings. A lower S_I found in black South African and African American populations without T2D might reflect differences in the underlying pathogenesis of T2D compared to white populations, but when T2D develops these differences are no longer apparent. We therefore need to explore the correlates of low S_I in black African populations and whether a greater sensitivity to these correlates exist.

1.5 ETHNIC DIFFERENCES IN HYPERINSULINAEMIA

Ethnic differences have been observed in the insulin response to glucose, with hyperinsulinemia frequently found in black African populations (39,44–49,52,54,61). Tables 1.1, 1.2 and 1.3 summarizes ethnic differences in stimulated plasma insulin levels.

Earlier studies emanating from South Africa, reported that compared to white adults, black men (63) and women (64) had a decrease in stimulated insulin response after an oral glucose challenge and lower C-peptide levels during the euglycemic period of a HIEG (40). However, these studies are conflicted with small sample sizes and one study used lean men that were manual labourers (63) and therefore external validity might be compromised. A study conducted in pre-menopausal women over a range of BMI found that black SA women had a greater acute insulin response to an intravenous glucose

load (AIRg) compared to their white counterparts (39). These findings were replicated in studies in African Americans. In fact, a meta-analysis that included 74 cohorts of which 19 included black African cohorts, including 1 South African cohort, found a greater AIRg in black African, compared to white and Asian individuals (997 ±36.2 vs 396 ±7.5 vs 265 ±13.7 pmol/L, respectively), but this was restricted to normal weight individuals with normal glucose tolerance (52). However, a more recent report corroborated these results in overweight/obese women over a range of glucose tolerance, but no T2D (49). Chung *et al*, reported that compared to their white counteparts, pre- and post-menopausal African American women had an elevated insulin response to oral and intravenous glucose stimulation, as well as to a mixed meal test, which represents more physiological conditions. These findings have also been confirmed in healthy, normal weight children, with African children demonstrating an elevated AIRg compared to other ethnicities (45,56,58). The reason for this ethnic disparity in stimulated insulin response needs to be further explored.

1.6 Reasons for hyperinsulinemia in black African populations

1.6.1 Low insulin sensitivity

Majority of the studies that showed an elevation in stimulated insulin response in black African adults also reported a reduced S_I (39,45–48,52,54). Based on the hyperbolic relationship between AIRg and S_I, a negative feedback loop dictates that a change in one variable results in a reciprocal and proportional change in the other variable, to ensure euglycemia. Indeed, in those with normal glucose tolerance, an elevation in AIRg could be explained by a reduced S_I (48). Haffner *et al*. suggested that a reduced S_I,

independent of age, sex, BMI, waist-to hip ratio, physical activity and diet, could explain the higher fasting and early phase insulin response, in black Africans, as a compensatory mechanism to maintain euglycemia (48). However, it is also possible that hyperinsulinemia may precede the low S_I found in black African populations.

1.6.2 LOW INSULIN CLEARANCE

Plasma insulin levels reflect the balance between insulin secretion and insulin clearance. Further, different methods, which will be discussed in detail in the method development chapter, are used to quantify insulin clearance. Some studies do not distinguish between hepatic and peripheral insulin clearance and reports the total insulin clearance, while others distinguish between hepatic and peripheral insulin clearance. Studies that evaluated insulin clearance in black African adults, using different methodologies, demonstrated a reduced clearance compared to white populations (Table 1.2) (44,49,50,59), with similar findings observed in children (56,58,65). While some studies only reported a reduced insulin clearance as the main contributor to a high insulin response (44,66), others showed that both reduced insulin clearance and high insulin secretion were contributors to hyperinsulinaemia (49,56). Further, a longitudinal study in dogs emphasized that both these factors are important to maintain high insulin responses but they do not occur simultaneously (67). Dogs were fed a high fat diet over 12 weeks and as S_I deteriorated over the first 6 weeks, insulin secretion increased by 60%. However, from week 6 to 12, eventhough S_I did not deteriorate further, the high level of insulin secretion could not be sustained. Rather, plasma insulin levels remained stable due to a reduction in hepatic insulin extraction. This suggests that a reduction in hepatic insulin extraction is a mechanism to alleviate the burden on the pancreatic β-cells, and may therefore protect against the development of T2D. On the contrary, a longitudinal study in African Americans and Hispanics found that a

reduced insulin clearance predicted the onset of T2D, independent from insulin secretion, adiposity, lifestyle factors and high-density lipoprotein cholesterol (68). This paradox allows us to speculate whether a reduced insulin clearance observed in black African populations may only provide temporary respite for the β-cells and that the hyperinsulinemia that ensues may be the factor that escalates the development of T2D.

Nonetheless, the reasons for low hepatic insulin clearance in black African populations needs to be considered. Notably, a lower hepatic insulin clearance is present in African American children, compared to white children, even before the onset of obesity (58), perhaps indicating a genetic component. Hepatic fat has been shown to be a major correlate of low hepatic insulin clearance in studies conducted in white adults, without T2D (69,70), but the role in black African populations has not been well-studied. This is particularly relevant given that black Africans typically present with lower hepatic fat compared to their white counterparts (49,53). The two studies that examined the association between hepatic fat and hepatic insulin clearance in black African populations produced conflicting results, probably due to differences in study populations and methods of assessment. The first study conducted in African American women without T2D, found no association between hepatic fat and basal hepatic insulin extraction during a mixed meal tolerance test (49). In contrast, a UK study conducted in black men of West African descent with T2D found a trend for an association between hepatic fat and insulin clearance, determined in response to a hyperglycemic clamp (53). Although based on limited data, one may hypothesise that hepatic fat might not be an important factor in the low insulin clearance observed in black African adults without T2D. Further studies are required to confirm this hypothesis.

Another factor to consider is the contribution of peripheral insulin clearance to hyperinsulinemia. The few ethnic comparison studies that measured peripheral insulin

clearance found no difference between African Americans and their white or Hispanic counterparts (58,59). Nevertheless, the proportion of total insulin cleared peripherally was greater in African American women compared to white women (59). Moreover, in black African men, peripheral insulin clearance was associated with glucose tolerance status, such that those with IGT had reduced peripheral insulin clearance compared to those with normal glucose tolerance (71). Based on these findings, the role of peripheral insulin clearance in the pathogenesis of T2D is of interest but it is a component that has been largely understudied. Further, although it has been shown that peripheral insulin clearance is associated with higher S_I in black African men (71), the association with AIRg is not clear and also no previous studies have evaluated the relationship with β-cell function.

1.6.3 HIGH INSULIN SECRETION

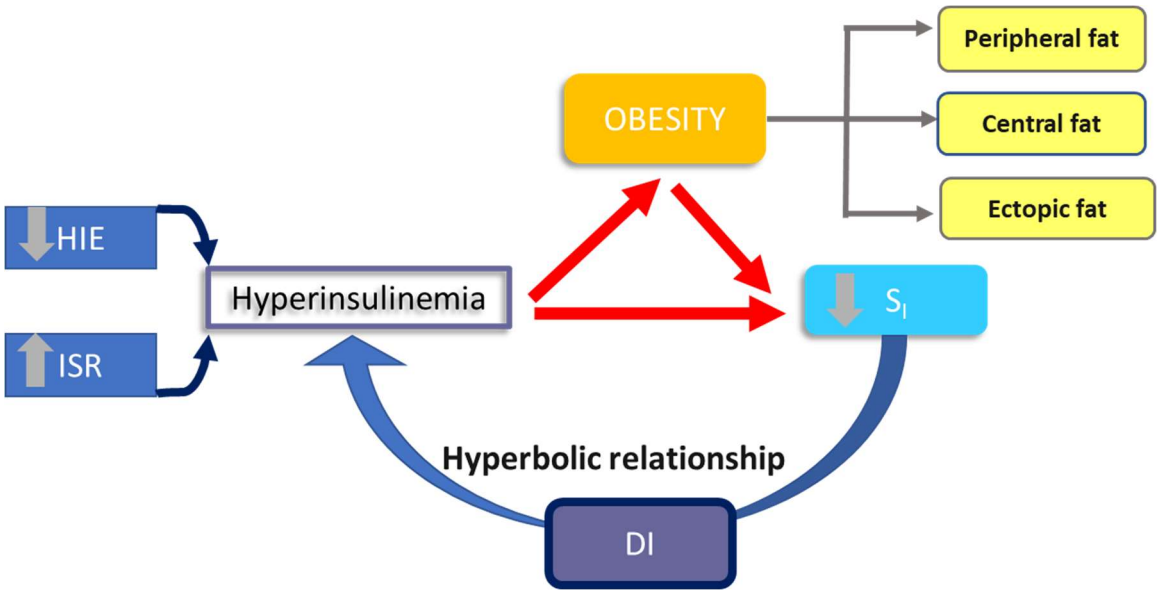
Black African premenopausal women without T2D, have both a greater first phase β-cell sensitivity and insulin secretion rate (ISR) compared to white populations, but no ethnic differences are found in the second phase response (49,54). This indicates that in black African populations, the pancreatic β-cell might have a greater capacity to store insulin compared to white populations. Moreover, some studies have demonstrated that the high stimulated insulin response found in black African populations is excessive for the level of S_I (47,56). The reasons for this are not clear. Whether, the unique phenotype found in black African women of less VAT and ectopic fat deposition and more peripheral fat may explain this high insulin secretory capacity has not been fully elucidated.

1.7 IMPORTANCE OF HYPERINSULINEMIA

Hyperinsulinemia, referring to an elevated insulin response due to elevated insulin secretion and/or reduced insulin clearance, may compensate for the low S_I observed in black African populations, however, it could also precede low S_I. Moreover, because obesity can be associated with both hyperinsulinemia (72) and reduced S_I (30,73), it may confound these associations. In addition, lifestyle factors may also influence these two factors; accordingly, the study of children provides a good model to evaluate whether hyperinsulinemia precedes low S_I, independent of obesity and other lifestyle factors. Compared to white children, normal-weight black African children demonstrated a high AIRg and first phase insulin secretion, despite similar S_I (58). A similar finding was reported in older children, however obesity was already present (57). Accordingly, these findings suggest that hyperinsulinemia may be the primary event that may cause low S_I in black African children.

Indeed, an *in-vitro* study showed that insulin receptors in isolated cells develop diminished autophosphorylation, secondary to hyperinsulinemia (74). Furthermore, hyperinsulinemia may also induce obesity. Insulin is an anabolic hormone that stimulates the uptake of free fatty acids and glucose into adipose tissue depots to stimulate TGs synthesis and DNL, respectively (12). During DNL, insulin stimulates acetyl-CoA carboxylase to convert acetyl CoA to malonyl CoA. Further, during TG synthesis, insulin activates diacylglycerol transferase, the enzyme responsible for conversion of diacylglycerol to TGs. Additionally, insulin inhibits lipolysis through inactivation of hormone sensitive lipase, and inhibits fatty acid oxidation, which together promote TG storage (12). Indeed, in mice models, knockout of insulin receptors in white and brown adipose tissue subsequently protected the mice from age and diet-induced

obesity (75). Further, in a longitudinal study (follow-up of a mean of 16.7 years) of normal glucose tolerant adults, hyperinsulinemia, high AIRg, defined by a FSIGT, was a risk factor for long term weight gain, especially in insulin sensitive individuals (76). Similarly in Pima Indian children that were followed up for a mean of 9.3 years, fasting hyperinsulinemia predicted weight gain independent of other factors known to influence weight gain (72). Based on this evidence it is plausible to hypothesize that in black African individuals, hyperinsulinemia may precede both obesity and low S_I. Further support for this hypothesis is found from interventional studies that suppressed insulin secretion using insulin secretion inhibitors, such as octreotide, for 20 weeks in morbidly obese adults, without T2D (77). Lower plasma insulin concentrations were associated with a concomitant weight loss. Taken together, I hypothesise that in black African populations, hyperinsulinemia may be the primary event that increases the propensity for obesity and lowered S_I, which then exacerbates hyperinsulinemia further through the hyperbolic relationship (Figure 1.1).



S_I – Insulin sensitivity, DI – Dispsosition index, HIE, Hepatic insulin extraction, ISR -Insulin secretion rate

Figure 1.1: *Proposed relationships between hyperinsulinemia, insulin sensitivity and obesity in Black African populations*

***Table 1.1**: South African studies: Exploring differences in insulin sensitivity and response in black and white adults*

Study	Health Status	Sample size, (sex)	Participant characteristics Age (y) BMI (kg/m^2)	Tests/ Variables measured	S_I	Insulin response/ Insulin secretion	HIE	β-cell function
Goedecke, 2009 (39)	Non-T2D	*NW*: B: 13 (F), W: 14 (F) *OB:* B: 16 (F) W: 14 (F)	*NW:* B:24±2; 23.4±1.0 W:26±2; 22.3±0.9 *OB* B: 28±1; 38±0.9 W: 31±2; 35±0.9	FSIGT: S_I x10^{-5} (min-^{1}/(pmol/l) AIRg – pmol DI - x10^{-5} OGTT: Ins/Glc0-30min	*NW:* **B<W** **B: 2.65±0.44** W:6.07±0.47 *OB:* **B<W** **B: 1.89±0.45** W: 3.78±0.61	AIRg *NW:* ***B>W*** B: **171.3 ±42.5** W: 58.6**±41.0** *OB:* ***B>W*** B:**328.0 ±38.2** W: 78.2 ±41.0 Ins/Glc0-30 *OB:* ***B>W*** B: **507 ±65** W: 248 ±65 fIns *(OB)* ***B>W*** B: **90.6 ±7.2** W: 52.5 ±7.7	ND	FSIGT -DI: *NW*: B=W *OB*: B=W
Goedecke, 2015 (42)	Non-T2D	B:15 (F) W:15 (F)	OB: B: 36±5; 37.9±5.1 W: 36±4; 35.2±3.5	2-step HIEG OGTT Periph S_I -M/l Hep S_I- %suppression EGP	Periph S_I – low dose B=W Periph S_I – high dose B=W Hep S_I: **B>W** **B: 56, W: 17**	fIns **B>W** **B:90.7±39.1** W:64.4±15.4	ND	ND

Keswell, 2016 (43)	Non-T2D	B: 288 (F) W:197 (F)	B:**22(22-23); 31.7(19.4-44.3)** W:32(24-39); 26.6 (22.4-33.2)	HOMA-IR	B=W (Adjusted age, FMI)	fIns: B=W (Adjusted age, FM)I	ND	ND
Rubenstein, 1969 (63)	Non-T2D	B: 7 (M) W:8 (M)	B: 30±6; 21.5 W:27±7; 23.2	OGTT: Ins: µU/ml Ins AUC: µU/min IUCL: (ml/min)	Glc AUC: B=W	30min Ins: **B<W** **B:61** W:115 Ins AUC: **B<W** B**: 7980**, W:11100 IUCL: **B<W** **B:0.22±0.06** W:0.34±0.09 fIns: B=W	ND	ND
Shires, 1985 (64)	Non-T2D	B: 10 (F) W: 8 (F)	OB	OGTT(tolbutamide and glucagon) HIE: Cpep AUC/Ins AUC	ND	**Ins: B<W** **C-pep: B<W**	B=W	ND
Van der Merwe, 2000 (40)	Non-T2D	B:10 (F) W: 10 (F)	OB: B:34.8±0.8; 36±0.3 W:34.3±0.8; 34.4±0.3	HIEG: Periph S_I M/I - mmol/kg.min^{-1}/pmol/l x100	Periph S_I: **B<W** B**: 0.12±0.01** W: 0.24±0.02	2h Ins: B=W fIns: **B<W** **B: 87±12** W:155±9	ND	ND

Summary measures expressed as mean±Standard deviation, T2D – Type 2 Diabetes, B - Black, W - White, F – Female, M – Male, NW – normal weight, OB – Obese, BMI – body mass index, FSIGT – Frequently sampled intravenous glucose tolerance test, HIEG – hyperinsulinemic euglycemic clamp, OGTT – Oral glucose tolerance test, IV – Intravenous, HOMA-IR – Homeostatic Model Assessment of Insulin Resistance, S_I, insulin sensitivity, AIRg – acute insulin response to glucose, EGP – Endogenous glucose production, fIns – Fasting insulin, M – glucose disposal rate, AUC – Area-under-curve, Ins – insulin concentration, Glc – Glucose, C-pep – C-peptide concentration, HIE – hepatic insulin extraction, IUCL – Insulin urinary clearance, ND – Not done; FMI – Fat mass index

Table 1.2: *USA and UK studies: Exploring differences in insulin sensitivity and response in black and white adults*

Study	Health Status	Sample size, (sex)	Participant characteristics Age (y); BMI (kg/m^2)	Tests/ Variables measured	S_I	Insulin response	Insulin clearance	S_I/Insulin response
Chandler-Laney, 2010 (54)	Non-T2D	B: 25 (F), W: 32 (F)	*Pre-Menopausal* B:25±3;%BF 36 ±11 W:26±3; %BF 37 ±9	FSIGT S_I B-cell sensitivity$_{glc}$ (Φ) DI = S_I X Φtot	S_I **B<W** B: **3.5±3.6** W:4.9±2.7	1st Phase ϕ **B>W** B: **345±202** W: 213±111 2nd Phase ϕ B=W B: 10.0±5.4 W:11.5±4.5	ND	DI: B=W
Chandler-Laney, 2010 (54)	Non-T2D	B: 18 (F), W: 31 (F)	*Post-Menopausal* B:55.5±6.3, W:44.6±7.3 W:55.7±4.2; 38.3±8.7		S_I **B<W** B: **2.81±2.02** W:5.79±5.30	1st Phase ϕ **B>W** B: **241±103** W: 203±134 2nd Phase ϕ B=W B:11.4±5.4 W: 11.0±4.6	ND	DI: B=W
Chow, 2011 (47)	Non-T2D	B: 17 (F) W:17 (F)	36±10; 30.0±6.7	FSIGT S_I AIRg	S_I: **B<W B:3.7±1.6** W: 5.23±2.7	AIRg **B>W B: 642±379** W:263±206 fIns: B=W	ND	DI: **B>W B:2203±1442** W: 1108±660 AIRg higher at every level of S_I
Chung, 2018 (49)	Non-T2D (NGT/IGT)	*Pre-Menopausal:* B: 52 (F), W: 35 (F) *Post-Menopausal*: B: 19 (F),	*Pre-Menopausal:* B:39±7; 30.7±6.0 W: 38±7; 29.9±5.0 *Post-Menopausal*: B: 53±4; 30.1±4.6 W: 57±4; 27.7±4.4	OGTT: IGI FSIGT: S_I AIRg HIE: (Polidori2016) ISR MMT:	S_I: *Pre-Menopausal:* B=W B:2.3 (1.6-3.2) W:2.8 (1.8-4.4) *p=0.06 (trend)*	AIRg/IGI/MMT: *Both groups* **B >W** 1st Phase ϕ *Both groups* **B>W** 2nd Phase ϕ	Basal/ Total HIE: *Whole cohort* **B<W** (11%/9% lower)	DI: *Pre-Menopausal:* **B>W B:1867** W:1481 *Post-Menopausal: B=W*

		W: 13 (F)		Ins AUC/Glc AUC0-30 HIE: Cpep AUC/Ins AUC0-30 ISR ß-cell sensitivity$_{glc}$	*Post-Menopausal:* **B<W** **B:2.4(1.7-3.0)** W:4.5(2.4-3.0)	*Pre-Menopausal*: not B=W *Post-Menopausal:* **B>W** 1st Phase ISR: *Both groups* **B>W**	MMT -IC: *Whole cohort* **B<W** (20% lower)	
Ellis, 2013 (46)	Non-T2D	B: 23 (F), W: 30 (F)	B:24.3±4.1; 26.6±5.8 W:26.0±3.5; 25.2±4.3	FSIGT (glc tracers) Perip S_I, Hep S_I AIRg	Perip S_I: **B<W** **B: 7.46±3.8** W: 10.5±4.5 Hep S_I: **B<W** **B: 3.42±1.34** W: 4.46±1.71	AIRg **B>W** **B: 1178.0±799.8** W: 481.5±384.3 (adj %Fat) fIns: **B>W** **B: 12.2 ±4.3** W:10.3±4.6	ND	ND§ DI: B=W B: 8788 W:5056
Goree, 2010 (45)	Non-T2D	B: 27 (F) W:33 (F)	*Pre-menopausal:* B: 24.8±3.3; 27.6±6.2 W: 25.7±3.5; 26.1±6.0	FSIGT S_I AIRg	S_I **B<W** **B: 3.63±3.6** W:4.7±2.7	AIRg **B>W** **B: 1089±713** W:493±375 fIns: **B>W** **B: 11.9±3.7** W: 10.6±4.6	ND	DI **B>W** **B:3051±1883** W:2015±1340
Goree, 2010 (45)	Non-T2D	B: 15 (F) W: 31 (F)	*Post-menopausal:* B:56.6±5.1; 30.5±5.5 W:55.7±4.2; 26.2±5.1	FSIGT S_I AIRg	S_I **B<W** **B:3.3±2.** W:5.0±3.8	AIRg **B>W** **B: 933±536** W:443±341 (AIRg adj for S_I) fIns: B=W B: 12.6±4.9 W:12.2±7.2	ND	DI **B>W** **B:2573±4253** W:1768±1716
Haffner, 1996 (78)	Non-T2D (NGT/IGT)	B:122 (M), 166 (F) W:115 (M), 114 (F)	By center LA: B: 54.2±0.7; 29.2±0 W: 54.7±0.8; 27.9±0.6 OL:	FSIGT S_I AIR–pmol.ml$^{-1.}$ min^{-1} fIns – pmol/L	S_I: **B<W** **B:1.31±0.13** W:1.69±0.19	AIR **B>W** **B:358±19** W:217±22	ND	ND

			B: 54.6±0.7;29.5±0.5 W: 56.1±0.7; 27.4±0.5			(adj for age, sex, clinic, BMI, WHR, PA, diet) 2h-Ins: **B>W** **B:45.1±28.8** W:35.2±25.0 fIns: **B>W** **B:81.6±3.2** W:65.4±2.9		
Hakim, 2019 (53)	T2D	B: 18, (M) W: 19 (M)	B:54.9 **±9.3; 29.8±3.5** **W:58.5±6.3; 31.5 ***	2-step HIEG - glc tracers Periph S_I (M) Hep S_I: % suppression EGP	Periph S_I: B=W Hep S_I: B=W	ND	IC: B=W	ND
Ingram, 2010 (60)	Non-T2D	B: 14 (M), 29 (F) W:14 (M),29 (F)	B:37.6±10, **31.8±5.2*** W:39±11, 29.3±5.8	HIEG	Periph S_I B=W	ND	ND	ND
Kodoma, 2013 (52)	Non-T2D, T2D	*NGT:* B: 688 W: 2177 A: 205 *IGT:* B: 68 W: 224 A: 196 *T2D* B: 45 W: 53 A: 157 *(M+F but split not reported)*	*NGT (excluded OB & GDM, Fam Hx T2D)* B: 25.9 ±3.6; 24.7±1.0 W:31.2±2.7; 24.6±0.7 A:32.6±2.9; 21.5±0.4 *IGT* B: 48±4.6; 35.6±3.5 W: 55.4±2.3;29.8±0.8 A: 43.7±3.6; 25.2±1.3 *T2D* B:49.4±4.5; 34±2.3 W:57.8±4.9; 35.1±5.8 A:47.6±2.9; 24.9±1.6	FSIGT S_I $x10^{-5}min^1/(pmol/L)$ AIRg – pmol/L	S_I: *NGT: B<W/A* **B: 4.4±0.1** W:7.1±0.1 A:11.9±0.5 *IGT: B<A* **B:2.1±0.2** W: 3.0±0.1 A:3.6±0.2 *T2D: B=W<A* B:0.76±0.11 W: 0.73±0.12 A: 2.6±0.20	AIRg *NGT:* **B>W/A** **B: 997±36.2** W:396±7.5 A:265±0.13.7 *IGT: B=W/A* B:365±35 W: 285±21.4 A:270±17.4 *T2D: B=W>A* B:120±19.9 W: 120±21.3 A: 36±5.5	ND	DI ND§ *NGT: B=W=A* *IGT: B=W=A* *T2D: B=W=A*
Lingvay, 2014 (51)	Non-T2D, T2D	B: 16 (F) W:26 (F) H:26 (F)	*NW Non-T2D:* B: 35±10; 24.2±0.7 W/H: 37±4; 21.1±0.8 *OB Non-T2D:* B:39; 37.5±2.5	FSIGT S_I AIRg DI	*NW Non-T2D:* *B=W/H* B: 4.2±0.4 W/H: 8.3±1.1 *OB Non-T2D:*	AIRg: *NW Non-T2D:* B=W B: 161 W/H:204	ND	DI: *NW Non-T2D:* B=W B:652±249 W/H:1312±265

			W/H: 42.2; 33.5±2.5 *T2D:* B:43±5; 38.8±2.3 W/H:53±2; 34.4±1.1		**B<W/H** **B: 1.9±0.4** W/H: 3.3±0.3 *T2D:* B=W/H B: 1.2±0.3 W/H:1.9±0.6	*OB Non-2D:* **B>W** **B:1353** W/H:367 *T2D* B=W B:613 W/H:98		*OB Non-T2D:* **B>W** **B: 2057±367** W/H: 1088±116 *T2D:* B=W B: 321±134 W/H: 125±55
Osei, 1994 (44)	Non-T2D	B:14 (M), 18 (F) W:15(M),15 (F)	BF:27.8±3.3; 24.2±0.9 WF: 27.9±1.9; 23.4±0.5	FSIGT-Tolbutamide S_I: OGTT HIE: Cpep AUC/Ins AUC	S_I: **B<W** B:**4.53±0.46** W:7.17±0.88	fIns **B>W** **BF:7.68±0.8** WF:5.88±0.59 fC-pep B=W	Basal HIE: **B<W** **B: 6.98±0.5** W:10.4±1.3 Postprandial HIE: **B<W** **B: 4.33±0.53** W:7.81±0.83	ND
Osei, 1997 (66)	Non-T2D	AA:16 (M), 50 (F) GI:20 (M), 11 (F) NG:22 (M), 28 (F) W:14 (M), 25 (F)	3 black African groups: AA, GI, NG vs W AA: 33.3±0.9; 28.3±0.9 GI: 37.2±1.6; 29.3±0.7 NG: 34.1±1.0; 29.5±2.2 W:28.7±1.0; 28.3±1.3	OGTT: AUC HIE: Cpep AUC/Ins AUC	ND	Mean Ins AUC: **NG/GI/AA>W** (No difference btw B groups) Mean Cpep AUC: NG=GI=AA=W fIns: **NG>GI/AA/W** 21.2*/8.2/11.9/7 fC-pep: NG=GI=AA=W	Basal HIE: **NG/GI/AA< W*** 4.5/6.6/6.43 vs 9.54 Postprandial HIE: **52/21/36% < W***	ND
Piccinini, 2017 (59)	Non-T2D	B: 18 (F) W: 29 (F)	B: 25 ±4; 27 ±6 W: 26 ±4; 25 ±4	FSIGT S_I AIRg ISR HIE (Polidori, 2016) CLp (Polidori, 2016)	S_I: B=W B: 4±4 W:5±3	AIRg B: 1158 ±755 W: 492±386 *(significance not reported)* Ins AUC $_{0\text{-}20min}$ **B>W** B: **215±134** W:102 ±72	HIE **B<W** B:**17±15 %** W: 44±21% CLp B=W	ND§ B: 4632 W: 2460

						Ins $AUC_{20\text{-}60min}$ B=W FIns B=W		
Ryan, 2002 (50)	Non-T2D	B: 9 (F) W: 26 (F)	B:55±2; 35±1.1 W:60±1; 32±0.9	HIEG:	M: **B<W** **B:35.6±3.8** W:56.9±3.2 M/I: **B<W** **B: 0.07±0.010** W:0.12±0.008	ND	MCR: **B<W** **B:432±29** W:512±11	ND
Smith, 2010 (61)	Non-T2D	B: 34 (F) W: 83 (F)	B: 39±1.9, 31.4±0.8; W:44±0.9, 32.0 ±0.5	FSIGT S_I AIRg	S_I: B=W	AIRg: **B>W** **B:567±75** W:422±36	ND	ND§ B:1633 W:1194

Summary measures expressed as mean±standard deviation or median (interquartile range), T2D – Type 2 Diabetes, B: Black Africans living in USA, UK, unless otherwise specified such as in Osei1997: AA – African Americans, GI – Ghanaian immigrants, NG- Native Ghanaians; H – Hispanics, A – Asians, W – White, M – Male, F-Females, BMI – body mass index, %BF – percent body fat, S_I – Insulin sensitivity in $x10^{-4}$ min^{-1}/µU/mL, unless otherwise specified, AIRg – Acute insulin response to glucose in $mU/L^{-1}.min^{-1}$, unless otherwise specified, IGI – Insulinogenic Index, AUC – Area-under-curve, fIns – fasting insulin in µU/mL, unless otherwise specified, fC-pep – Fasting C-peptide, HIE – Hepatic insulin extraction, MCR – Metabolic insulin clearance rate; § - DI – Disposition Index, was not mentioned in paper and thus no statistical test was done, but it was calculated by author of thesis (AIRg x SI)

Table 1.3: *USA studies: Exploring differences in insulin sensitivity and response in black and white children*

Study	Health Status	Sample size, (sex)	Participant characteristics Age (y); BMI (kg/m^2)	Tests/ Variables measured	S_I	Insulin response/ Insulin secretion	Insulin clearance	β-cell function
Arslanian, 1997 (62)	Non-T2D	B: 6 (M), 6 (F) W: 7 (M), 4 (F)	B:10.0± 0.2; 17.3 ±0.6 W:10.7±0.3;18.1 ±0.5	HIEG clamp S_I mg/kg min/ µU/mL ISR - µU/mL	S_I B=W	1st phase ISR **B>W** **B: 76.9±6.8** W: 52.1±6.4 2nd phase ISR B=W	ND	ND
Chandler-laney, 2010 (54)	Non-T2D	B: 33 (F) W: 29 (F)	B:9.8±1.6; %BF 24.4±1.6 W:10.3±1.5; %BF 26.0±7.7	FSIGT S_I B-cell sensitivity$_{glc}$: Φ (Toffolo, 1995) DI = S_I X Φtot	S_I **B<W** B: **3.07±1.52** W:5.71±2.11	1st Phase ϕ B**>W** B: **312±130** W: 174±82 2nd Phase ϕ B=W	ND	DI: B=W
Goran, 2001 (55)	Non-T2D	B: 25 (M), 26 (F) W:39 (M), 29 (F)	B:9.3±1.2, BF(kg) 12.1±9.4 W:10.±1.2, BF(kg) 12.7±9.1	FSIGT (tolbutamide) S_I AIRg	S_I: **B<W** **B:3.9±2.6** W:7.2±5.0	AIRg: B=W B: 1811±1320 W: 782±649 fIns: B=W B: 14.6±8.2 W:13.8±8.3		DI: **B>W** **B: 5756±3710** W: 3793±2222

Goran, 2002 (65)	Non-T2D	B: 15 W:14 H:28 (M/F)	B: 10.1±1.5; 21.5±4.7 W: 10.8 ±1.9; 20.7±5 H: 10.0±1.9; 24±5.8	FSIGT (tolbutamide) S_I AIRg ISR -nmol/min HIE (Combined model)	<u>S_I:</u> **B<W** B: 4.1±0.6 W:6.3±0.6 H:4.5±0.5	<u>AIRg:</u> **B>W/H** **B: 1210±116** W: 747±122 H:938±85 <u>First phase ISR:</u> B=W=H <u>Second Phase ISR</u>**:** **B<W/H** **B: 200±53** W: 206±56 H:289±41	<u>HIE:</u> **B<W** **36.6±2.9** W:49.1±3.0	<u>DI:</u> B=W=H
Goree, 2010 (45)	Non-T2D	B: 34 (F), W: 14 (F)	B: 9.3±1.5; 19.3±4.1 W:9.6±1.6; 17.6±1.8	<u>FSIGT</u> S_I AIRg	<u>S_I:</u> **B<W** **B: 3.5 ±2.2** W: 5.3±1.7	<u>AIRg:</u> **B:1473±751** W :592±287 <u>Fasting Ins:</u> **B: 14.7±5.7** W:1 0.9±3.2	ND	ND
Gower, 2002 (56)	Non-T2D	B: 19 (M), 23 (F) W: 14 (M) 20 (F)	BF:11.6±1.8;23.2±7 WF:11.4±1.6;21.9±5	<u>FSIGT</u> S_I x10^{-5}min-^{1}/(pmol/L) AIRg – pmol/L x 10min B-cell sensitivity to glucose (ϕ) (Toffolo, 1995) HIE: Cpep AUC/Ins AUC	<u>S_I</u> **B<W** B: **6.12 ±3.82** W: 11.10 ±7.00	<u>AIRg</u> **B>W** **B: 11766 ±16050** W: 3516 ±2142 <u>ISR</u> **B>W** **B: 3549±1942** W: 1734±756 <u>1st Phase ϕ</u> **B>W** **B: 325±187** W: 159±98 <u>2nd Phase ϕ</u> B=W	<u>HIE</u> **B<W** **B:3.77±1.78** W:5.99±2.18	ND
Michaliszyn, 2017 (57)	Non-T2D	B: 32(M), 53(F) W:35(M),43(F)	B: 14.6±0.2; 34.1±0.6 W:14.8±0.2; 34.1±0.7	<u>OGTT:</u> S_I – ml min^{-1} m^{-2} ISR	<u>S_I:</u> B=W B:361.4±7.3 W:349.6±7.8	<u>Early ISR 30min:</u> **B>W** <u>ISR late phase:</u> **B<W**	<u>Basal, Stimulated IC:</u> **B<W**	ND

Piccinini, 2018 (58)	Non-T2D	B:34 (M),21 (F) W:42 (M), 46 (F) H:29 (M), 31 (F)	B: 7-12; 18±3 W: 7-13; 18±3 H: 7-12; 15±1	<u>FSIGT</u> S_I AIRg ISR HIE (%) and CLp – mL/kg/min (Polidori, 2016)	<u>S_I</u> B=W=H B: 5±4 W:7±3 HA:5±5	<u>AIRg</u> **B>W, H>W** **B: 1026 ±737** W: 585±365 H: 1194 ±1119 <u>Early ISR 0-20min</u> **B>W/H** **B: 271±176** W:105 ±59 H: 202±110 <u>ISR late phase</u> **B<H, W<H** **B: 250 ±157** W: 289±212 H: 371±227	<u>HIE</u> **B<W/H** B: **19%** W:33% H: 28% <u>CLp</u> B=W=H	ND

Summary measures expressed as mean±standard deviation, T2D – Type 2 Diabetes, B- Black, W-White, H - Hispanics, F - Female, M – Male, NW – normal weight, OB – Obese, BF – Body fat, FSIGT – Frequently sampled intravenous glucose tolerance test, HIEG – hyperinsulinemic euglycemic clamp, OGTT – Oral glucose tolerance test, HOMA-IR – Homeostatic Model Assessment of Insulin Resistance, S_I, insulin sensitivity, AIRg – acute insulin response to glucose, ISR – insulin secetion rate, DI – Disposition index, fIns – Fasting insulin, M – glucose disposal rate, AUC – Area -under curve, Ins – insulin concentration, Glc – Glucose, C-pep – C-peptide concentration, HIE – hepatic insulin extraction IC – Insulin clearance, ND – Not done; FMI – Fat mass index

1.8 ETHNIC DIFFERENCES IN THE DETERMINANTS OF INSULIN SENSITIVITY

1.8.1 BODY COMPOSITION

Dual X-ray absorptiometry (DXA) can distinguish between fat, bone and non-bone lean tissue and has therefore been used to measure not only total body fat and fat-free soft tissue mass but is also use to estimate regional fat mass such as trunk fat, leg fat, abdominal SAT (aSAT) and VAT (79). An advantage of DXA is that it has low levels of radiation and its accuracy is maintained across a wide range of body sizes, although adjustments may be required in obese individuals (80) and ethnic specific equations are required for the estimation of VAT and aSAT (81).

Studies in predominantly white populations have found an inverse association between total body fat and S_I (82,83). However, this relationship appears to be altered by ethnicity. A study conducted in African American and white American women with a wide range of age and BMI, found that body fat percent was inversely associated with S_I, only in white women but not in African American women (54). Another study conducted in obese premenopausal African American women with normal glucose tolerance and IGT found that body fat percent was not associated with S_I in a multiple regression model that contained age, lean body mass, SAT and VAT and total fat weight (84). However, the failure to show an association may be due to the collinearity commonly shown between these correlated variables. Regardless, a South African study found that body fat percent was inversely associated with S_I in both black and white premenopausal women with normal glucose tolerance, but was stronger in white women ($r=-0.498$, $p=0.008$), compared to black women ($r=-0.379$, $p=0.042$) (85).

These findings might suggest that body fat percent may be a less important correlate of S_I in women of African descent compared to white women. Perhaps muscle mass or body fat distribution may be more important contributors to S_I in women of African descent.

1.8.2 MUSCLE MASS

Skeletal muscle is the major site of glucose uptake (86). Muscle mass may therefore be an important correlate of S_I. A cross-sectional study conducted in predominately white adults (mean age 41 year old and mean BMI 25.6 kg/m^2) reported an inverse association between skeletal muscle mass (normalized to total body weight) and insulin resistance (HOMA-IR) and the prevalence of diabetes (87). These findings were corroborated by a longitudinal study in older (mean age 51.6 years), normal-weight (mean BMI 24.2 kg/m^2) Japanese Americans without T2D (88). This study reported that a lower baseline thigh muscle mass predicted insulin resistance, determined be HOMA-IR and the quantitative S_I check (QUICKI), after 10 years of follow-up (88). The reasons why low skeletal muscle mass is associated with insulin resistance and T2D is not clear but could due to lower mitochondrial oxidative capacity (89), or reduced secretion of myokines that are important in glucose and lipid metabolism (90). However, in a study conducted in African American and white women without T2D a greater muscle mass was associated with lower S_I and a greater muscle mass partly explained the lower S_I in African Americans compared to their white counterparts (91). These discrepant findings may be explained by the method used to determine muscle mass and whether it represents fat free muscle mass or general muscle mass that may still contain extramyocellular lipids as in the latter study. Intriguingly, a higher total body skeletal mass has been reported in African American women across all ages compared to white, Hispanic and Asian women (92).

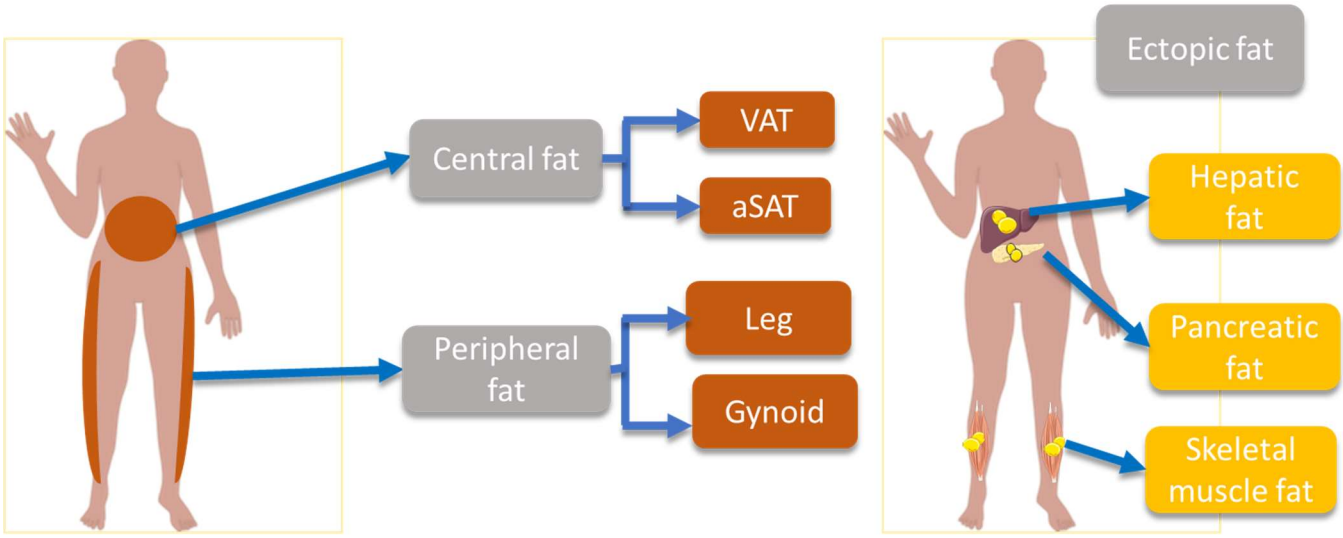
Contrastingly, in South African studies, no difference was found in fat-free soft tissue mass (muscle mass) between obese black and white premenopausal women (40,42,85). Rather, sarcopenia was prevalent in older black South African women which was associated with lower levels of physical activity (93). These findings may suggest that black populations from Southern Africa, compared to those from West African descent, may be more susceptible to lower muscle mass, which may predispose to lower S_I. However, no study assessed the direct association between fat-free mass and S_I in black obese South African women.

Furthermore, skeletal muscle is not homogenous and consist of different fiber types with different glucose disposal and fat oxidative capacity, which may influence S_I. Three different muscle fiber types have been identified based on histochemistry methods (94). Type I fibers, also referred to as slow twitch fibers based on a slower contractile ability, have greater oxidative potential and greater mitochondrial density, compared to other fiber types. Type IIb fibers are also referred to as fast twitch fibers based on a greater contractile ability, are better at utilizing glycogen as fuel, compared to Type I fibers, and are therefore also referred to as glycolytic fibers. Type IIa is an intermediate muscle fiber type with fast twitch fibers, but with greater oxidative capacity compared to type IIb fibers. A greater proportion of type I fibers is associated with a greater S_I in those without T2D (95–97). Moreover, obese adults without T2D have more Type I fibers compared to those with T2D (98). Compared to Type II fibres, Type I fibers have higher protein levels of insulin receptor, GLUT4, hexokonase II and glycogen synthase, which infers greater glucose uptake ability which likely explains the positive association with S_I (98). Interestingly, ethnic differences in muscle fiber type have been observed, with lower levels of type I fibers found in African Americans compared to their white counterparts (99), which may in part, explain the lower S_I

found in this population. However, African Americans are mainly of West African descent (99) and may therefore have a different muscle mass and muscle fiber type distribution to those black populations of Southern African descent.

1.8.3 Overview of adipose tissue and non-adipose tissue depots

The accumulation of fat occurs when an energy surplus exists due to energy intake exceeding energy utilization (100). Excess energy from either dietary fat or carbohydates is stored as TGs in adipose tissue depots (26). A schematic diagram of the various adipose tissue depots relevant to this thesis is depicted in Figure 1.2. The adipose tissue can be divided into SAT (representing 80% of total fat) and VAT depots. SAT depots can be further divided into peripheral and aSAT depots (101). Peripheral fat depots include the arms and legs (102), of which the gluteal-femoral (gynoid) depot is of great interest. The landmarks for delineating leg fat, using dual-energy X-ray absorptiometry (DXA), are two diagonal lines passing through the right and left femoral neck forming a “V” and that meets in the midline between the legs (103). The gluteal-femoral region (gynoid), a subdivision of the leg, extends from the greater femoral trochanter downwards to the middle of the thigh (104) (Figure 1.3). Accumulation of fat in non-adipose tissue depots is termed ectopic fat and can be found in skeletal muscle, liver, pancreas and the heart (101). However, this thesis will focus only on ectopic fat accumulation in the first three organs. These various adipose tissue depots differ with regards to their size and ability to expand (32), which may explain their differential associations with S_I.



VAT – Visceral adipose tissue, aSAT – Abdominal subcutaneous adipose tissue

Figure 1.2: *Different adipose tissue depots in the body relevant to this thesis*

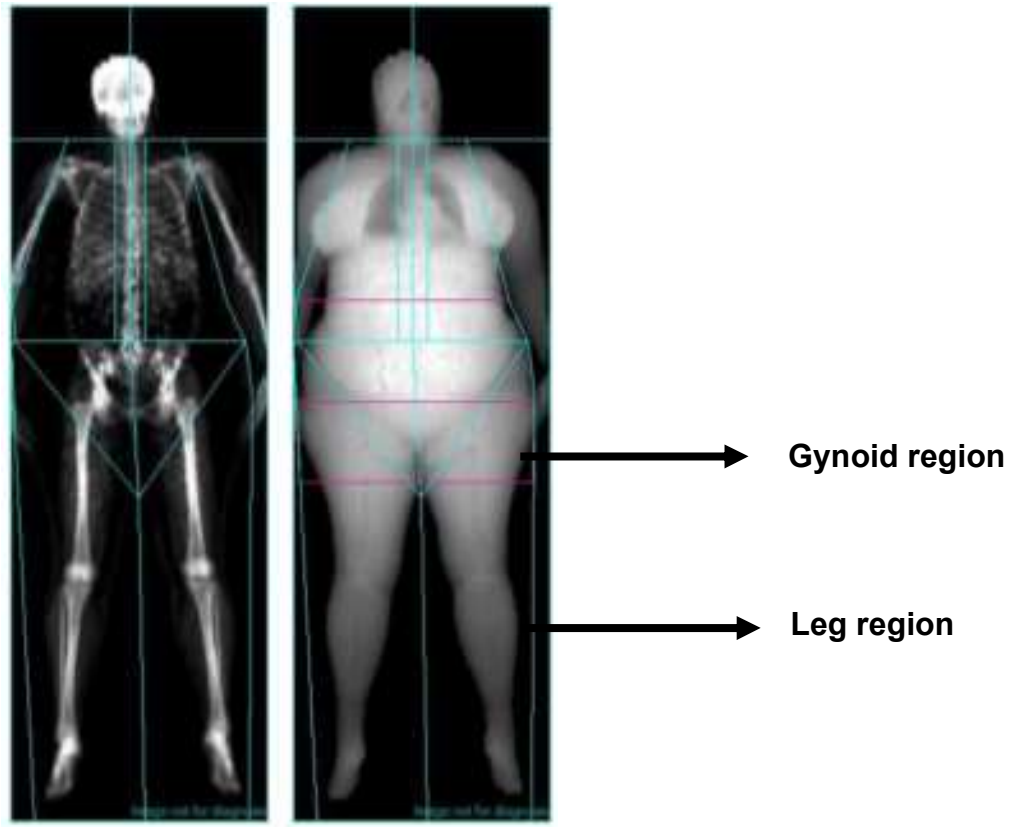


Figure 1.3: *Dual-energy X-ray absorptiometry landmarks for delineation of leg and gynoid regions (Source MRC DAPA measurement toolkit website: https://www.measurement-toolkit.org/anthropometry/objective-methods/whole-body-dexa-scan [Accessed 29 Jan 2020]*

The SAT depots act as a buffer by storing excess free fatty acids as TGs (105). A generalised or partial absence of adipose tissue, such as in those with lipodistrophy, is associated with insulin resistance (106). This emphasizes the importance of the SAT depot for safe storage of excess free fatty acids. Contrastingly, insulin resistance is also associated with excessive accumulation of fat in adipose tissue depots, explained by the expandibility hypothesis. This hypothesis proposes that adipose tissue has a limit to the amount of fat that can be stored without adverse metabolic effects (27). Individual variability occurs in the ability to expand adipose tissue, which may be regulated by genetic and environmental factors (107). Adipose tissue that has reached its storage capacity may become 'dysfunctional', characterized by hypertophic adipocytes that have outgrown their vascular supply and therefore becomes hypoxic (105). Further, this 'dysfunctional state' is marked by release of free fatty acids instead of the safe storage of TGs, increased secretion of pro-inflammatory cytokines (27), reduced secretion of insulin-sensitizing adipokines, and increase secretion of anti-insulin-sensitizing adipokines (108). The increased mobilization of free fatty acids may be redirected to visceral and ectopic sites such as skeletal muscle, liver and pancreas (109).

1.8.4 PERIPHERAL SUBCUTANEOUS ADIPOSE TISSUE

Studies have consistently shown that higher peripheral fat associates with lower insulin resistance across a wide range of age, BMI and ethnicity (82,110–115). A study conducted in premenopausal black South African women showed that DXA-determined leg fat mass correlated negatively with HOMA-IR (43). Studies conducted in predominately white populations using the gold-standard HIEG echoed these findings (82,114). The one study conducted in young-to-middle aged adults (82) and

the other in older (46-75 year old) women (114) showed that peripheral adipose tissue was associated with higher S_I. The positive association between peripheral adipose tissue (determined both by CT (114) and DXA (110,112,113)), and S_I exists independent of central fat depots in overweight to obese adults. A possible reason for this observed finding could be due to the a higher lipoprotein lipase activity of lower body fat depots, compared to abdominal depots, which indicates a greater uptake of free fatty acids (116,117). This will enable peripheral fat depots to store fat more readily and prevent the release of fatty acids into the circulation (118) where it could be diverted to ectopic sites and therefore protects against insulin resistance. Moreover, a lower inflammatory profile of peripheral fat depots, compared to aSAT, observed in white adults, may contribute towards the beneficial association with S_I (119).

The peripheral SAT depots, in particular the gynoid region, seem to be the preferential storage depot of black African women. Indeed, a greater gynoid fat mass (adjusted for total fat mass) has been found in black South African women compared to white women matched for body fat (120). Few studies have been conducted in black African populations that assessed the association between leg/gynoid fat mass and S_I (43,115,120). Mixed results have been found with a protective association observed in overweight African American premenopausal women (115) and in overweight-to-obese South African premenopausal women (43) but a detrimental effect in another small South African study (120). The beneficial effect of leg/gynoid fat mass on S_I may depend on the the expandibility of this depot and when the storage limit has been reached, it may become dysfunctional and cease to be protective. Notably, the gynoid fat depot of the black South African women in the latter study showed reduced expression of lipogenic and adipogenic genes which may indicate that the storage

capacity had been reached (120). Nevertheless, a longitudinal study reported that a reduction in relative gynoid fat mass over time was independently associated with lower S_I at follow-up (121). Evidence points to leg/gynoid fat mass as being protective against deteriorating S_I in both white adults and those from African descent. However, we are left with a conundrum as to the reason for a low S_I in black African populations despite greater gluteo-femoral depots. Interventional studies in black South African women are therefore warrated to investigate this further.

1.8.5 ABDOMINAL SUBCUTANEOUS ADIPOSE TISSUE

The aSAT depot does not only differ from the peripheral SAT depot by location, but also in lipolytic potential. Abdominal SAT depots are more lipolytic compared to peripheral SAT (118,122). Abdominal SAT depot is also distinct from VAT and is characterized by larger adipocytes (123,124) and higher LPL-activity (124), which suggests a greater ability to store fat compared to VAT. Indeed, aSAT is a much larger fat depot compared to VAT. Moreover, aSAT is the main contributor to systemic free fatty acids, which may be linked to peripheral insulin resistance through their effects on peripheral (muscle) insulin signaling (125). However, discrepant findings have been reported, with studies showing a negative (82,126), positive (111) and no association (114,127) between aSAT and S_I. Similarly, studies conducted in black African populations produced contradictory results. No association was found in some studies from USA (54,84) and from South Africa (43), while a negative association was found in another study from USA (128). The reasons for these disparate findings are not clear, but could relate to differences in sample size, or analysis strategy (adjusting for multiple body fat measures or only BMI). Alternatively, the inability to distinguish between deep aSAT and superficial aSAT (129), which may have different functions

(85,130) could influence the association of total aSAT with S_I. Indeed, an American study conducted in lean and obese adults, ethnicity not mentioned, with normal glucose tolerance, found that deep aSAT (r-0.51, P<0.001), but not superficial aSAT (r-0.29, p>0.05), was associated with S_I, independent of total body fat and VAT (83). Moreover, the association between deep aSAT and S_I (r-0.64) was similar to the association between VAT and S_I (r-0.61), which may indicate that the deep aSAT depot may be more similar to VAT in function. However, the inflammatory profile of these depots may not be the reason for the above findings since a study conducted in black and white South African women, found a similar inflammatory profile between deep and superficial aSAT, which was distinct from the VAT depot (130). Conversely, another SA study found that both deep and superficical aSAT was negatively associated with S_I in both black and white women (85). Although deep aSAT was a stronger correlate of lower S_I in black women compared to superficial aSAT, superficial aSAT may still be detrimental to S_I if its storage capacity has been reached. Notably, black African populations have greater aSAT depots compared to their white counterparts of which superficial aSAT, compared to deep SAT, is the largest contributor (85). Clearly, the evidence linking aSAT to lower S_I is inconclusive, which may be explained by the heterogeneity in the fat storage threshold of this depot together with the concomitant extent of fat accumulation in non-SAT depots.

1.8.6 VISCERAL ADIPOSE TISSUE

Shen *et al*.identified that VAT can be located within 3 main body cavities: intrathoracic, intraabdominal and intrapelvic (131). The VAT depot most commonly linked to T2D and cardiovascular disease is intra-abdominal VAT, in particular omental and mesenteric fat (129). Despres *et al*. proposed various theories as to why VAT is

associated with deteriorating S_I (132). One of these theories is the portal vein theory which proposes that VAT secretes free fatty acids directy into the portal vein because it is highly lipolytic and resistant to the effects of insulin. The liver is therefore directly exposed to the high flux of free fatty acids and through its lipotoxic effects may result in hepatic and peripheral insulin resistance. Another theory, is that VAT is highly immunogenic and able to secrete inflammatory cytokines, such as IL-6 and TNFα that have been implicated in insulin resistance. A further theory is that VAT is a marker of dysfunctional SAT that has reached its fat storage capacity (132).

The association between VAT and S_I has been extensively studied (82,83,85,111,114,126,128,133–142). A negative association between VAT and S_I has been found consistently in normal weight (133,136), overweight and obese (134–136,138,139) adults without T2D. Although these were select populations, studies that included adults over a wide range of BMI reported a similar relationship between VAT and S_I (83,139–141). While an association is apparent between VAT and S_I regardless of the level of BMI, a large population-based study did report that BMI modifies this association with a stronger association found in obese compared normal-weight adults (126). In contrast, one study conducted in predominantly white adults (mean age 36.6 years) found that VAT was not associated with S_I, if adjusted for aSAT (82). Nevertheless, the negative association between VAT and S_I found in cross-sectional studies have been reiterated by a longitudinal study conducted in Japanese Americans (135). This study reported that baseline VAT was positively associated with HOMA-IR and negatively associated with Matsuda S_I index after 10-11 years of follow-up, even after adjusting for baseline BMI, total fat and aSAT.

Studies in populations of African descent are also generally supportive of the relationship between VAT and S_I. For example, studies including African American

men and women (128,137) or only women (84) confirmed a negative association between VAT and S_I. However, a South African study found no association between VAT and S_I in black premenopoasual women over a range of BMI, while a negative association was observed in white women (85). Notably, a recent study reported that the accumulation of VAT was a predictor of T2D in middle-aged black South African women after 13 years of follow-up (143). This study showed that over the follow-up period VAT increased by a greater margin (41%) compared to that of aSAT (16%). These findings were echoed by an earlier South African study reporting that premenopausal black women were more prone to VAT (increased by 28%) than aSAT (8%) accumulation after 5.5-years of follow-up (121). The heterogeneity in findings in black African populations regarding the association between S_I and VAT might be explained by the level of VAT. A curvilinear association has been described between S_I and VAT such that at higher VAT, no changes in S_I are observed (144). Therefore, no association will be apparent if VAT is beyond a certain point. However, overweight and obese black African women have lower VAT, compared to Hispanic (128) and white women (39), but are more insulin resistant. Perhaps indicating that black African populations might have a greater sensitivity to VAT accumulation. Further research is required to explore this phenomenon in black African populations.

1.8.7 SKELETAL MUSCLE FAT

The accumulation of fat in non-adipose tissue sites such as the skeletal muscle have been associated with reduced S_I (82,145–150). The mechanism that excess fat in the muscle could lead to insulin resistance is based on the lipotoxicity theory (24). Fatty-acid derivatives such as long-chain fatty acyl CoA and ceramides interfere with the insulin signalling pathway (24,151). Lipid intermediaries activate PKC, which

phosphorylates IRS-1 on a serine residue. This renders IRS-1 unable to activate PI3K, which is an important step in the translocation of GLUT4 to the plasma membrane, as well as for glycogen synthesis. In addition to a defect in the insulin signalling pathway, earlier research in obese individuals with and without T2D has also implicated defects at the level of the insulin receptor such as reduced numbers and reduced kinase activity (152). Chronic hyperinsulinemia may be considered a plausible explanation for this finding (153).

Despite studies using different methods to determine skeletal muscle fat content, including CT (82), muscle biopsy and histochemistry methods (149,150) and MRS (145–148), a consistent negative association has been found with S_I. However, the location of the fat in the muscle is important because intramyocellular lipids (IMCL) were found to be a stronger correlate of insulin resistance compared to extramyocellular lipids (EMCL) (147). MRS is therefore the optimal method to differentiate between IMCL and EMCL. Another aspect to consider is the fibre composition within muscle, as discussed in section 1.8.2, which may confer differential associations with S_I. Indeed a study conducted in lean offspring of parents with T2D found that soleus IMCL, but not tibialis anterior IMCL, was the main correlate of reduced S_I (147). Further, the association between IMCL content and S_I may also be modified by the underlying fatty oxidation capabilities such that a positive association is observed when fat oxidation is high compared to a negative association when fat oxidation is low, which is also termed the ‘athlete’s paradox’ (154).

Ethnicity may also alter the relationship between skeletal muscle lipid content and S_I, but conflicting evidence exists. In a study that compared soleus IMCL content, measured by MRS, between African Americans and European Americans (21 and 46 years of age), IMCL content and S_I were similar between the groups (60). However,

IMCL correlated with S_I in white and not African American adults, which led the authors to suggest that IMCL might not be pathological in African Americans. Another American study in obese adolescents, showed that although soleus IMCL was lower in African Americans compared to Hispanics and white individuals, S_I was similar in all the groups (155). A South African study found no difference in soleus IMCL content between obese black and white women, but only in black women was soleus IMCL content correlated with a lower S_I. Potential reasons for the discrepancies in findings could be that Ingram *et al*.(60) included men and women over a wide range of BMIs (21-46 kg/m^2), while the South African study only focused on obese women. Nevertheless, cross-sectional studies show that black African populations may be more sensitive to the accumulation of IMCL (42,155). Although another study found that IMCL was only associated with S_I in white but not African American women and girls (156). Future interventional studies that manipulate IMCL such as an exercise or dietary interventions, may provide greater insight into this association between S_I and skeletal mucle fat in black African women.

1.8.8 HEPATIC FAT

The liver plays an important role in glucose and lipid homeostasis both in conditions of low and excess energy intake (157). Postprandially, when energy sources are high, insulin will inhibit EGP and activate glycogen synthase to ensure glucose is converted to glycogen and stored. However, after maximal glycogen storage capacity has been reached, the excess glucose will be converted into free fatty acids via DNL. These free fatty acids can be esterified and stored in the liver as TGs or secreted as very low-density lipoprotein (VLDL) and transported to adipose tissue and other tissue. Free fatty acids can also enter the mitochondria and undergo β-oxidation in the liver (158).

Under normal physiological conditions, minimal fat is stored in the liver. However, hepatic TG accumulation may occur if the net carbohydrate and free fatty acid flux into the liver is increased. Excess carbohydrates and free fatty acids may be derived from the diet or from increase fatty acid secretion from adipose tissue (159), a consequence of adipose tissue that exceeded its storage capacity as discussed previously. Further, the liver itself may increase TG synthesis. Hyperglycemia may stimulate lipogenesis through activation of the transcriptional factor, carbohydrate response element-binding protein (ChREBP) (159). Another mechanism that may explain hepatic fat accumulation is when fatty acid oxidation is decreased. This may occur as another consequence of dysfunctional adipose tissue which secrete less adiponectin (160).

Hepatic fat accumulation associates strongly with hepatic insulin resistance in cross-sectional studies in non-obese (161,162) and obese individuals (163,164), without T2D and in those with T2D (165). Furthermore, in obese adults with non-alcoholic fatty liver disease (NAFLD), hepatic fat accounted for 34% of the variability in hepatic insulin resistance (163). It is possible that hepatic insulin resistance may cause hepatic fat accumulation, but evidence is also available that suggests hepatic fat accumulation causes hepatic insulin resistance. Insulin resistance in the liver is characterized by an inability of insulin to suppress EGP, while DNL remains intact. Elevated glucose levels can stimulate pancreatic insulin secretion (166). Subsequent increased insulin levels stimulate DNL which promotes lipid accumulation in the liver. In support, targets have been found where these 2 pathways diverge downstream from the insulin receptor (166) which explains the molecular basis for insulin's dual action in the liver. On the other hand, hepatic fat may also cause hepatic insulin resistance and the possible mechanism is the accumulation of lipid intermediaries such as diacylglycerol, that interrupts the insulin transduction pathway, which has been shown in human (167)

and mice (168) studies. Notably, discrepant findings of the association between hepatic fat and hepatic insulin resistance have been found in black populations. A significant positive association was found in non-obese, young Jamaicans without T2D (169) and in obese pre-menopausal South African women without T2D (42), whereas no association was observed in black men of West African descent residing in the UK whom have T2D (53). The reason for the inconsistent findings are not clear and whether differences in the measurement of hepatic fat or hepatic insulin resistance or population characteristics such as T2D status may explain it, requires further study. Further, ethnic discrepancies have been noted in hepatic fat accumulation with lower levels observed in black African populations compared to other ethnicities (42,53,155,170–172). However, despite black African populations exhibiting lower hepatic fat, they may have similar whole body S_I compared to white populations (42) but only in the black Africans where hepatic fat associated with lower S_I. This may indicate a greater sensitivity of S_I to the effects of hepatic fat, but further research is required to explore the direction of causality in black Africans.

The association between hepatic fat accumulation and peripheral insulin resistance, and whether the development of hepatic insulin resistance may precede this association requires further consideration. An elegant murine study was able to investigate the chronological effect of a high fat diet over 16 weeks on muscle, liver and adipose tissue S_I as well as associated fat accumulation in these organs (168). Notably, hepatic insulin resistance occurred prior to muscle insulin resistance and was associated with an increase in hepatic fat content. Interestingly, the insulin suppression of lipolysis was not altered, only glucose uptake into the adipose tissue was reduced, which coincided with hepatic insulin resistance. Similar findings were observed in lean men after 5 days of high fat diet, such that hepatic insulin resistance

occurred prior to any changes in peripheral S_I (173), however, adipose tissue S_I was not measured. Based on these limited studies, it appears that hepatic insulin resistance seems to precede peripheral S_I and is associated with increased dietary fat intake and hepatic fat accumulation, rather than increased free fatty release from adipose tissue.

Hepatic fat accumulation may therefore not only induce hepatic insulin resistance but may also have an impact on systemic insulin resistance. Indeed, an association between hepatic fat and peripheral insulin resistance has been reported (163,165,174–177). These findings were obtained regardless of the method used to quantify hepatic fat. These methods varied from a liver biopsy (175) to magnetic resonance spectroscopy (MRS) (174,177). However, these studies were conducted mainly in white individuals. Due to the ethnic differences in adipose tissue distribution and ectopic fat deposition, these findings may not be applied to black Africans.

1.9 INSULIN SECRETION

Causal factors that have been implicated in the impairment of insulin secretion are a combination of reduced β-cell glucose sensitivity, glucotoxicity, lipotoxicity, inflammation and β-cell exhaustion (178). β-cell exhaustion occurs when the demand for insulin is high. This involves increases in the synthesis and packaging of insulin into storage vesicles, which can strain the endoplasmic reticulum and result in an increase proinsulin:insulin ratio. Nevertheless prior to β-cell failure and the development of IGT, normal plasma glucose levels are maintained through increasing plasma insulin levels (179).

Studies use various terms to describe β-cell function such as insulin response or insulin secretion. However in this thesis the insulin response refers to the fasting or stimulated plasma insulin levels and reflects both insulin clearance (hepatic and peripheral) and insulin secretion. Insulin secretion will refer to measures that reflect fasting or stimulated plasma C-peptide levels or the ISR, derived from C-peptide modelling. Importantly, the ability to maintain euglycemia depends on the ability to match the insulin response or insulin secretion to the prevailing level of S_I. Indeed, a hyperbolic relationship between the AIRg and S_I, derived after a FSIGT, has been described (180). The product of AIRg and S_I is also known as the disposition index (DI) and remains constant when the insulin secretory response completely compensates for the level of S_I. In this thesis, DI will be used as an index of β-cell function. Of note, DI is an overall measure of insulin action because it encompasses both the insulin response and S_I. A deterioration in DI will occur when the first phase insulin secretion/response is unable to fully compensate for the level of S_I, which will result in IGT and eventually T2D. Importantly, defects in β-cells function have been observed early in the pathogenesis of T2D (181,182). In addition, the sensitivity of the β-cells to the stimulus of glucose may also affect β-cell function. This is a measure of insulin secretion in response to the incremental change in glucose. Indeed, β-cell glucose sensitivity was a predictor of deteriorating glucose tolerance in healthy Europeans (183).

1.10 ASSOCIATIONS OF HEPATIC FAT WITH INSULIN CLEARANCE, RESPONSE AND SECRETION

Accumulation of fat in the liver has been associated with reduced insulin clearance and a higher insulin response (184). The exact mechanisms for this are not known but *in vivo* studies in rats (185) and dogs (186) have shown that free fatty acid infusion into the portal vein was associated with reduced hepatic insulin clearance. However, these results were not replicated in non-obese adults without T2D (187). The human study infused glycerol into the systemic circulation, which may explain the lack of association with hepatic insulin clearance.

Majority of studies conducted in humans that assessed the association between hepatic fat and insulin clearance found a negative association (69,70,165,188), but not all (189) (Table 1.4). Different methods were used to quantify hepatic insulin clearance. Two studies used mathematical modelling that was able to distinguish between hepatic and peripheral insulin clearance (49,189). Others used the metabolic clearance rate formula that provides an estimate of total insulin clearance (69,188) or the C-peptide to insulin ratios (53,70) to approximate hepatic insulin clearance.

Populations of African descent have shown reduced insulin clearance (44,49,50,59), although paradoxically have a lower hepatic fat than white populations (49,155) (42,171). Indeed, ethnic differences have been observed in the association between hepatic fat and insulin clearance. A study conducted in pre- and post-menopausal women without T2D found that hepatic fat was associated with lower basal hepatic insulin clearance only in white women, but not African American women (49). These findings were mirrored in men with early T2D, (53). This study reported that under basal conditions, hepatic fat was associated with lower total insulin clearance only in the white men but not in the black men of West African descent, while under stimulated conditions no association was found in both groups (53). However, no studies in Africa have measured both hepatic fat and hepatic insulin clearance and assessed their

relationship. Studies are therefore required to determine whether findings from other black populations residing in USA and UK are translatable to those living in Africa.

Table 1.4: *Studies, including ethnic comparison studies, that assessed the association between hepatic fat and insulin clearance*

Study (Country)	Sample size	Participants	Hepatic fat measurement	Insulin clearance measurement	Association HF and IC
Chung, 2018 (USA) (49)	Premenopausal: B (n=52), W (n=35) Post-menopausal: B (n=19), W (n=13)	F, Non-T2D Pre- and Postmenopausal OW/OB	MRS	FSIGT: Polidori (2016) model MMT: Cpep $AUC_{0\text{-}30m}$/ Ins $AUC_{0\text{-}30m}$ and Piccinini (2016) model	Basal HIE: B: No ~ W: Negative ~ Stimulated HIE: Not mentioned
Finucane, 2014 (UK) (70)	W (n=70) NAFLD (n=29) Non-NAFLD (n=41)	M + F, Non-T2D 71.3 yo, BMI NAFLD: 28.6 kg/m^2 Non-NAFLD: 25.2 kg/m^2 (Not BMI matched)	MRS	OGTT: 1-(Ins AUC/Cpep AUC)	HIE: Negative ~ (å age, sex)
Gastadelli, 2007 (Italy) (165)	Non-T2D (n=14) T2D (n=43)	M+F Non-T2D: 43yo, NW T2D: 54 yo, NW +OB	MRS	HIEG Insuin infusion rate/ steady state [Insulin]	Total IC: Negative ~ (å age, sex, BMI) (groups combined)
Hakim, 2019 (UK) (53)	B (n=18), W (n=18)	M, T2D,	MRI	Hyperglycemic clamp: ISR AUC/Ins AUC + ([Insulin]Final-[Insulin]Basal)x MRTInsulin	Basal total IC: B: No ~ W: Negative ~ Stimulated Total IC: B: No ~ (neg trend) W: No ~
Kotronen, 2007 (Finland) (69)	W (n=80)	M+F, Non-T2D, 18-60yo, OW	MRS	HIEG: Insulin infusion rate/ steady state [Insulin]	Total IC Negative ~ (å age, BMI, sex)
Kotronen, 2008 (Finland) (188)	W (n=68) T2D (n=34) Non-T2D (n=34)	M+F T2D: 49yo, 31.8 kg/m^2 Non-T2D:45yo, 29.9 kg/m^2 (age, BMI matched)	MRS	HIEG: Insulin infusion rate/ steady state [Insulin] and Cpep AUC/Ins AUC	Total IC: Negative ~ (å VAT)

Utzschneider, 2018 (USA) (189)	NAFLD (n=13): W (n=9), H (n=2), Other (n=2) Non-NAFLD (n=15): W (n=12), B (n=1), Other (n=2)	M+F, Non-T2D, OB, NAFLD: 49.7yo Control: 50.6yo (BMI matched)	CT	OGTT + HIEG Polidori (2016) model	Basal HIE: No ~ Stimulated HIE: No ~ Peripheral IC: No ~

HF – Hepatic fat, IC – Insulin clearance, HIE – Hepatic insulin clearance, ~ - associations, å – adjusted, B – Black, W – White, H – Hispanic, NAFLD – Non-alcoholic fatty liver disease, T2D – Type 2 Diabetes, M – Male, F – Female, NW – Normal-weight, OB – Obese, OW – Overweight, HIEG – Hyperinsulinemic euglycemic clamp, FSIGT – frequently sampled intravenous glucose tolerance test, MMT – Mixed meal tolerance test, OGTT – Oral glucose tolerance test, AUC – Area-under-curve, [] -Concentration, MRTInsulin – Mean residence time of insulin, VAT – Visceral adipose tissue

Hepatic fat accumulation may also affect the secretion of insulin from the pancreas, as explained by the Twin-cycle hypothesis (190). This hypothesis postulates that hepatic fat increases due to a positive energy balance that leads to hepatic insulin resistance. This will result in increased glucose production by the liver and elevated plasma glucose levels. Consequently, basal insulin secretion will increase, which will further increase hepatic fat through the effect of insulin on DNL. Increased VLDL secretion transports TGs to the rest of the body. Due to its proximity to the liver, the pancreas is susceptible to fat accumulation. This process culminates in both elevated plasma glucose and free fatty acids causing a reduction in β-cell function and β-cell glucose sensitivity. Additionally, an *in vitro* study showed that hepatokines secreted by fatty livers can induce inflammation in both the β-cells and the fat cells within the pancreas, that results in reduced insulin secretion in response to glucose (191). This study also highlighted that in cell culture, pancreatic fat does not alter insulin secretion on its own, but only after being exposed to free fatty acids and fetuin-A. However, studies have shown a lack of association between hepatic and pancreatic fat (192,193), which may indicate that other mechanisms may be involved in the interaction of pancreatic fat accumulation on insulin secretion and β-cell function.

1.11 ASSOCIATION BETWEEN PANCREATIC FAT AND INSULIN SECRETION/B-CELL FUNCTION

Various mechanisms have been proposed to explain the reduction in insulin secretion and/or failure of β-cell function in the progression to T2D. The β-cells experience increased stress when demand for insulin is high, such as in black African populations that present with hyperinsulinemia. Endoplasmic reticulum and oxidative stress ensues, which may lead to reduced β-cell function (194). This results in incomplete folding of pro-insulin. Moreover, elevated blood glucose, known as glucotoxicity, has been shown to reduce insulin secretion (195). Another factor to consider is β-cell exhaustion, which occurs when continuous high insulin secretory responses depletes the available pool of insulin and results in a reduced insulin response. However, this seems to be transient, with studies showing an improvement in insulin secretion after the pancreas has been rested with drugs (i.e diazoxide) known to inhibit insulin secretion (196). In addition, the harmful effects of lipids and lipotoxicity on the β-cells is another factor to consider. Lipid intermediaries such as ceramides induce β-cell apoptosis (197). However, controversy exists regarding whether fat accumulation in the pancreas directly influences the β-cells ability to secrete insulin or whether it is just a marker of adipose tissue dysfunction. The lipotoxic effect on β-cells has been demonstrated in *in vitro* (198,199) and animal studies (200). One *in vitro* study revealed that prolonged exposure of the β-cell to free fatty acids can lead to decreased insulin gene expression, decreased stimulated insulin response and an increase in the apoptosis rate (198). Another *in vitro* study showed that β-cell death via apoptosis is due to endoplasmic reticulum stress that activates inflammatory pathways (199). A study conducted in rats demonstrated that chronically elevated systemic free fatty

acids correlated with fat in the pancreatic islet cells and with reduced glucose stimulated insulin secretion (200).

In contrast to the *in vitro* studies, studies in humans have found no consensus on the association between pancreatic fat and insulin secretion or β-cell function. Table 1.5 summarizes human studies that evaluated the association between pancreatic fat and insulin secretory function and β-cell function (51,191–193,201–211). These studies used different definitions of insulin secretion. Some used plasma C-peptide levels, which is more reflective of insulin secretion, while others used plasma insulin levels, which reflects both insulin secretion and insulin clearance (insulin response). In children without T2D, one multi-ethnic Canadian study reported an inverse association between the insulin response and pancreatic fat (206), while a bi-national study from Sweden and Austria found no association (203). The latter study adjusted for BMI as well as other fat depots such as VAT, SAT and hepatic fat, while the first study did not, which may explain the disconcordant findings. In adults without T2D but over a range of glucose tolerance, an inverse association was only observed in those with IGT (191,208) or with elevated hepatic fat (207), while no association was found in normal glucose tolerant women with a history of gestational diabetes (202) and in adults with or without T2D (192,193,201,210). Insulin response/secretion was derived after an oral glucose tolerance test (OGTT) for most of previously mentioned studies, except Van der Zijl *et al.* who used a HIEG method. A study that evaluated whether the association between insulin secretion/response and pancreatic fat differed between ethnicities found an inverse association only in white men, but not black African men with T2D (211). Contrastingly, a positive association was found between AIRg, derived from a FSIGT, and pancreatic fat in both black African and white adults without T2D. However, the association in black Africans had a steeper slope, while an inverse

association was found in Hispanics (209). Studies that investigated the association between pancreatic fat and β-cell function (insulin secretion/response normalised to the level of S_I) also found discrepant results. In one study, there was an inverse association in those without T2D, while those with T2D showed no association (192). No association was found between pancreatic fat and β-cell function in black Africans and Hispanic teenagers and young adults without T2D, independent of ethnicity (204). Another ethnic comparison study found a positive linear association in black African women, while in white and Hispanic women a non-linear association was noted (51). This study grouped lean and obese women with and without T2D together. Taken together, significant associations between pancreatic fat and insulin response/secretion were only apparent in those with IGT or elevated hepatic fat, but not in those with normal glucose tolerance or with T2D. Further, ethnicity seems to influence the nature of these associations, such that in black African populations a greater positive association was evident between both the insulin response and β-cell function and pancreatic fat, compared to Hispanics, which may be due to lower pancreatic fat deposition observed in black African populations. All these studies were cross-sectional and therefore it is difficult to evaluate causality. A longitudinal study that followed Japanese adults for 5 years found no association between pancreatic fat, determined by CT, and T2D onset (212). However, interventional studies that induced substantial weight loss either through dietary restriction (~15 kg weight loss) (213) or after bariatric surgery (13.6%) (214) in individuals with T2D, found that the first phase insulin response improved concomitant with a reduction in pancreatic fat. Although the association of pancreatic fat with insulin response and β-cell function has been studied across various populations, no study has been conducted in black

Africans living in Africa to determine whether pancreatic fat follows similar patterns to those in black populations residing in other continents.

Table 1.5: *Studies that evaluated the association between pancreatic fat and insulin secretion and/or β-cell function*

Study	Country; ethnicity	Sex; Age	Health status	Pancreatic fat measurement	Insulin secretion/ β-cell function	Association (~) PF with insulin secretion	Association (~) PF with β-cell function
Begovatz, 2015 (210)	Germany; White	M+F, adults OW/OB	Non-T2D, T2D	MRS	OGTT	No ~	No ~
Cohen, 2014 (206)	Canada; White, Black, Asian, Hispanic, Mixed race	M+F, 8-18yo 78% OW/OB	Non-T2D	MRS/MRI	OGTT	Neg ~ * (å sex, puberty, ethnicity)	Neg ~ (å sex, puberty, ethnicity)
Gerst, 2017 (191)	Germany, not mentioned	M+F,50-75yo	Non-T2D NGT, IGT/IFG	CT	OGTT	Neg ~ * (IGT) No ~ (NGT)	-
Heni, 2010 (208)	Germany; White	M+F, adults, OW/OB	Non-T2D NGT, IGT/IFG	MRI	OGTT	Neg ~ (IGT/IFG) No ~ (NGT)	-
Hakim, 2018 (211)	UK; Black, White	M,	T2D	MRI	MMT, HIEG	Black: No ~ White: Neg ~ (MMT, 1st phase)	-
Jaghutriz, 2018 (207)	Germany; not mentioned	M + F, adults	Non-T2D (high risk)	MRI	OGTT	Neg ~ * (only in those with high HLC, FFAs)	-
Komada, 2018 (201)	Japan; Japanese	M+F, adults Non-OB	NGT	MRS	OGTT	No ~ *	No~
Le, 2011 (204)	USA; Black, Hispanics	M+F, 13-25yo, OW	Non-T2D	MRI	FSIGT	-	No ~ (å ethnicity, sex, VAT, SAT, HLC, BF, FFA)
Lingvay, 2014(51)	USA; Black, White, Hispanics	F, adults	Non-T2D, T2D	MRS	FSIGT	-	<u>Combined non-T2D/T2D</u> Black: Neg ~(linear) (å BMI, VAT, SAT) White/Hispanic:Neg ~ (non-linear)
Nowotny, 2017 (205)	Germany; not mentioned	M+F, adults	Non-T2D	MRS	OGTT	No ~	-
Popp, 2018(202)	Germany, not mentioned	F; 35.5yo 37% OW/OB	No-GDM, GDM	MRI	OGTT* FSIGT*	No ~	No ~

Staaf, 2017 (203)	Sweden/Austria; Not mentioned	M+F; 10-18yo OB, non-OB	Non T2D NGT/IFG/IGT	MRI	OGTT	No ~ (å age, BMI, VAT, SAT, HLC)	No ~ (å age, BMI, VAT, SAT, HLC)
Szczepaniak, 2012 (209)	USA; Black, White, Hispanics	M+F;18-65yo	Non-T2D	MRS	FSIGT	Black: pos ~ * White: pos ~ * Hispanic: neg ~ * (å VAT, HF)	-
Tushuizen, 2010 (192)	Netherlands; White	M, 35-65yo	Non T2D vs T2D	MRS	OGTT	*Non-T2D/T2D:* No ~	Neg ~ (Non T2D) No ~ (T2D) å (BMI, fglc, TG)
Van der Zijl, 2015 (193)	Netherlands; White	M+F; adults, OW	Non T2D NGT/IGT/ IFG	MRS	HIEG	No ~	No ~ (å age,BMI)

*Insulin secretion – measures derived using plasma C-peptide levels, * - plasma insulin levels were used to measure insulin response, B-cell function – insulin secretion/response normalized to the level of insulin sensitivity; PF – Pancreatic fat, M – Male, F- Female, OW – Overweight, OB -Obese, NonT2D- Non- Type 2 Diabetes, IGT – Impaired glucose tolerance, IFG – Impaired fasting glucose, OGTT – Oral glucose tolerance test, FSIGT – Frequently sampled intravenous glucose tolerance test, MMT – Mixed meal tolerance test, HIEG – Hyperinsulinemic euglycemic clamp,* å *– Adjusted, Neg– Negative association, Pos – Positive association, HF – Hepatic fat content, SAT – Abdominal subcutaneous adipose tissue, VAT – Visceral adipose tissue, BMI – Body mass index, fglc – fasting glucose, TG – triglycerides, FFA – fat free fatty acids*

1.12 ROLE OF BODY FAT DISTRIBUTION ON INSULIN RESPONSE OR ß-CELL FUNCTION

Based on the expandibilty hypothesis, if the maximum expandibility of SAT is reached, an increase in VAT may be observed (27). Accordingly, the effect of VAT, or the VAT-aSAT ratio on the insulin response or β-cell function should also be considered.

Indeed, a negative association between DI and VAT has been found in black African (54,128) and white adults (189). However, this was not a consistent findings with no association found in other studies conducted in both black African and white adults (45,140) as well as in black African children (55). Reasons for these discrepant findings may be the level of VAT or the direction of association between VAT and the components of DI. As mentioned, DI is a composite measure of both S_I and AIRg and therefore the association between VAT and S_I, as discussed in section 1.8.6, may also explain the above findings. For example if a negative association is found between S_I and VAT and a positive association between AIRg or ISR and VAT, no association between DI and VAT may be apparent due to matched compensation. Further, aSAT was not associated with DI (45,128,165,215) in black African and white populations, when VAT was also placed in the model. The lipolytic and immunogenic profile of VAT, compared to aSAT may explain these findings.

Another consideration is that VAT may associates directly with AIRg or ISR and thus requires further mentioning. Interestingly, ethnic differences have been observed such that a positive association was found between AIRg and VAT in white adults (83,114,140,215), while a negative association has been observed in African American adults after adjusting for S_I, but no association was found between AIRg and

VAT-aSAT ratio (128). However, another study in African American women found no association between AIRg and VAT or aSAT in a multivariate model, but collinearity could have occurred through the inclusion of body fat weight and body fat percent into the same model. In addition, the association of VAT or VAT-aSAT ratio with β-cell function were not examined in this study. Importantly AIRg reflects both insulin clearance and ISR and how these components relate to VAT or aSAT is also of interest. Although a positive association has been found between insulin secretion [derived after both an OGTT (140) and FSIGT (138)] and VAT in white populations, no evidence is available in black African populations. In addition, women of black African descent have lower VAT compared to white women (61,85) which may contribute towards the ethnic specific association between VAT and the insulin response. Finally, none of these studies incorporated whether the association between the insulin response and VAT or VAT-aSAT ratio could be mediated through insulin clearance, which may be an important compensatory mechanism in black African women.

Further, associations between insulin secretory function and VAT are confounded by ectopic fat accumulation in the liver and pancreas, since it has been postulated that VAT accumulation may precede ectopic fat deposition in the liver and pancreas (132). One ethnic-comparison study found that in African American adults, VAT was not associated with AIRg, independent from pancreatic fat such that VAT did not add significantly to the model. A similar finding was found in white individuals, while in Hispanics, VAT, together with hepatic and pancreatic fat were all independent predictors of AIRg (209). Another study conducted in African American and Hispanic adolescents and younger adults (13-25 year old) reported that VAT confounded the associations between hepatic and pancreatic fat and between pancreatic fat and inflammatory markers, but did not directly ascertain whether VAT associates with AIRg

or DI independent from hepatic and pancreatic fat. Therefore further studies are needed to establish whether fat partitioning observed in African Americans are similar in black populations living in Africa and whether VAT is a more important correlate of insulin secretory function compared to hepatic and pancreatic fat.

1.13 EXERCISE TRAINING

Physical inactivity is a risk factor for the development of obesity and T2D (216). Low levels of physical activity have been reported in African American women with 57% engaging in less than an hour a week of walking for exercise (217). Whereas in a cross-sectional South African study conducted on urban black women, 67% were deemed physically active according to the Global Physical Activity Questionnaire (218). Nevertheless, majority of the physical activity time was attributed to low intensitiy activity, accumulated during walking, which is used as a mode of transport (219). Moreover, this low intensity activity might explain the low cardiorespiratory fitness observed in the black South African women, which was below the 5th percentile for females between 30-39 year of age (219). Similarly, in the USA, the National Health and Nutrition Survey reported that African American women across a range of BMI (20-49 year old) had lower VO_{2max} (32.9 ml/kg/min) compared to white (35.9 ml/kg/min) and Hispanic women (36.0 ml/kg/min) and with increasing obesity the decline in VO_{2max} was more pronounced in the African American women (220). Further, low cardiorespiratory fitness has also been associated with insulin resistance in black South African women (219). Clearly, obese women of African descent would benefit

from an exercise intervention that has been shown to improve both cardiorespiratory fitness and S_I.

Lifestyle interventions that include exercise together with diet are a cornerstone in the management of T2D (221), but also in the prevention of T2D (222–224). Large population-based diabetes prevention studies have shown that a combination of diet and increased physical activity in those with IGT can reduce the risk of T2D by 58% (224) in the US Diabetes Prevention Program, by 43% (225) in the Finnish Diabetes Prevention Study and by 42% (222) in the Chinese Diabetes Prevention Study (Da Qing). Interestingly, in the Da Qing study, exercise without dietary intervention reduced the risk of T2D by 46% (222). The importance of exercise training and its metabolic benefits in improving glucose homeostasis has been noted and hence The American Diabetes Association recommends 2.5 hours of moderate aerobic activity per week for the prevention of T2D (100).

While a healthy diet is an important component of a healthy lifetsyle and the prevention of obesity and T2D, this thesis has focused solely on the effect of exercise training on improving metabolic outcomes. The impact of diet on obesity and insulin resistance is beyond the scope of this thesis and will therefore not be discussed further.

1.13.1 Overview of the effect of exercise on glucose and fat metabolism

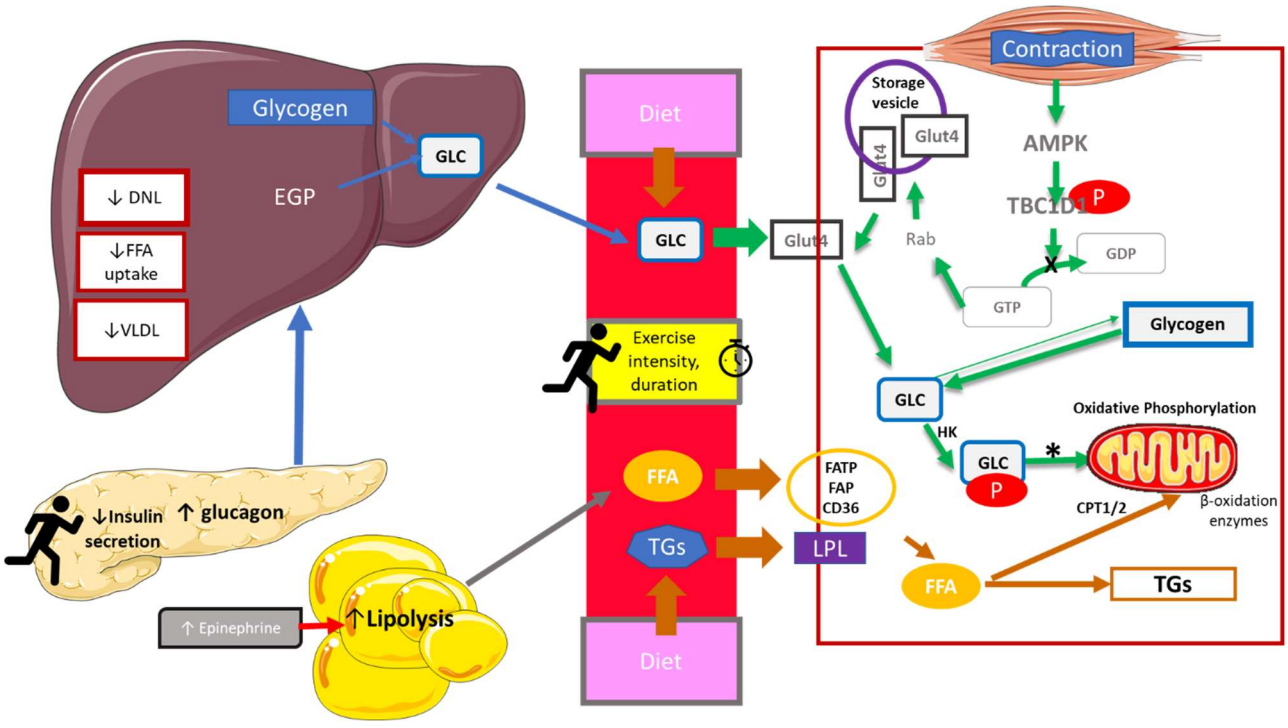
It is important to provide a brief overview of the effect of exercise on glucose and fat metabolism including glycogen depletion to identify the cellular mechanisms that may

explain changes in S_I and hyperinsulinemia in response to exercise training (Figure 1.4).

During exercise, the muscles require fuel to sustain contractions. The type of fuel, whether glucose or fat, depends on the intensity of exercise, session duration, exercise modality, diet prior to exercise, duration since last meal and the ability to oxidize fat (226). During exercise, glucose disposal and utilization in the muscle can occur independent from insulin. Muscle contraction initiates the uptake of glucose through an insulin-independent pathway, AMP kinase signalling, that result in increased GLUT4 translocation to the plasma membrane (227). The glycogenolysis pathway will dominate to further supplement the availability of glucose with less glucose converted to glycogen. This will deplete the glycogen stores in the muscle. If more glucose is required, the liver will assist by increasing glucose production either through breakdown of glycogen or through EGP under the influence of glucagon. Upon entering the cell, glucose will be phosphorylated to glucose-6-phospate by hexokinase (HK). Glucose-6-phospate will undergo a series of metabolic reactions (indicated by an asterisk* in figure 1.3) to be converted to pyruvate and then to acetyl Coenzyme A (if oxygen is available), which will be transported into the mitochondria for complete oxidation (228). During moderate intensity and longer duration of exercise, fat oxidation will increase. (226). In the fasted state, free fatty acids will be derived from adipose tissue lipolysis under the influence of epinephrine (228). Further, during exercise, insulin levels are low and therefore DNL in the liver will be inhibited and VLDL secretion reduced (229).

Some of the mechanisms that may explain the improvement in S_I after exercise training are an increase in GLUT4 transporters in the cell membrane (230) and

improved phosphorylation of insulin receptor substrates and other key molecules in the insulin signalling cascade (231). In addition, glycogen depletion may also contribute to an exercise-induced improvement in S_I (232). Moreover, exercise training may increase mitochondrial biogenesis and enhance mitochondrial function in both skeletal muscle (233) and adipose tissue (234), with an augmentation in oxidative capacity. In particular, an improvement in fatty acid oxidation (235) may occur either through the increased uptake of FFAs, via fatty acid transporter proteins, into the cell and/or the improvement of FFAs transport into the mitochondria via the carnitine palmitoyl transferase 1 and 2 (CPT1/2) complex and/or through the upregulation of enzymes involved in β-oxidation.



AMPK- Adenosine monophosphate activated protein kinase, CPT1/2 - Carnitine palmitoyl transferase 1 and 2, *DNL – De novo lipogenesis, EGP – Endogenous glucose production, FFA – free fatty acids Glc – glucose, GDP – Guanosine-5-diphosphate, GTP – Guanosine-5- triphosphate, HK – Hexokinase, , LPL – Lipoprotein lipase, TBC1D1 – Rab-GTPase activating protein TGs – triglycerides,*

Figure 1.4: *Overview of glucose and fat metabolism during exercise, schematic adapted from Brouwers et al (2017)* (229) *and Richter et al (2013)* (230)

1.13.2 FACTORS AFFECTING THE INSULIN SENSITIVITY RESPONSE TO EXERCISE TRAINING

The effect of exercise on S_I has been extensively studied with systematic reviews already published in those with and without T2D (227,236–238). Therefore, in this section, a brief overview of factors that can influence the S_I response to exercise training will be given followed by a discussion on the effect of exercise training on S_I in black populations of African descent.

An acute exercise bout increases S_I, an effect that can last up to 72 hours (239). However, to sustain the insulin-sensitizing effects of exercise for longer, a chronic exercise training program is required, which may lead to adaptations that promote a greater glucose disposal (238). The underlying metabolic health of a study population and the volume and intensity of an exercise training intervention may influence the insulin sensitizing responses to exercise training. It has been reported that those who are more insulin resistant show greater improvements in S_I (240). In support, Madsen *et al*. showed that only those with T2D showed an improvement in S_I compared to no improvement in those without T2D. Further, the effect of volume and intensity of exercise training on S_I have been studied in a randomized controlled trial (241). Overweight-obese older adults (mean age >50yo) without T2D participated in a 6-month intervention of either a low volume/moderate intensity or low volume/high intensity or a high volume/ high intensity exercise training compared to a control group. S_I improved significantly in all 3 groups, but the greatest improvement (~85%) was seen in those groups with a longer exercise duration (~170 minutes/week) compared to the low volume/low intensity group (~40%) (~115 minutes/week). In addition, greater improvements in S_I are found with increasing exercise dose, however this study did

not demonstrate a minimum or maximum threshold for an improvement in S_I (242). Another aspect to consider is the effect of high intensity interval or sprint interval training compared to moderate intensity continuous training on S_I. While, both sprint interval training and moderate intensity continuous training over 12 weeks, in non-obese men, increased S_I similarly, in the sprint interval group this was accomplished with a lower time commitment of 30 mintutes per week compared to 150 minutes per week (243). However, in another study in obese girls, 12 weeks of high intensity interval training increased S_I to a greater degree compare to moderate intensity exercise (244). Nevertheless, high intensity training may not improved S_I in all populations (245).

The mode of exercise training is another factor to consider when evaluating the effect of exercise training on the improvement of S_I. The effect of resistance training on S_I in adults without T2D has been studied less extensively compared to the effect of aerobic exercise. A study that randomized young (18-35 years old), normal-weight women either to a resistance training group, an aerobic training group or a control group for 6 months found that S_I improved similarly in both the exercise groups, but not in the control group (246). Similarly, a study in older overweight/obese men found that S_I improved regardless of the mode of exercise after 12 weeks (247). Contrastingly, in older women (54-78 years old), no improvement in S_I was found in both the resistance or aerobic exercise training groups after 6 months (248). However, this study attributed the lack of improvement in S_I to the frequency of exercise which was 3 times per week. Another study conducted in overweight adults (18-70 years old) reported that a combination of resistance and aerobic training was more effective in improving S_I and sustaining this improvement after 14 days of no exercise, compared to either type of exercise on its own (249). A mechanism to explain the improvement in S_I after

resistance training may be an increase in skeletal muscle mass. However, limited evidence exists in those without T2D. For example, after 8 months of resistance training in overweight-obese adults without T2D, lean body mass increased significantly but no changes in S_I were observed (249). However, resistance training has shown to improve key mediators of uptake of glucose in both insulin-sensitive and insulin-resistant muscles such as the GLUT4 protein content and enzymes in the insulin signalling cascade, which may occur independent of muscle mass changes (250). Taken together, these studies suggest that both aerobic and resistance training can improve S_I, and a combination is recommended for the prevention and treatment of T2D (100).

The majority (235,242,249,251–271), but not all (271–277) exercise-training studies show an improvement in S_I. The discrepancies observed in these studies may perhaps be explained by the impact of exercise training on body composition and body fat distribution. An improvement in S_I after exercise training may be mediated through a reduction in body weight. Indeed, most studies that did not report a significant reduction in body weight also did not report a change in S_I (253,271,274,275). However, a study conducted in adults with T2D that participated in 2 weeks of sprint interval or moderate-intensity continuous training reported an improvement in S_I without a concomitant reduction in weight (252). Nonetheless, this study also demonstrated a reduction in body fat mass, VAT and SAT. Lack of change in body weight or BMI may not always correlate with body fat changes. Therefore it is important to evaluate the effect of exercise training on fat-free soft tissue mass, body fat mass, as well as on regional fat depots when assessing the impact on S_I.

The VAT depot has been shown to be an important correlate of insulin resistance in cross-sectional studies, as well as in a recent longitudinal study (143). Therefore a

reduction in VAT may contribute to an improvement in S_I after exercise training. Moreover, VAT responds more readily to adrenergic-stimulated lipolysis induced by exercise (278) and thus may be preferentially reduced after exercise training. Indeed, studies that measured VAT and reported an improvement in S_I also observed a concomitant reduction in VAT (235,251,269,271). However, these studies also reported concomitant reductions in aSAT (235,251,269,271) and body fat mass (235,251,271), which could also explain the improvement in S_I. However, studies that assessed the contribution of VAT to the improvement in S_I, independently from body fat mass and/or other body fat depots have shown conflicting results. One study reported that VAT was not an independent predictor of S_I when accounting for reductions in body fat mass, aSAT and subfascial thigh fat (235), while another study found that VAT contributed to the improvement in S_I, independently from other fat depots (251). However, differences in study populations may have contributed to these discrepancies, the latter study (251) included both overweight and obese participants while the former study (235) included only obese individuals. Importantly, both these studies combined exercise training with calorie restriction. Nevertheless, a Danish study randomized obese adults without T2D into an exercise training without calorie restriction group, a calorie restriction only group or an exercise training combined with calorie restriction group. They showed that in the exercise training alone group, the relative reduction in VAT was greater compared to the reduction in body fat mass, but in all 3 groups, the reduction in VAT correlated closely with the reduction in body fat mass (273). This study did not perform a multivariate analysis to determine the independent contribution of a reduction in VAT to S_I but reported that after exercise training alone, the reduction in VAT, aSAT and the gluteal-femoral depot were similar. The evidence with regards to whether a reduction in VAT may be a more important

correlate of an improvement in S_I after exercise training above a reduction in body fat mass or other regional fat depots is inconclusive.

Few exercise-intervention studies that evaluated the effect of exercise training on S_I have been conducted in black African populations (Table 1.6). Two studies that reported an improvement in S_I, conducted the FSIGT <72 hours after the last exercise bout (279,280); the results may therefore reflect the effect of an acute exercise bout rather than chronic adaptation. Ortmeyer *et al.* included African American and white post-menopausal women, but found no interaction by ethnicity and therefore reported results in the combined group (281). In this study, an improvement in S_I was found after 6 months of high-intensity aerobic exercise training combined with calorie restriction that coincided with a reduction in body weight, VAT and SAT. A study conducted in pre-menopausal, overweight-obese African American women without T2D reported no change in S_I after 14 weeks of high-intensity interval training (245). In addition, no improvement in VO_{2peak} and no change in body weight and SAT were observed, but a reduction in VAT was noted. Notably, there is a dearth of randomized-controlled exercise intervention studies in populations of black African descent that are able to assess the independent effects of exercise training on S_I together with body composition and body fat distribution, and crucially, no exercise intervention study has been conducted in Africa.

Table 1.6: *Summary of exercise intervention studies from USA that included African Americans individuals and measured insulin sensitivity*

Study	Participants	Study groups	Exercise intervention: Dx, volume, intensity, mode	S_I Method	Δ S_I	Other changes
Arad, 2015 (245)	F, 29±4 yo, OW/OB, NonT2D Black only	CON (n=9) EX (n=11) Controlled High Fat Diet 10d prior to testing	14 weeks, Three 24 min sessions/week 75-90% HRR, High intensity interval	HIEG Clamp >72h after last session	No Δ	No Δ: VO_{2peak}, BW, SAT, IMAT, IC ↓VAT
Brown, 1997 (280)	F, 51±2 yo, OB, NonT2D Black only	EX (n=12) No control	7 consecutive days 65% HRR Aerobic exercise	FSIGT >14-18h	↑ (58%)	No Δ: VO_{2peak}, BW, BF
Many, 2013 (279)	F+M, 14-18yo, OB, NonT2D Black (8), Hispanic (3)	EX (n=11) No control	8 weeks 180min/week 40-55% VO2peak Aerobic exercise	FSIGT 24-48h	↑ (37%)	No Δ: VO_{2peak} ↓: BW, BF
Orthmeyer, 2017 (281)	F, 50-75yo, OW/OB, NonT2D/IGT Black/White (combined)	CR (N=34) CREX (N=37)	6 months Three 45min sessions/week 85% HRR Aerobic exercise	HIEG clamp 36-48h	↑	↑VO_{2peak} ↓: BW, BF, VAT, SAT

Dx – Duration of exercise training, S_I – Insulin sensitivity, Δ – Change,F- Female, M – Male, OW – Overweight, OB -Obese, NonT2D- Non- Type 2 Diabetes, IGT – Impaired glucose tolerance, B – African American, H – Hispanic, W – White, CON – control group, EX- exercise group, HRR – Heart rate reserve, HIT – High intensity training, HIEG – Hyperinsulinemic euglycemic clamp, BW – Body weight, BF – Body Fat, SAT – Abdominal subcutaneous adipose tissue, VAT – Visceral adipose tissue, IMAT – intramuscular fat, IC – insulin clearance, ↑ - increase, ↓- decrease

1.13.3 EFFECT OF EXERCISE TRAINING ON HYPERINSULINEMIA

The preservation of the insulin response and β-cell function is critical in preventing or delaying the onset of T2D and therefore investigating the effect of exercise training on these key components is warranted. During an acute bout of exercise, insulin secretion will depend on the intensity of the exercise (282). During both low and moderate intensity exercise, insulin secretion is inhibited to allow the liver to produce more glucose under the influence of glucagon. This ensures euglycemia while glucose is taken up by the muscle, via an insulin-independent pathway. Similarly, during high intensity exercise, insulin secretion is reduced, however, the rate of glucose production is much higher compared to lower intensity exercise. During the post-exercise period, glucose uptake reduces more than glucose production which results in hyperglycemia.

Insulin secretion will therefore increase during this period to normalize glucose levels (282). However, whether chronic adaptations occur in the insulin response to glucose after exercise training, as well as whether the exercise-induced changes in both the insulin response and β-cell function differ according to diabetic status requires discussion. Studies that reported on the effect of exercise training on insulin response/ secretion or β-cell function are summarized in Table 1.7.

Studies that have shown an improvement in S_I after exercise training also reported a reduction in the insulin response (251,260,264,265,280,283,284). The majority of these studies were conducted in those without T2D (251,260,265,283). T2D status may modify the exercise-induced change in the insulin response because those with T2D may have already impaired insulin responses. Indeed, a study conducted in those with T2D, differentiated between low and moderate insulin secretors and found that after 3 months of exercise training, the moderate secretors had an increased insulin response, whereas in the low secretors showed no change (285). Interestingly, in this study the insulin response was augmented in the moderate secretors without an improvement in S_I. Moreover, in those with T2D the residual insulin response is therefore an important factor in the exercise training response and can perhaps explain discrepant findings in those with T2D. For example, after 8 weeks of high intensity training in those with and without T2D, no changes occurred in the insulin response (263). While in another study conducted in those with T2D and prediabetes reported a reduction in the insulin response after 12-16 weeks of moderate-high intensity training (284). Further, the mechanism responsible for a lowered insulin response after exercise training could be due to lower insulin secretion. A study that evaluated both the insulin response and the insulin secretory capacity, derived from C-peptide measures, after 12 weeks of vigorous training in obese prediabetic adults,

found that only the insulin response decreased but not the insulin secretory response, indicating an increase in insulin clearance only (284). However, in Heiskanen *et al*, the ISR, obtained through C-peptide deconvolution and normalized to glucose concentration (0-30min), decreased after exercise training in both those with T2D and those without (252).

As previously mentioned, the insulin response normalized to the prevailing level of S_I is a better marker of β-cell function. The β-cell function in response to exercise will therefore depend on the net change in S_I and the insulin response. An increase in β-cell function was observed in studies that reported an increase in S_I together with a reduction (264,284) and no change in insulin response (263). These studies indicate that the degree of change in S_I is an important contributor to the improvement in β-cell function. Exercise intensity and volume also influences the degree of change in S_I and β-cell function. Compared to low volume/high intensity and high volume/high intensity exercise training, low volume and moderate intensity exercise training resulted in the biggest increase in β-cell function in adults without T2D due to a large increment in S_I and no change in the insulin response (261). In addition, combining aerobic and resistance training will sustain increases in β-cell function for at least 14 days post-exercise compared to aerobic or resistance training alone (249), due to an increase in S_I that only reduced by less than 50% after detraining. Indeed, the influence of exercise training on the insulin response and β-cell function seems to be driven by changes in S_I. Black African populations frequently present with hyperinsulinemia and a reduction in the acute insulin response was found in African American women after acute exercise (280), but not in African American and Hispanic adolescents (279). However S_I increase after exercise training in both the latter 2 studies

The effect of exercise training on insulin clearance requires further mention, especially since this may be a mechanism through which exercise training may reduce the insulin response. Although studies in Table 1.7 evaluated the effect of exercise training on insulin response or β-cell function, they did not evaluate the effect on insulin clearance. Nevertheless, two other studies assessed the effect of exercise training on insulin clearance (245,286). One study, in which ethnicity was not mentioned, was conducted in overweight-obese adults with IGT and reported that insulin clearance did not change after 12-weeks of combined aerobic and resistance training, but increased after using metformin or metformin in combination with the exercise training program (286). In contrast, S_I improved to a greater extent in the exercise training group compared to both the metformin groups. This study speculated that exercise training does not affect insulin clearance unless combined with metformin. The other study conducted in pre-menopausal African American women without T2D found no change in insulin clearance after 14 weeks of high-intensity interval training (245). Nevertheless, both these studies determined the insulin clearance after a hyperinsulinemic euglycemic clamp, which compared to a FSIGT derived measure, correlates poorly with direct measures of insulin clearance (287). More studies are therefore necessary to assess the effect of exercise training on insulin clearance derived from FSIGT measures in a black African population who typically present with lower insulin clearance compared to white populations (59)

In the following sections the effect of exercise training on ectopic fat will be discussed to determine whether these changes may explain changes in S_I and hyperinsulinemia.

Table 1.7: *The effect of exercise training on insulin response/secretion and β-cell function*

Study (Country)	Participants	Study groups	Exercise intervention: Dx, volume, intensity, mode	Measurement of Insulin response/ B-cell function	Δ Insulin response/ secretion	Δ β -cell function	Other Δ
AbouAssi, 2015 (USA) (249)	Black (n=17), White (n=79), Other (n=2), M+F, 47-51yo, OW/OB, NonT2D	RES (n=38) AER (n=27) AER+RES (n=23) (matched for age, sex, BMI, race)	8 months RES:180min/wk, 8 Exercises, 3 sets/day, 8-12 reps/set, progressive AER:132min/wk, 65-80% VO2peak	FSIGT -AIRg, DI Post-Ex: 16-24hrs and 14 days	*16-24hrs post Ex* RES: No Δ AER: ↓ AER+RES: No Δ	*16-24hrs post Ex* RES: No Δ AER**:** No Δ AER/RES: ↑ *14 days post Ex* AER: ↓ (trend) AER+RES:↑ (trend)	↑ VO2max (AER> other groups) ↑SI (AER+RES only) ↓BMI BF (AER, AER/RES)
Brown, 1997 (Canada) (280)	Black, F, 51±2yo, OB, NonT2D,	EX (n=12)	7 consecutive days 65% HRR aerobic exercise	FSIGT- AIRg Post-Ex: 14-18h	Yes ↓	ND	↑ SI, No Δ: VO2peak, BW, BF
Bruce, 2006 (Canada) (283)	M+F, 36yo, OB, Non-T2D,	EX (n=9)	8 weeks, five 60min sessions/wk, 60 min, Mod-intensity aerobic exercise	OGTT - AUCIns Post-Ex: 36-48h	Yes ↓	ND	↑: VO2max, SI, FAO No Δ: BW, BMI, IMAT ↓: DAGs, adiponectin
Dela, 2004 (Denmark) (285)	M, middle aged, OW/OB, T2D	*Mod secretors:* EX (n=9) vs NoEX (n=7) *Low secretors:* *EX (n=5) vs NoEX (n=3):*	3 months, five 30-40min sessions/week, 75% VO2max aerobic exercise	Hyperglycemic clamp Arginine stimulation Post-Ex: 16h	Ins and Cpep responses **EX:** Mod secretors: ↑ Low secretors: no Δ **NoEX** Mod secretors: no Δ Low secretors:	ND	↑VO2max No Δ: SI, BW, BF
Heiskanen, 2018 (Finland) (252)	White, 40-55yo: NonT2D: M, OW T2D: M+F, OW/OB	NonT2D (n= 28) T2D/IGT (n=26)	2 weeks, SIT: 4-6 sessions 30s cycle/4min rest CT: 6 sessions of 40-60min, 60% of Wmax	OGTT Post-Ex: NM	T2D: ISR basal ↓ NonT2D: ISR early↑ T2D/NonT2D: ISR/Glc0to30min ↓	ND	↑VO2max ↑SI ↓:PF, SAT, VAT, BF Both groups

		(SIT vs CT combined)					
Kahn, 1992 (USA)(265)	M, 61-82yo, FM 19.4%, NonT2D	EX (n=13)	6 months, five 45min session/wk, 80-85%HRR Progressive, aerobic exercise	FSIGT: AIR (glucose and arginine stimulation) <u>Post-Ex:</u> 60h	AIRg Yes ↓ AIRmax ↓ Glc sensitivity No Δ	No Δ	↓BW, BF ↑VO2max ↑SI No Δ glucose tolerance
Kirwan, 1993 (USA) (260)	M+F, 60-70yo, NGT	EX (n=12)	9 months, four 45min sessions/wk, ~80%HRmax, aerobic exercise	HIEG <u>Post Ex:</u> 16h	↓ Ins response		↑VO2max ↑ SI
Larson-Meyer, 2006 (USA) (251)	White (n=30)Black (n=15)Asian (n=1), *Non-T2D* (48): M+F, 25-50yo, OW	CON (n=11) EXCR (n=12) CR (n=12):25% LCD (n=11): 15%WL	6 months EXCR: 5 sessions/wk, aerobic exercise, ↑ energy expenditure by 12.5% and CR of 12.5%	FSIGT <u>Post-Ex:</u> 48h	↓ (all 3 intervention groups)	ND	↑SI (EXCR, LCD) ↓BW, BF, VAT, HF
Madsen, 2015 (Denmark) (263)	M+F, T2D: 56yo; NonT2D: 52yo	T2D (n=10) nonT2D (n=13)	8 weeks, 3 sessions/wk, High intensity interval training 10x6s cycle	OGTT: IGI, DI <u>Post-Ex</u> 48-72h	T2D/nonT2D: IGI: No Δ	T2D: Yes↑ NonT2D: No Δ	T2D: ↓2h glucose, HOMA-IR T2D and nonT2D: ↓BMI, central fat ↑VO2max
Malin, 2013 (USA) (284)	M+F, 66.8yo, OB, Prediabetes	IFG (n=8) IGT (n=8) IFG+IGT (n=8)	12 weeks, 5 sessions /wk, 60min/day, ~85% HRmax, aerobic exercise	OGTT: <u>Post Ex:</u> 72h	Ins/Glc: Yes↓ (1^{st} and 2^{nd} phase) Cpep/Glc: No Δ (1^{st} and 2^{nd} phase)	↑ 1^{st} and 2^{nd} phase	↑SI ↓:BMI, BF, Abd fat, VAT
Many, 2013 (USA) (279)	Black (n=8), Hispanic (n=3) F+M, 14-18yo, OB, NonT2D	Ex (n=11)	8 weeks, 180min/wk 40-55% VO2peak aerobic exercise	FSIGT <u>Post-Ex:</u> 24-48h	No Δ	ND	↑SI
Slentz, 2009 (USA) (261)	M+F, 40-65yo, OW/OB, NonT2D	NoEX (n=58) Lo/Mod (n=57) Lo/Vig n=(58)	8 months, Lo/Mod: 40-55% VO_{2peak}, 176min/wk	FSIGT <u>Post-Ex:</u> NM	Lo/Mod No Δ Lo/Vig ↓ Hi/Vig ↓↓ (15.2%)	Lo/Mod ↑↑ Lo/Vig ↑(p=0.06) Hi/Vig ↑	Lo/Mod ↑↑SI Lo/Vig ↑SI Hi/Vig ↑SI ↑VO2max

		Hi/Vig (n=64)	Lo/Vig: 65-80% VO_{2peak}, 113min/wk Hi/Vig: 65-80% VO_{2peak}, 172min/wk aerobic exercise				(all Exercise groups)
Solomon, 2013 (Denmark) (264)	M+F, 61yo, OW/OB, IGT (n=56), T2D (n=49)	EX (n=105)	12-16 weeks, 4-5 sessions/wk, ~60min/d, ~75% VO2max, aerobic exercise	OGTT (GSIS) HIEG	↓ 1st and 2nd phase	↑ 1st and 2nd phase	↑SI, VO2max ↓;BMI, BF

Dx – Duration of exercise training, Δ – Change, F- Female, M – Male, OW – Overweight, OB -Obese, NonT2D- Non- Type 2 Diabetes, IGT – Impaired glucose tolerance, RES – Resistance training group, AER – Aerobic training group, CON – control group, EX- exercise group, CR – Calorie restriction, EXCR – Exercise and calorie restriction group, LCD – Low calorie diet, SIT – Sprint interval training, CT – continuous training, Lo/Mod – low volume moderate intensity exercise group, Lo/Vig – low volume, vigorous intensity exercise group, Hi/Vig – High volume, vigorous intensity exercise group, HRR – Heart rate reserve, Wmax – maximum work load, HR – Heart rate, HIT – High intensity training, FSIGT – Frequently sampled intravenous tolerance test, OGTT, Oral glucose tolerance test, HIEG – Hyperinsulinemic euglycemic clamp, AIRg – Acute insulin response to glucose, DI – Disposition index, IGI – Insulinogenic index, AUCIns – Area-under-curve Insulin, GSIS – Glucose stimulated insulin secretion, ND – Not done, S_I – Insulin sensitivity, BW – Body weight, BF – Body Fat, SAT – Abdominal subcutaneous adipose tissue, VAT – Visceral adipose tissue, PF – Pancreatic fat, FAO – fatty acid oxidation, DAGs – diacylglycerol, HF- Hepatic fat, HOMA-IR Homeostatis Model assessment of Insulin Resistance, ↑ - increase, ↓ - decrease

1.13.4 Effect of exercise training on skeletal muscle fat

During exercise the main source of lipids used in oxidation, are from plasma long-chain fatty acids derived from VAT and SAT lipolysis, 10% from intramyocellular lipids and less than 5% from VLDL (288). The net effect of exercise training on lipids depends on the metabolic status, lean vs obese, trained vs untrained as well as the diet before and during exercise (289). Another important factor to consider is the intensity of exercise, with maximal fat oxidation occurring during moderate intensity exercise. Further, the longer the duration of exercise, the more reliant the muscle will be on lipids as a fuel source (226). A recent study also highlighted that the type of exercise is important, with an increase in IMCL observed with 2 weeks of sprint interval training (4-6 30 second cycling sessions with 4 minutes of recovery), but no change observed with 2 weeks of moderate-intensity continuous training program (40-60 minutes), regardless of the metabolic status of the participants (290).

Considering all the factors that may influence the exercise response of intramuscular fat, it is not surprising that studies have reported mixed results. Table 1.8 summarizes studies that evaluated the effect of exercise training on intramuscular fat and S_I. Studies in those without T2D reported no changes (251,253,270,273,274,283,291), an increase (277,292,293) and a decrease (294) in intramuscular fat. The discrepancies between these studies may be explained partly by the exercise duration. The studies that showed a change in intramuscular fat ranged from 2 weeks to 16 weeks, while the studies that did not report a change

range from 4 weeks to 6 months. As most studies used a moderate intensity aerobic exercise training, the exercise intensity and mode were unlikely explanations for the discrepant findings . Another important factor to consider are the methods use for intramuscular fat assessment, namely histochemistry after a muscle biopsy or magnetic resonance spectroscopy (MRS), which are able to distinguish between IMCL and EMCL content, with one study using magnetic resonance imaging (MRI) that measures total intramuscular fat. Although the above-mentioned studies focused on those without T2D, differences in study population can still occur. For example, an increase in IMCL was observed in studies that included young males (BMI was not mentioned) (277), or lean older adults (293). However, the third study that showed an increase in IMCL was conducted in older adults that were overweight or obese (292).

Findings from studies that were conducted on those with T2D also lacked consistency. One study, using a combination of aerobic and resistance training, reported an increase in IMCL, (270), while a decrease was observed in another study using only aerobic exercise training (291). No change in IMCL was reported in another 12-week study that used combined aerobic and resistance training with a concomitant Paleolithic diet (295). The reason for the heterogeneity in findings is not clear but differences in the intervention duration, the study population and diet may contribute to the discrepant findings.

No study found an association between the changes in intramuscular fat and changes in S_I, although an improvement in S_I occurred in most of the studies

(251,253,270,273,283,291,292,294–296). Two studies (274,293) that did not report an improvement in S_I, used HOMA-IR as a surrogate marker of S_I and the other study used an insulin tolerance test (277). In addition, two of the studies were of a shorter duration, 2 weeks (277) and 4 weeks (293). Importantly, an improvement in S_I was observed in studies without a concomitant change in intramuscular fat (251,253,270,283,291,295). This suggests that changes in intramuscular fat content are not essential for improvements in S_I. Perhaps changes in lipid intermediaries such as diacylglycerols (292), improvements in oxidative capacity (283,291,294,296,297), or metabolic flexibility (270) may be more important correlates of S_I improvements. However, majority of these studies investigating the effect of exercise training on both S_I and skeletal muscle fat were conducted in predominately white populations. In addition, no study focused only on premenopausal women. A gap therefore remains, to evaluate the effect of exercise training on both S_I and skeletal muscle fat in black South African premenopausal women known to have a greater sensitivity to skeletal muscle fat accumulation compared to their white counterparts (42).

1.13.5 Effect of exercise on hepatic fat

Studies that evaluated the effect of exercise training on hepatic fat showed mixed results, which were dependent on cohorts with and without NAFLD). A recent systematic review showed that the majority of studies reported a reduction in hepatic fat after exercise training (298). However, these studies were mostly conducted in those with NAFLD and/or T2D (256,257,272,299–303). Notably, the

studies that did not show any change in hepatic fat content after exercise were conducted in those without NAFLD (253,304). Although, one study reported that after 12 weeks of a combined aerobic and resistance training the reduction in hepatic fat in those without NAFLD (relative Δ -28.3%) and those with NAFLD (relative Δ -34.5%) were similar (256). Further, an increase in hepatic fat has also been observed after exercise training (295). This study was conducted in adults with T2D that combined exercise training with a Paleolithic diet. However, this increase was driven by 3 subjects whereas most of the group (n=10) showed a reduction in hepatic fat. It seems that exercise training is beneficial to reduce hepatic fat in those with NAFLD and T2D. However, in those without NAFLD, the effect of exercise training on hepatic fat is more variable. In those that showed a reduction in hepatic fat, the effect was modest and larger reductions were observed with longer duration of exercise training (6 months) that were combined with calorie restriction.

Although a reduction in hepatic fat after exercise training coincided with an increase in hepatic and peripheral S_I, no association was found between these two factors (251,256). The change in hepatic fat was rather associated with changes in either body weight, VAT or total body fat mass (251,256,305). Although an association between hepatic fat and S_I has been reported in cross-sectional studies, this association is not so apparent in intervention studies, probably due to the heterogenous response of hepatic fat to exercise training (295). While cross-sectional studies to evaluate the association between hepatic fat and S_I have been conducted in black South African women (42,171), interventional studies are

needed to provide greater insight into the causality of hepatic fat on S_I in this population. Furthermore exercise intervention studies have not addressed whether changes in insulin clearance and hyperinsulinemia can be explained by changes in hepatic fat in black African populations.

1.13.6 Effect of interventions on pancreatic fat

Intervention studies have been performed to assess the role of pancreatic fat on β-cell function, with a focus to reverse T2D. These studies induced weight loss either through bariatric surgery (13.6%) (214) or low calorie diets (15%) (213). Both these studies reported a change in pancreatic fat from 6.6 to 5.5% 8 weeks after bariatric surgery (214) and from 8.0 to 6.2% after 8 weeks of low calorie diet (213), which in both these studies coincided with an improvement in acute insulin response in those with T2D. Contrastingly, those without T2D, had a comparable weight reduction to those with T2D after bariatric surgery, but had no reduction in pancreatic fat and no improvement in the acute insulin response (214). This could indicate that a reduction in pancreatic fat might be needed for an increase in acute insulin response. Notably, in those without T2D, the acute insulin response was not impaired and may be the reason why no change was observed. Interestingly, in the intervention study, the importance of a reduction in pancreatic fat on β-cell function was only observed in those with T2D, while in cross-sectional studies an association was only apparent in those without T2D (Table 1.5).

The effect of exercise training on pancreatic fat has only been studied once before in a Finnish population and included adults with T2D and healthy men (252). The

participants completed 2 weeks of either high intensity interval training or moderate intensity continuous training. A similar reduction in pancreatic fat was found in both the healthy men (from 4.4 to 3.6%) and in those with T2D/prediabetes (from 8.7 to 6.7%). However, this also coincided with a reduction in SAT and VAT, but body weight was not altered after the intervention in both groups. While β-cell measures improved similarly in both the healthy men and those with T2D it was not associated with reduction in pancreatic fat.

Pancreatic fat has never been assessed in a black South African population. Importantly, a cross-sectional study showed that in black South African women both skeletal muscle and hepatic fat accumulation was associated with lower S_I. A study is therefore justified to explore the response of pancreatic fat to exercise training and whether this change will be associated with changes in the insulin response and β-cell function in this cohort who are at high risk for T2D.

Table 1.8: *Effect of exercise training on skeletal muscle fat and association with insulin sensitivity*

	Study, (Country)	Participants	Study groups	Exercise intervention: Dx, volume, intensity, mode	Skeletal muscle measurement	Δ IMAT	Δ S_I	Association with Δ S_I	Other changes
1	Bruce, 2004 (Canada) (291)	*T2D*: M, 48.2yo, BMI 32.3 *Non-T2D*: M, 46.3yo, BMI 28.8	T2D (n=7) Non_T2D (n=6) **EX only**	8 weeks, 3x/wk, AER:70%VO_{2peak}, 60min HIIT ~80-85%$VO_{2peak,}$ 50min (combined)	Histochemistry – vastus lateralis (IMAT)	*T2D:* ↓ *NonT2D:* No Δ	T2D+nonT2D ↑ (HIEG) 36-48h post-Ex	ND	↑muscle oxidative capacity
2	Bruce, 2006 (Canada) (283)	*Non-T2D:* M+F, 36yo, OB	EX (n=9) **EX only**	8 weeks, 5x/wk, 60 min, Mod-intensity aerobic training	Histochemistry Vastus-lateralis (Muscle TAGs)	No Δ	↑ (OGTT-ISI) 36-48h post-Ex	ND	↓ceramides, ↓trend DAGs ↑FAO
3	Christiansen, 2009 (Denmark) (273)	*Non-T2D*: M+F, 18-45yo, OB	EX (n=19) CR (n=19) EXCR (=21)	12 weeks, 3/wk 60-75min 70% HRR, aerobic training	MRI (Thigh) (IMAT)	EX: No Δ CREX: ↓ CR: ↓	HOMA-IR ↓trend 24h post Ex	ND	↓BW
4	Dube, 2008 (USA) (292)	Non-T2D (IGT/NGT): M+F, 60-75 yr, OW/OB	EX: n=25 **EX only**	16 weeks, 4-5/wk, 45min, Mod Intensity, aerobic training	Histochemistry (muscle NM) (IMCL)	Yes↑	↑ (HIEG) 48h post-Ex	No	↓DAGs, ceramides ↑glycogen, capillary density
5	Dube, 2011 (USA) (296)	*Non-T2D*: M+F, 60-75yo, OW/OB EX:IGT/NGT CR: IGT	EX (n=8) CR (n=8)	16 weeks, 4-5/wk, 45 min, Mod intensity, aerobic training	Histochemistry (muscle NM) (IMCL)	EX: ↑ CR: ↓	↑ (HIEG) 36-48h post-Ex	No	↓: BW, BF, ↓DAGs, ceramides, ↑Muscle glycogen, muscle oxidative capacity
6	Johnson, 2009 (Australia) (274)	*Non-T2D*: M+F, 49.1yo, OB	EX (n=12) noEX (n=7)	4 weeks, 3/wk, 30-45min, 50-70%	MRS Vastus lateralis (IMCL)	EX: No Δ NoEX: No Δ	HOMA-IR No Δ 72h post Ex	ND	No ΔBW

				VO_{2peak}, aerobic training					
7	Larson-Meyer, 2006 (USA) (251)	White (n=30), Black(n=15), Asian (n=1), *Non-T2D*: M+F, 25-50yo, OW	CON (n=11) EXCR (n=12) CR (n=12):25% LCD (n=11): 15%WL	EXCR: 6 months, 5/wk, aerobic training, ↑Energy expenditure by 12.5% DIET: 12.5% CR	MRS-soleus (IMCL)	CR/EXCR/LCD: No Δ	↑(FSIGT) 48h post Ex	No	↓: BW, AIRg , VAT, SAT, BF,
8	Meex, 2008 (Netherlands) (270)	*T2D*:M, 59.4yo, BMI 30.0 *NonT2D*: M, 59yo, BMI 29.7	T2D (n=18) Non T2D (n=20) **EX only**	12 weeks, 3/wk, 45min, 55% Wmax, aerobic and resistance training	Histochemistry-vastus lateralis (IMCL)	*NonT2D*: No Δ *T2D:* ↑ (trend)	T2D: PeripSI/HepSI ↑ NonT2D:HepSI ↑/ PeripSI ↑(trend) (HIEG) 72h post Ex	ND	↑mitochondrial function, metabolic flexibility
9	Pruchnic, 2004 (USA) (293)	Non-T2D:M+F, 67yo, OW	EX (n=13) **EX only**	12 weeks, 3-5/wk, 30-40min, 60-70%HRmax, aerobic training	Histochemistry-vastus lateralis (IMCL)	↑	No Δ HOMA-IR	ND	↑FAO but not associated with ΔIMAT
10	Otten, 2018 (Sweden) (295)	T2D, M+F, 30-70yo, OW/OB	EXPD (n=13) PD (n=13)	EXPD: 12 weeks, 3x/wk, 60 min low/mod/high intensity, aerobic and resistance training + paleolithic diet	MRS – soleus, tibialis anterior (IMCL)	EXPD: No Δ PD: ↓	*EXPD*: PeripSI↑ HepSI No Δ AT-SI ↑ (HIEG) 48h post Ex	PeripSI: No	↓Plasma CRP No Δ: Adipose tissue IL6, TNFα:
11	Schrauwen-Hinderling, 2003 (Netherlands) (277)	Non-T2D: M, 23yo, BMI 22.6, highly trained	EX (n=8)	2 weeks- 1 rest day, aerobic (2h) and interval training (45 min), intensity NM	MRS -vastus lateralis (IMCL)	↑	No Δ (ITT) 48h post Ex	No	No Δ oxidative capacity

12	Shojaee-Moradie, 2006 (UK) (253)	EX:*Non-T2D* (10), M, 47yo, OW	EX (n=10) noEX (n=7)	6 weeks, 3/wk, 20 min, 60-80% of VO2max, Mode NM	MRS (tibialis anterior) (IMCL)	*EX/NoEx*: No Δ	Perip SI ↑ HepSI ↑ (HIEG) 72h post Ex	ND	↓Circulating FFA
13	Solomon, 2008 (Denmark) (268)	*Non-T2D*: IGT M+F, EX: 66yo, OB EXCR: 67yo, OB	EX (n=12) EXCR (n=11)	12 weeks, 5/wk, 60 min 75% $VO_{2max,}$ aerobic training	Histology - vastus lateralis (IMCL)	↓ both groups	*EX/EXCR:* ↑ (HIEG) >48h post-Ex	No	↓BW, BF

Dx – Duration of exercise training, Δ – Change, IMAT – intramuscular fat, S_I – Insulin sensitivity, F- Female, M – Male, OW – Overweight, OB -Obese, NonT2D- Non- Type 2 Diabetes, IGT – Impaired glucose tolerance, NGT – Normoglucose tolerant, RES – Resistance training group, AER – Aerobic training group, CON – control group, EX- exercise group, CR – Calorie restriction, EXCR – Exercise and calorie restriction group, LCD – Low calorie diet, SIT – Sprint interval training, CT – continuous training, Lo/Mod – low volume moderate intensity exercise group, Lo/Vig – low volume, vigorous intensity exercise group, Hi/Vig – High volume, vigorous intensity exercise group, HRR – Heart rate reserve, Wmax – maximum work load, HR – Heart rate, HIT – High intensity training, FSIGT – Frequently sampled intravenous tolerance test, OGTT, Oral glucose tolerance test, HIEG – Hyperinsulinemic euglycemic clamp, IMCL – intramyocellular lipid content, ISI – Insulin sensitivity, HOMA-IR Homeostatis Model assessment of Insulin Resistant, ND – Not done, Periph – Periperal, Hep -Hepatic, BW – Body weight, BF – Body Fat, SAT – Abdominal subcutaneous adipose tissue, VAT – Visceral adipose tissue, FAO – fatty acid oxidation, DAGs – diacylglycerol, FFA – free fatty acids, EX – exercise, ↑ - increase, ↓ - decrease

1.14 RATIONALE FOR THIS STUDY

Although great strides have been made to unravel the mechanisms involved in the pathogenesis of T2D in black African women, who have a high risk for developing T2D (306), the underlying mechanisms still remain poorly understood. Evidence has evolved to show that ethnic differences exist in the pathogenesis of T2D with black African women frequently having a lower S_I and higher insulin response compared to their white counterparts (39,48,49). Despite both a lower hepatic insulin clearance and higher insulin secretion being observed in black African populations compared to other ethnicities, the relative contributions of these two factors to a high insulin response is still under debate. Further, how peripheral insulin clearance relates to hyperinsulinemia and β-cell function has not been previously reported.

Intriguingly, black African women may also demonstrate an insulin response that exceeds the level of S_I with a notably higher β-cell function, compared to white women (47,56). Clearly, exploring the correlates of these key components in black, obese South African women is warranted. Previous studies in Africa have focused mainly on the correlates of S_I (42,43,121,143,171) and all, but one, used HOMA-IR to quantify S_I (43,121,143,171). In addition, while the insulin response and DI, have been described before in black South African women (39), its correlates were not evaluated. Notably, black African women exhibit a unique phenotype that contrasts to the established pathophysiology of insulin resistance typically shown

in white populations. Compared to their white counterparts, black African women have less VAT (43), an established determinant of insulin resistance (141), and more peripheral SAT (43), typically regarded as protective (110). Further, black African women have less hepatic fat and equivalent intramuscular fat compared to their white counterparts (42). However, the majority of evidence are derived from cross-sectional studies, from which causality cannot be inferred. Moreover, although two longitudinal studies were conducted on black South African women to determine how changes in body fat distribution predict IGT/T2D onset (143) or reduced S_I (121), only fasting indices were used.

Further, studies exploring the correlates of insulin secretory function and β-cell function in black African populations have reported ethnic differences in the association between body fat distribution, pancreatic and hepatic fat and the insulin response and β-cell function (49,51,54,128,209). Nevertheless, correlates of both hepatic and peripheral insulin clearance have rarely been studied in black African populations.

Importantly, an intervention that perturbs S_I and hyperinsulinemia and/or body fat distribution and ectopic fat will provide greater insight into the role of these components in the pathophysiology of T2D in African populations. Indeed, exercise training has been shown to alter body composition and regional and ectopic fat distribution, as well as S_I, insulin secretion and clearance. Exercise training therefore provides an ideal model to understand how changes in regional and ectopic fat distribution relate to changes in S_I and hyperinsulinemia and how this

may contribute towards the pathogenesis of T2D in black African populations. It is particularly relevant given that women of black African descent exhibit lower levels of cardio-respiratory fitness compared to white populations. A sedentary lifestyle may contribute towards this lower cardio-respiratory fitness (219), although it may not be the only explanation for this phenomenon in black Africans. Nevertheless, only few studies have evaluated the effect of exercise training on S_I, insulin response and insulin clearance and regional and ectopic fat depots in African Americans. Importantly, no exercise intervention study has previously been conducted in obese, black Africans residing in Africa, and is therefore warranted.

CHAPTER 2: AIMS AND HYPOTHESES

2.1 OVERALL AIM

The overall aim of this PhD was to investigate the correlates of low S_I and hyperinsulinemia, characteristic in black African populations. This was assessed by using a cross-sectional design and an exercise intervention study, with emphasis on the role of ectopic fat deposition (skeletal muscle, hepatic and pancreatic fat), body fat distribution and body composition.

2.2 CROSS-SECTIONAL STUDY

2.2.1 AIM

In a sample of obese black South African women, the aims of the study were to: i) identify the correlates of DI by examining the relationships between DI and its direct (S_I and AIRg) and indirect components (hepatic and peripheral insulin clearance and ISR), and to assess the associations of ectopic fat (skeletal muscle, hepatic and pancreatic fat) and body fat distribution with the variance in DI; ii) explore the correlates of the insulin response (insulin secretion and clearance) and S_I; and iii) assess the relationships between the various regional adipose tissue and ectopic fat depots.

2.2.2 OBJECTIVES

- To evaluate the associations between DI and its direct components (S_I and AIRg) and its indirect components (insulin secretion, hepatic and peripheral insulin clearance).
- To determine the relative contribution of insulin secretion and hepatic and peripheral insulin clearance to AIRg, independent of S_I.
- To determine the relationship of DI with body fat distribution and ectopic fat depots.
- To determine the associations of S_I and hyperinsulinemia with body fat distribution and ectopic fat.
- To determine the associations between various ectopic fat depots and body fat distribution.

2.2.3 HYPOTHESES

- The main correlates of a higher DI in obese black South African women will be a higher AIRg, which will be associated with a higher ISR, while a lower hepatic and higher peripheral insulin clearance will also contribute to the variance in DI, but to a lesser extent.
- A higher ISR will be the primary contributor to higher AIRg above lower hepatic and higher peripheral insulin clearance, independent of S_I
- A higher DI will be associated with lower VAT, skeletal muscle, hepatic and pancreatic fat.
- The above associations will be explained by:

- a higher AIRg and ISR associated with lower VAT, hepatic and pancreatic fat
- A higher S_I associated with lower VAT, hepatic and skeletal muscle fat.
- Whereas hepatic insulin clearance will not be associated with VAT and hepatic fat and peripheral insulin clearance will not be associated with skeletal muscle fat accumulation

- VAT will be positively associated with hepatic and pancreatic fat, and hepatic fat will be positively associated with pancreatic and skeletal muscle fat.

2.3 EXERCISE INTERVENTION STUDY

2.3.1 AIM

The aim is to evaluate the effect of 12-week combined aerobic and resistance exercise training on S_I, hyperinsulinemia and DI and whether changes in these metabolic outcomes can be explained by associated changes in body composition, body fat distribution, skeletal muscle, hepatic and pancreatic fat.

2.3.2 OBJECTIVES

- To determine the changes in S_I, insulin response, insulin secretion, insulin clearance and DI after 12-weeks of exercise training compared to the control group.

- To examine the changes in body composition, body fat distribution, skeletal muscle, hepatic and pancreatic fat after 12-weeks of exercise training compared to the control group.
- To explore if changes in S_I are associated with changes in body composition, body fat distribution, skeletal muscle and hepatic fat.
- To determine if changes in insulin response, secretion and clearance are associated with changes in hepatic and pancreatic fat.
- To examine if changes in DI are associated with changes in pancreatic fat.

2.3.3 HYPOTHESES

- Exercise training will increase S_I and reduce AIRg, which will also be associated with a reduction in insulin secretion.
- Exercise training will increase DI due to a greater change in S_I compared to AIRg.
- Exercise training will reduce VAT as well as skeletal muscle, hepatic and pancreatic fat.
- The increase in S_I will be associated with reductions in VAT, skeletal muscle and hepatic fat.
- The reduction in AIRg and insulin secretion will be associated with reductions in pancreatic fat, hepatic fat and VAT.
- The increase in insulin clearance will be associated with a reduction in hepatic fat.
- The increase in DI will be associated with a reduction in pancreatic fat.

CHAPTER 3: PERSONAL CONTRIBUTION TOWARDS PHD

3.1 RECRUITMENT AND SCREENING

I was integrally involved in the recruitment and screening of the participants.

3.2 MEASURING OF INSULIN SENSITIVITY, SECRETION AND CLEARANCE

A FSIGT was used to obtain glucose, insulin and C-peptide blood samples at various time points. As a medical doctor, I was responsible for all aspects of the FSIGT, including calculations, infusions, blood sampling and monitoring. The minimal model was used to estimate S_I, insulin response and DI. I ran the models and did standard checks. Mathematical modelling was used to estimate ISR, hepatic and peripheral insulin clearance. I ran three different models to find the best fit for the data.

3.3 ECTOPIC FAT QUANTIFICATION

MRS and MRI were done to measure fat in the liver, pancreas, soleus and tibialis anterior muscles. I was integrally involved in generating the spectral peaks in LC Model (MRS) for skeletal muscle, hepatic and pancreatic fat quantification, in

assessing the most appropriate method for ectopic fat quantification for this thesis and in drawing region of interests (ROIs) on the MRI images for skeletal muscle fat quantification.

CHAPTER 4: METHOD DEVELOPMENT

4.1 ECTOPIC FAT QUANTIFICATION

A 3 Tesla whole-body human MRI scanner (MAGNETOM Skyra; Siemens Medical Solutions, Erlangen, Germany) was used to perform both the MRI and MRS sequences. Quantifying the fat fraction, using MRI-Dixon and MRS, relies on the chemical shift technique based on the different resonate frequencies of the hydrogen protons in water and fat.

4.1.1 MRS

MRS signals are derived from the specific resonance frequencies of hydrogen protons which are depicted as a metabolite peak (307). Water and fat are identified by their resonance frequencies expressed as parts per million (ppm). Although, fat and water both have hydrogen protons, the resonance frequencies differ based on the configuration of the protons in the compound. Water has 2 symmetrical hydrogen protons that exhibit a single resonance frequency peak (Figure 4.1). However, fat has different configurations of the hydrogen protons depending on the fatty acid chain (e.g. methylene (CH_2) or methyl (CH_3) groups) and therefore exhibit distinct peaks (Figure 4.2). Due to more water in the tissue, the water peak is much larger than the fat peaks and therefore needs to be suppressed to visualize the fat peaks (307).

The resonance frequencies of CH_2 and CH_3 are at 1.3 ppm and 0.9 ppm, respectively. They are the largest fat peaks due to the abundance of these moieties, and therefore these peaks were used for the fat measurement in the liver and pancreas. The area under each peak is directly proportional to the concentration of proton species in the compound. The fat peak is expressed in relation to water. The fat fraction is expressed as $\frac{Area\ fat\ peak}{Area\ fat\ peak + Area\ water\ peak}$.

In skeletal muscle, MRS can distinguish between IMCL and EMCL content. IMCL and EMCL have different locations and orientations towards the magnetic field and therefore, differences in resonance frequencies occur. The CH_2 and CH_3 peaks for IMCL are found at 1.3 and 1.5 ppm, respectively, and at 0.9 and 1.1 ppm, respectively for EMCL.

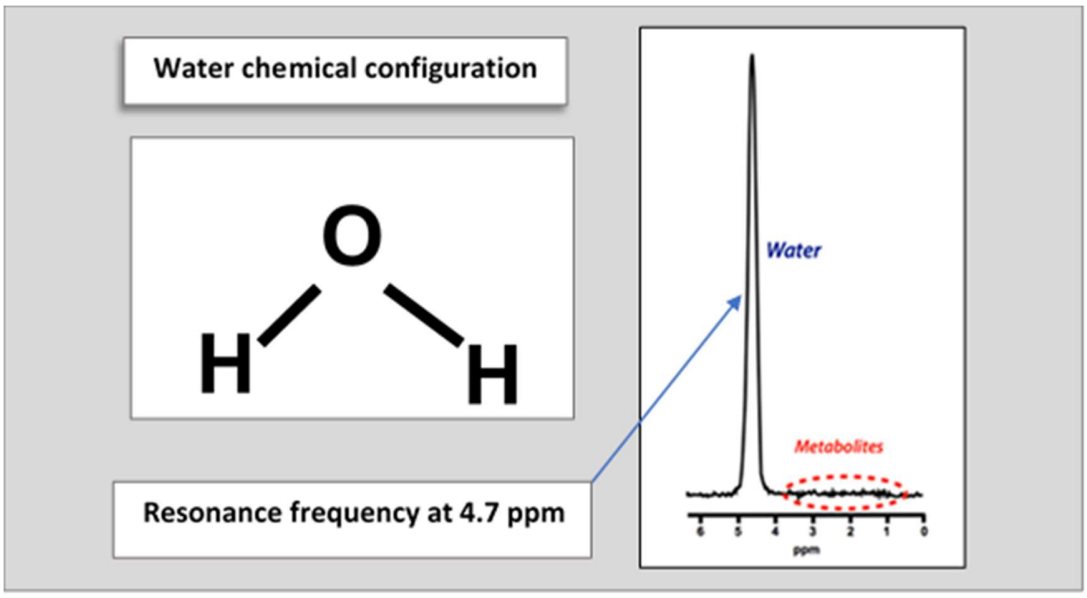


Figure 4.1: *Graphical display of the chemical configuration of water and its spectral peak*

MRS data was acquired using the point-resolved spectroscopy sequence (PRESS) with the following parameters: relaxation time (TR)/echo time (TE) 3000/33 ms, bandwidth 2000 Hz, 10 averages for the abdominal scans and 80 averages for the calf scans. The voxel dimensions were 20×20×20 mm^3 for liver, 15×15×15 mm^3 for pancreas and 15×15×15 mm^3 for both soleus and tibialis anterior muscles (Figure 4.3).

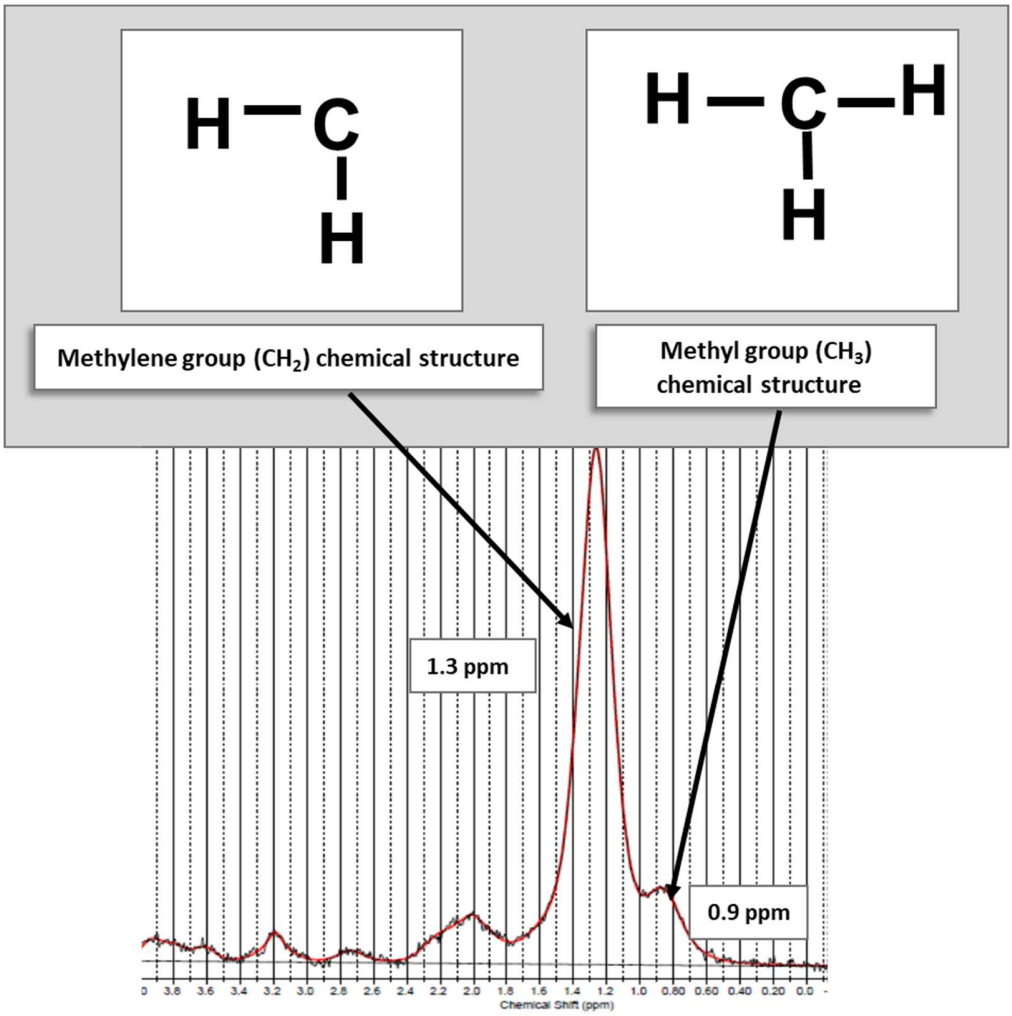


Figure 4.2: *Graphical display of the chemical configuration of methylene and methyl groups of fatty acids and its spectral peaks*

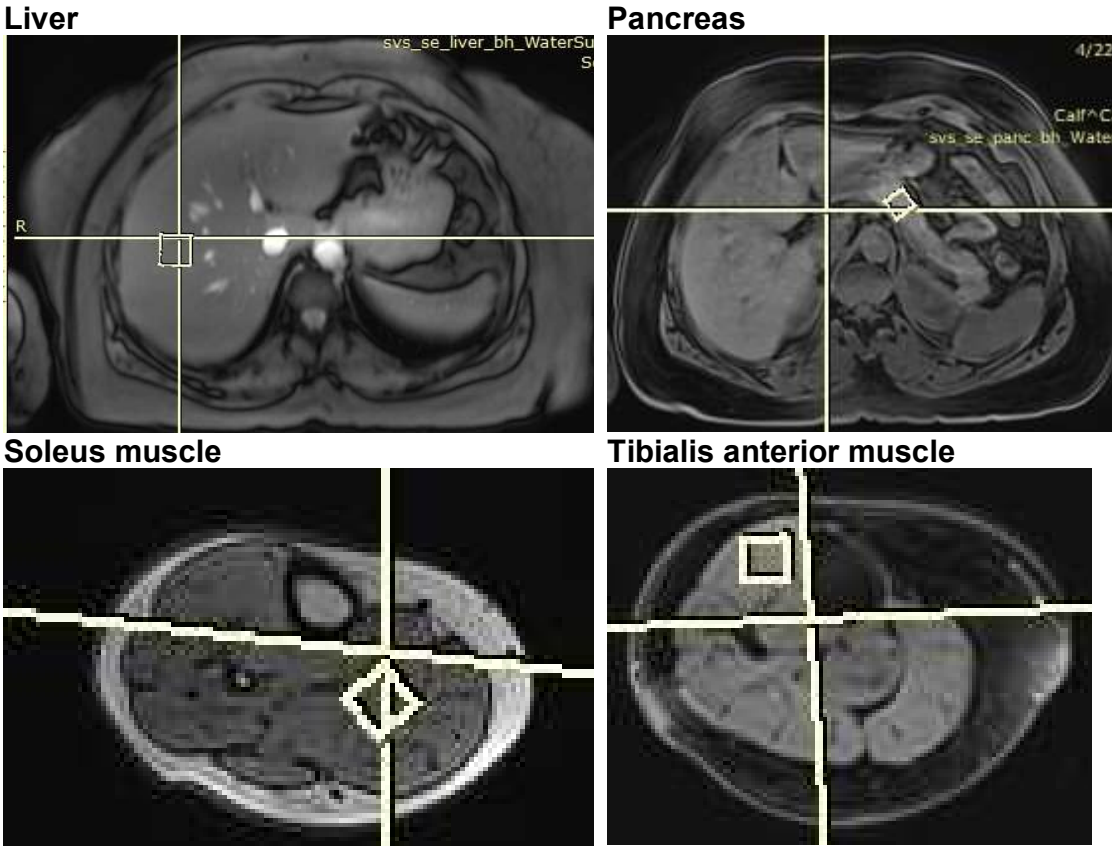


Figure 4.3: *MRS voxel placement in the liver (top, left picture), pancreas (top, right picture), soleus (bottom, left picture) and tibialis anterior (bottom, left picture) muscles*

4.1.2 MRI - DIXON

The Dixon method refers to the separation of fat and water based on the difference in resonance frequency between water and fat. During the Dixon MRI sequence, the fat (F) and water (W) signals are separated by acquiring images at different

TEs. The TE where both the fat and water signals are in the same direction is called in-phase ($IPsignal = Wsignal + Fsignal$) and the TE where the fat and water signals are in opposite directions, with the water signal dominating, is called opposed phase ($OPsignal = Wsignal - Fsignal$) (Figure 4.4). Based on these two phases, fat ($Fsignal = \frac{IPsignal - OPsig}{2}$) or water only ($Wsignal = \frac{IPsignal + OPs}{2}$) images can be obtained. Further, by combining the fat and water only images the fat fraction signal can be acquired ($Fsignal = \frac{Fsignal}{Fsignal + Wsig}$) (308).

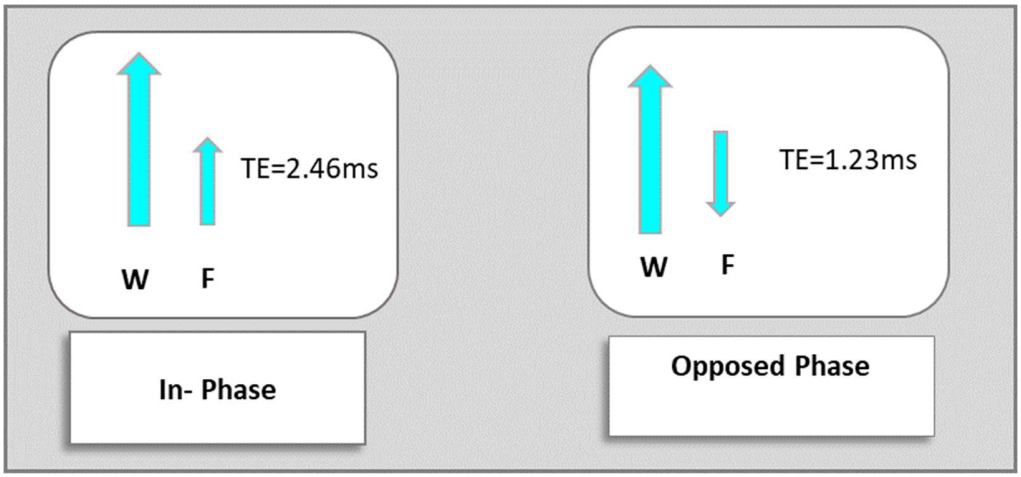


Figure 4.4: *In- and Opposed phases of MRI-Dixon technique*

We used a three-point Dixon volume interpolated breath-hold gradient recalled echo sequence: TR 3.97 ms, TE1 1.23 ms, TE2 2.46 ms, flip angle 9 degrees, number of averages 1, bandwidth 1040Hz/px, field of view 450 X 366 mm, matrix size 195 x 320 x 144 and a slice thickness of 2 mm.

After the images were acquired the hepatic, pancreatic and skeletal muscle fat were quantified using a MATLAB algorithm to separate the water and fat signals and to create a fat fraction map calculated as the fat signal over the sum of the water and fat signals. OsiriX (Pixmeo SARL, Geneva, Switzerland) software was used to manually draw a ROI in the liver (Figure 4.5) and skeletal muscle (Figure 4.6) and HOROS V1.1.7 (HOROS Project) software was used to manually draw a ROI in the pancreas.(Figure 4.7) The ROI was drawn on the in-phase image due to better visualization of anatomy. The ROI was then copied to the fat fraction image where the average pixel value from the ROI were converted to a fat fraction (fat fraction=pixel value from ROI/65535; a value between 0 and 1 was obtained where 0 in no fat and 1 is only fat) (309).

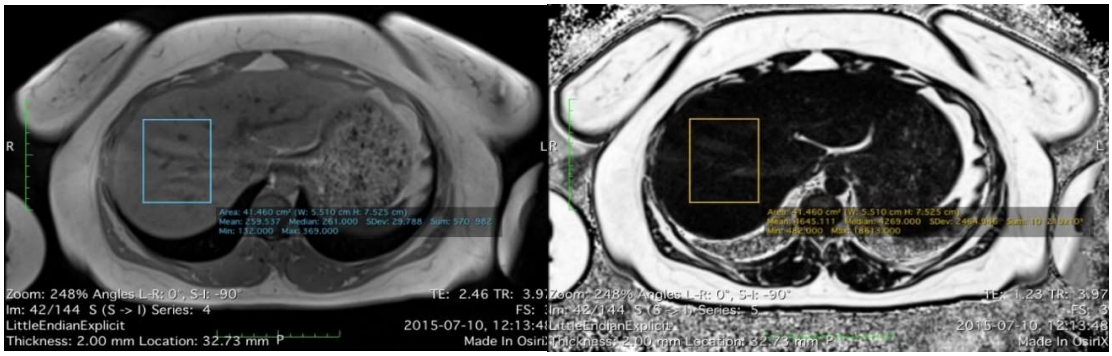


Figure 4.5: *Region of interest in right lobe of liver in fat fraction image (left picture) and in-phase image (right picture).*

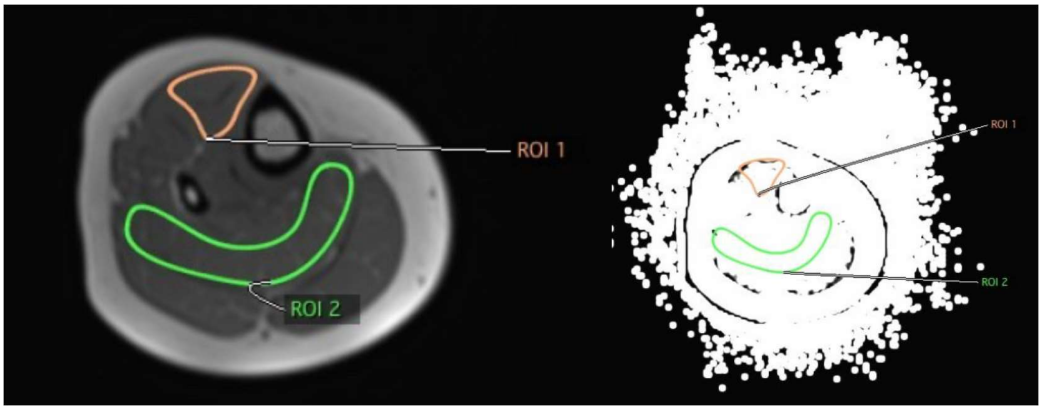


Figure 4.6: *Region of interest in tibialis anterior (orange ROI 1) and soleus (green-ROI 2) muscles in a transverse image through the calf in both the in-phase (left) and fat fraction (right) images.*

4.1.2.1 HEPATIC FAT

A rectangular ROI (5.5 cm width, 7.5 cm height) was drawn on one slice, in the right lobe of the liver (Figure 4.5) by a single reviewer that was blinded to group allocation. This single ROI was duplicated on 3 superior and 3 inferior slices from this initial slice, taking care to avoid ducts and blood vessels. The average from the seven slices were calculated together with standard deviations.

Studies have shown that the right lobe, compared to the left lobe, correlated better with a liver biopsy when evaluating hepatic steatosis (310). However, this difference is less obvious in those without NAFLD (311). Also, the fat fraction variability was found to be less in the right lobe (1.15%) compared to the left lobe (1.74%) and the left lobe tends to underestimate the hepatic fat content (310). This supports our rationale of estimating the fat fraction only in the right lobe of the liver.

Pilot analysis was completed using one subject to compare the liver fat fraction obtained by the in-house MATLAB algorithm with the fat fraction obtained from the build-in Phillips scanner mDixon QUANT algorithm and almost identical values were obtained. This confirmed that our in-house MATLAB algorithm was adequate to determine fat fractions.

4.1.2.2 SOLEUS AND TIBIALIS ANTERIOR SKELETAL MUSCLE FAT

Various methods have been used to identify ROIs in skeletal muscle (312–319) . The most recent studies (320,321) use a single slice to determine the fat fraction in the thigh and calf muscles. Hogrel *et al*. reported that a single slice provided a good representation of the fat in the thigh muscle compared to using 7 slices, but the slice should include the largest cross-sectional area of the muscle of interest (315). However, Hogrel *et al*. included non-obese male and females across a wide spectrum of age. The mean BMI (kg/m^2) was 21.4 and 22.6 for younger (20-30 yo) and older females (70-80 yo), respectively (315). In contrast, another study also conducted in non-obese population reported that fat infiltration in calf muscles were inhomogeneous (317).

Due to inconsistency in the literature regarding number of slices, we opted to use 7 consecutive slices over a 14 mm area (slice thickness 2 mm). The largest diameter of the muscle was found visually by scrolling up and down the sagittal image and confirmed using the measurement tool in OsiriX. An irregular shape

ROI was drawn by a single reviewer using the method adapted from Machann *et al*. (322) (Figure 4.6). The ROI captured the intramuscular fat and excluded the fatty septa between muscles.

A pilot study was performed on 5 randomly selected participants to determine the best method of drawing the ROI. Initially an ROI was drawn individually on each of the seven slices, then an ROI was drawn on the most proximal slice and copied to the 6 distal slices and lastly an ROI was drawn on the middle slice (of the 7 identified slices) and copied to 3 slices superior and inferior to this slice. The latter method produced the most consistent result with smaller standard deviations and thus less variability. The ROI was therefore, duplicated on 3 superior and 3 inferior slices and the average was calculated. Calculation of the fat fraction was performed by the MRI physicist that was blinded to group allocation.

4.1.2.3 PANCREATIC FAT

Pancreatic fat quantification is technically challenging due to the small size of the organ and due to irregular borders especially in those with type 2 diabetes. Various methods referring to the number of slices used, number of ROIs drawn, manual vs automated methods and with or without thresholding, have been used to determine the fat fraction in the pancreas (176,202–204,208,213,323).

In our study, pancreatic fat was determined by drawing one circular 1cm^2 ROI in the head, body and tail of the pancreas (Figure 4.7) using HOROS V1.1.7. A single reviewer, blinded to group allocation, drew the ROI and calculated the fat fraction.

An ROI was drawn on both the water and fat images and the following formula was used to calculate the fat fraction, (Fat fraction= $\frac{Fsignal}{Fsignal+Wsignal}$). The fat fractions from the pancreas head, body and tail were averaged to obtain the mean fat fraction for the whole pancreas. This method consisting of several smaller ROIs, compared to one big ROI encompassing the whole pancreas, is used to avoid inclusion of VAT (211). Also, greater precision was achieved with using the 3 smaller ROIs with or without thresholding compared to using one big ROI (324).

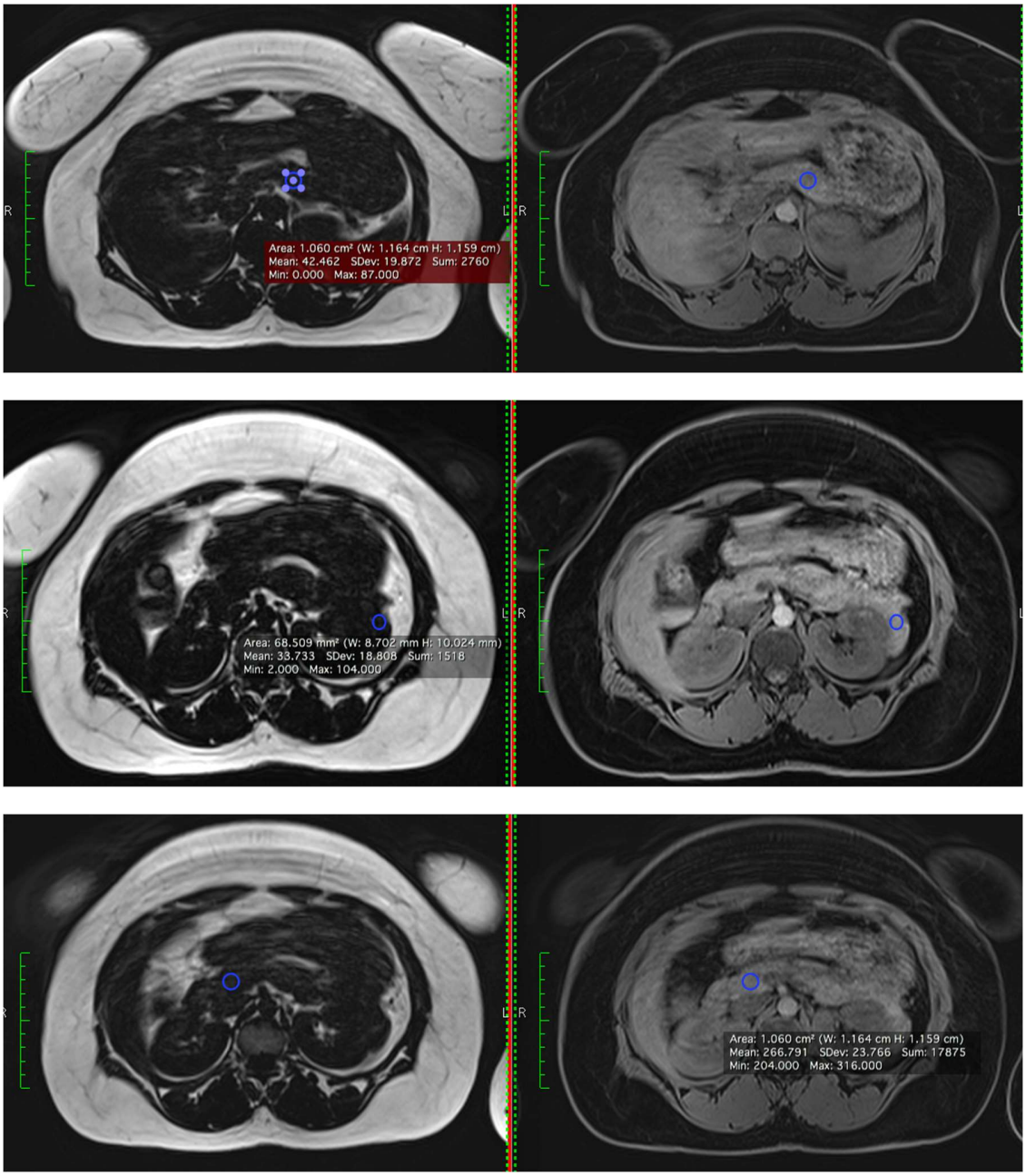


Figure 4.7: *Region of interest in head (top picture), body (middle picture) and tail (bottom picture) of the pancreas, in the fat fraction images (left pictures) and in-phase image (right pictures) of a transverse MRI scan through abdomen.*

4.1.2.4 ABDOMINAL VAT AND SAT

MRI and computed tomography (CT) are considered the gold standard in determining abdominal VAT and SAT. However, CT exposes individuals to ionizing radiation whereas MRI does not. Further, while DXA is a more cost-effective method compared to MRI, it has been found to underestimate VAT by ~30% in older men (325). MRI fat quantification was therefore used in our study. Further, because MRI fat quantification can be expressed as an area (cm^2) derived from a single slice or multiple slices, or as a volume (cm^3), requiring multiple slices, we needed to determine whether to use a single slice or multiple slices. VAT quantified by a single slice, located at approximately the level of L3, showed excellent correlation with VAT volume ($r>0.95$) in black and white men and women (326) but this was based on cross-sectional data. However, after weight loss in overweight and obese adults, the reduction in VAT and SAT was poorly estimated using a single slice compared to using multiple slices (327). Further, after a 6-month exercise intervention in premenopausal women with a wide range of BMI, using multiple slices was more precise in detecting a reduction in VAT compared to using a single slice (328). We, therefore, calculated the VAT and SAT volumes, using multiple slices. Firstly, the slice at the L4-5 vertebral position was located, and VAT and SAT were manually delineated using HOROS V1.1.7 (Figure 4.8). The area of VAT and SAT in this slice was measured. The same method was repeated in the 15^{th} slice proximal from the first slice and then repeated 3 more times. The volume of VAT and SAT over a 15cm region of the abdomen was determined using

the following formula: $V = (t + h)\sum_{ith}^{N} A_i$, where t – thickness of each slice, h – between slice interval, N – number of total slices, A_i – cross-sectional area of each slice. The sum of the cross-sectional areas of 5 slices were multiplied with 3 (2 mm(t) + 28 mm (h) = 30 mm or 3 cm) (327).

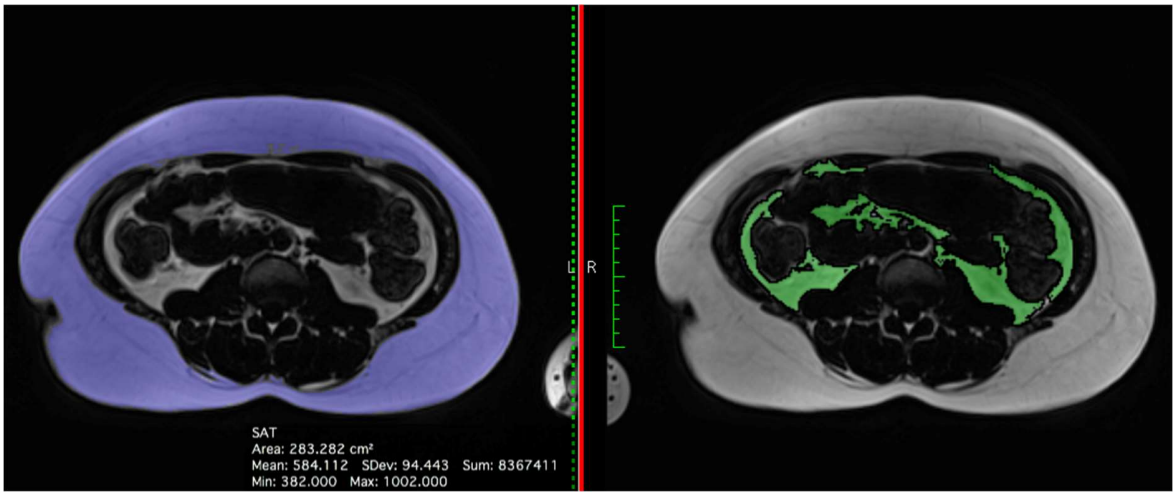


Figure 4.8: *Abdominal subcutaneous adipose tissue (left picture) and visceral adipose tissue (right picture) delineation on a transverse MRI scan through abdomen*

4.1.2.5 VALIDITY OF MRI METHODS TO DETERMINE HEPATIC, SKELETAL MUSCLE AND PANCREATIC FAT AND SAT AND VAT

The 3-point Dixon method has been validated before using a fat/water phantom and was found to be accurate and reproducible even at low levels of fat in liver, pancreas and skeletal muscle (329). Coefficients of variation for hepatic, skeletal muscle and pancreatic fat, and SAT and VAT quantification previously published are 10-17%, 3.2%, 3.5%, 3.5% and 8.8%, respectively (309,318,324,330).

4.1.3 COMPARING BASELINE AND CHANGES IN HEPATIC AND PANCREATIC FAT BETWEEN MRS AND DIXON MRI METHODS

In order to choose the best method to quantify hepatic and pancreatic fat we investigated the discrepancies between the 2 fat quantification methods in relation to baseline and change in hepatic and pancreatic fat contents. Simple correlations and a Bland Altman analysis were done on baseline data. The baseline MRI and MRS hepatic fat data were non-normally distributed and even natural log transformation was unable to normalize the data. However, after exclusion of 2 outlying values visible in figure 4.8 A and outside the 95% limits of agreement in figure 4.8 C, natural log transformation was able to normalize the data. While a significant linear association was found between natural log transformed MRI and MRS hepatic fat, poor agreement was found between the 2 methods as illustrated by the line of perfect agreement (Figure 4.8 B). This was confirmed in the Bland Altman analysis that showed that on average the MRI-derived hepatic fat was higher compared to MRS values (mean difference: $e^{0.801}$=2.23). There was also a significant systematic bias between these 2 methods with the line of perfect agreement (red dotted line at zero) outside the 95% confidence interval (antilog:1.97 to 2.51) of the average difference between MRI and MRS-derived values (Figures 4.8 D).

Pancreatic fat at baseline determined by MRS varied considerably from 0.72% to 67.5%, while the range for MRI-determined pancreatic fat (mean of the fat in the body, head and tail of the pancreas) was 4.7% to 12.9% (figure 4.9 A). MRI and

MRS pancreatic fat needed to be natural log transformed to achieve a normal distribution. A significant linear association was found between MRI and MRS, but again poor agreement was found (Figure 4.9 B). The Bland Altman analysis showed that on average MRI-derived pancreatic fat was higher compared to MRS-derived pancreatic (mean difference: $e^{-0.022.}$=0.98) and that a systematic bias existed with the line of perfect agreement outside the 95% confidence interval of 0.84 to 1.14 (antilog) (Figure 4.9D).

The relative changes in hepatic and pancreatic fat, in response to the 12-week intervention, determined by MRI and MRS are shown in Figure 4.10. In the exercise group, MRI-determined hepatic fat was reduced (Δ -13.1%) while the MRS-derived values showed an increase (Δ 33.6%). A similar discrepancy was observed in the control group, albeit in opposite directions. Further, in the exercise group, pancreatic fat determined by both MRI and MRS showed a reduction, but greater variability occurred in the MRS measures. In the control group, contradictory results were obtained and again the MRS-derived pancreatic fat showed great variability.

Taken together, a systematic bias existed between MRI and MRS derived measures of hepatic and pancreatic fat at baseline and the relative changes in hepatic and pancreatic fat in response to the 12-week intervention showed contradictory findings between these two methods. Fat derived from MRS showed outlying values and considerable variability. A possible reason could be that the MRS sequences require longer scanning times compared to MRI sequences and

movement of the voxels could have occurred when breath-holding was inadequate. We therefore included only the MRI-derived hepatic, pancreatic, as well as skeletal muscle fat in our pre-post analysis. The baseline analysis included MRI-determined hepatic and pancreatic fat, but skeletal muscle fat determined by both MRS and MRI was included because only MRS can discriminate between IMCLand EMCL content. MRS- determined skeletal muscle fat will also be less affected by breathing compared to pancreas and the liver.

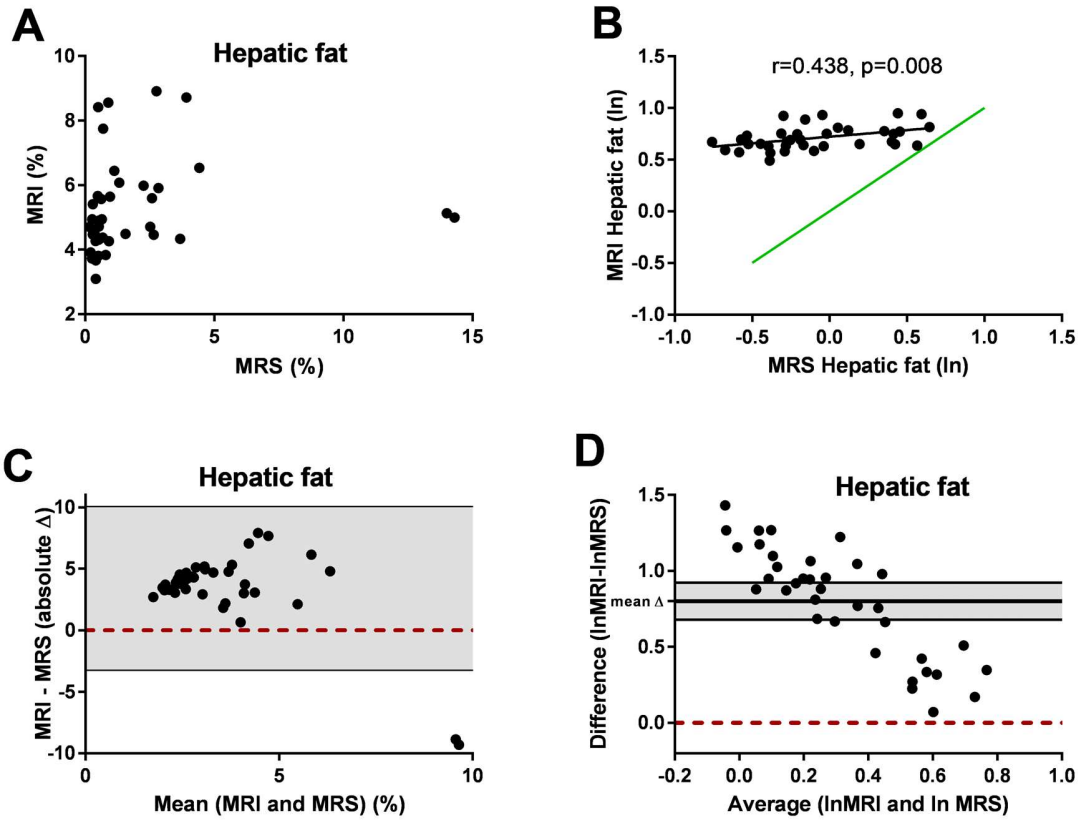


Figure 4.9: *Comparison of baseline hepatic fat derived from MRI and MRS in A) scatterplot of untransformed data, B) Correlation of transformed data with regression line (black) and line of perfect agreement (green), C) Bland Altman plot*

of untransformed data with limits of agreement, and D) Bland Altman plot of natural log transformed data (ln) with mean difference and 95% confidence interval.

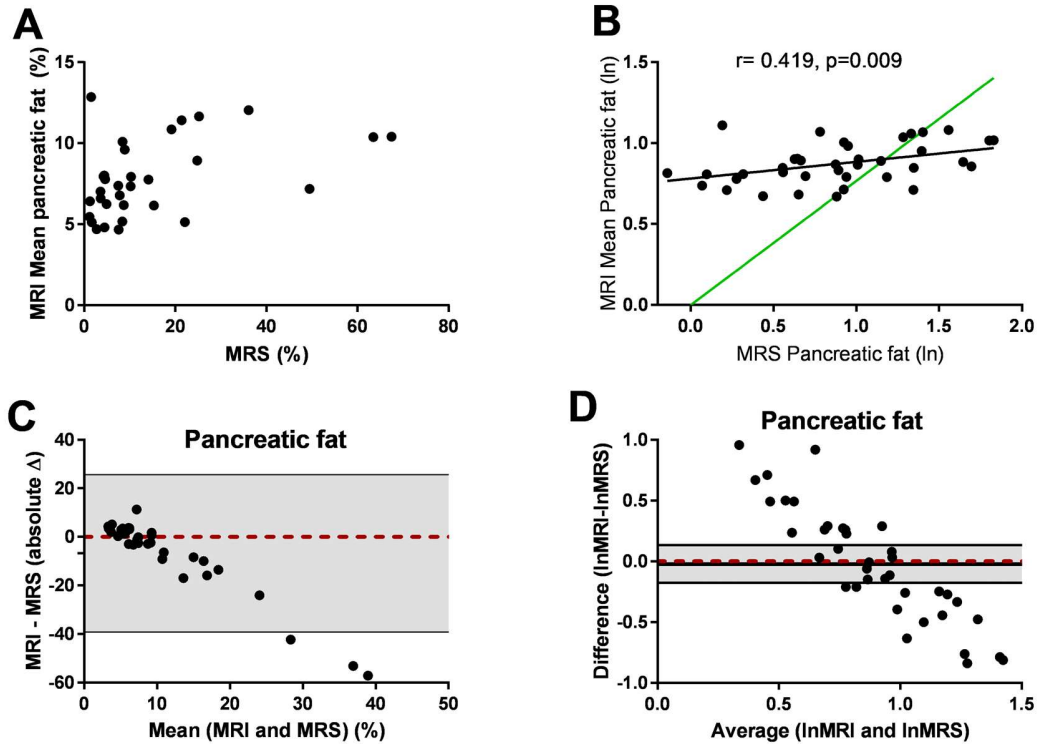


Figure 4.10: *Comparison of baseline pancreatic fat derived from MRI and MRS in A) scatterplot with untransformed data, B) Correlation of transformed data with regression line (black) and line of perfect agreement (green), C) Bland Altman plot of untransformed data with limits of agreement and D) Bland Altman plot of natural log transformed data (ln) with mean difference and 95% confidence interval*

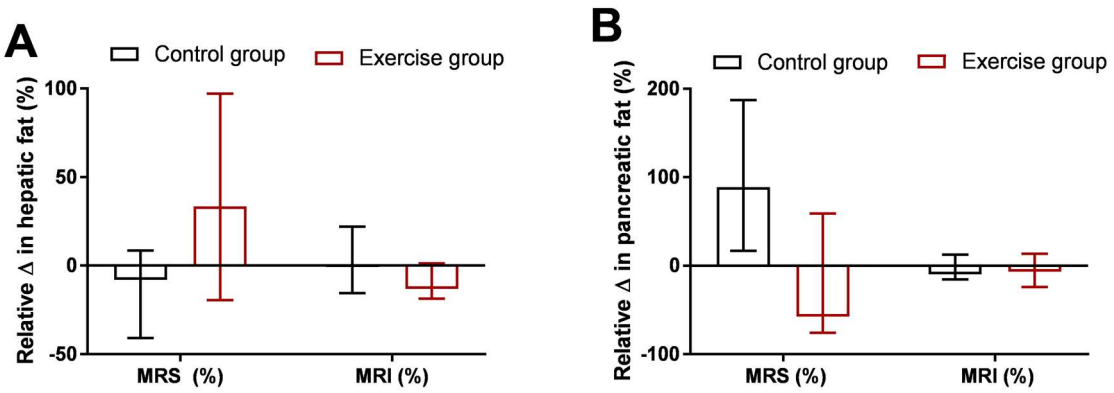


Figure 4.11: *Comparing the relative changes between MRS and MRI with regards to (A) Hepatic fat and (B) Pancreatic fat in the control and exercise groups*

4.2 DETERMINATION OF INSULIN SENSITIVITY, INSULIN SECRETION AND INSULIN CLEARANCE

4.2.1 INSULIN SENSITIVITY

Various methods are available to measure S_I. The HIEG clamp, developed by de Fronzo and Andres (331), is the gold standard. It gives a direct measure of peripheral S_I under steady-state conditions, but it is labour intensive and costly.

The minimal model developed by Bergman and Cobelli (332) is an indirect method of measuring S_I and requires glucose and insulin plasma levels obtained during a FSIGT. The FSIGT is less labour intensive than the clamp method and steady state conditions are not required. Additionally, glucose effectiveness (Sg), AIRg and the DI can also be calculated. A disadvantage of the FSIGT is that it cannot distinguish between hepatic and peripheral S_I because it unable to isolate endogenous glucose produced by the

liver from exogenous glucose (333). Nevertheless, a good correlation has been found between FSIGT and hyperglycemic clamp-derived S_I (r=0.88, p=0.001) and insulin response (r=0.75, p=0.005) in normo-glycaemic women (334).

The minimal model calculates S_I, a measure of the fractional disappearance of glucose for a given insulin concentration by uncoupling the glucose and insulin responses. This minimal model encompasses a glucose (describes glucose dynamics) and insulin (describes insulin dynamics) minimal model (332). The following parameters are derived from this model (Figure 4.11): (a) At 0 min of the FSIGT test, glucose is injected into a single compartment with a subsequent rise in the plasma glucose concentration (G(t)) from baseline glucose (G_B). (b) This results in glucose stimulating its own uptake (Sg) and a simultaneous triggering of the pancreatic β-cells to release endogenous insulin into the interstitium (remote insulin compartment). Insulin levels (I(t)) increase from baseline insulin levels (I_B). (c) After insulin enters the interstitium it can then promote glucose uptake. The action of insulin (X) is proportional to the interstitial insulin. The uptake of glucose is a composite measure of both the hepatic and peripheral glucose uptake, as well as the inhibition of hepatic glucose production. (d). Exogenous insulin is infused at 20 min at a time that Sg is declining, and therefore the glucose uptake after 20 min is assumed to be due to insulin. The movement of insulin into the interstitium and out of the interstitium is noted as P3 and P2, respectively. Other measures obtained from the minimal model are: AIRg, the incremental area under the insulin curve in response to intravenous glucose over the first 10 minutes, and thus reflects the first phase insulin response; DI, a measure of the insulin response to glucose relative to the prevailing level of S_I (AIRg x S_I) which gives an index of whether the insulin response is adequate for the level of S_I (180). A graphical output from the minimal model is shown in figure 4.12. The fractional

standard deviation (FSD) was used as a measure of how well the parameters derived from this model were estimated.

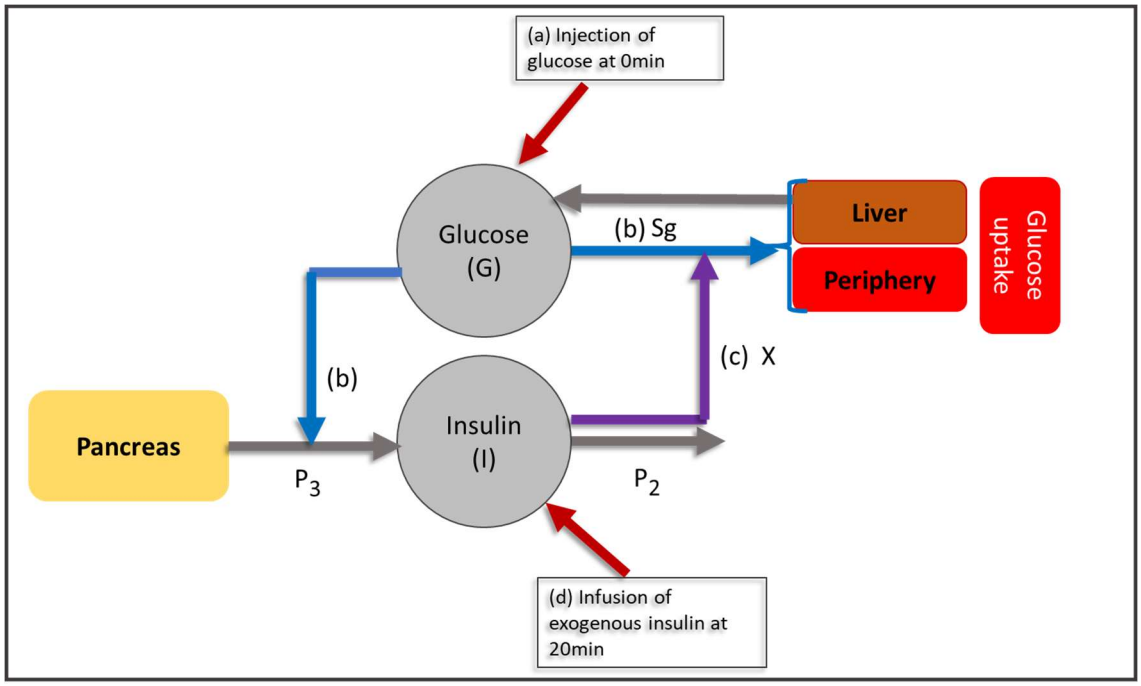


Figure 4.12: *Schematic illustration of the minimal model that is used to determine insulin sensitivity. Sg – glucose effectiveness, X – insulin action, P_3 and P_2 kinetic insulin parameters*

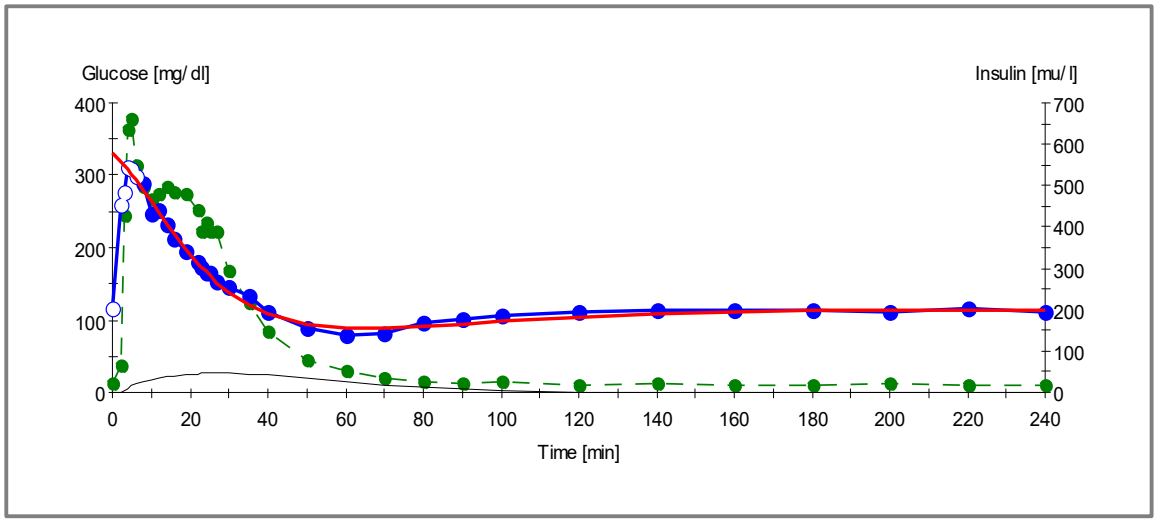
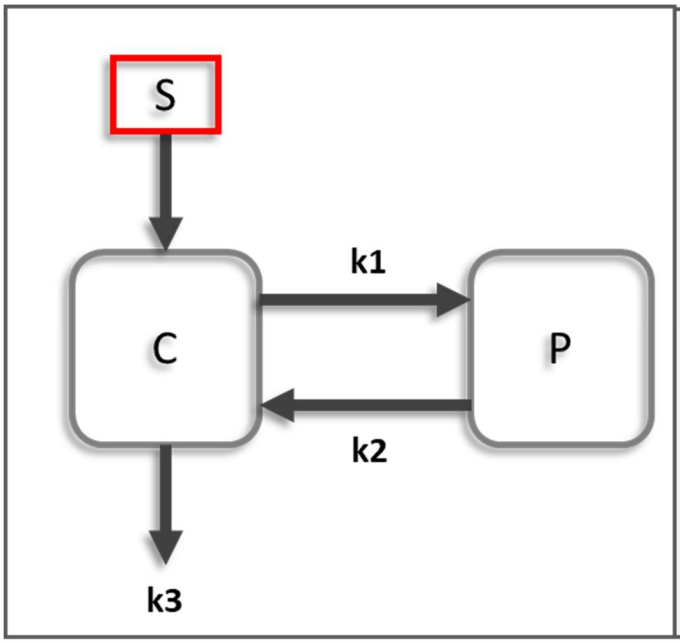


Figure 4.13: *Output from the Minimal Model. Green – Insulin response, Blue – Glucose response, Red – Glucose model fit*

4.2.2 INSULIN SECRETION

AIRg, is a measure that reflects glucose-stimulated plasma insulin levels and not insulin secretion *per se*. Plasma insulin levels reflects both insulin secretion and insulin clearance. Insulin is secreted by the pancreatic β-cells into the portal circulation and during first pass through the liver up to 70% and 54% of insulin is degraded in the liver under fasting and stimulatory conditions, respectively (18). As a result, the amount of insulin that reaches the systemic circulation can be up to half to two-thirds less than the amount secreted by the pancreas. Nevertheless, C-peptide is co-secreted with insulin, in equimolar quantities, by the pancreas and is not degraded by the liver. In addition, C-peptide has a longer half-life (30-35 min) compared to insulin (5-10 min) and has therefore been used to estimate insulin secretion.

C-peptide deconvolution is used to determine ISRs by using peripheral C-peptide levels, but the clearance kinetics of C-peptide must be known. The kinetics of C-peptide after administration of synthetic human C-peptide in adults with and without T2D was described by Faber *et al.* (335). Subsequently, Eaton *et al*. (336) proposed the two-compartment model of C-peptide (Figure 4.13) to determine ISR.



C – Central compartment (plasma)
P – Peripheral compartment (tissue)
S – Bolus of/endogenous C-peptide into C (secretion rate of C-pep into C)
k1 – rate of transfer of C-peptide from C to P
k2 – rate of transfer of C-peptide from P to C
k3 – rate of irreversible clearance of C-peptide from C

Figure 4.14: *Schematic of two-compartment C-peptide model for estimating insulin secretion rate*

This method of estimating ISR was a 2-step process. Firstly, the kinetic parameters (k1, k2, k3) need to be obtained. Therefore, synthetic human C-peptide was administered to diabetic patients with no endogenous C-peptide secretion. Information obtained from these C-peptide decay curves was used to determine the kinetic parameters (k1, k2, k3) by solving equations using non-linear least squares regression. Secondly, stimulated endogenous insulin and C-peptide levels were obtained using an OGTT. The kinetic parameters, together with the plasma levels of C-peptide are then used as inputs into the following formula for the calculation of the ISR

$$S(t) = -e^{-k2t}\left[k1C(t1)e^{k2t1} + k1k2\int_{t1}^{t} e^{k_{2s}}C(s)\,ds\right] + \frac{d}{dt}C(t) + (k1+k3)C(t)$$

(336). However, this method required two sets of experiments and the administration of human biosynthetic C-peptide, which can be costly and time-consuming. Subsequently, the Van Cauter and Polonsky group showed that standard kinetic parameters can be used, taking into account sex, age and body surface area, to

determine ISR without obtaining individual kinetic C-peptide parameters (337). Following this, various other models have been proposed to measure ISR such as a one-compartment model that combines the kinetics of insulin and C-peptide into one model (338). Nevertheless, after validating the use of peripheral C-peptide levels under various conditions, it was found that the two-compartment C-peptide model was better at estimating ISR compared the one-compartment model (339). Hence, in our study the two-compartment C-peptide model was used with standard kinetic parameters, to obtain an estimate of ISR.

4.2.3 INSULIN CLEARANCE

Direct measures of estimating hepatic insulin clearance such as using hepatic vein and artery catherization are not feasible in humans due to its invasive nature (340). An indirect measure of hepatic insulin clearance uses the ratio of plasma C-peptide/insulin levels. The use of this ratio depends on the following assumptions: C-peptide and insulin must be secreted in equimolar amounts, C-peptide must not be degraded by the liver, the clearance of C-peptide must be constant over a range of physiological conditions (341). These assumptions have been tested and shown to be reliable, but only under steady-state conditions and are thus not a reliable proxy for hepatic insulin extraction under non-steady state conditions (342). In addition, this measure has not been validated to my knowledge in a black African population and in response to an insulin-modified FSIGT. Accordingly, C-peptide/insulin ratio was only used in our study during fasting conditions that reflect a steady-state condition, and not during the FSIGT.

Mathematical modelling is another method to estimate insulin clearance. The following models have been used with FSIGT data, and can estimate hepatic insulin clearance either as a constant (71,343) or that varies over the course of the FSIGT (18). We first used the latter model in our study. This model combined the two-compartment C-peptide model with a new one-compartment minimal model of insulin delivery and kinetics (18) (Figure 4.14). This model assumed that above a certain threshold of plasma glucose levels (h), insulin is secreted (ISR) into a central compartment (CP1). The insulin secreted is in response to the rapid change in glucose concentration ($Glucose_{max}$-$Glucose_o$) and is proportional to the insulin in an already available pool in the β-cells (X). After this initial phase, new insulin is provided (Y) to the releasable pool in the β-cell after a delay, and is controlled by the glucose concentration. X reflects the balance between ISR and new insulin provided to the releasable pool (Y). C-peptide and glucose plasma levels during the FSIGT are used as the input into this model. The one-compartment model of insulin, estimates the post-hepatic insulin delivery rate (IDR), normalized to the volume of distribution (Vd), using insulin kinetics that reflects both secretion and extraction of insulin. The other parameters from this model are the constant rate of insulin disappearance (n), exogenous insulin delivered between 20-25 min and the insulin level above basal (Insulin) in the accessible compartment. After the ISR and IDR are obtained, the hepatic insulin extraction is calculated: $HIE = 1 - \frac{IDR\ (t)}{ISR\ (t)}$.

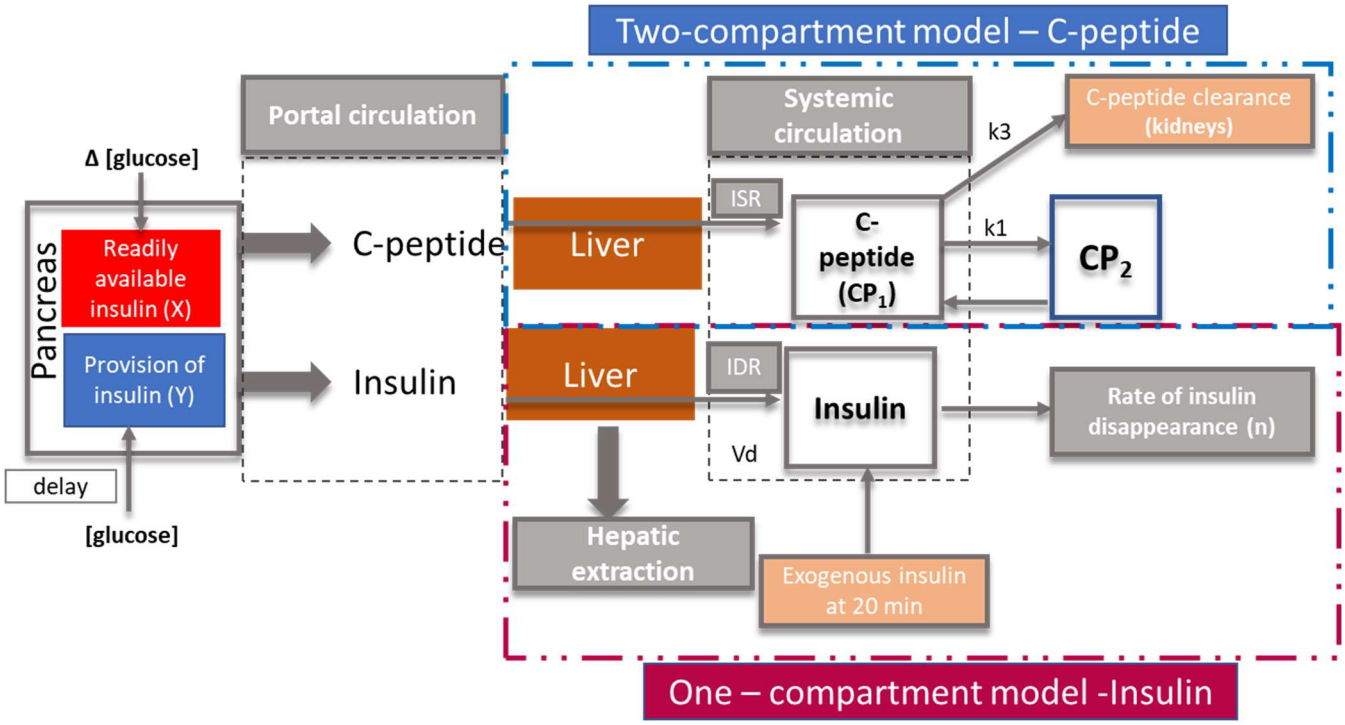


Figure 4.15: *Schematic illustration of the combined two-compartment model of C-peptide and one-compartment model of insulin from Toffolo et al.* (18) *to determine the insulin secretion rate (ISR), insulin delivery rate (IDR) and hepatic insulin extraction*

Each participant's glucose, insulin and C-peptide data were analysed using WinSAAM mathematical software programme (344). This software program estimates the parameters necessary to solve the model followed by checking how well the model fits the data. Depending on the fit of the model, the parameters might need to be adjusted or specified up front using Bayesian constraints to optimize the fit. A FSD of <0.5 indicates that the model fits the data well and that the parameters are well estimated. Examples of modelled C-peptide, insulin, ISR and hepatic insulin extraction graphs are shown in figure 4.15.

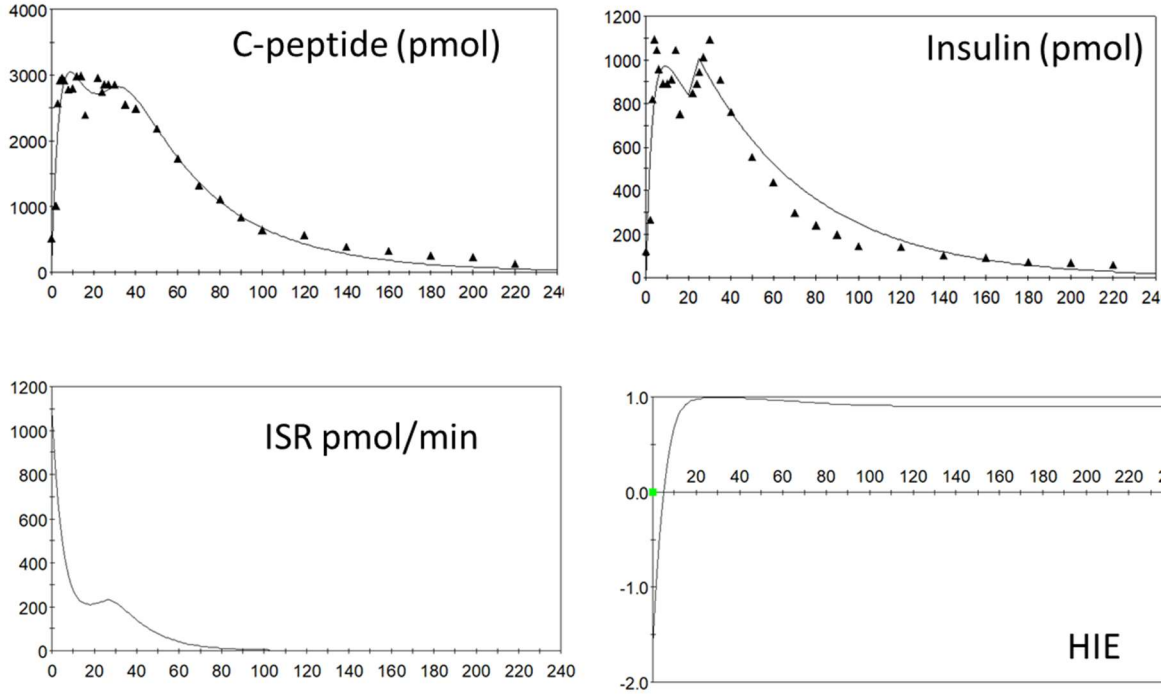


Figure 4.16: *Graphical output from the Toffolo model showing the C-peptide and insulin observed data (closed triangles) over time with the model predicted values (black solid line). The model estimated insulin secretion rate (ISR) and hepatic insulin extraction (HIE) over time in the bottom graphs*

In 18 participants, the Toffolo model were unable to estimate key parameters. We, therefore, could not use the ISR and hepatic insulin extraction derived from the Toffolo model. Possible reasons why the Toffolo model fitted our data poorly were the complexity of the model, estimated 11 parameters, or the model was not applicable to our population that have high insulin and C-peptide levels in response to glucose.

Subsequently we choose a simpler model with less estimated parameters and that has been used in black men of African descent (71), as well as in black African American children (58) and women (59). This model was first described by Polidori *et al.* and can estimate both hepatic and peripheral insulin clearance (71). This model assumes that hepatic insulin delivery comes from 2 sources, the portal vein (which

reflects endogenously secreted insulin - ISR) and from the systemic circulation (Figure 4.17). The insulin delivered to the liver from the systemic circulation is assumed to be equal to the hepatic plasma flow multiplied by the plasma insulin concentration. Polidori *et al*. assumed that the hepatic plasma flow was constant (0.576 L/min/m^2) (71). Hepatic plasma flow quantification requires invasive techniques which are not feasible in majority of settings. However, to determine whether the changes in hepatic insulin extraction is due to cellular changes and not changes in hepatic blood flow, hepatic blood flow therefore needs to be considered. A study that inserted 3 catheters - in the hepatic vein, subclavian vein and the brachial artery - and used a continuous infusion of indocyanine dye, determined that hepatic blood flow increased after an oral glucose load but did not changed significantly after an intravenous glucose infusion (345). This finding may justify using a constant hepatic plasma blood flow with a FSIGT. However, the previous mentioned study used healthy, lean males, so the validity of the assumption of constant hepatic blood flow in our study population (obese, black African females) cannot be certain. ISR are obtained from C-peptide deconvolution. Insulin can either be degraded peripherally by tissues such as the kidney, muscle and adipose tissue or by the liver. Peripheral insulin degradation is assumed to be proportional to plasma insulin levels with the parameter CLp. Lastly, the model assumes that hepatic insulin clearance can either follow linear or saturable kinetics. The linear model assumes a constant hepatic insulin clearance and is explained by the fractional hepatic extraction (HIE) parameter, while the saturable model assumes hepatic insulin clearance varies with the ISR and plasma insulin levels which are explained by the maximal insulin degradation rate (V_{max}) and the hepatic insulin delivery rate at which 50% of insulin degradation occurs (K_m).

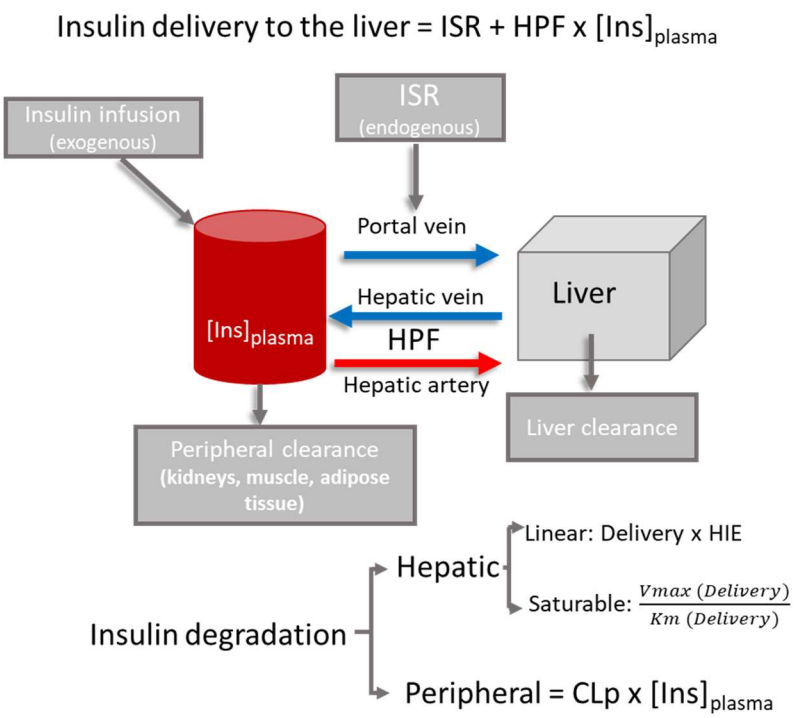


Figure 4.17: *Graphical display of the Polidori model (adapted from* (71)*), ISR – insulin secretion rate, HPF – hepatic plasma flow, Ins – Insulin, HIE – hepatic insulin extraction, CLp – peripheral insulin clearance*

WinSAAM was used to estimate the parameters of both the linear (volume of distribution (Vd), HIE, CLp, ISR) and the saturable models (Vd, Vmax, Km, CLp, ISR). The Polidori model only looks at the disposal of insulin and not the effect of glucose on insulin. Therefore, only the insulin and C-peptide levels over the FSIGT are used as inputs into the model. Both the linear and saturable models were run on all participants. An FSD was obtained for the estimated parameters to ensure parameters were well estimated. The model that fits the data the best was chosen as the preferred model. The Akaike Information Criterion (AIC) was used to determine the best fit (346): $AIC = N\ X \ln\left(\frac{SSR}{N}\right) + 2n$ with N - Number of insulin time points, n – number of parameters estimated + 1, SSR - Sum of squares of residuals with SSR=

$\sum_{(observed\ value-calculate\ \ value)} 2$. The model with the lowest AIC was chosen as the preferred model. An additional measure was used to determine how well the model fitted the data, the normalized root mean square error (NRMSE) = $100\ X\ \frac{(\sqrt{SSR})/N}{Insulin_{max-}Insulin_{min}}$. The NRMSE normalizes the square root of SSR to the change in insulin concentration (71).

The linear model provided a single parameter to explain hepatic insulin clearance (HIE) (post-glucose load) compared to two parameters (Vmax and Km) provided by the saturable model. In order to compare hepatic insulin extraction between subjects as well as before and after the intervention, the output from the linear model was used. Prior to the intervention (baseline), both the linear and saturable model were run on 43 participants (excluded: 2 did not have a FSIGT test) and the linear and saturable models were preferred in 26 (60.5%) and 14 (32.6%), respectively, whereas in 3 participants no model could be resolved. Both models were run on 35 participants that completed the intervention but 5 had to be excluded from pre- and post-intervention analysis because no pre-FSIGT test was done (n=1), or no model was resolved on pre-FSIGT data (n=3), or the post FSIGT test was prematurely halted at 50 minutes (n=1). In a further 2 participants, the preferred pre-and post-intervention models was discrepant and therefore the insulin clearance parameters could not be compared. In 20 (linear model) and in 8 (saturable) participants the same model was preferred for both pre- and post-intervention.

CHAPTER 5: METHODS

The methods in chapter 5 have been published in part (347):

Goedecke JH, Mendham AE, Clamp L, Nono Nankam PA, Fortuin-de Smidt MC, Phiri L, et al. An Exercise Intervention to Unravel the Mechanisms Underlying Insulin Resistance in a Cohort of Black South African Women: Protocol for a Randomized Controlled Trial and Baseline Characteristics of Participants. JMIR Res Protoc. 2018 Apr 18;7(4):e75.

5.1 RESEARCH DESIGN

We performed a randomised controlled exercise intervention study. Participants meeting all inclusion criteria were block (2-4 participants) randomised (Microsoft Office Excel, 2013) into an exercise (n=23) and control group (n=22). The CONSORT participant flow diagram is shown in Figure 5.1. The schematic overview of all testing procedures is shown in Figure 5.2. All testing procedures occurred before and after the intervention. Post-intervention FSIGT was performed ≥72 hours after the last exercise bout to exclude the acute effects of exercise on the primary outcome.

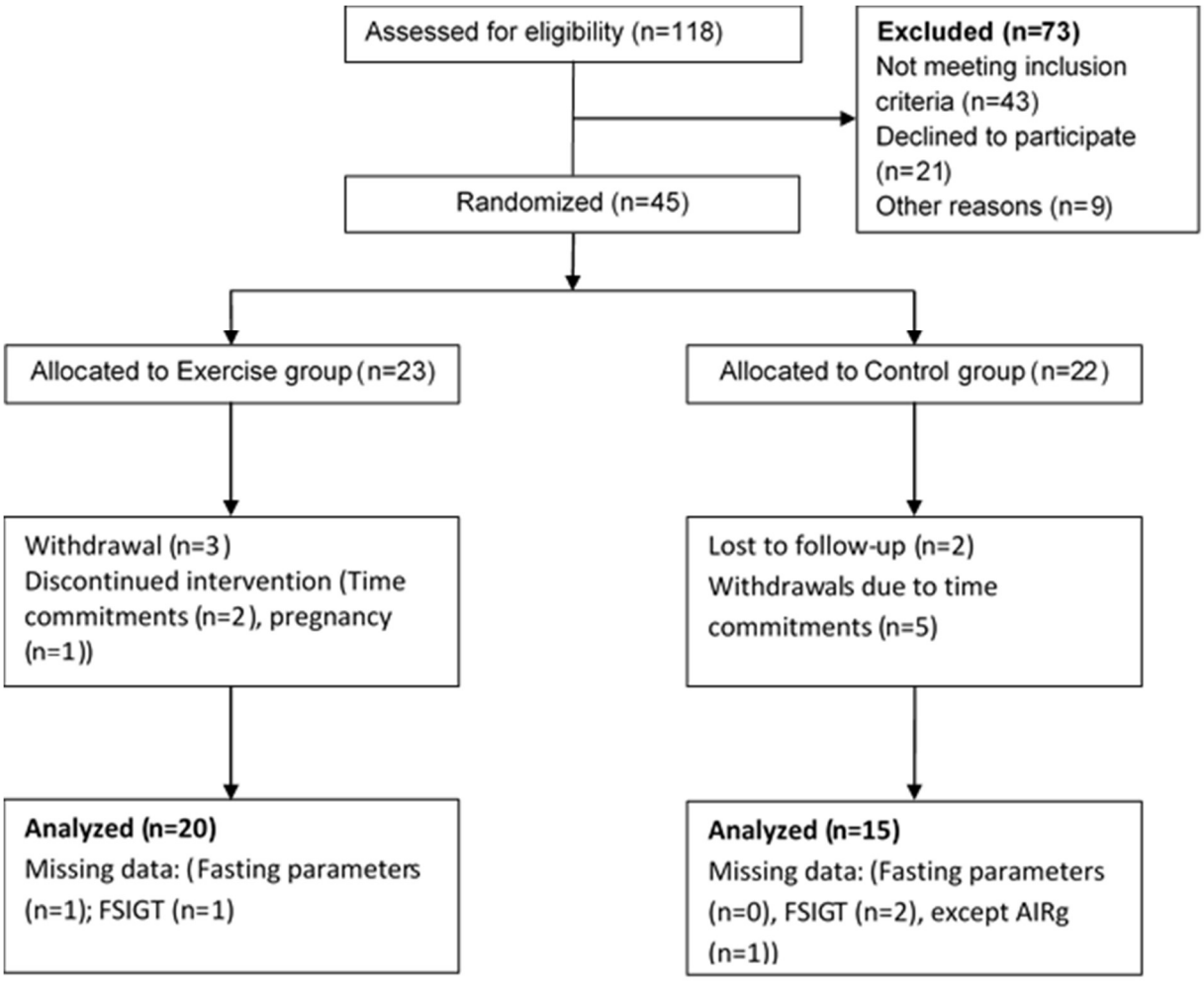


Figure 5.1: *CONSORT participant flow diagram*

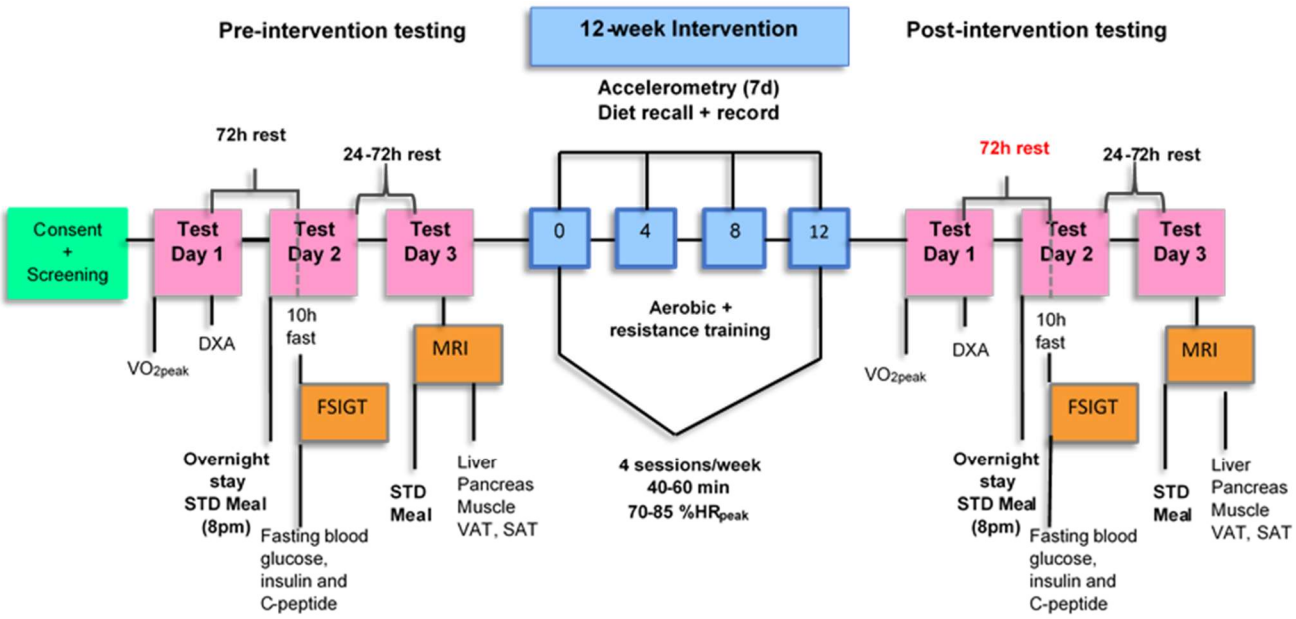


Fig 5.2. *Schematic overview of testing procedures and timelines (Adapted from Goedecke et al, 2018)*

5.2 STUDY PARTICIPANTS

Black South African women were recruited from a low socioeconomic community in Cape Town. Recruitment and testing occurred from July 2015 to December 2016 through advertisements and flyers distributed in local communities and universities. Inclusion criteria were: obese (BMI 30-40 kg/m^2), both parents of *isiXhosa* descent (self-reported), 20-35 years old, stable weight (<5 kg change in weight in the last 6 months), using injectable contraception (depot medroxyprogesterone acetate, 400 mg) for a minimum of 2 month prior to testing. Participants were excluded if they had any known diseases (e.g. HIV, hypertension, diabetes (random blood glucose >11.1mmol/L, HbA1c >6.5%)), were pregnant or lactating, smoking or had any other orthopaedic or medical problems that prevented or restricted exercise participation. University of Cape Town Human Research Ethics Committee provided ethical

permission (HREC REF:799/2015) (Appendix A). The trial was registered in the Pan African Clinical Trial Registry (PACTR201711002789113). Written informed consent (Appendix B) was obtained prior to screening and testing procedures.

5.3. SCREENING PROCEDURES

Volunteers completed a screening questionnaire (Appendix C) that collected information on ancestry, weight gain, current level of physical activity, contraception use, smoking history, medical and surgical history, current medication usage and HIV status. Weight was measured with no shoes and minimal clothes using a digital calibrated scale to the nearest 0.1kg. Height was measured with a stadiometer to the nearest 0.1cm. The BMI was calculated as weight (kg) ÷ height $(m)^2$. Blood pressure was measured using an automated blood pressure monitor (Omron 711, Omron Health Care, Hamburg, Germany) with an appropriately sized cuff. The participant had to remain seated for at least 5 minutes after which blood pressure was measured three times at 1-min intervals. The participant was excluded if the mean blood pressure was ≥140/90 mmHg. An HIV Rapid test was performed to ensure all participants were HIV-negative. The participants received pre-and-post HIV test counselling and were referred to the local clinic if HIV test was positive. Blood samples were also drawn to test for Hb (to exclude anaemia), and to measure random blood glucose and HbA1c (to exclude diabetes). A physical activity readiness questionnaire (PARQ) was completed by each participant to assess their risk of taking part in the exercise intervention. They answered “no” to all questions for inclusion into the study. A pregnancy test will also done to exclude any participants who were not aware that they were pregnant. Reasons for participant exclusions were: a positive HIV test (n=2), unwillingness to further participate in the study (n=21), a BMI <30 kg/m^2 (n=12), BMI

>40 kg/m^2 (n=12), too young (n=1), not on contraception (n=15) and taking medication (n=1). Participants that met all the inclusion criteria and none of the exclusion criteria were enrolled in the study.

5.4 EXERCISE INTERVENTION

The exercise group participated in a 12-week aerobic and resistance training intervention. A biokineticist facilated and supervised every session. The exercise session duration progressed from 40-60 min, 4 days per week. Table 5.1 display the session duration and the resistance exercise training (repetitions, sets and weights used) progression. The programme included aerobic-based exercises (dance, running, skipping and stepping) performed at a moderate-vigorous intensity (75-80% maximal heart rate, HR_{peak}). Aerobic-based exercises were done continuously moving from one exercise to another with no prescribed rest in between. Resistance exercise training consisted of progressive (body weight, bands and free weights) strengthening exercises (squats, lunges, bicep curls, push-ups and shoulder press) to achieve a prescribed intensity of 60-70% HR_{peak}. The prescribed resistance training intensity was a guide to ensure that the repetition tempo was maintained. The program was designed to use low weights with high repetition. Body weight and resistance band exercises were done during the first 2 weeks. Free weight strengthening exercises were added to body weight and resistance band exercises in the following weeks (Table 5.1). A heart rate monitor (Polar Electro, Kempele, Finland) was used to ensure participants achieved the desired exercise intensity and attendance was recorded for each session. The relative intensity of the exercise program did not change but as the

cardiorespiratory fitness of participants increased, the duration and intensity of the activities increased (Table 5.1). The control group was instructed to continue with habitual activity and not to initiate any exercise programs during the study period. Both the control and the exercise group were asked to continue with habitual dietary patterns which was monitored on a monthly basis. The participants were not blinded to the aims of the study. They were informed that the aim of the study was not to induce changes in body weight, but rather changes in health status. Weight loss was therefore not anticipated by the participants.

Table 5.1: *Details of the combined aerobic and strength exercise training program*

Weeks	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Session frequency	4 times a week											
Total session duration (min)	40	40	40	40	45	45	50	50	55	55	60	60
Aerobic training												
Session duration (min)	30	30	28	28	33	30	35	32	37	37	40	40
Intensity	75-80% HR_{peak}											
Strength training												
Session duration (min)	10	10	12	12	12	15	15	18	18	18	20	20
Reps and sets	10 x 3	15 x 3	10 x 3	15 x 3	10 x 3	15 x 3	20 x 3	20 x 3	10 x 3	15 x 3	10 x 3	15 x 3
Weights	-	-	1kg	1kg	1.5kg	1.5kg	1.5kg	1.5kg	2kg	2kg	2.5kg	2.5kg
Intensity	60-70% HR_{peak}											

Min – minutes, HR – heart rate, Reps – repetitions, Weights – refers to the weight of the dumbbells

5.5 STUDY MEASUREMENTS

5.5.1 CARDIORESPIRATORY FITNESS

Peak oxygen consumption (VO_{2peak}) was measured using a walking treadmill-based (C, Quasar LE500CE, HP Cosmos, Nussdorf-Traunstein, Germany), graded exercise test adapted for sedentary participants that are not familiar with gym-based equipment (348). The test started at 3 km/h at 2% gradient and the gradient was increased by 2% after every 2 minutes until a gradient of 16% were reached. Thereafter, the speed and gradient were increased alternatively (0.5 km/hour and 1%, respectively) to volitional exhaustion. VO_{2peak} was measured by assessing ventilation and oxygen and carbon dioxide concentrations in the expired air using a metabolic gas analysis system (CPET, Cosmed, Rome Italy) and the heart rate was monitored continuously (Polar Electro Oy, Kempele, Finland) to determine peak heart rate (HR_{peak}).

5.5.2 Quantification of S_I and β-cell function

Prior to the testing day, participants slept overnight at the laboratory and consumed a standardized meal, typical of their usual intake, at 20h00 (Energy: 2,456 kJ, 21 g protein (14% energy), 49 g carbohydrate (33% energy) and 32 g fat (48% energy)), followed by an overnight fast (10-12 h). In the morning, fasting blood samples were drawn for measurement of HbA1c, insulin, glucose and C-peptide, and the FSIGT was performed. Glucose (50% dextrose; 11.4 g/m^2 X body surface area) was infused at 0 min over a 60 s period followed at 20 min by an infusion of human insulin (0.02 unit/kg; NovoRapid, Novo Nordisk Limited, Cape Town, RSA) over 5 min at a constant rate. Thirty-two blood samples were drawn for the measurement of insulin, glucose and C-peptide at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240 time points over 240 min, following the start of glucose administration. S_I, AIR_g and DI (S_I X AIR_g), glucose effectiveness (minimal model of glucose kinetics) (332), ISR (337) and hepatic and peripheral insulin clearance (71) were derived from mathematical modelling. The incremental area-

under curve (AUC) above baseline for ISR, glucose, insulin and C-peptide were calculated using the trapezoidal rule. The first 10 minutes and the entire period of the FSIGT are referred to as the first phase AUC and the total AUC, respectively.

5.5.3 BIOCHEMICAL ANALYSES

HbA1c was analysed using high-performance liquid chromatography (Meharini Diagnostics, Florence, Italy). Plasma glucose was measured using a colorimetric assay (Randox (Pty) Ltd, Gauteng, South Africa). Serum insulin and C-peptide were determined by an immunochemiluminometric assays (IMMULITE 1000 immunoassay system, Siemens Healthcare (Pty) Ltd, Midrand, South Africa). Pre- and post-intervention samples for each participant were analysed in the same run. Coefficient of variation for assays ranged from 6 to 8.5%.

5.5.4 ANTHROPOMETRY AND WHOLE-BODY FAT COMPOSITION

Anthropometric measurements included weight, height, hip and waist circumferences using standardized methods (103). Whole body composition was measured by dual-energy x-ray absorptiometry (DXA; Discovery-W®, software version 12.7.3.7; Hologic, Bedford, MA) in the mornings after an overnight fast, for the analyses of total body fat mass, fat-free soft tissue mass, trunk, and leg fat mass. All values are expressed as a percentage relative to sub-total fat mass (whole body minus the head).

5.5.5 ECTOPIC FAT, SAT AND VAT QUANTIFICATION

A standardized meal (Energy: 2553 kJ, protein: 20.9 g; carbohydrates: 83.0 g; fat: 22.2 g) was consumed 2-4 h prior to skeletal muscle, hepatic and pancreatic fat and SAT and VAT volume determination. Image acquisition and post-processing of MRI and MRS images were described in detail in Chapter 4.

5.5.6 PHYSICAL ACTIVITY AND DIETARY MONITORING

Energy expenditure of both the exercise and control groups was estimated using accelerometery (ActiGraph GTX3+, LLC, Pensacola, Florida, worn on the right hip for 24 hours a day over a 7-day period) at baseline, 4, 8 and 12 weeks. The activity counts from the ActiGraph were collected from 3 orthogonal axes. The vector magnitude was calculated as the square root of the sum of the 3 squared vectors ($\sqrt{X^2 + Y^2 + Z^2}$) (349). Activity counts were categorized into sedentary, light, moderate and vigorous based on cutpoints from Matthews *et al*. (350). Data were recorded in 60 second epochs. Non-wear days were defined as 60 continuous minutes without any counts (351). Physical activity data were analyzed using the ActiLife Software version 6 (ActiLife software; Pensacola, Florida, USA). Energy expenditure was compared between the exercise (non-exercise days) and the control group. Dietary intake was measured by a registered dietician using a 24-hour dietary intake recall and a 3-day dietary record (2 weekdays and one weekend day) at baseline, 4 and 8 and 12 weeks. The South African Food Composition Database System (SAFOOD, the South African Food Composition Database, Version 2016, South African Medical Research Council, Parow, Cape Town, South Africa) was used for the nutrient intake analysis (352).

5.6 STATISTICAL ANALYSIS

Data was analysed using STATA 12.0 (College Station, TX, USA). The distribution of continuous variables was evaluated using histograms, standardized normal probability plots and the Shapiro Wilk test. Normally and non-normally distributed data were

expressed as mean ± standard deviation (SD) and median and interquartile range (25^{th}-75^{th} percentiles), respectively.

Baseline data

The study population was divided into tertiles based on the DI: DI_{Low}, $DI_{intermediate}$ and DI_{high}. Differences between DI tertiles was determined using one-way ANOVA with a Bonferroni *post hoc test* or Kruskal Wallis test with the Dunn *post hoc* test for normally and non-normally distributed data, respectively. Pearson (normally distributed variables) and Spearman (non-normally distributed variables) correlation analysis were conducted to firstly assess the associations between DI and its direct components (S_I, AIRg), indirect components through AIRg (first phase ISR, hepatic insulin extraction), peripheral insulin clearance and secondly to assess associations of all the aforementioned DI components with body composition, body fat distribution and ectopic fat deposition. This thesis did not correct for multiple comparisons and thus a possibility exists of obtaining false positive associations. However, this thesis wanted to explore all the possible significant underlying associations between these various component and therefore wanted to minimize false negative associations. These variables are highly correlated and therefore correcting for multiple comparisons may be to stringent. Linear regression was performed with the transformed DI (square root) as dependent variable and natural log transformed ectopic fat variables and VAT as independent variables. Additionally, linear regression was also used to assess the relative contribution of first phase ISR and hepatic insulin extraction to AIRg, with and without adjusting for S_I).

Pre- and Post-intervention Analysis

A per protocol analysis was performed using participants that had both pre- and post-intervention data available (n=20 in exercise and n=15 in control group). Mixed model analysis was performed to determine the main effect between groups (exercise and control groups) and within groups (before and after intervention) and cumulative effect of between and within group factors (interaction), with Fisher's least significant difference *post-hoc* test. The random effects refer to inter-subject variability, while the fixed effects refer to differences between the groups (control vs exercise). Mixed model analysis was also used for the diet and energy expenditure data to determine differences between the control and exercise groups at baseline, week 4, 8 and 12 and to determine within group differences (between baseline and week 4, 8 and 12). Linear regression was used to determine the associations between changes in main outcomes and changes in possible predictors firstly, in the combined group and secondly, to check for an interaction by intervention group (control vs exercise group). If the homoscedasticity or the normality of residuals assumptions were not met, robust regression was used. In order to check whether the variation in the exercise training compliance influenced our study findings we calculated a composite measure, the exercise dose (AU), which is the product of the number of sessions (maximum = 48 sessions) attended and the mean $\%HR_{peak}$ attained over the 12-week training period. The exercise dose was converted into a categorical variable with the following categories: No exercise (n=15) (control group), moderate exercise dose (n=10) (≤ median (3070.6), total number of sessions (range) 25-42, $\%HR_{peak}$ 78.3-83.4) and high exercise dose group (n=10) (> median (3070.6), total number of sessions 38-49, $\%HR_{peak}$ 75.5-83.1). Whether the exercise dose modified the changes in metabolic outcomes, regional and ectopic fat deposition were assessed using mixed models. Linear regression was used to assessed whether exercise dose modified the

associations of the metabolic outcomes with regional and ectopic fat deposition. A p-value of <0.05 was regarded as significant. The sample size calculation was based on the change in S_I as one of the primary outcomes of this study. Based on the mean change (±SD) in S_I ($x10^{-5}$ min/pmol) determined by FSIGT, in the control group (Δ-0.35 ±1.7) and exercise group (Δ 1.7±2.5) (261) with a power of 80% and a significance level of $p<0.05$, 18 participants per group were needed. The number included in the study was 20 per group to account for 10% attrition.

CHAPTER 6: CROSS-SECTIONAL STUDY

6.1 RESULTS

6.1.1 PARTICIPANT CHARACTERISTICS: OVERALL AND BY DI TERTILES

A hyperbolic association was evident between AIRg and S_I in the whole group and by each of the DI tertiles (Figure 6.1).

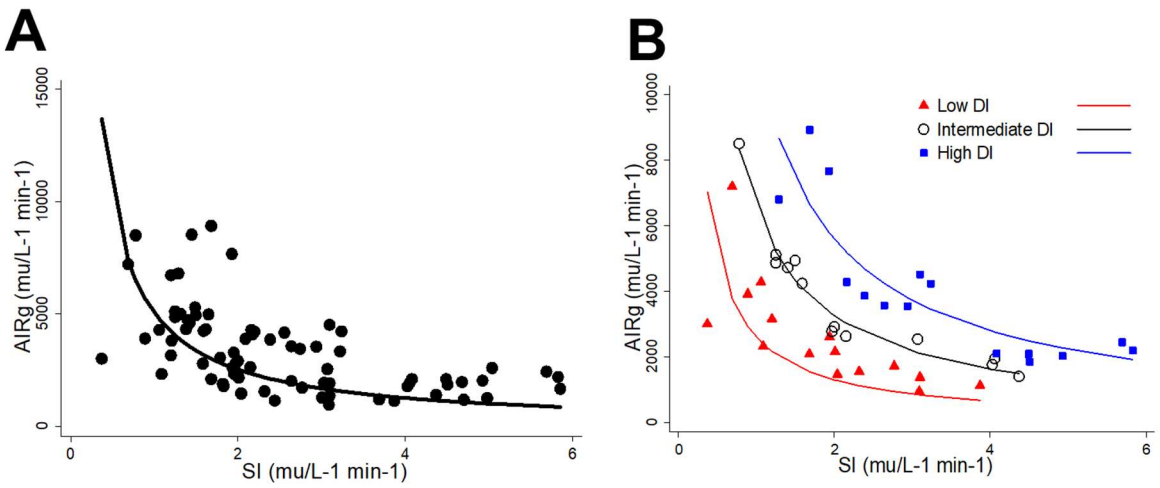


Figure 6.1: *Hyperbolic association between insulin sensitivity (S_I) and acute insulin response to glucose (AIRg) in all participants (A) and by disposition index (DI) tertiles (B)*

The participants' characteristics described by DI tertiles are displayed in Table 6.1. In the whole cohort the participants had a median age of 23 years (range 20 to 35 years) and were obese (BMI 33.9 ±2.8 kg/m^2). The median pancreatic, hepatic, total soleus and tibialis anterior fat were 7.3 (6.2-9.3)%, 4.9 (4.4-5.8)%, 10.2 (8.8-11.6)% and 4.2 (3.2-5.7)%, respectively.

Table 6.1: *Characteristics of participants by disposition index tertile*

	DI_{Low} (≤5073.4)	$DI_{Intermediate}$ (>5073.4 - ≤7933.2)	DI_{High} (>7933.2)	P value
	n=15	n=13	n=15	
Age and anthropometry				
Age (years)	24 (21-28)	22 (21-28)	23 (21-24)	0.455
Body weight (kg)	85.1 ±8.8	87.8±6.3	87.1 ±13.7	0.400
Body mass index (kg/m^2)	33.3 ±2.6	35.3 ±2.1	33.6 ±3.5	0.147
Waist circumference (cm)	103.1 ±5.3	103.9 ±8.5	105.8 ±11.0	0.705
Waist-hip ratio	0.89 (0.84-0.95)	0.89 (0.87-0.91)	0.90 (0.85-0.94)	0.899
Body composition and fat distribution				
Body fat mass (%)	50.1 ±2.8	51.3 ±3.1	50.4 ±4.5	0.660
Fat free soft tissue mass (kg)	36.9 (33.8-40.3)	37.4 (35.8-38.8)	38.7 (34.9-40.6)	0.791
Trunk fat mass (%)	47.9 ±4.7	47.3±4.0	46.5 ±4.7	0.684
Leg fat mass (%)	40.0 ±5.2	40.6 ±4.3	40.8 ±5.2	0.901
Android fat mass (%)	8.5 ±1.4	8.1 ±0.8	7.9 ±0.8	0.284
Gynoid fat mass (%)	18.5 ±1.8	18.7 ±2.0	18.4 ±2.5	0.942
VAT (cm^3)	1113.3 ±317.9	931.6 ±355.1	669.8 ±229.1*	**0.002**
aSAT (cm^3)	5303.9 ±1116.8	5741.4 ±1105.2	5629.1 ±2191.8	0.747
VAT-aSAT	0.20 (0.16-0.24)	0.15 (0.11-0.21) ǂ	0.13 (0.09-0.15) *	**0.003**
Ectopic fat				
Pancreatic fat (%)	8.0 (7.3-9.0)	7.4 (6.6-10.4)	6.2 (5.2-6.4) *†	**0.006**
Hepatic fat (%)	5.6 (4.5-6.1)	5.9 (4.5-8.4)	4.5 (3.9-4.9) *†	**0.023**
Soleus fat (%)	10.7 (10.1-11.4)	9.7 (8.8-11.6)	9.4 (6.8-10.3)	0.111
Tibialis anterior fat (%)	4.1 (3.3-5.7)	4.2 (3.5-5.4)	5.0 (2.6-6.1)	0.953
SIMCL (%)	2.9 (2.5-3.9)	2.9 (2.5-4.8)	2.1 (1.3-3.1) †	0.055
SEMCL (%)	4.5 (4.2-12.8)	5.1 (3.6-4.5)	4.3 (3.6-5.6)	0.410
TIMCL (%)	0.5 (0.3-0.8)	0.4 (0.3-0.5)	0.4 (0.3-0.5)	0.288
TEMCL (%)	2.9 (1.1-4.7)	2.6 (1.6-3.0)	2.5 (1.5-4.2)	0.815
Fasting metabolic parameters				
Fasting glucose (mmol/L)	5.4 ±0.9	5.0 ±0.9	5.2 ±0.6	0.411
Fasting insulin (pmol/L)	85.2 (63.9-119.1)	82.5 (78-113.7)	57.7 (38.6-88.5)	0.107
Fasting c-peptide (pmol/L)	605.7 (572.6-744.8)	733.2 (491.5-835.8)	466.7 (382.3-787.8)	0.218
C-peptide/insulin$_{basal}$	6.8 (6.3-8.4)	8.4 (6.4-9.3)	9.0 (7.7-10.9)	0.087
ISR_{basal} (pmol/min)	78.7 (74.4-96.8)	94.7 (62.9-102.4)	62.4 (49.-104.6)	0.567
HOMA2 IR%	2.1 (1.7-2.9)	2.0 (1.8-2.7)	1.4 (1.0-2.2)	0.149
HOMA2 B%	134.6 (97.5-204.3)	182.6 (130.4-213.2)	108.7 (85.2-149.1)	0.133
HbA1c and FSIGT-derived measures				
HbA1c	5.24 ±0.36	5.19 ±0.36	5.20 ±0.43	0.829
Disposition index (AU)	3780 (2989-4527)	6596 (6070-7119) ǂ	9995 (9247-13882) *†	**<0.001**
S_I (mU/l)$^{-1}$ min^{-1}	1.94 (1.1-2.8)	1.97 (1.4-3.1)	3.1 (2.2-4.5) *†	**0.008**
AIR_g mU l^{-1} min^{-1}	2159 (1465-3159)	2909 (2544-4870)	3561 (4278-4514)	0.064
Sg (min^{-1})	0.017 ±0.008	0.024 ±0.008	0.031 ±0.008 *	**<0.001**
$ISR_{first\ phase}$(pmol/min)	2692 (1909-4023)	3624 (2826-4999)	4049 (2758-4877)	0.060
ISR_{total} (pmol/min)	4246 (3231-8095)	5589 (4813-7913)	4272 (3261-6500)	0.365

HIE (%) §	58.5 ±13.0	51.3 ±8.3	49.6 ±10.1	0.213
CLp (ml/min/kg) §	75.7 (69.5-115.9)	105.4 (87.8-136.5)	133.4 (105.9-170.4) *	**0.041**

*Mean (±standard deviation) for normally distributed variables and median (interquartile range) for non-normally distributed variables; DI – Disposition index, VAT – visceral adipose tissue, aSAT – abdominal subcutaneous adipose tissue, SIMCL- soleus intra-myocellular fat, SEMCL – soleus extra-myocellular fat, TIMCL – tibialis anterior intra-myocellular fat, TEMCL – tibialis anterior extra-myocellular fat, ISR – insulin secretion rate, Homeostatic model assessment 2 of insulin resistance (IR) and beta cell function (B), SI – Insulin sensitivity, AIRg – acute insulin response to glucose, Sg – Glucose effectiveness, HIE – hepatic insulin extraction; CLp – peripheral insulin clearance. P value derived from ANOVA or Kruskal Wallis, Significant post hoc differences: * High DI compared to low DI; † High DI compared to intermediate DI; ǂ Intermediate DI compared to low DI with P≤0.05 deemed as statistically significant. § HIE and CLp numbers by DI tertiles: High (n=7), Intermediate (n=9) and Low (n=10) DI.*

No differences were found in fasting glucose or HbA1c between DI groups. The group with the highest DI, was the most insulin sensitive (*post hoc* p=0.006) and had the highest Sg (*post hoc* p<0.001) compared to those with the lowest DI. Moreover, over the whole FSIGT period the high DI tertile group had the lowest glucose AUC compared to the low DI tertile group (p=0.042) (Figure 6.2). AIRg and first phase ISR showed a gradual decline across DI tertiles (interaction p=0.060 and p=0.064, respectively). Interestingly, while no difference was observed in hepatic insulin extraction across the DI tertiles, peripheral insulin clearance declined moving from the highest to lowest DI tertile (*post hoc* p=0.005). While, groups did not differ in BMI, waist circumference, total fat mass, aSAT and peripheral fat mass, those with the highest DI had the lowest VAT and lowest VAT-aSAT ratio, as well as the lowest pancreatic and hepatic fat compared to those with the lowest DI.

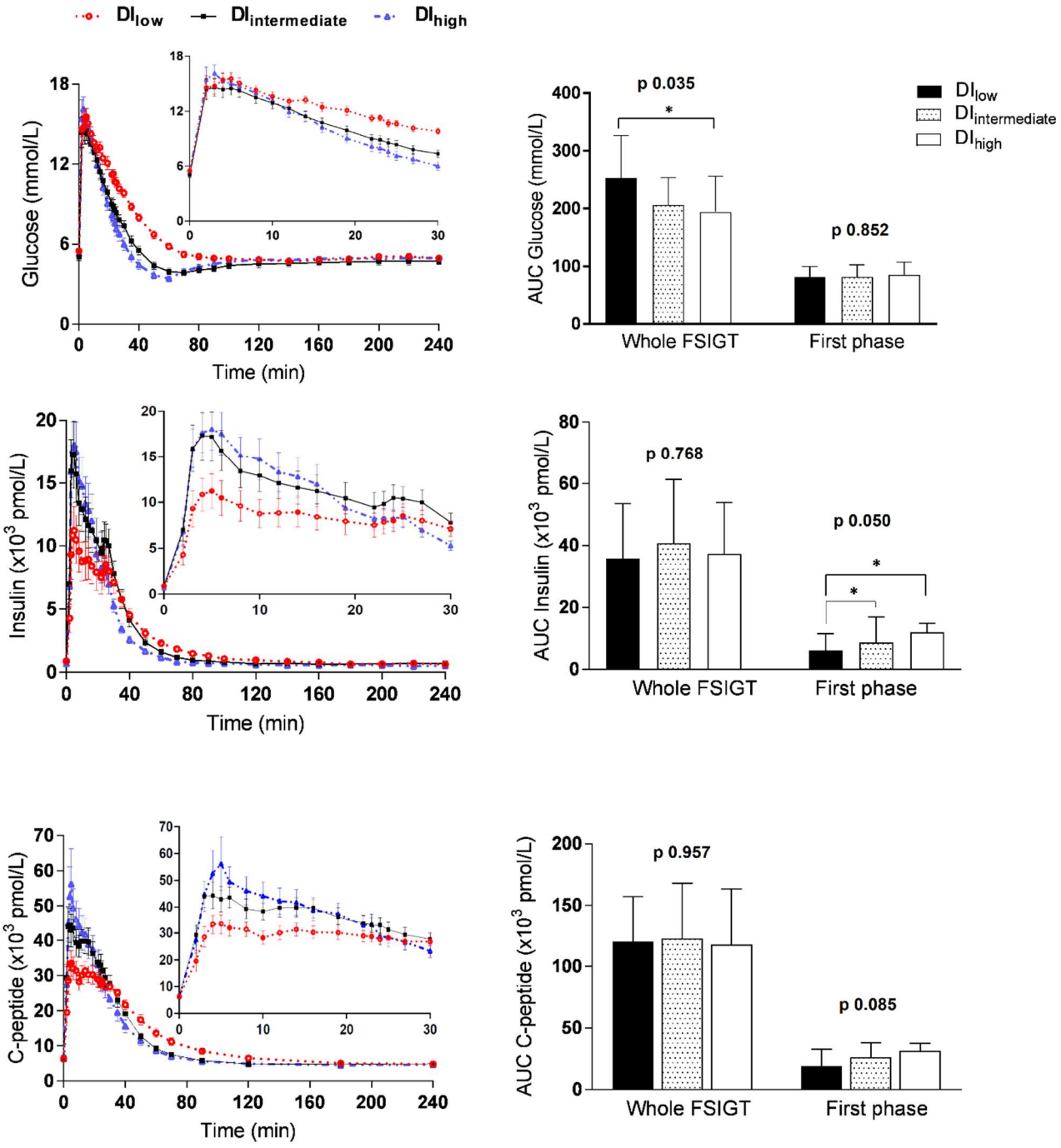


Figure 6.2: *Average time course and area under the curve for glucose, insulin and C-peptide by disposition index (DI) tertiles. Insert shown is an enlargement of the average time course from 0 to 30 minutes.*

6.1.2 Describing how DI and its components relate to each other

Various components contribute to DI and is the main outcome measure. This thesis, therefore, wanted to describe how DI relates to each of these components and how the components relate to each other. The correlation matrix of DI and its components is depicted in Table 6.2. As expected, DI was positively associated with both its immediate components: S_I and AIRg, as well as with first phase ISR (rho 0.365, p=0.016). DI was also positively associated with peripheral insulin clearance (rho 0.528, p=0.006), with a negative trend with hepatic insulin extraction (rho -0.305, p=0.079). Higher S_I was associated with lower first phase ISR and a higher hepatic insulin extraction, but was not associated with peripheral insulin clearance. Further, higher first phase ISR was associated with lower hepatic insulin extraction and higher peripheral insulin clearance. However, the different insulin clearance components were not associated with each other (p=0.191).

Further, this thesis determined the underlying correlates of a high AIRg, above the compensatory relationship with S_I. Therefore, evaluating the relative contribution of insulin secretion and insulin clearance to high AIRg, firstly without and then adjusting for S_I. This thesis started by evaluating the correlates of AIRg using simple correlations, which showed that a high AIRg was strongly associated with a high first phase ISR (rho 0.920, p<0.001)) and a low hepatic insulin extraction (rho -0.744, p<0.001), but was not associated with peripheral insulin clearance (p=0.236) (Table 6.2). Secondly, linear regression models were used to determine the relative contributions of hepatic insulin extraction and first phase ISR to AIRg, with and without adjusting for S_I (Table 6.3). Peripheral insulin clearance did not add significantly to the multivariable model and was therefore not included. In the univariate models, early

phase ISR and hepatic insulin extraction explained 70.7% and 55.3% of the variance in AIRg, respectively, while S_I explained 32% of the variance in AIRg (data not shown). In the multivariable model, hepatic insulin extraction and early phase ISR were independently associated with AIRg and together accounted for 88.5% of the variance, with only a further 3.2% explained by the addition of S_I to the model.

Table 6.2: *The relationships between disposition index (DI) and its components*

	DI		S_I		AIRg		$ISR_{first\ phase}$		HIE	
DI (AU)	1									
S_I ($(mU/l)^{-1}\ min^{-1}$)	0.459 (0.020)	n=43	1							
AIRg ($mU\ l^{-1}\ min^{-1}$)	0.372 (0.014)	n=43	-0.632 (<0.001)	n=43	1					
$ISR_{first\ phase}$ pmol/min	0.350 (0.021)	n=43	-0.598 (<0.001)	n=43	0.933 (<0.001)	n=43	1			
HIE (%)	-0.350 (0.079)	n=26	0.406 (0.040)	n=26	-0.744 (<0.001)	n=26	-0.514 (0.007)	n=26	1	
CLp (ml/min/kg)	0.528 (0.006)	n=26	0.032 (0.877)	n=26	0.241 (0.236)	n=26	0.305 (0.129)	n=26	-0.299 (0.139)	n=26

Data expressed as Spearman correlation coefficient, rho (p value) with p ≤0.05 regarded as statistically significant; DI – Disposition index, S_I – Insulin sensitivity, AIRg – acute insulin response to glucose, ISR - insulin secretion rate, HIE – hepatic insulin extraction, CLp – peripheral insulin clearance

Table 6.3: *The contribution of first phase insulin secretion and hepatic insulin extraction to acute insulin response to glucose in those with linear model preferred (n=26)*

Model	**Variables**	**Ln AIR_g**			
		β ± SE	**Partial beta**	**P**	**Model R^2**
1.	ln $ISR_{first\ phase}$	1.14 ±0.12	-	<0.001	0.787
2.	Hepatic insulin extraction (%)	-0.014 ±0.002	-	<0.001	0.553
3.	ln $ISR_{first\ phase}$	0.883 ±0.112	0.687	<0.001	0.865
	Hepatic insulin extraction (%)	-0.007 ±0.002	-0.344	0.001	
4.	ln $ISR_{first\ phase}$	0.773 ±0.141	0.602	<0.001	0.877
	Hepatic insulin extraction (%)	-0.007 ±0.002	-0.347	0.001	
	ln S_I	-0.106 ±0.566	-0.136	0.165	

Data expressed as linear regression slope (β) ± SE (Standard error), Ln – natural log, AIRg – acute insulin response to glucose, ISR- insulin secretion rate, S_I – Insulin sensitivity

6.1.3 Associations of DI and its components with body fat distribution and ectopic fat

This thesis explored body fat distribution and ectopic fat as correlates of DI. To appraise these associations, the relations of the components of DI with body fat distribution and ectopic fat were also evaluated. Firstly, DI was not associated with body fat percent (p=0.855). The associations of DI with ectopic fat depots are shown in Figure 6.3. A higher DI correlated with lower pancreatic fat and showed a tendency to associate with lower hepatic fat (p=0.076). A higher DI was also associated with a lower total soleus fat, as well as with both a lower soleus IMCL and EMCL, with a stronger association evident with soleus IMCL, than EMCL. DI was not associated with tibialis anterior fat, leg fat mass or aSAT (data not shown) but was associated with the VAT-aSAT ratio (β -167.4, p<0.001). In the univariate analysis VAT explained 32% of the variance in DI while soleus IMCL explained 18.7%, EMCL 10.9% and pancreatic fat explained 12%. Multivariable models were constructed to assess whether abovementioned associations occurred independent of age and fat mass percent. The addition of age and fat mass percent did not add significantly to the models as such the negative associations of DI with pancreatic fat (β -62.9, p=0.030), VAT (β -0.037, p<0.001), soleus fat (β -50.0, p=0.049), soleus IMCL (β -41.3, p=0.012) and soleus EMCL (β -32.0, p=0.047) remained.

Additionally, to explore whether the associations between DI and the ectopic fat depots were independent from VAT, this thesis adjusted for VAT in bivariate regression models for each of the ectopic fat depots. (Table 6.4). When adjusting for VAT, the associations between DI and pancreatic fat and soleus IMCL were no longer significant, but soleus EMCL remained significantly associated with DI. However,

when soleus IMCL was also placed in the model with soleus EMCL and VAT, the association between DI and soleus EMCL was diminished (p=0.079). VAT remained a significant correlate of DI regardless of the ectopic fat depot placed in the model.

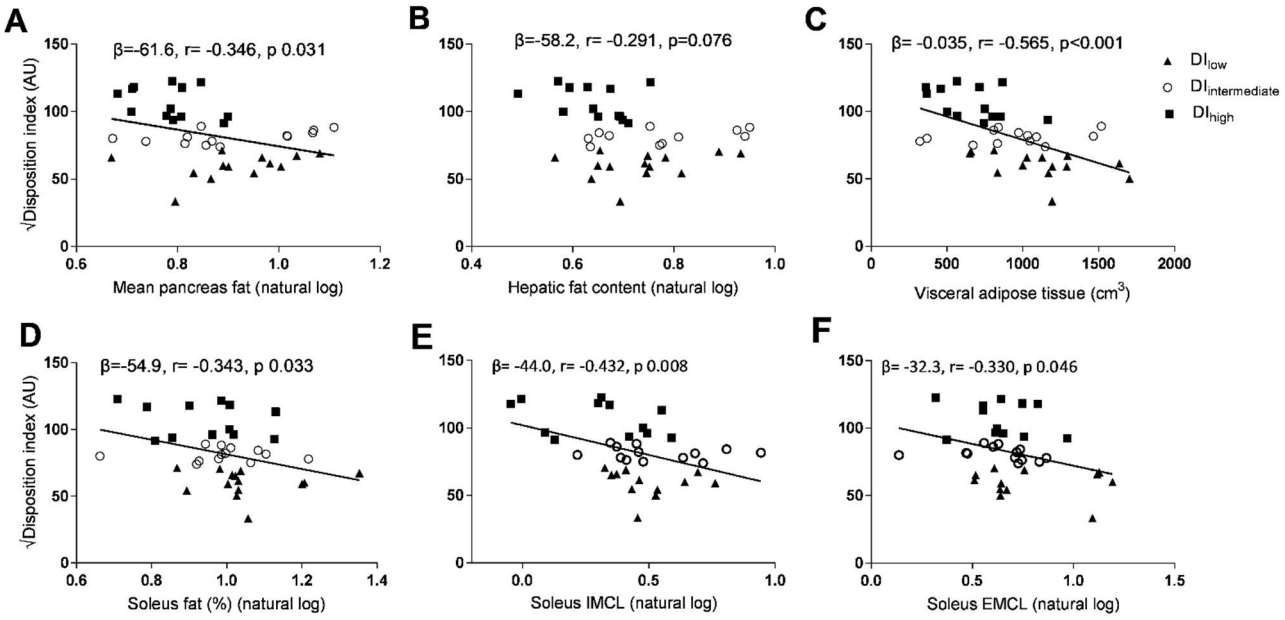


Figure 6.3: *Disposition index (DI) associations: with (A) pancreatic fat (B) hepatic fat, (C) visceral adipose tissue, (D) soleus fat (E) soleus intramyocellar fat (IMCL) and (F) soleus extramyocellular fat (EMCL) in those with low DI (solid black triangles), intermediate DI (open circles) and high DI (solid black square). Regression line indicates significant association (p<0.05) in the whole cohort, β - linear regression slope, r - correlation coefficients*

Table 6.4: *Associations between disposition index and ectopic fat adjusted for visceral adipose tissue*

		Disposition index (square root)		
		β ± SE	**P value**	**R^2**
1.	Ln Pancreatic fat	-19.2 (26.8)	0.479	0.348
	Visceral adipose tissue (cm^3)	-0.03 (0.009)	0.001	
2.	Ln Hepatic fat	-31.7 (27.4)	0.255	0.379
	Visceral adipose tissue (cm^3)	-0.04 (0.009)	<0.001	
3.	Ln Soleus fat	-35.8 (23.2)	0.132	0.348
	Visceral adipose tissue (cm^3)	-0.03 (0.009)	0.002	
4.	Ln Soleus IMCL	-27.3 (16.0)	0.097	0.363
	Visceral adipose tissue (cm^3)	-0.03 (0.009)	0.010	
5.	Ln Soleus EMCL	-29.02 (14.1)	0.047	0.386
	Visceral adipose tissue (cm^3)	-0.03 (0.008)	0.001	
6.	Ln Soleus IMCL	-23.2 (15.5)	0.146	0.427
	Ln Soleus EMCL	-26.0 (13.9)	0.071	
	Visceral adipose tissue (cm^3)	-0.02 (0.009)	0.012	

Data expressed as linear regression slope (β) and the standard error (SE). Pancreatic, hepatic, total soleus fat and soleus intramyocellular (SIMCL) and extramyocellular (SEMCL) fat were log transformed (Ln)

Next, this thesis explored the associations between the components of DI and ectopic and regional fat. Firstly, the association with fat mass percent requires mention. None of the components of DI (S_I, AIRg, first phase ISR, hepatic insulin extraction and peripheral insulin clearance) were associated with fat mass percent ($p>0.05$) (data not shown). Secondly, AIRg and first phase ISR, were not associated with pancreatic fat (rho -0.202, p=0.217 and rho-0.215, p=0.189, respectively), hepatic fat (rho 0.057, p=0.735 and rho-0.039, p=0.816, respectively) or soleus fat (rho-0.179, p=0.275 and rho-0.214, p=0.191, respectively). Additionally, hepatic insulin extraction was not associated with hepatic fat (rho -0.253, p=0.244) and peripheral insulin clearance was not associated with soleus fat (rho-0.036, p=0.872). Moreover, S_I was not associated with fat deposition in the liver (rho-0.259, p=0.177), soleus muscle (IMCL rho-0.280, p=0.094 and EMCL rho-0.021, p=0.904) or tibialis anterior muscle (IMCL rho-0.184,

p=0.270 and EMCL rho-0.248, p=0.123). Rather, significant associations were found between the components of DI and central fat measures (Table 6.5). A lower S_I was associated with higher VAT but not aSAT or VAT-aSAT ratio, while AIRg was not associated with VAT but rather with aSAT and VAT-aSAT ratio. However, when adjusting for S_I, a higher AIRg was associated with a lower VAT and VAT-aSAT ratio but the association with aSAT was no longer significant. First phase ISR was not associated with any of the central fat depots, but after adjusting for S_I, an inverse association emerged between first phase ISR and VAT, and a tended towards an association with the VAT-aSAT ratio (p=0.054). Further, hepatic insulin extraction was associated only with the VAT-aSAT ratio, and not with VAT, with and without adjusting for S_I. Peripheral insulin clearance was not associated with central fat depots (VAT rho 0.104, p=0.630 and aSAT rho 0.113, p=0.599).

In terms of peripheral fat deposition, S_I was positively associated with leg fat mass (%FM) (rho 0.411, p=0.007). However, both AIRg and first phase ISR were negatively associated with leg fat mass (%FM) (rho-0.395, p=0.010 and rho-0.425, p=0.005, respectively), but these associations were diminished to non-significance when adjusted for S_I. Further hepatic insulin extraction and peripheral insulin clearance were not associated with leg fat mass (%FM), with or without adjustment for S_I (data not shown).

6.1.4. Associations between body fat distribution and ectopic fat

Lastly, the associations between the various regional and ectopic fat depots were investigated to inform how these depots related to each other. VAT was positively associated with pancreatic fat and soleus IMCL, but not soleus EMCL, and showed a

positive trend with hepatic fat (p=0.06) (Table 6.6). Interestingly, aSAT was positively associated with hepatic fat and inversely associated with total soleus fat. Pancreatic fat was positively associated with hepatic fat (rho 0.540, p<0.001). None of the ectopic or regional fat depots were associated with fat mass percent (data not shown), except aSAT (r = 0.623, p<0.001).

Table 6.5: *Correlations of the components of DI, insulin sensitivity, secretion and clearance, with central adipose tissue depots*

	Model 1				Model 2		
	S_I (Ln_I) $(mU/l)^{-1}$ min^{-1}	**AIR_g (Ln_I) mU l^{-1} min^{-1}**	**$ISR_{first\ phase}$ (Ln) (pmol/min)**	**HIE (%)**	**AIR_g (Ln) mU l^{-1} min^{-1}**	**$ISR_{first\ phase}$ (Ln) (pmol/min)**	**HIE (%)**
VAT cm^3	**-3.07×10^{-4}**	-0.74×10^{-4}	-0.36×10^{-4}	0.007	**-2.780×10^{-4}**	**-1.95×10^{-4}**	0.011
	(0.011)	(0.511)	(0.720)	(0.262)	**(0.006)**	**(0.039)**	(0.070)
aSAT cm^3	-0.33×10^{-4}	**0.50×10^{-4}**	0.23×10^{-4}	-0.001	0.34×10^{-4}	0.08×10^{-4}	-0.0002
	(0.248)	**(0.049)**	(0.302)	(0.432)	(0.114)	(0.694)	(0.918)
VAT-aSAT ratio (Ln)	-0.320	**-0.427**	-0.228	**26.06**	**-0.617**	-0.327	**24.86**
	(0.179)	**(0.042)**	(0.233)	**(0.044)**	**(<0.001)**	(0.054)	**(0.044)**

Unadjusted (Model 1) and in adjusted for S_I (Model 2). Data expressed as the linear regression slope (P-value). S_I – Insulin sensitivity, AIRg – acute insulin response to glucose, ISR- insulin secretion rate, HIE – hepatic insulin extraction, VAT – visceral adipose tissue, SAT – abdominal subcutaneous adipose tissue. Ln – natural log transformed variable

Table 6.6: *Correlation matrix between regional and ectopic fat depots*

	VAT (cm^3)	aSAT (cm^3)	Pancreatic fat (%)	Hepatic fat (%)	Soleus fat (%)	TA fat (%)	SIMCL (%)	SEMCL (%)
VAT (cm^3)	1							
aSAT (cm^3)	**0.338 (0.029)**	1						
Pancreatic fat (%)	**0.417 (0.007)**	-0.070 (0.662)	1					
Hepatic fat (%)	0.300 (0.060)	**0.323 (0.042)**	**0.540 (<0.001)**	1				
Soleus fat (%)	0.128 (0.438)	**-0.349 (0.029)**	**0.329 (0.044)**	0.048 (0.779)	1			
TA fat (%)	-0.015 (0.926	-0.201 (0.220)	0.266 (0.107)	0.022 (0.898)	**0.503 (<0.001)**	1		
SIMCL (%)	**0.403 (0.014)**	-0.044 (0.796)	**0.395 (0.017)**	0.093 (0.595)	**0.585 (<0.001)**	**0.547 (<0.001)**	1	
SEMCL (%)	0.121 (0.474)	-0.004 (0.981)	0.087 (0.613)	-0.127 (0.468)	**0.426 (0.009)**	**0.392 (0.017)**	0.234 (0.153)	1
Leg fat mass (%)	**-0.450 (0.003)**	**-0.623 (<0.001)**	-0.038 (0.812)	-0.293 (0.067)	**0.334 (0.033)**	0.107 (0.504)	0.024 (0.885)	0.125 (0.448)

Data expressed as correlation coefficient (p value). P≤0.05 is deemed statistically significant. VAT - Visceral adipose tissue; aSAT - abdominal subcutaneous adipose tissue; TA - tibialis anterior, SIMCL - soleus intramyocellular fat; SEMCL - soleus extramyocellular fat.

6.2 DISCUSSION

β-cell function is a critical factor in the pathogenesis of T2D. Nevertheless, the correlates of β-cell function, prior to onset of T2D, are incompletely understood, especially in black African populations who present with a phenotype of low S_I, hyperinsulinemia and low ectopic fat deposition. This study not only showed associations between DI and its classic components, but we extend on existing evidence by demonstrating that DI was positively associated with peripheral insulin clearance. Notably, the major correlate of AIRg was first phase ISR above insulin clearance. Another key finding was that a higher DI was associated with lower VAT, pancreatic fat and soleus fat and a trend for lower hepatic fat. However, VAT emerged as the strongest correlate of DI, above and independent of the other ectopic fat depots. In addition, this thesis showed that AIRg, typically high in black African populations,

was associated with lower VAT deposition. This study further adds by showing that a lower VAT also associated with a higher ISR and lower hepatic insulin extraction. Thus, our findings suggest that VAT may be the main culprit in low DI in this cohort, not only through its association with a lower S_I, but also through its relationship with the AIRg's downstream components, ISR and hepatic insulin extraction.

Our study, not only distinguishes between hepatic and peripheral insulin clearance, which has only been done in a few studies in adults without T2D (59,71,189), but also reported a positive association between DI and peripheral insulin clearance which to the best of my knowledge has never been shown before in any population. The reason for this relationship is unclear. Nevertheless, considering DI is based on the product of S_I and AIRg, we can postulate two scenarios. Firstly, a higher DI may be due to hyperinsulinemia relative to the level of S_I. However, hyperinsulinemia has been associated with lower insulin clearance in both adipose tissue (353) and skeletal muscle (354), due to reduced affinity of insulin receptors at these sites, and is therefore an unlikely explanation for our finding of a higher peripheral insulin clearance relative to higher DI. Secondly, a higher DI could be due to a greater S_I in relation to the level of AIRg. In this scenario, a higher peripheral insulin clearance may be due to enhanced binding of insulin to insulin receptors in peripheral tissues. Although a positive association has been demonstrated between S_I and hepatic insulin clearance (33), no association between insulin internalization, a measure of insulin clearance, and S_I was observed in rat adipocytes (353), whereas the association between S_I and insulin clearance in skeletal muscle is unknown. However, in support of the second scenario, we showed that those with the highest DI and peripheral insulin clearance were also more insulin sensitive, but only exhibited a slightly higher AIRg compared to those with lower DI and peripheral insulin clearance. Nevertheless, peripheral insulin clearance

was not associated with S_I and AIRg, which may suggest that only when compensation between S_I and AIRg is not matched, peripheral insulin clearance becomes more important. Further, we may also consider that the observed association between DI and peripheral insulin clearance is in compensation for a lower hepatic insulin extraction. However, we found no association between peripheral and hepatic insulin extraction, which is in accordance with Polidori *et al*. (71), who suggested that these components are differentially regulated. We also evaluated the associations of peripheral insulin clearance with ectopic fat deposition as possible explanation for the association with DI, but found that peripheral insulin clearance was not associated with soleus or tibialis anterior fat. Skeletal muscle fat has been associated with reduced S_I (82), which may affect peripheral insulin clearance in the muscle. However, this requires further study, considering that the muscle is only secondary to the kidney in the proportion of insulin cleared in the periphery (17).

Preservation of DI is critical for delaying the onset of T2D. Therefore, we investigated, firstly, whether ectopic fat accumulation may explain the variance in DI. Secondly, we assessed the associations of ectopic fat with the components of DI, with a particular focus on hepatic and peripheral insulin clearance, which have not been studied together in this context before. Our study demonstrated that a higher DI was associated with lower pancreatic and soleus fat and a tendency towards a lower hepatic fat, but these associations were not independent from VAT. A similar observation was found in overweight African American and Hispanic adolescents (13 to 25 years old) without T2D (204). In contrast a positive association between DI and pancreatic fat, adjusted for BMI and VAT, was shown in black African American women (51). However, this study included both participants with and without T2D, which may explain the discrepancy in findings. Further, two previous studies

conducted in black African adults without T2D assessed the association of AIRg and pancreatic fat, with one showing a positive association (209) and another showing no association (204). While our study is in accordance with the latter study, we also extend the literature by showing that pancreatic fat was not associated with first phase ISR determined by C-peptide deconvolution, or with hepatic insulin extraction. Differences in the accuracy of the techniques used to determine pancreatic fat and VAT may be considered to explain the lack of association between pancreatic fat and ISR and the diminished association between pancreatic fat and DI, after adjusting for VAT. However, the technique we used to detect pancreatic fat in this thesis showed good accuracy with a CV of 3.5% (324). Notably, a positive association was found between pancreatic fat and VAT in our study. Our findings may therefore suggest that pancreatic fat may only be a marker of VAT accumulation and may not be detrimental to DI in this cohort.

The ability to maintain DI and prevent deteriorating glucose tolerance depends on the balance between AIRg and S_I (180). However, there is no consensus on the mechanism of maintaining a higher AIRg, which is frequently observed in black African populations (39,45–47). Some studies reported that a lower hepatic insulin clearance alone is responsible for a higher AIRg (44,66), while others showed that both lower hepatic insulin clearance and higher ISR contribute towards a higher AIRg (47,58,59). Notably, AIRg may be out of proportion for the level of S_I, and to assess the relative contribution of first phase ISR and insulin clearance to AIRg in this context, adjustment for S_I is required. No association was found between AIRg and peripheral insulin clearance. Accordingly, a lower hepatic insulin extraction and higher first phase ISR were the main independent contributors towards a higher AIRg, independent of S_I, but first phase ISR explained more of the variance in AIRg. Lowering hepatic insulin

clearance is an important compensatory mechanism to reduce the strain on the pancreatic β-cells, which has been shown in canines (67). However, this research has shown that a higher ISR can occur independent of a lower hepatic insulin extraction and lower S_I. This may indicate that despite a lower hepatic insulin clearance, pancreatic β-cells continue to secrete insulin at a higher rate, which may be detrimental to the longevity of the β-cell in this cohort. Black African children present with hyperinsulinemia prior to obesity and a low S_I (58), so whether a higher ISR is driven by a genetic predisposition or epigenetic alterations, remains to be determined. Although the inputs into these mathematical models may overlap, this is unlikely to explain the findings because three different models were utilized to estimate abovementioned variables; the two-compartmental model of C-peptide for ISR, the linear model from Polidori *et al*. for hepatic insulin extraction and the minimal model of insulin kinetics for AIRg and S_I. Also, first phase ISR, hepatic insulin extraction and S_I were independently associated with AIRg. Further, we also need to consider that a higher AIRg, independent from S_I, may be explained by reduced insulin independent glucose uptake, measured by glucose effectiveness. We examined these associations in bivariate analyses and demonstrated a positive association between glucose effectiveness and AIRg or first phase ISR, independent from S_I. This makes it unlikely that a higher AIRg, independent from S_I, is due to reduced glucose effectiveness.

This study further examined the associations between the components of AIRg and ectopic fat. Previously, reduced hepatic insulin extraction and increased insulin secretion have been associated with hepatic fat accumulation (184). The association between fasting hepatic insulin extraction and hepatic fat had been explored in African American women before (49). However, the association between stimulated hepatic insulin clearance, which is a more physiological response, and hepatic fat has not

been previously investigated in black African populations without T2D. We showed that hepatic fat was not associated with hepatic insulin extraction and insulin secretion in obese black South African women. Therefore, in black African populations, hepatic fat may not be an important correlate of alterations in hepatic insulin extraction and insulin secretion prior to T2D, and also not in those with early T2D (53). However, we should also consider that a lack of statistical power my explain this finding in our study since we only included those in whom the linear model was preferred. Instead, we found that a lower first phase ISR and higher hepatic insulin extraction was associated with higher VAT and VAT-aSAT ratio, respectively. This highlights a novel mechanism to explain the association between hyperinsulinemia and VAT and to the best of our knowledge, no previous study has evaluated the effect of central fat depots on hepatic insulin extraction. In addition, evidence on the associations between central fat depots and ISR, determined by C-peptide deconvolution are also limited in black African populations. A previous study showed ethnic and age differences between first phase ISR and VAT: a positive association in white women and a negative association in African American women and with a stronger negative association in post-menopausal African American women (54). However, we found that an inverse association between VAT and first phase ISR, independent of S_I, was already evident in young pre-menopausal South African women. The negative associations between hyperinsulinemia indicators and VAT and VAT-aSAT ratio may reflect a reduced capacity of aSAT to store fat with a redirecton of fat to VAT and other ectopic sites, in line with the overflow hypothesis (109). Therefore, we should consider that VAT may only be a marker of ectopic fat accumulation and that VAT emerges as the strongest correlate due to a smaller measurement error in quantifying VAT compared to other ectopic sites. Nonetheless, possible reasons for the negative association between

VAT and ISR could be mediated through inflammatory (355,356) and/or lipotoxic pathways that involves lipid intermediaries rather than TGs (198). This may also explain why no association was found between ISR and pancreatic fat that reflects the TG content in the pancreas. Interestingly, black African women have lower VAT compared to other ethnicities (39,47,61), but they have a greater propensity to increase VAT over time, with the greatest increase occurring in the 20-29-year age group (357). Taken together, the ability to compensate for a lower S_I with a higher insulin response may be compromised with an increase in VAT with increasing age and adiposity, and this inhibitory effect may start from a young age in black South African women. However, further studies are needed to confirm this hypothesis.

This study also assessed the association between S_I and ectopic fat and found no significant association between S_I and skeletal muscle or hepatic fat. These findings suggest that these ectopic fat depots are not important contributors to S_I in our study. However, considering that whole body S_I was measured in this study this could have diluted the associations with hepatic and soleus fat. Indeed, a small South African study found that in black South African women, hepatic fat was associated with hepatic S_I but not peripheral S_I (42). Furthermore, total soleus fat and soleus IMCL were associated with peripheral S_I, but not hepatic S_I (42). Another reason that may explain the lack of association between S_I and hepatic fat may be reduced statistical power. The liver scans from five participant could not be used due to technical challenges experienced during MRI acquisition. Nevertheless, lower S_I was associated with higher VAT, which may contribute to a decline in DI in this cohort.

A major strength of this study is that ISR and both hepatic and peripheral clearance were evaluated, determined using mathematical modelling, which has never been done before in an African cohort. Further we assessed pancreatic, hepatic and skeletal

muscle ectopic fat depots, as well as VAT and aSAT volumes using MRI. However, limitations of this study were that we did not determine glucose tolerance, and we did not distinguish between hepatic and peripheral S_I. In addition, these findings are applicable to pre-menopausal, obese black South African women and may not be extrapolated to, all black South African women, men or other ethnicities.

In conclusion, an original finding from this study was that DI was associated with peripheral insulin clearance, which may be explained by a greater S_I, instead of hyperinsulinemia. Further, while both hepatic insulin extraction and insulin secretion contributed towards hyperinsulinemia, insulin secretion was more important. Ectopic fat was not an important independent correlate of DI and its components. Rather, a key finding was that higher VAT was the principal correlate of a lower DI, above other ectopic fat depots. Additionally, the associations of higher VAT on the downstream components of DI, a lower first phase ISR and higher hepatic insulin extraction, exposes a novel mechanism of low DI prior to T2D onset. Accordingly, the prevention of VAT accumulation, especially in young black African women may be an important target for DI preservation.

CHAPTER 7: EXERCISE INTERVENTION

7.1 RESULTS

7.1.1 COMPARING PARTICIPANTS AT BASELINE

The exercise and control group participants were well matched at baseline. They consumed a similar diet and had similar levels of energy expenditure (Table 7.1). Further, they did not differ in age, cardiorespiratory fitness and metabolic outcomes (Table 7.2). Body composition, body fat distribution (Table 7.3) and ectopic fat deposition (Figure 7.2) also did not differ between the groups.

7.1.2 EXERCISE ADHERENCE AND EFFECT OF EXERCISE ON CARDIORESPIRATORY FITNESS

Mean number and percentage of exercise session attended were 38±6 (range 25-49, maximum number of sessions = 48) and 79% (range 52-100%), respectively, at a mean intensity of 79.6±4.0 $\%HR_{peak}$. An improvement in cardiorespiratory fitness (VO_{2peak}) was observed in the exercise group (2077±211 to 2278±231 ml/min, $p<0.001$ and 24.9±2.42 to 27.6±3.39 ml/kg/min, $p<0.001$), while no change was observed in the control group (2099±282 to 2032±196 ml/min, $p=0.286$ and 23.9±2.97 to 23.0±2.64 ml/kg/min, $p=0.309$). The change in the exercise group was significantly different from the change in the control group for the absolute (interaction $p=0.001$) and relative (interaction $p<0.001$) VO_{2peak}.

7.1.3 DIETARY AND PHYSICAL ACTIVITY

Energy intake, macronutrient composition of the self-reported diets, and total energy expenditure (non-exercise days in the exercise group) did not differ within or between groups at 4, 8 and 12 weeks (Table 7.1).

Table 7.1: *Energy intake and energy expenditure (non-exercise days for exercise group) over 12-week intervention*

	Control					Exercise					Group @time
	Baseline	Week 4	Week 8	Week 12	Time @group P value	Baseline	Week 4	Week 8	Week 12	Time @group P value	Joint P
Energy intake (kJ/day)	8138 (6493-9434)	7489 (7110-9217)	7963 (6306-8920)	8429 (7335-9509)	0.390	8369 (7015-10566)	7838 (6934-9781)	8516 (7363-9659)	8443 (7595-9919)	0.426	0.686
Fat (% of total EI)	31.0 ±5.6	29.2 ±5.1	29.5 ±7.3	27.8 ±6.4	0.379	30.4 ±6.1	31.0 ±6.0	31.3 ±5.5	32.2 ±5.9	0.719	0.188
Protein (% of total EI)	14.3 ±1.9	13.2 ±2.3	13.8 ±2.8	13.6 ±2.1	0.642	13.2 ±2.5	13.9 ±2.9	13.4 ±2.0	13.7 ±3.0	0.761	0.578
Carbohydrate (% of total EI)	54.0 ±5.7	56.9 ±6.0	56.0 ±7.6	57.8 ±7.2	0.214	55.1 ±5.4	53.9 ±5.9	54.2 ±5.3	52.7 ±8.3	0.548	0.076
Total energy expenditure (kJ)	7355 (6527-8503)	7446 (6587-8772)	7422 (6417-8636)	7413 (6470-8493)	0.386	6796 (6460-7816)	6874 (6339-7906)	6492 (6308-8315)	6877 (6394-7544)	0.223	0.160

Mean (±SD) for normally distributed variables. Non-normally distributed variables are described with median (interquartile range) and were log-transformed prior to mixed model analysis. EI – Energy intake. Time@group p value – reflects the interaction between time points within each group. Group @Time joint p value reflects the interaction between groups at each time point combined

7.1.4 Effect of exercise training on metabolic outcomes

Results of metabolic outcomes pre- and post-intervention are shown in Table 7.2. S_I improved in the exercise group (*post hoc* p=0.005), improving in 15 out of 19 participants, with no change in the control group (*post hoc* p=0.711) (Figure 7.1). Nevertheless, exercise training did not alter the AUC of $glucose_{total}$ (group x time p=0.529). Similarly, the AUC for insulin (group x time p=0.853) and C-peptide (group x time p=0.956) over the entire FSIGT period did not change (Figure 7.2). Accordingly, no changes were observed in AIRg (Figure 7.1), ISR, hepatic and peripheral insulin clearance. As S_I improved with no reciprocal change in AIRg, DI increased after exercise training (*post hoc* p=0.028).

This thesis further assessed whether the changes in S_I, AIRg and DI differed by adherence to the exercise intervention, characterized as the exercise dose (Figure 7.3). The exercise group where split into moderate and high exercise dose groups, compared to the control group. Notably, a significant increase in S_I was only observed in the high exercise dose group compared to the control group (p=0.009). Although the moderate exercise dose group showed a similar increase in S_I (median change (25-75th percentile) 0.59 (0.01 to 0.95) compared to the high exercise dose group (median change 0.53 (0.35 to 0.75) (p=0.132), there was greater variability in response. Equally, a significant increase in DI was only observed in the high exercise dose compared to the control group (p=0.009). However, no significant changes were observed in AIRg after the intervention regardless of the exercise dose.

Table 7.2. *Age and metabolic outcomes of participants before and after the 12-week intervention period*

	Control group		Exercise group		C/E‡	Group	Time	Group x Time
	Pre	Post	Pre	Post		p value		
Age (years)	23 (21-27)	-	22 (21-24)	-	15/20	0.748	-	-
HbA1c (%)	5.2 ±0.35	5.3 ±0.38	5.2 ±0.32	5.2 ±0.29	15/19	0.676	0.794	0.884
HbA1c (mmol/mol)	33 ±2.2	34 ±2.4	33 ±2.0	33 ±1.84	15/19	-	-	-
Fasting glucose (mmol/L)	5.0 ±0.66	5.1 ±0.79	5.5 ±0.84	5.1 ±0.98	15/19	0.102	0.829	0.217
Fasting insulin (pmol/L)	78.6 (58.4-85.2)	79.2 (64.5-117.6)	88.5 (38.6-114.6)	75.3 (63.2-102.6)	15/19	0.942	0.979	0.773
Fasting C-peptide (pmol/L)	575.9 (484.9-787.8)	586.7 (448.5-916.9)	620.6 (362.4-804.3)	643.8 (458.4-852.3)	15/19	0.949	0.900	0.943
ISR_{basal} (pmol/min)	74.8 (63.0-102.4)	76.3 (58.3-119.2)	80.7 (47.1-102.4)	83.7 (59.6-110.8)	13/19	0.681	0.795	0.715
HOMA2 %IR	1.8 (1.4-2.2)	2.0 (1.1-2.9)	2.2 (1.0-2.9)	1.9 (1.4-2.6)	15/19	0.939	0.993	0.943
HOMA2 %B	143.5 (98.9-235.6)	132.1 (117.9-208.6)	131.5 (89.9-182.7)	145.7 (97.6-199.7)	15/19	0.287	0.907	0.227
S_I $x10^{-4}(mU/l)^{-1}$ min^{-1}	2.01 (1.29-3.24)	1.83 (1.65-32.64)	2.04 (1.20-2.77)	2.17 (1.45-3.69)*	13/19	0.094	0.711	**0.037**
AIR_g $x10^3$ $(mU/l)^{-1}$ min^{-1}	2.43 (2.10-4.28)	2.70 (1.91-4.21)	3.16 (1.72-4.72)	3.33 (1.68-4.33)	14/19	0.609	0.404	0.804
DI $x10^3$	7.80 (4.53-8.78)	5.92 (5.34-8.25)	6.10 (3.61-7.12)	6.53 (5.56-9.22)*	13/19	0.151	0.294	**0.035**
Sg $x10^{-2}$ (min^{-1})	2.4 ±0.97	2.2 ±0.66	2.3 ±0.98	2.6 ±0.79	13/19	0.795	0.574	0.226
ISR_{total} (pmol/min)	4272 (3572-5798)	5560 (4536-7588)	4887 (3229-8248)	5442 (4207-9827)	13/19	0.359	0.057	0.325
$ISR/Glucose_{total}$	23.7 (17.0-28.0)	29.0 (20.0-39.9)	24.2 (11.8-38.6)	27.8 (18.9-51.5)	13/19	0.393	0.010	0.151
$C\text{-peptide}/Insulin_{basal}$	8.1 (6.47-9.37)	7.5 (7.31-8.71)	7.0 (6.39-9.09)	7.7 (7.01-8.41)	15/19	0.957	0.916	0.711
HIE (%)	56.4 ±8.3	53.6 ± 13.6	52.4 ±9.0	54.2 ±10.7	10/11	0.358	0.408	0.328
CLp (ml/kg/min)	86.2 (74.7-131.9)	89.3 (76.7-94.6)	105.4 (97.8-158.6)	101.0 (73.1-155.4)	10/11	0.138	0.734	0.718

*Mean (±SD) for normally distributed variables and median (interquartile range) for non-normally distributed variables, Non-normally distributed variables were transformed. P-values are derived from Mixed Model analysis, ISR – Insulin secretion rate, HOMA – Homeostasis model of assessment, IR – insulin resistance, B - beta cell function, S_I – Insulin sensitivity, AIRg – Acute insulin response to glucose, DI – Disposition index, Sg – Glucose effectiveness, AUC – Area-under-curve , HIE – hepatic insulin extraction, CLp – peripheral insulin clearance. *Significant post hoc difference (pre- vs post-intervention) within group p≤0.05, †Significant post-hoc difference (Exercise vs Control) between groups p≤0.05, ‡C/E – Number of participants in Control (C)/Exercise (E) group*

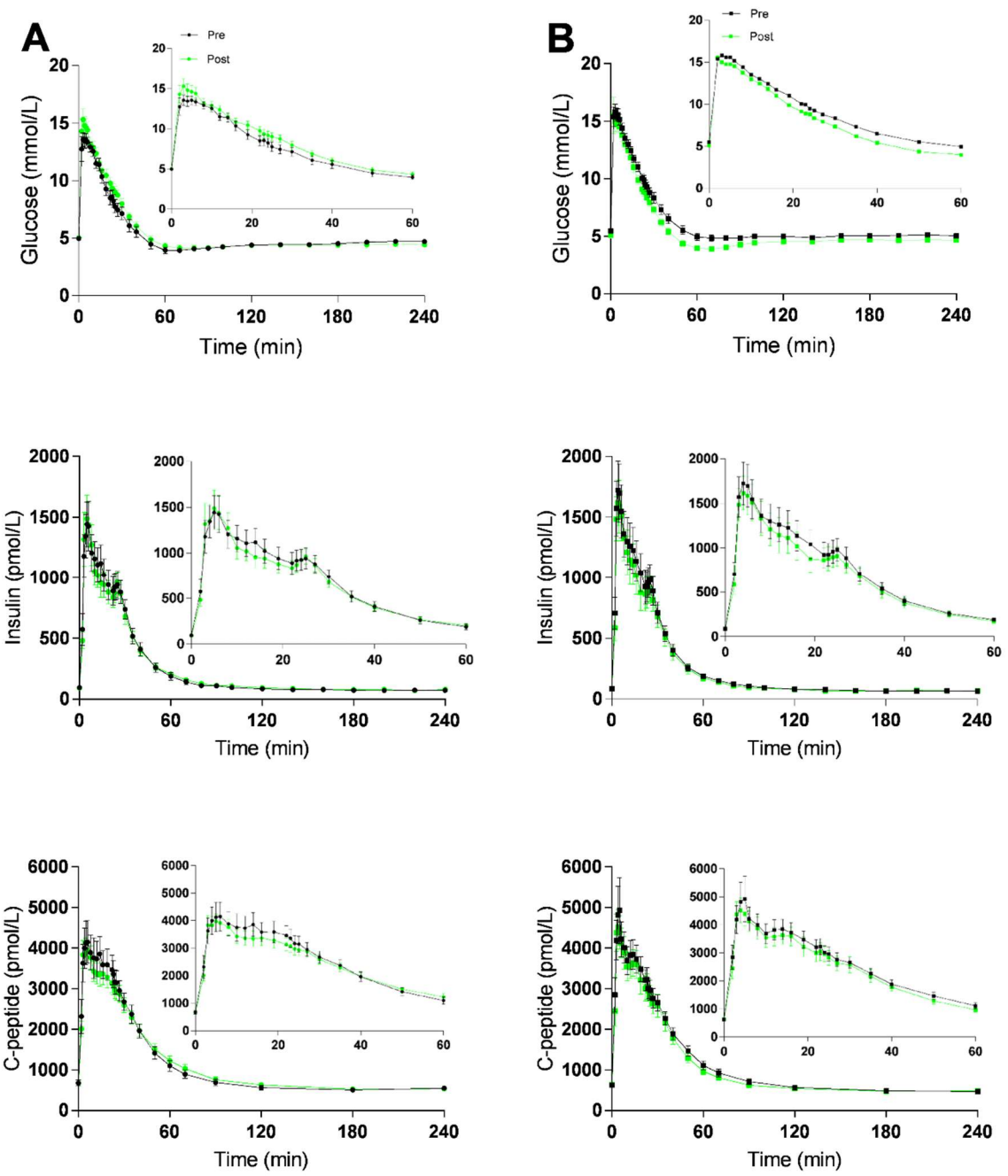


Figure 7.1: *Average time course of glucose, insulin and C-peptide in the (A) control (circles) and (B) exercise group (squares), before (black symbols) and after (green symbols) the exercise intervention. The insert graph is an enlargement of the 0 to 60 min time period.*

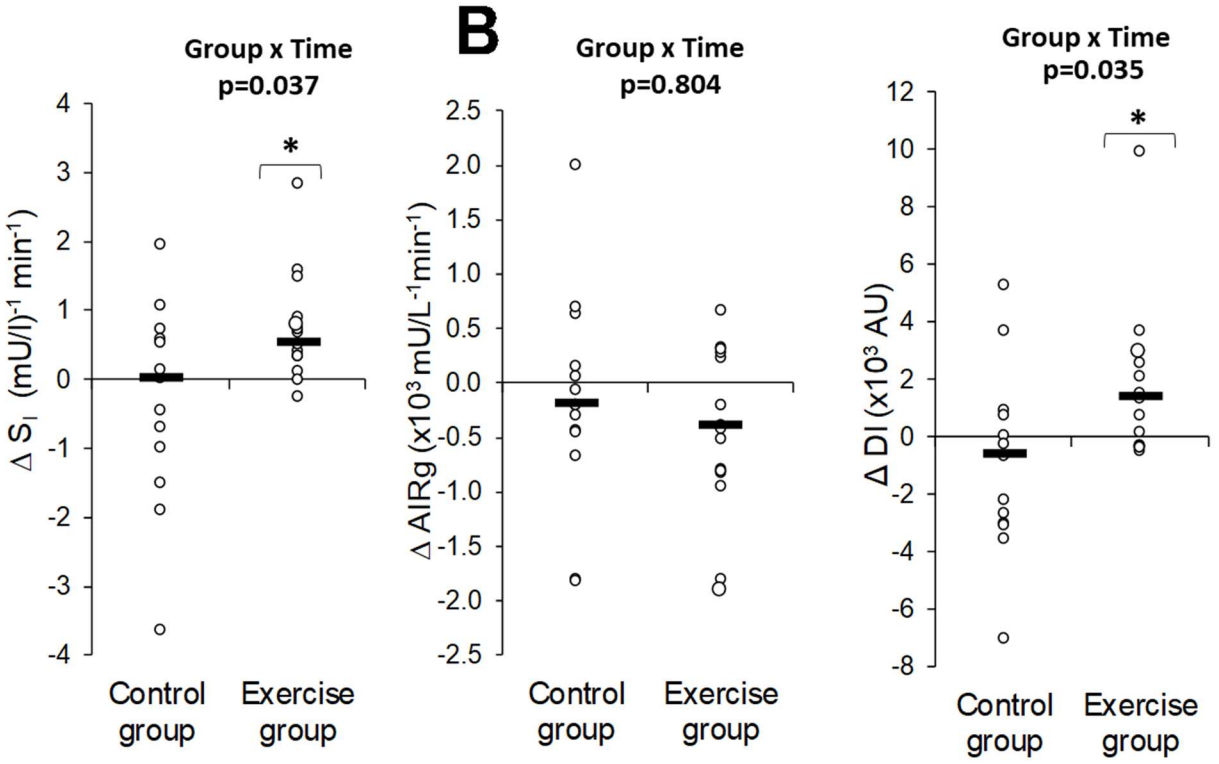


Figure 7.2. *Changes in (A) insulin sensitivity (S_I), (B) acute insulin response to glucose (AIR_g) and (C) disposition index (DI) in the control and exercise groups. Thick black line – median, empty circles – individual changes. *Post hoc test p≤0.05*

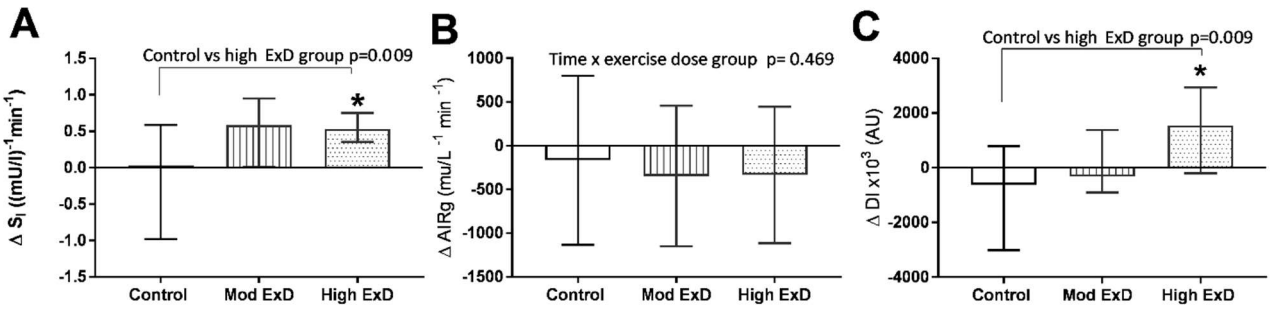


Figure 7.3. *Comparison of changes in (A) insulin sensitivity (S_I) (B) acute insulin response to glucose (AIR_g) and (C) disposition index (DI) in the control (white bar), moderate exercise dose (ModExD) (vertical stripe bar) and high exercise dose (High ExD) (dotted bar) groups. *Post hoc test for post compared to pre vaue p≤0.05 when the interaction from mixed model test was significant. Between group p-value was also derived from mixed model analysis.*

7.1.5 Effect of exercise training on body composition, body fat distribution and ectopic fat content

Body composition and body fat distribution pre- and post-intervention are shown in Table 7.3. Exercise training resulted in a small but significant reduction in body weight (change: -0.8 kg, *post hoc* p=0.038), whereas the control group gained body weight (change: 0.99 kg, *post hoc* p=0.030) and aSAT (change: 278.3 cm^3, *post hoc* p=0.008). While exercise training did not alter relative fat mass (%), fat-free soft tissue mass (kg), aSAT and VAT (cm^3), there was a small but statistically significant decrease in gynoid fat mass (change: -0.27 %FM, p<0.001) in the exercise group only.

Ectopic fat pre-and post-intervention are shown in Figure 7.3. After exercise training pancreatic (7.8 (6.4-10.4) % to 6.9 (6.4-10.4) %), hepatic (4.9 (4.5-6.4)% to 4.8 (3.9-5.6)%), soleus (10.3 (9.5-11.5) % to 9.6 (8.0-10.9) %) and tibialis anterior (3.9 (3.2-5.6) % to 3.8 (3.1-5.1) %) fat remained unaltered. Similarly, the control group showed no changes in ectopic fat (group x time p>0.05).

Table 7.3. *Body composition and body fat distribution of participants before and after the 12-week intervention period*

	Control group		Exercise group		C/E‡	Group	Time	Group x Time
	Pre	Post	Pre	Post		p value		
Body weight (kg)	87.8 ±10.9	88.8 ±11*	84.1 ±8.7	83.3 ±9.7*	15/20	0.267	0.030	0.003
Body Mass Index (kg/m^2)	33.4 ±2.7	33.8 ±2.8*	34.1 ±2.8	33.8 ±3.1*	15/20	0.430	0.038	0.003
Waist circumference (cm)	100.0 (97.0-112.0)	103.0 (98.5-116.5)*	103.2 (97.9-108.1)	99.5 (94.0-103.3) *†	15/20	0.911	0.013	<0.001
Waist-to-hip-ratio	0.88 (0.83-0.93)	0.89 (0.86 -0.95)	0.89 (0.87-0.94)	0.87 (0.86-0.91)	15/20	0.202	0.078	0.015
FFSTM (kg)	37.7 (35 - 41)	38.2 (35 - 41)	37.1 (34 - 39)	37.1 (34 - 40)	14/20	0.293	0.223	0.324
Body fat mass (%)	49.8 (47-54)	50.9 (48-52)	49.9 (49-52)	49.9 (48-51)	14/20	0.981	0.480	0.471
Trunk fat (%FM)	45.8 ±4.7	45.6 ±4.7	48.0 ±4.6	47.7 ±4.7	14/20	0.170	0.568	0.845
Leg fat (%FM)	42.5 ±5.0	42.9 ±5.3	39.8 ±5.0	39.8 ±4.8	14/20	0.103	0.386	0.607
Android fat (%FM)	8.0 ±1.3	7.9 ±1.5	8.3 ±1.0	8.1 ±1.0	14/20	0.572	0.163	0.860
Gynoid fat (%FM)	19.4 ±2.3	19.6 ±2.3	18.5 ±1.7	18.2 ±1.6*†	14/20	0.129	0.323	0.002
Visceral fat (cm^3)	903.1 ±431.0	953.1 ±403.9	920.0 ±322.1	906.2 ±346.9	13/20	0.850	0.177	0.178
Abdominal subcutaneous fat (cm^3)	5298.9 ±1853.8	5584.4* ±1956.9	5489.3 ±1053.4	5447.7 ±1260.7	13/20	0.850	0.008	0.018

*Mean (±SD) for normally distributed variables and median (interquartile range) for non-normally distributed variables, Non-normally distributed variables were transformed. P-values are derived from Mixed Model analysis. FFSTM – Fat free soft tissue mass, FM – Fat mass. *Significant post hoc difference (pre- vs post-intervention) within group p≤0.05, †Significant post-hoc difference (Exercise vs Control) between groups p≤0.05, ‡C/E – Number of participants in Control (C)/Exercise (E) group*

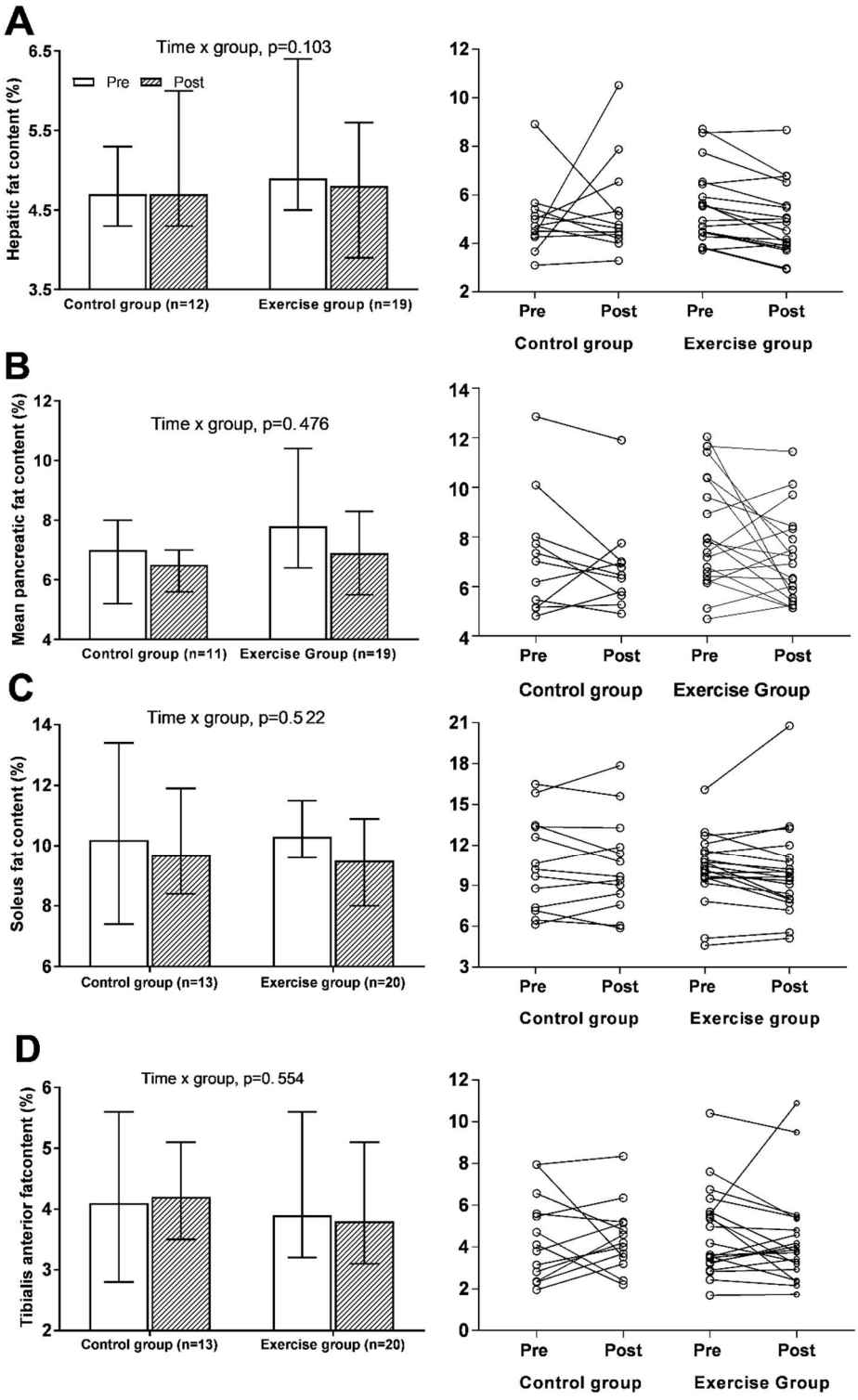


***Figure 7.4**: Individual changes from pre- to post-exercise training in (A) hepatic, (B) pancreatic, (C) soleus and (D) tibialis anterior fat in control and exercise groups.*

The changes in body composition, regional and ectopic fat were also stratified by exercise dose (control vs moderate vs high exercise dose) (Figure 7.5). A significant reduction in body weight was observed in the high exercise dose group ($p<0.001$) but not in the moderate exercise dose group ($p=0.925$), with this change being significantly different from both the moderate exercise dose ($p=0.017$) and control groups ($p<0.001$). Nevertheless, no significant changes were observed in fat mass (%) after the intervention period. Interestingly, a significant reduction in gynoid fat mass was only observed in the moderate exercise dose group ($p=0.001$) and not in the high exercise dose group ($p=0.102$), which was significantly different compared to the control group ($p=0.001$). There was a tendency for a reduction in VAT in the high exercise dose group ($p=0.082$), but this change was not significantly different from the other 2 groups. No differences in other measures of body fat distribution or ectopic fat deposition were found between the exercise dose groups (Figure 7.5).

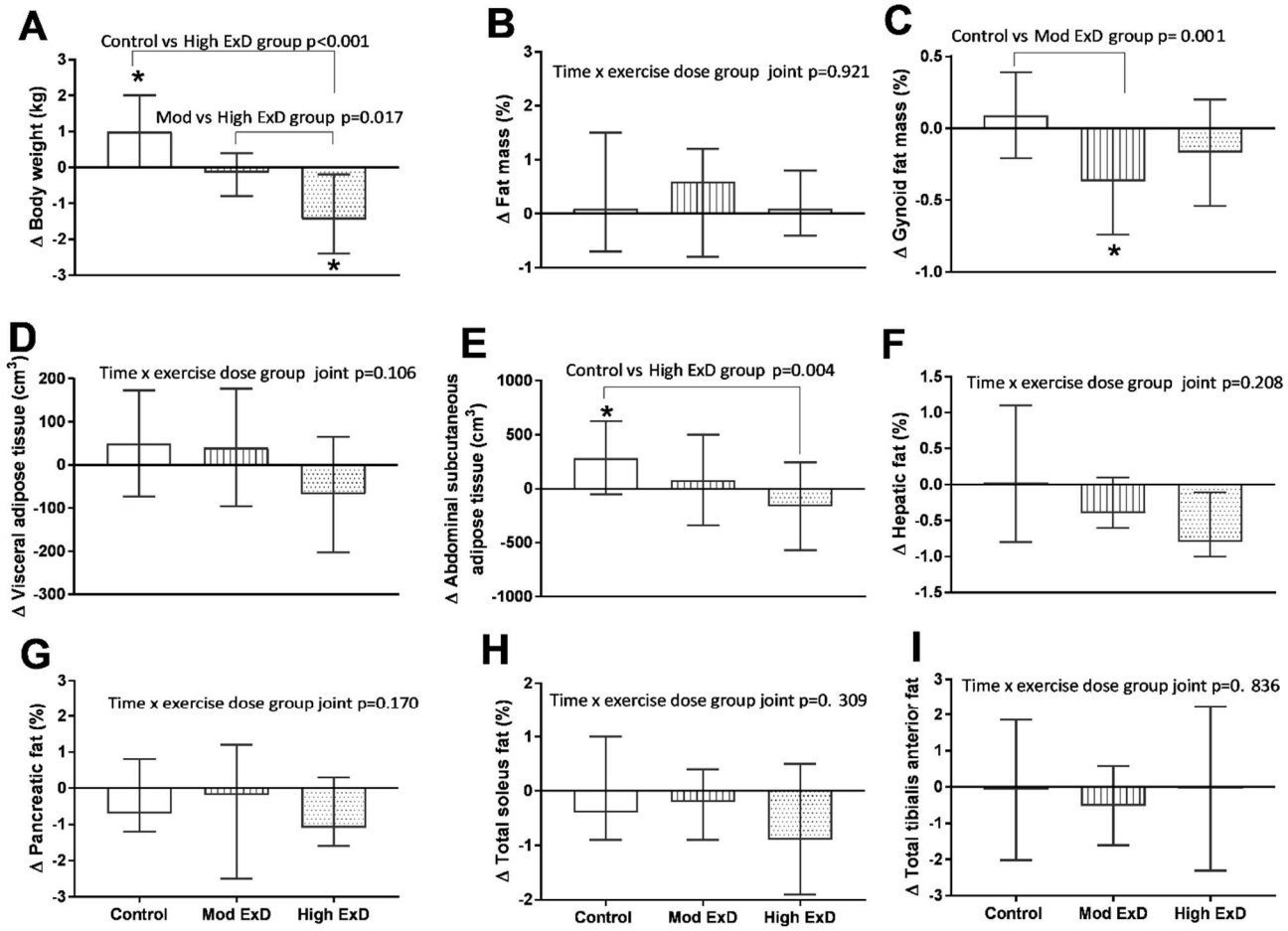


Figure 7.5 *Comparison of changes in (A) Body weight (B) Fat mass (%) (C) Gynoid fat mass (%), (D) Visceral adipose tissue, (E) Abdominal subcutaneous adipose tissue, (F) Hepatic fat, (G) Pancreatic fat, (H) Total soleus fat, (I) Total tibialis anterior fat in the control (white bar), moderate exercise dose (ModExD) (vertical stripe bar) and high exercise dose (High ExD) (dotted bar) groups. *Post hoc test for post compared to pre vaue p≤0.05 when the interaction from mixed model test was significant. Between group p-value was also derived from mixed model analysis.*

7.1.6 Associations between changes in S_I, AIRg, ISR, insulin clearance and DI and changes in body composition, body fat distribution and ectopic fat and VO_{2PEAK}.

Even though body fat distribution, except gynoid fat mass (%FM), and ectopic fat did not change significantly after exercise training, the variability in the participants' responses allowed us to assess the associations between the changes in metabolic

outcomes and fat depots in response to the intervention. The exercise-induced increases in S_I and DI were not associated with changes in body fat mass (%), gynoid fat mass (%FM), android fat mass (%FM) and ectopic fat (data not shown). Nevertheless, only the control group (significant interaction by group, $p<0.05$) showed significant associations between changes in S_I and changes in trunk fat mass (%FM) (β -0.628, $p<0.001$) and leg fat mass (%FM) (β 0.745, $p=0.002$) (Figure 7.6). Further, in the combined group (no interaction by group, $p>0.05$), an increase in S_I was associated with a reduction in VAT and an increase in DI was associated with a reduction in trunk fat mass (%FM) (Figure 7.6). No significant associations were observed between changes in AIRg, ISR_{total} and insulin clearance and changes in body fat distribution and ectopic fat (data not shown). No significant associations were found between any of the metabolic outcomes and change in absolute or relative VO_{2peak} (data not shown).

In addition, the exercise dose (control vs moderate vs high exercise dose) did not modify the observed associations between the changes in S_I, AIRg, DI and first phase or total ISR and changes in regional and ectopic fat deposition above those already observed between the control and exercise group in Figure 7.6.

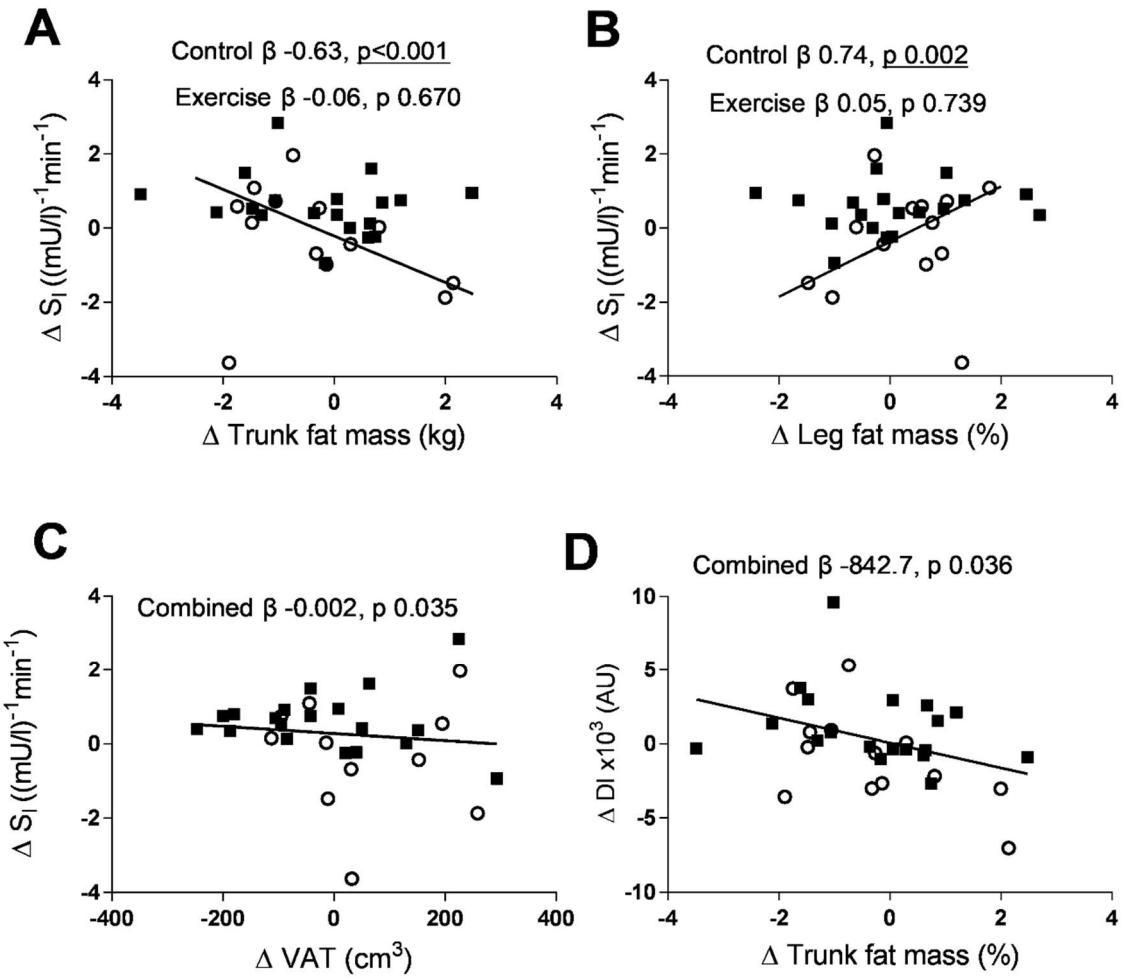


***Figure 7.6**: Associations between changes in insulin sensitivity (S_I) and changes in (A) trunk fat mass, (B) leg fat mass and (C) visceral adiposity (VAT); (D) changes in disposition index (DI) and changes in trunk fat mass in the control group (open circle) and exercise group (closed square). β – refers to the linear regression coefficient. A regression line (solid black) is indicated if an association is significant (p≤0.05) in the combined group or if a significant interaction by group was present, the line will represent the group with underlined p-value.*

7.2 DISCUSSION

The underlying mechanisms for lower S_I and hyperinsulinemia observed in black African populations are incompletely understood. This is the first exercise intervention study conducted in Africa to evaluate whether body fat distribution and ectopic fat may

explain this phenomenon. While few exercise intervention studies have been conducted in African Americans (245,279–281), the effect of exercise training on insulin secretion and clearance, as well as on hepatic and pancreatic fat were not studied. This study demonstrated that a 12-week exercise intervention was efficacious to increase S_I and cardiorespiratory fitness and occurred despite minimal weight loss. However, exercise training did not alter any of the components of hypersinulinemia and DI increased suggesting as dissociation between AIRg and S_I. Further, exercise training did not result in significant changes in central, pancreatic, hepatic and skeletal muscle fat, but reduced gynoid fat mass (%FM). Accordingly, body fat distribution and ectopic fat were not important correlates of the observed increase in S_I and DI. Nevertheless, exercise training reduced gynoid fat mass which may induce long-term benefits.

We showed that S_I improved significantly after exercise training, which occurred simultaneously with an increase in VO_{2peak}, despite minimal weight loss (~1 kg). Contrastingly, a study conducted in a similar cohort of obese, black African American, premenopausal women, showed no significant improvement in S_I or cardiorespiratory fitness after 14 weeks of high-intensity interval training (75-90% of heart rate reserve) (245). These discrepant findings are opposed to reports that showed that high-intensity interval training increases S_I equally or to a greater degree compared to moderate-intensity continuous exercise, despite being of lesser volume (227). Nevertheless, the improvement of S_I in our study was dependent on the exercise dose. Exercising at a higher exercise dose produced a more consistent improvement in S_I compared to a moderate exercise dose.The exercise dose used in this thesis is a composite measure of both the exercise volume and intensity and notably, the

exercise intensity, measured by the mean HR_{peak} achieved, was similar between the moderate (80.2 ± 3.7%) and high exercise dose groups (79.0 ±4.4%), but the volume differed (mean number of sessions per week 2.8 compared to 3.6 respectively). This is in agreement with another study in which predominately white adults (African Americans represented 18% of study population) undertook three exercise training programmes that differed based on volume and/or intensity (241). When comparing the two exercise training groups with a similar prescribed exercise intensity (65-80% VO_{2peak}), the group with the bigger increment in S_I also completed a greater number of sessions per week. It is plausible that more cumulative exercise sessions may induce greater adaptations in the expression and activity of proteins in the insulin signaling cascade which would augment S_I (358). While, not all research supports this notion (359), moderate-vigorous exercise training of no less than 3 days per week may be required to improve S_I in obese, black South African women. Further, based on the results of focus group discussions conducted in black South African women, the intervention utilized a combination of exercises, such as skipping and dancing, to ensure likeability and adherence to the training (360). The suitability of this intervention was highlighted by the fact that only three participants did not complete the exercise training, with one withdrawing due to pregnancy.

An important point to highlight is the magnitude of increase in S_I (6%) in this thesis. Greater improvements in S_I has been reported in studies in obese African American adults (58%) after 7 days of consecutive moderate-intensity aerobic training (280), and in obese African American adolescents (37%) after 8 weeks of moderate-intensity aerobic exercise training (279). However, in these studies, the FSIGT were performed 14-18 hours (280) and 24-48 hours (279) after the last exercise session. This contrasts to this thesis, in which the post-intervention FSIGTs were performed at least 72 hours

after the last exercise training bout. Considering that S_I remains elevated for up to 72 hours after the last exercise bout (239) due to, amongst other factors, the depletion of muscle glygogen post-exercise (361), the large changes observed in the aforementioned studies may merely reflect acute changes in the muscle. This may have amplified the observed change in S_I, and explain the smaller observed improvement in S_I in this thesis.

Another reason for the small increment in S_I in this thesis may be attributed to the concomitant marginal weight loss (~1%) in response to the exercise intervention. A multi-ethnic study that divided the cohort into those with less than 3% reduction in body weight and those with greater than 3% reduction in body weight after 8 months of aerobic exercise training, found that both groups showed an improvement in S_I but a smaller increment was observed in those who attained less than 3% of weight loss (359). A reduction in the mobilization of free fatty acids that accompanies major weight loss (12%) was found to be a major correlate of the improvement in S_I (60%) (254), which may suggest that a smaller reduction in body weight may evoke a smaller reduction in free fatty mobilization and an associated smaller increase in S_I. However, further research is required to confirm this.

The exercise training intervention, although eliciting only a small reduction in body weight, improved the VO_{2peak}. However this improvement in VO_{2peak} was not associated with the increase in S_I. A common mechanism may improve both these factors, such as an enhancement in mitochondrial oxidative capacity after exercise training (233). Therefore, a reduced statistical power perhaps may explain the lack of an association. Moreover, obesity may be a common feature in both those with a low VO_{2peak} (362) and in those with a low S_I (73). Nevertheless, improvements in VO_{2peak}

and S_I may occur without alterations in adiposity. Indeed, in obese men, VO_{2peak} and S_I improved independent of a reduction in body weight or fat mass after 12-weeks of moderate-intensity endurance training (255), contemporaneous with reduced systemic and skeletal muscle oxidative stress markers and an increased skeletal muscle antioxidant capacity. Therefore in this thesis, alterations in the oxidative stress:antioxidant balance and/or reduced activation of inflammatory pathways such as nuclear factor kappa β, mitogen activated protein kinase p38 and Jun NH2-terminal kinases (363) may be possible reasons to explain the improvement in S_I. However, further research is required to address this hypothesis.

The lack of change in the ISR and hepatic insulin extraction was unexpected. Other exercise training studies have shown a reduction in stimulated insulin response (261,265) or insulin secretion (252) in those with normal glucose tolerance. However, in those with impaired glucose tolerance, only a reduced stimulated insulin response, but no change in insulin secretion, was reported (264,284). These studies were conducted in predominantly white older men and women known to have relatively lower baseline plasma insulin levels than those of black African ancestry. Notably, exercise-intervention studies that demonstrated reductions in AIRg either showed reduced body fat mass (264,265,284) or reduced VAT (261). While no change in AIRg was observed in those without a significant change in VAT despite an improvement in S_I (261). These findings may suggest that either a reduction in AIRg is important for a reduction in VAT to occur or that a reduction in VAT is important for a change in AIRg to occur. In support, we showed in the combined group that a reduction in DI and ISR_{total} was associated with a reduction in trunk fat mass (%FM). However, to determine whether causality exists and the direction of this causality between VAT and AIRg or trunk fat mass and DI and ISR_{total}, further research is required. While our

12-week supervised moderate-to-high intensity exercise training program was unable to reduce hyperinsulinemia, despite increasing S_I, it produced a modest decrease in body weight (~1 kg) with no changes in central and ectopic fat depots. Notably, even in lifestyle intervention studies such as the Diabetes Prevention Program that included both exercise training and dietary changes, black African American women demonstrated smaller reductions in body weight compared to black African American men and white and Hispanic men and women (364). It is possible that the inability to show greater exercise-induced changes in body weight in black women could be due to higher fasting and/or stimulated insulin levels. Insulin is a potent suppressor of lipolysis in adipose tissue and therefore limits free fatty acid mobilization (365). Even during exercise, excess insulin impedes the β-adrenergic stimulation of lipolysis (365). Lower compliance to exercise training amongst black women should also be considered as possible explanation for blunted weight loss. However, in our study, exercise was supervised and although adherence varied, a mean of 79% of sessions were attended. Thus, longer exercise training duration in combination with a dietary intervention, possibly a low carbohydrate and low glycemic diet (366), might be required to reduce hyperinsulinemia, fat mass and central/ectopic depots in obese women of black African ancestry.

Another key finding of our study was that exercise training increased DI, a proxy of β-cell function due to the increase in S_I without a compensatory decrease in hyperinsulinemia. The increase in DI may delay the onset of T2D over the short term, but we should also consider that sustained greater insulin secretion above the level of S_I may predispose to β-cell exhaustion over the longer term (367). Cross-sectional studies have shown that a greater AIRg observed in black African populations is out of proportion to the level of S_I (47,56). Further, the current interventional study showed

that changes in S_I and AIRg may occur independent from each which contest that hypersinulinemia, observed in black African populations, is merely a compensation for lower S_I (52). In support, young black African children present with hyperinsulinemia prior to obesity and while S_I is still equivalent to white children (58). Hyperinsulinemia may therefore not only precede lowered S_I, but may perhaps also be the cause. Evidently, *in vitro* cell culture studies have shown that hyperinsulinemia may directly impact S_I through its effect on the insulin receptors (153) and/or indirectly through obesity-related sequelae (368). Whether a higher DI, in the context of hyperinsulinemia out of proportion to the level of S_I, may be beneficial to slow down progression or whether it could accelerate the progression towards T2D in black African populations, needs to be evaluated.

The exercise intervention did not reduce central abdominal fat depots and thus could not explain the increase in S_I in this thesis. Notably, significant associations were observed in the control group only between changes in S_I and changes in trunk fat mass (%) and leg fat mass (%), as well as in the combined group, changes in S_I was associated with changes in VAT. Nevertheless, exercise training ameliorated these associations. This indicates that the changes in S_I in the exercise group possibly occurred via other mechanisms such as changes in systemic and skeletal muscle oxidative stress (255), mitochondrial biogenesis and mitochondrial function (269), as mentioned above. Another reason for the improvement of S_I may be a reduction in fatty acid availability, which has been shown to occur without concurrent VAT changes (253). Furthermore, the significant reduction in gynoid fat mass (%) after exercise training is of major interest. Previous studies reported lower adipogenesis (120), and higher inflammation (41), fibrosis and hypoxia (369) in the gluteal fat depots of black

South African women, compared to white women. Accordingly, the reduction in gynoid fat mass may have major beneficial long-term metabolic effects.

Notably, this is the first study to evaluate the effect of exercise training on pancreatic fat in obese black African women without type 2 diabetes and we found no changes in pancreatic fat. In addition, the increase in DI observed in our study after exercise training, could not be explained by changes in pancreatic fat. In accordance with our findings, Heiskanen *et al.* did not find any association between improved β-cell function, derived from an oral glucose tolerance test, and a decrease in pancreatic fat (252). Similarly, a cross-sectional study in black African men with early type 2 diabetes failed to show an association between pancreatic fat and β-cell function (211). Evidence suggests that pancreatic fat accumulation can occur from many sources. This includes excess dietary free fatty acids, hyperinsulinemia that promotes *de novo* lipogenesis, elevated plasma free fatty acid due to adipose tissue insulin resistance and VLDL secretion from the liver (370). We did not measure markers of adipose tissue insulin resistance or free fatty acid flux and are therefore unable to comment on these factors. However, hyperinsulinemia was unaltered by exercise training and dietary fat intake did not change, which could contribute to unaltered pancreatic fat. Alternatively, pancreatic fat accumulation may be a marker of obesity and may not be detrimental to β-cell function in our cohort.

Similar to pancreatic fat, we found no change in hepatic fat after exercise training. Exercise intervention studies in black African populations that present with lower levels of hepatic fat compared to their white counterparts (42) or to Hispanics (204) are rare. A multi-ethnic study that included healthy, overweight African Americans (n=15) together with white (n=30) and Asian (n=1) adults reported a reduction in hepatic fat after 6 months of exercise training in combination with calorie restriction (251). Hepatic

fat has been linked to hyperinsulinemia and consuming a carbohydrate-rich diet (371). These factors did not change in our study and could, in part, provide a plausible explanation for no changes occurring in hepatic fat content. However, in response to training, hepatic fat content showed high inter-individual variability with 14 participants showing a reduction and 5 showing an increase in hepatic fat. Measurement error could be responsible for the observed increase in hepatic fat. However, heterogeneity in the hepatic fat response to exercise was also reported in Swedish adults with T2D (295). This study suggested that an exercise induced increase in hepatic fat might last longer in some individuals. Further we found no association between the change in S_I and hepatic fat, indicating that hepatic fat might not be an important correlate of S_I in this population, a finding also echoed by our cross-sectional analysis.

The effect of exercise training on intramuscular fat has been extensively studied, but again a paucity of studies include black African populations. Our study showed no changes in both soleus and tibialis anterior fat content after exercise training. Arad *et al,* made similar observations in black African overweight/obese premenopausal women, although they measured total body skeletal muscle fat (245). The improvement in S_I in our study could therefore not be explained by changes in skeletal muscle fat content. However, exercise training has been shown to improve skeletal muscle mitochondrial oxidative capacity and muscle GLUT4 mRNA and protein (372) and can attenuate the detrimental effect of lipid intermediaries on cellular insulin signalling pathways (292). Hence, the improvement in S_I in response to exercise training might be due to skeletal muscle adaptions relating to lipid metabolism and mitochondrial function instead of fat content in the muscle; however, this requires further investigation.

Taken together, this thesis showed that in obese black South African women exercise training increased S_I independent of changes in hyperinsulinemia and ectopic fat. These findings suggest that ectopic fat might not be the primary correlate of insulin resistance in this cohort, rather intrinsic factors in the muscle and adipose tissue may be more important correlates of the exercise-induced changes in S_I. Moreover, hyperinsulinemia may not solely occur as compensation for reduced S_I. Further, the exercise-induced reduction in gynoid fat mass may improve adipose tissue function and confer long-term benefits. Finally, exercise training attenuated weight gain and aSAT accumulation, observed in the control group, and are thus a valuable tool to curb escalating obesity in this cohort.

CHAPTER 8: THESIS CONCLUSIONS

DI reflects the compensatory relationship between AIRg and S_I (180). However, prior to T2D diagnosis, the DI will start deteriorating when the ability of the insulin response to match the level of S_I is impaired (373). Nevertheless, compared to white populations, black African populations demonstrate an unique phenotype, characterized by a low S_I and high insulin response, due to a combination of low hepatic insulin extraction and high insulin insulin secretion (39,44,48). This thesis therefore aimed to understand whether regional and ectopic fat distribution may explain this unique phenotype in an obese black South African sample. To address these aims we conducted a cross-sectional investigation, and for the first time in a South African cohort, completed a randomized controlled exercise intervention study, which strengthened the quality of evidence. In addition, this thesis used advanced methods to measure its main outcomes, S_I, AIRg, insulin secretion and clearance as well as its main correlates, regional and ectopic fat depots.

The outcomes of this thesis are summarized in Figure 8.1. Firstly, the associations between DI and its components were investigated to understand the importance of these components in maintaining DI (Figure 8.1 A). Secondly, the associations of DI and its components with regional and ectopic fat were explored (depicted by arrow labelled “B” in Figure 8.1). Thirdly the effect of exercise training on S_I, AIRg, ISR, insulin clearance, DI (C1-labelled arrow in Figure 8.1) and on regional and ectopic fat (C2-labelled arrow in Figure 8.1). Finally, the effects of exercise training on the associations between metabolic outcomes and regional and ectopic fat accumulation were assessed (D-labelled arrow in Figure 8.1).

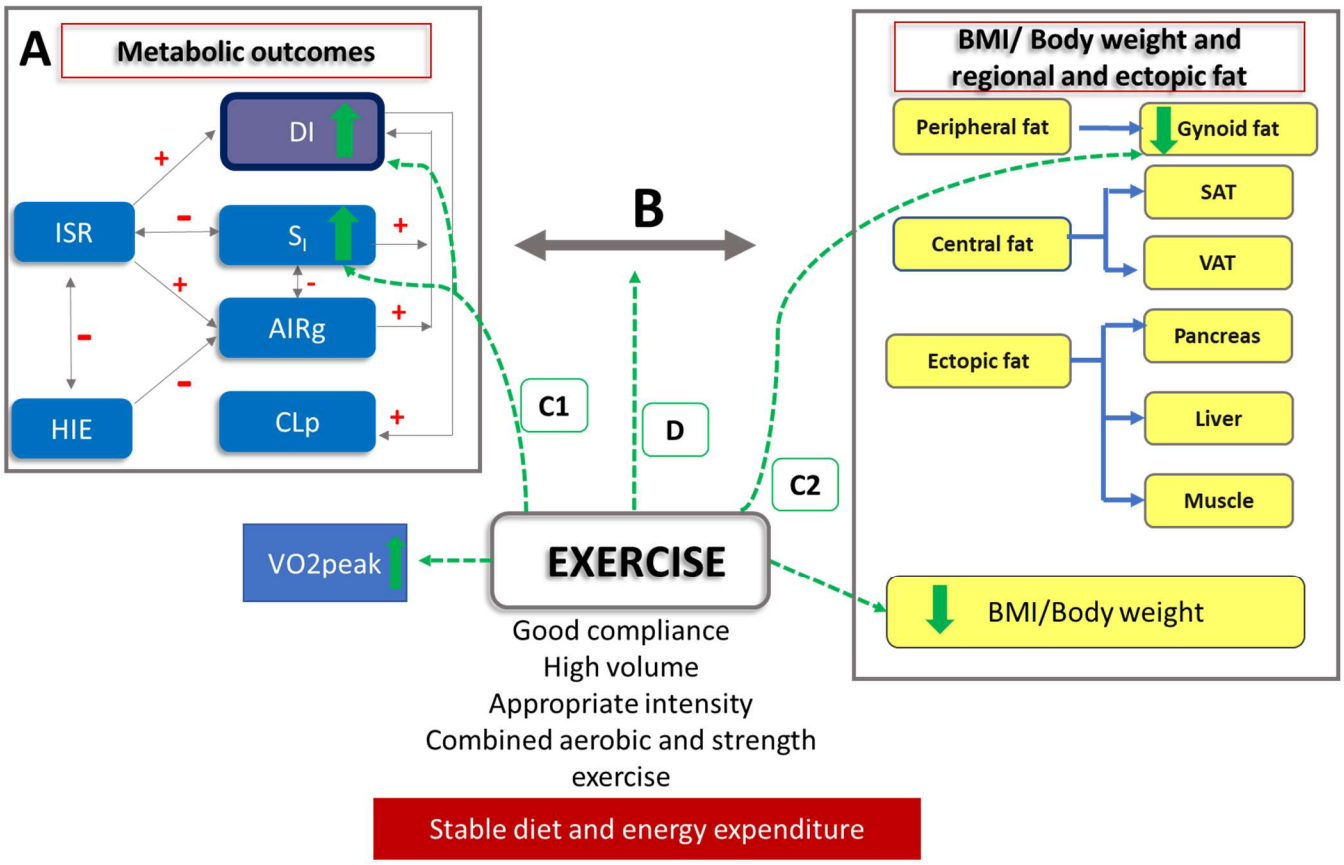


Figure 8.1: *Summary of findings for the cross-sectional (A, B) and exercise intervention studies (C, D). DI -disposition index, S_I - insulin sensitivity, AIRg - acute insulin response, ISR - insulin secretion rate, HIE - hepatic insulin extraction, CLp - peripheral insulin clearance, SAT – abdominal subcutaneous adipose tissue, VAT – visceral adipose tissue.*

One of the main findings from the cross-sectional study (Figure 8.1 A) was that peripheral insulin clearance was positively correlated with DI. However, peripheral insulin clearance did not associate with AIRg, rather a lower hepatic insulin extraction and higher first phase ISR were independent correlates of higher AIRg, adjusted for S_I. Considering that a hyperbolic relationship was evident between S_I and AIRg at baseline; this thesis found that after exercise training, S_I and DI increased (Figure 8.1 C1), but without a commensurate decrease in AIRg. The increase in DI after exercise training could be attributed to the increase in S_I (Figure 8.1 C1). DI, therefore, may not always reflect β-cell function due to the uncoupling of AIRg and S_I.

The second focus of the cross-sectional study was to evaluate the associations between the metabolic outcomes (DI, S_I, AIRg, ISR, hepatic and peripheral insulin clearance) and regional and ectopic fat accumulation (Figure 8.1 B). Based on findings from this thesis, a higher DI was present when pancreatic fat, soleus fat and VAT was lower, but VAT was the strongest correlate of DI. VAT is therefore an important factor in the pathogenesis of T2D, not only due to its association with a lower S_I, but also due to its association with lower AIRg and first phase ISR, independent from S_I. In addition, the association between higher hepatic insulin extraction and higher VAT-aSAT ratio highlights another mechanism through which VAT may associate negatively with DI. These findings occur despite black women having less VAT compared to their white counterparts (39), which may indicate a greater sensitivity to VAT accumulation.

Nevertheless, we did not show similar relationships in the exercise intervention study. In fact, the exercise training-related increases in S_I and DI were not associated with changes in regional and ectopic fat (Figure 8.1 D). Possible reasons for these finding are illustrated in Figure 8.2. Exercise training is known to upregulate key proteins involved in the insulin signaling pathway (231) and has also been shown to improve fatty acid oxidation (235), which may explain an increase in S_I and thus DI. The reason why the exercise-induced increases in S_I and DI was not associated with a change in VAT in the exercise interventional study may be due to exercise training improving fatty acid oxidation and thus the utilization of free fatty acids in skeletal muscle. This may occur through mitochondrial biogenesis and improved mitochondrial function (233) rather than through a reduction in VAT or an increase in muscle mass, since this thesis showed no change in fat-free soft tissue mass after exercise training.

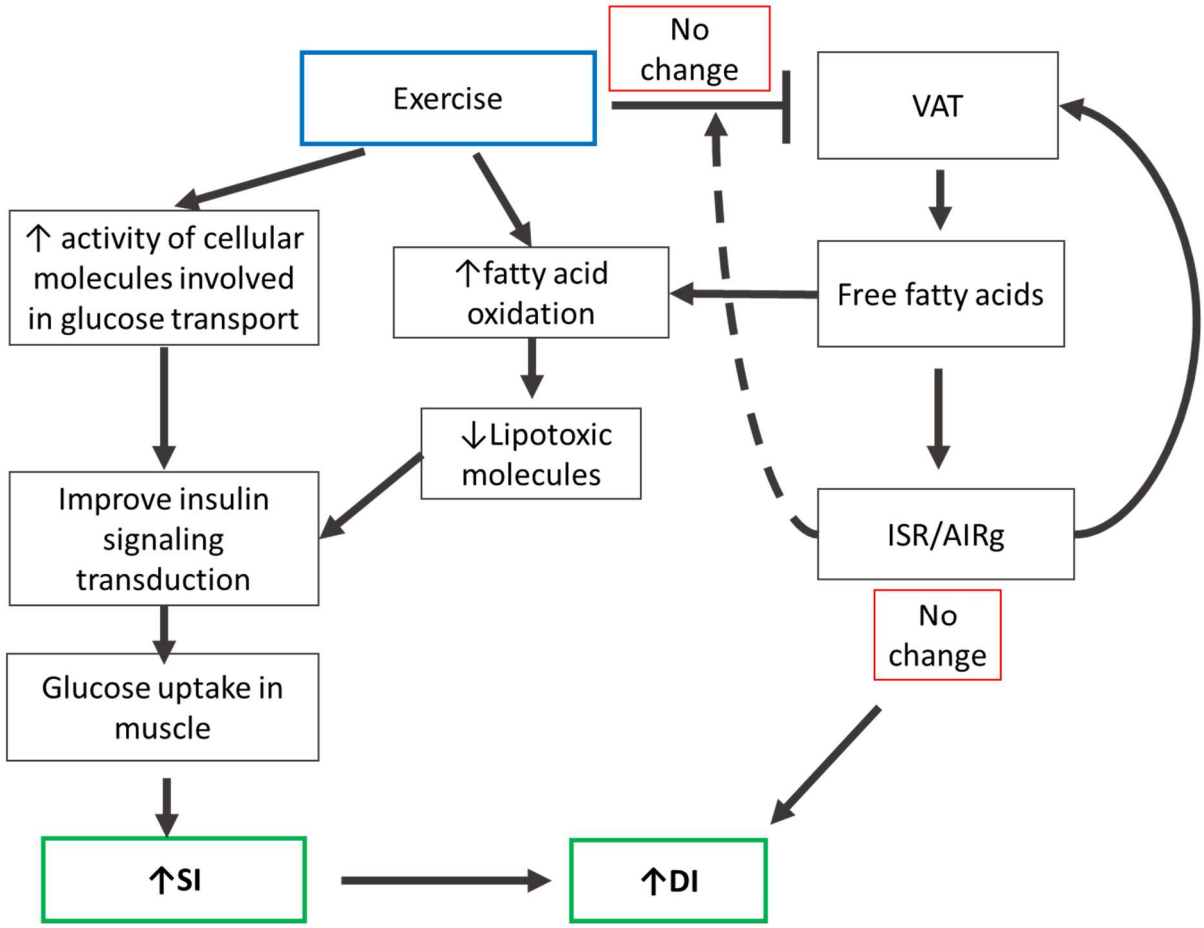


Figure 8.2: *Proposed mechanisms of exercise-induced changes in insulin sensitivity (SI) and disposition index (DI) and the lack of change in insulin secretion rate (ISR), acute insulin response to glucose (AIRg) and visceral adipose tissue (VAT)*

Another point to consider is that exercise training reduced gynoid fat mass. In black South African women, the lower adipogenic and lipogenic potential of the gynoid fat depot, compared to white women, has been found to associate with lower S_I (120). Although, the reduction in the gynoid fat mass was unrelated to the increase in S_I, molecular changes may have occurred that improve the inflammation (374), hypoxia and fibrosis (369), characteristically found in this depot in black South African women, which may have contributed to an improvement in S_I after exercise training.

Further, exercise training was beneficial to curb an increase in the truncal fat depots. Notably, the control group gained weight and in particular aSAT, over the interventional period of 12 weeks. Exercise training therefore, also curbed an overall weight gain which is especially relevant in young premenopausal black South Africans, as previous research has shown that young black South African women significantly increase their weight (by ~7kg) over a relatively short period (5.5 years) (121).

The lack of change in AIRg and its components, first phase ISR and hepatic insulin extraction, after exercise training may be explained by revisiting the paradigm that hyperinsulinemia precedes low S_I and obesity, discussed in section 1.7 and depicted in Figure 1.1. Based upon findings from this thesis, we need to add to this paradigm the association of VAT accumulation with a lower ISR, the association of the VAT-aSAT ratio with a higher hepatic insulin extraction and the associations of both VAT and VAT-aSAT ratio with lower AIRg and DI (red arrows in Figure 8.3). Secondly, this thesis posits a bi-directional association between VAT and ISR/insulin response such that hyperinsulinemia may promote obesity and thus VAT accumulation, which in turn may lead to a lower ISR and lower AIRg, which is unable to completely compensate for the level of S_I and therefore a low DI ensues. Based on this bi-directional relationship we suggest that the lack of response in AIRg after exercise training may, in part, be due to a lack of change in VAT. The lack of change in VAT is surprising since this depot is more sensitive to adrenergic stimulated lipolysis induced by exercise training (274), such that a reduction in VAT has been observed in overweight and obese people after moderate-vigorous exercise training even without dietary changes (375). However, the lack of change in VAT may be explained by the obesity phenotype observed in black African populations of low VAT and high aSAT and

gluteal fat, together with the presence of hyperinsulinemia that may have blunted the β-adrenergic stimulation of lipolysis that occurs during exercise (365) (Figure 8.2).

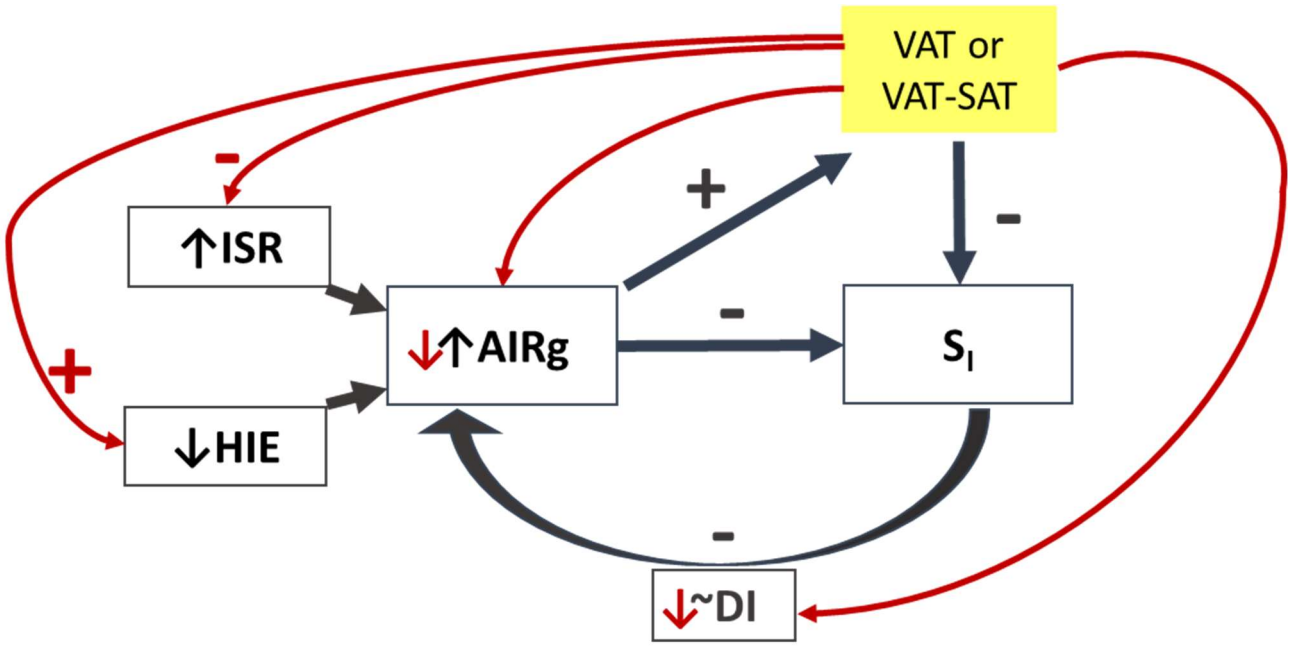


Figure 8.3: *Paradigm explaining associations of hyperinsulinemia (acute insulin response (AIRg), insulin secetion rate (ISR), hepatic insulin extraction (HIE) with insulin sensitivity (S_I) and visceral adipose tissue (VAT) alone or relative to abdominal subcutaneous adipose tissue (aSAT) and how this explains variability in disposition index (DI)*

A major strength of our study was the ability to not just evaluate the correlates of AIRg, a component of DI, but to also discriminate between ISR and insulin clearance components. Moreover, this thesis went further and differentiated between hepatic and peripheral insulin clearance. Previous studies that included African Americans, evaluated the correlates of insulin secretory function by using plasma insulin levels (51,84,128,209). This thesis therefore adds to these findings by also showing the associations of regional and ectopic fat depots with ISR and hepatic and peripheral insulin clearance. A two compartment C-peptide mathematical model was used to determine the ISR. The C-peptide kinetics derived from this model was validated and

found to be robust under various conditions (339). The two-compartmental C-peptide model was simplified by Van Cauter *et al.* by proposing the use of standard kinetic parameters that can be adjusted for body surface area, sex and age (337). Accordingly, the standard kinetic parameters were used in this thesis taking the obesity levels of our cohort into account. While the standard kinetic parameters for C-peptide kinetics were derived from white populations, it has been used in previous studies conducted in African American women with hyperinsulinemia (49,59). Further the mathematical model used to determine hepatic and peripheral insulin clearance was developed to determine insulin clearance in African immigrants living in the USA (71). A limitation of this model is the assumption of a constant insulin clearance over the duration of the FSIGT. In black African populations, the insulin response after an intravenous glucose load is substantially larger than in white populations during the first 10 minutes of the FSIGT (39), which suggests a marked reduction in insulin clearance. However, over the remainder of the FSIGT, the plasma insulin levels decline and to return to baseline levels. This suggests that insulin clearance may be more dynamic across the FSIGT. A constant insulin clearance measure may therefore not completely represent the normal physiological response.

Exercise training was chosen as the intervention strategy due to a large body of evidence showing that exercise training improves S_I (227). Moreover, based on focus group discussions, consisting of participants recruited from the same community as the participants from this thesis, revealed that physical activity would be the preferred intervention (376). A further consideration for the use of exercise training is the low cardiorespiratory fitness observed in black African populations (220). Indeed, the women from our study had a mean baseline VO_{2max} of 24.2 ml/kg/min, which falls below the 5^{th} percentile for women aged between 20 and 29 years (median cohort age

23 years old) and is rated as very poor (377). Further, the VO_{2max} of our participants was similar to the average VO_{2max} of healthy women double their age, between 50-59 years of age (378). A low hemoglobin could be an alternative explanation for a low VO_{2max} in this population but all the participants in this thesis had a hemoglobin within normal range. This low cardiorespiratory fitness is concerning since it increases the risk for all-cause mortality and cardiovascular disease (379). However, this thesis showed that after exercise training the VO_{2peak} increased by 10% in the exercise training group (Figure 8.1), while no change was observed in the control group. Although the post-exercise VO_{2peak} is still rated as very poor, it now falls below the 15^{th} percentile for women between 20 to 29 years of age. The VO_{2peak} trajectory in this thesis was in the right direction to lower risk for all-cause mortality and cardiorespiratory diseases, albeit, no direct association was observed between the increase in VO_{2peak} and increase in S_I and DI. However, an ongoing active lifestyle should be advocated to ensure further improvements in cardiorespiratory fitness. since high sedentary time, observed in black premenopausal South African women, has been associated with lower cardiorespiratory fitness (219).

Another strength of this thesis was the rigorous monitoring of the exercise intervention. Firstly, it was supervised and directed by a biokineticist. Secondly, the intensity of the exercise training was monitored at each session to allow for the adjustment of exercises to ensure the prescribed intensity was achieved. While the mean compliance was 79% it varied from 52% to 100% of sessions attended. The increase in S_I was associated with the exercise dose but it did not account for the variance in the ectopic fat responses after exercise training.

Self-reported dietary intake was monitored on a monthly basis by a registered dietician, which may be regarded as a strength. Dietary intake over 4 days, including

three weekdays and a weekend day, was recorded to account for dietary variation. No changes in dietary parameters were observed over the 12 week period, which suggested that our observed findings were not due to dietary changes. Nevertheless, the method of self-reported dietary intake has its limitations and is influenced by recall, bias, and may not be sensitive to small alterations in diet even though these alterations may affect substrate metabolism at rest and in response to exercise training. In addition, in the exercise group we reported energy intake as an average across 4 days but did not further stratify energy intake according to exercise compared to non-exercise days.

Another strength of this thesis was that the FSIGT was performed 72 hours after the last exercise training session to ensure that a training rather than the acute exercise effect was captured. This may explain the smaller improvement in S_I in this thesis compared to previous exercise training studies undertaken in African Americans where the measures of S_I were 14-18 (280) and 24-48 hours (279), after the last exercise session and could therefore rather represent the acute effect of exercise on S_I.

No changes were observed in central and ectopic fat depots after the exercise intervention. While this may be a true biological effect, the sensitivity of the MRI to detect small changes in these fat depots should be considered as a possible explanation for the lack of change in these fat depots. However, the 3-point Dixon method used in this thesis to quantify hepatic, pancreatic and skeletal muscle fat, as well as VAT and aSAT depots was previously validated and found to be a sensitive measure even when fat levels in abovementioned depots are low (329). Further, this thesis measured VAT and aSAT volumes over a 15 cm region, including 5 slices,

which is a more sensitive method to determine changes in these parameters, compared to using a single slice (328).

Other limitations to be considered, are that this thesis did not measure glucose tolerance and therefore was not able to discern whether the exercise training improved glucose tolerance, an important clinical marker of T2D risk. Further, no distinction was made between hepatic and peripheral $S_{I,}$ and thus cannot comment on the effect of hepatic insulin S_I on our findings. Another limitation was that we only included those in whom the linear model was preferred to compare HIE and CLp between subjects as well as over time. This reduced the sample size which may have contributed towards a type II error. Also, the focus of this study was on premenopausal black obese South African women and our findings might not be generalizable to all black South African women, older women, men or to other ethnic groups.

In conclusion, ectopic fat was not an important correlate of S_I, hyperinsulinemia or DI, a consistent finding in both the cross-sectional and interventional studies. Instead VAT and the VAT-aSAT ratio were important correlates of lower DI, explained not only by the association with lower $S_{I,}$ but also with lower ISR and a higher hepatic insulin extraction and not peripheral insulin clearance. Moreover, exercise training was beneficial to increase cardiorespiratory fitness, $S_{I,}$ as well as DI despite minimal weight loss and no reduction in central and ectopic fat depots. Cellular level alterations in the skeletal muscle and adipose tissue may explain these findings. The unique phenotype of this population, characterized by low cardiorespiratory fitness coupled with hyperinsulinemia and relatively low VAT and ectopic fat accumulation, may have dampened the exercise training-related response of these fat depots. Notably, gynoid fat mass reduced after exercise training, which may confer a beneficial effect on adipose tissue health. Also, exercise training ameliorated the detrimental associations

between truncal fat and metabolic outcomes, and a such remains fundamental in delaying the onset of T2D.

CHAPTER 9: FUTURE RECOMMENDATIONS

My research has shown that in obese black women without T2D, DI can vary considerably. (Table 6.1). The group with the lowest DI may be at the highest risk of developing T2D. However, longitudinal studies are needed to compare these groups and compare the incidence of T2D over time. Early detection of those at risk in the general population are required followed by appropriate interventions to delay the deterioration in β-cell function. Further, while screening for those at high risk of developing T2D are an important component in reducing T2D incidence it may require more expensive testing and resources to be done efficiently. Therefore, prevention of overweight and obesity should be another key focus, especially in black South African women who are disproportionately affected by obesity compared to their white counterparts (380).

According to the South African Diabetes Management Guidelines, the screening of T2D can occur at any age in individuals classified as high risk (221). High risk is defined as a BMI >25kg/m^2 (overweight) and having one or more additional risk factor such as physical inactivity, first degree relatives with diabetes, or conditions associated with insulin resistance, amongst others (221). Our study population although, young (median age 23 years of age), were all obese and physically inactive prior to exercise training, which make them all high-risk individuals. In addition, approximately half (48.8%) of the cohort and two-thirds (67%) of those in the lowest DI tertile were classified as insulin resistant (381). Further, our initial inclusion criteria for BMI 30-35 kg/m^2 had to be extended to <40 kg/m^2, due to a lack of volunteers that had Class I obesity. This emphasizes the magnitude of the obesity epidemic in young, black South African women. However, screening all obese and sedentary black South

African women may place enormous burden on the already under-resourced health services in South Africa. Therefore, risk stratification is important to identify those most at risk even amongst those deemed high risk.

My thesis has shown that a higher VAT was the strongest correlate of lower DI, independent of total body fat mass percent. Identification of those with the highest VAT levels are therefore warranted for risk stratification. Although, waist circumference is cost-effective and easy to use in low-resourced settings it also encompasses aSAT. Thus, in this thesis waist circumference was similar across the DI tertiles while VAT was not, showing the discord between these measures. In addition, the waist circumference to VAT ratio differs by ethnicity with African American women having lower VAT for the same waist circumference compared to white women (382). Waist circumference may therefore not be the best marker of higher VAT or reduced DI in an obese black African population. A risk score may need to be developed that is specific to obese black African populations to identify those at high risk for lower β-cell function, which encompasses both low S_I and insulin secretory function. This risk score should include a measure of visceral adiposity. A recent study used novel markers to define visceral adipose function (383). This study showed that in women, the visceral adiposity index, the lipid accumulation product and the product of triacylglycerol and glucose were independent risk factors for T2D and were stronger correlates of T2D compared to anthropometric and laboratory measures alone. The strength of this study was its size (n=9564) and longitudinal nature (median follow-up time 6.5 years). However, ethnicity was not mentioned in this study, but it was performed in the Netherlands, in a well-resourced environment and a predominately white population. Further, the visceral adiposity index includes measures of waist circumference, BMI, plasma triglycerides and high-density lipoprotein. These laboratory markers may not

be a feasible approach for screening in a South African setting where black African populations typically have lower levels of VAT, triglycerides and high-density lipoprotein (43), compared to their white counterparts. Although this thesis focused on understanding the underlying mechanism of S_I, insulin secretion and clearance it also highlights the need to explore appropriate screening measures that takes into account the unique phenotype of black African women.

After identification of those at high risk for T2D, lifestyle intervention needs to be implemented to delay or prevent T2D. This thesis showed that a 12-week exercise intervention was beneficial to increase S_I and VO_{2peak}, despite marginal weight loss (-0.8 kg) in a cohort with low baseline cardiorespiratory fitness. In addition, the exercise training prevented weight gain that was observed in the control group over the 12-week period. A longer duration of training may be needed, to ensure greater improvements in S_I, body compostion and ectopic fat deposition. Moreover, persistence in exercise training should therefore be encouraged even without obvious short-term benefits.

Hyperinsulinemia is already present in normal-weight African American children (58) which may increase propensity for obesity from an early age. Therefore, intervention strategies to combat rising prevalence of overweight and obesity should focus not only on encouraging an active lifestyle in the children but also educating parents on healthy dietary habits. A low glycemic diet which was more effective in reducing intra-abdominal fat in black American women compared to a low fat diet, may be advocated (366), in addition to exercise training. Perceptions of body size may be a barrier to the success of these lifestyle interventions, as overweight is seen as acceptable and weight loss is undesirable due to its association with HIV/AIDS (384). Nevertheless, the benefits of healthy lifestyle choices should be encouraged not just as part of school

curricula but also by local role models. Moreover, further research is required to test the uptake and efficiency of various health promotion campaigns in local communities.

While this thesis showed an improvement in S_I after 12-weeks of exercise training it was not explained by changes in body fat mass regional and ectopic fat accumulation, thus the underlying mechanisms remain elusive. Intrinsic changes in the skeletal muscle and adipose tissue, such as mitochondrial biogenesis, oxidative capacity, GLUT4 expression, lipid intermediaries, as well as inflammation and reactive oxygen species are putative mechanisms for improved S_I in obese black South African, and warrant further study.

This thesis studied obese black African premenopausal women without T2D and without HIV. However, the effect of exercise training on S_I and hyperinsulinemia has not been studied before in those with HIV. This would be of interest because of the lipodystrophic changes associated with antiretroviral medication, which may affect T2D risk. Further, age-related changes occur in S_I and AIRg (385) and thus our study findings may not be extrapolated to older individuals. The effect of exercise training on S_I and AIRg, as well as insulin secretion and clearance in older adults and those with HIV are therefore warranted.

Finally, Africa has the highest proportion of undiagnosed diabetes (59.7%), which is higher than the global figure of 50.1% (1). Delayed diagnosis of T2D when complications are already present will not only increase health expenditure, but also increase diabetes-related morbidity and mortality. Therefore, earlier screening and context-appropriate screening tools to monitor VAT, as well as longer duration exercise interventions together with dietary modifications are advocated in black South African women to combat onset of T2D.

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APPENDICES

APPENDIX A: ORIGINAL ETHICS PERMISSION LETTER

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

05 May 2016

HREC REF: 799/2015

A/Prof J Goedecke
Sports Science Institute
Human Biology

Dear A/Prof Goedecke

PROJECT TITLE: THE EFFECT OF EXERCISE TRAINING ON INSULIN SECRETION AND SENSITIVITY IN OBESE BLACK SOUTH AFRICAN WOMEN- LINKED TO 054/2015 (PhD candidate-Dr M Fortuin-de Smidt)

Thank you for your response letter, addressing the issues raised by the Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 May 2017.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the following student, Dr Melony Fortuin-de Smidt will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur

Yours sincerely

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

APPENDIX B: INFORMED CONSENT

INFORMED CONSENT

THE EFFECT OF EXERCISE TRAINING ON INSULIN SECRETION AND SENSITIVITY IN OBESE BLACK SOUTH AFRICAN WOMEN

Why is the study being done?

Within South Africa, there are many people living with diabetes (sugar disease), with black women being the most affected, especially those who are carrying extra body weight (obese). Many studies in other countries have shown that exercise training reduces the risk for diabetes. However, there are no studies in South Africa that have studied this. This study will help us understand the various factors that may cause diabetes in obese black women, including factors within the muscle, fat and blood, as well as lifestyle factors including food intake, activity levels and family history. Therefore, the aim of the study is to measure changes in the risk for diabetes in response to a 12-week exercise-training programme in obese black South African women, and to examine specific factors linked to the changes in diabetes risk. This study is important, as it will help us understand if exercise training does reduce the risk of diabetes in obese black South African women, and help us understand how this is done.

Who can participate?

If you fulfil the following criteria you will be able to take part in the study:

I. Aged 20-35 years;
II. Obese (weight in kg divided by height in metres squared: 30-38 kg/m^2)
III. No known diseases or not taking medication for any diseases;
IV. Not currently pregnant or breast feeding;
V. Self-reported Xhosa ancestry (both parents are Xhosa);
VI. No muscle or joint pains or medical problems that prevent you from exercising;
VII. Able to attend four exercise sessions per week for the 12 week study period;
VIII. Weight stable (weight not changed more than 5 kg or no change in your clothes size over the past 6 months);
IX. Using injectable contraceptives, to prevent pregnancy, for a minimum of 1 month prior to testing;
X. No smoking or taking of recreational drugs
XI. Not currently taking part in organized activity (exercise training);
XII. No previous adverse reactions to an anaesthetic (e.g. at the dentist).
XIII. No surgical procedures in the last 6 months.

How do we decide if you are eligible to take part in the study?

If you meet all the criteria listed above, you will then be asked to complete some tests on one day at the community facility in Khayelitsha to check that you meet the

inclusion criteria of the study. You will be asked to complete the following tests/measurements:

- **Complete a questionnaire** including information on your age, medication use, ancestry, medical history, exercise and diet history, physical activity readiness;
- **Weight and height** will be measured using a scale and a height measure;
- **Blood pressure**: After a 5 minute relaxation period, blood pressure will be measured 3 times in a row, separated by 5 minutes between readings using a standard blood pressure monitor;
- **Glycated haemoglobin (red blood cells that joined with blood glucose (sugar)) – A measure of the average blood glucose over a period of time:** A blood sample will be obtained;
- **HIV screening** will be performed. You will receive pre- and post-test counselling from a trained counsellor and a referral will be made to an appropriate HIV clinic if you are found to be HIV positive. If you test negative, you will be able to participate in the trial;

How many people will take part in the study?

Forty (40) obese women, who meet all the criteria above, can take part in the study.

How long will the study last?

The study will last 14 weeks in total. The first week (week 1) and last week (week 14) will include testing at the Sports Science Institute in Newlands. Weeks 2-13 (12 weeks) will include exercise training (4 times per week) in Khayeltisha.

What will happen if you decide to take part in the study?

If you meet all the criteria listed above and decide to take part in the study, you will be required to complete all the testing and training procedures outlined below. You will be randomly assigned to either an exercise group or a control group. Neither you nor the investigators will be able to choose which group you will be assigned to. If you are assigned to the exercise group, you will be required to complete 12 weeks of supervised aerobic training (training that increases your heart rate and breathing rate) for 1 hour on 4 days/week by a trained facilitator in a central facility in Khayelitsha. We request that you do not participate in any new additional training outside of this study. If you are assigned to the control group, you can continue with your normal life activities, and we request that you do not start a new exercise-training programme somewhere else for the 12 weeks.

You are under no obligation to take part in the study and are not required to give a reason if you do not wish to participate. If you decide to take part in the study, you are free to withdraw at any time and without giving a reason and without prejudice. If you decide to withdraw from the study, we will discuss with you what will happen to any information or samples that you have provided. If the incomplete samples and information can usefully contribute to the study, we will ask your permission to store them and use them in our analysis. Alternatively, on your request all your information and samples will be destroyed.

Procedures:
If you meet all the criteria above and are in the control OR experimental group, you will be required to complete 2 testing sessions before and another 2 sessions at the end of the 12 week study, as summarised in Table 1 and explained in detail below. All testing will be undertaken either at the Division of Exercise Science and Sports Medicine, based at the Sports Science Institute of South Africa (SSISA) in Newlands, or at CUBIC, Department of Radiology, Groote Schuur Hospital. Appropriately trained medical personnel will carry out all procedures.

Table 1. Summary of testing schedule the week before and after the 12 week exercise/control intervention

	Testing Week	
Testing session:	**Testing session 1**	**Testing session 2**
Location:	SSISA	Groote Schuur Hospital
Time of day:	8 am	Morning
Preparation:	Overnight fast and no exercise for 3 days prior	Overnight fast and no exercise for 3 days prior
Duration:	4 hrs	1 hr
Procedure:	-Insulin test -Inflammatory markers in blood -Body composition -Questionnaires -Physical activity	-MRS scan

Testing Session 1: Early morning before breakfast - At SSISA - 4 hours

You will be requested to come to the laboratory at the Sports Science Institute in the morning after an overnight fast. In other words, you must not eat or drink anything, except water, from 10pm the night before (at least 10 hours). You cannot take part in any exercise training for 72 (3 days) hours before this test.

Insulin test – a measure of insulin secretion and insulin sensitivity:
A small plastic tube will be placed into a vein in each arm. You will then be required to undergo a test that will measure how much insulin your body produces and how sensitive your body is to insulin. We will inject a concentrated glucose solution (~ 30-100 mL, depending on your weight) into one vein over a 1-minute period. Small amounts of blood (1 teaspoon) will be withdrawn from the other arm at regular intervals (1-2 minutes) for 20 min. After 20 min, insulin will be infused into your arm, which will assist your body to take up the glucose into the cells. Further blood samples (1 teaspoon each) will be drawn from your other arm for a further 3.5 hrs. During this

test, a maximum of 200 mL of blood will be drawn (1/3rd of the amount drawn when you donate blood). During the tests, you will be required to sit or lie quietly and DVDs will be provided for entertainment.

Questionnaires:
You will be asked questions related to your family history, your personal health and reproductive history, including the number of children you have, medications that you use and how much physical activity you do. You will also be interviewed by a dietician who will ask you questions about your diet and the foods that you normally eat. You will be given a form to record the foods that you eat at home for a period of three days.

Body composition:
After your insulin test, we will take measurements of your body, including weight, height, waist and hip circumference. In addition, you will be asked to undergo a scan, which will accurately measure your fat and muscle mass, as well as your bone density. This is called a dual x-ray absorptiometry (DXA) scan. The scan will take approximately 20 minutes to perform during which you will lie quietly on the scanning table in a medical gown provided.

Physical activity:
You will be given 2 motion sensor devices, to wear for 1 week before and 1 week after the 12 week study period and at week 4, 8 and 12 during the intervention. One device is an accelerometer that measures how fast you move and an Actipal that measures how little you move. Both devices are the size of a small matchbox and will be attached to your waist (accelerometer) and to your thigh (Actipal) with a lightweight belt. You should wear the monitors at all times, except when swimming, bathing, showering and sleeping.

Testing Session 2: Morning - At Groote Schuur Hospital- 1 hour

Magnetic resonance spectroscopy (MRS) scan
The MRS scans will be performed at Groote Schuur Hospital in Observatory, and will take approximately 1 hour.

The MRS scan will be used to measure the fat content of your calf muscle, liver and pancreas. MRS uses magnetic and radio waves, and will be used to generate a picture of your calf, liver and pancreas. You will be required to lie on a bed, which is moved into a wide-bore tubular structure. This is open at both ends. You will be required to lie still for 15 minutes while being in constant voice contact with the Radiographer.
If you have any of the following conditions, you may not have an MRS scan:
implanted medical devices such as aneurysm clips in the brain, heart pacemakers, and cochlear (inner ear) implants; lead-based tattoos; or pieces of metal close to or in an important organ (such as the eye); claustrophobia or fear of being confined in a small space.

12-week exercise/control intervention

If you are assigned to the **control group** we will request that you do not start any new exercise training programs during the 12-week study period, and continue with your daily activities and diet as usual. When you have completed the 14-week trial, you will be offered the opportunity to undergo the same exercise training as the exercise group, as described below. This is totally voluntary.

If you are assigned to the **exercise group**, you will be required to perform 12-weeks of supervised aerobic training at a moderate-vigorous intensity for one hour, four times per week. The exercise training will include cardiovascular exercises in the form of aerobic dance, boxing, running, skipping, stepping, and strengthening exercises using your own body weight or minimal equipment for resistance training (e.g. bands and weights). The frequency, duration and intensity of the exercise intervention are based on the recommendations from the British Association of Sport and Exercise Sciences (BASES), to ensure the prevention of injuries. The 60 min classes will include moderate exercise (70-75% of your maximum heart rate) and at least 30 min of vigorous activity (75-85% of your maximum heart rate), which will be monitored using heart rate monitors. The exercise classes will be supervised by a trained biokineticist, who will monitor your progress (using heart rate data) and adjust the classes accordingly to ensure adequate improvement in cardiorespiratory fitness throughout the 12-week programme. We request that you do not participate in any new additional training outside of this study and maintain your normal diet during the 12-week trial.

Monitoring in the control and exercise groups:

If you are in the control or the exercise group, you will be asked to wear the accelerometer and Actipal for 7 days at the beginning and every 4 weeks during the 12-week trial (week -1, 4, 8 and 12) (as described above). Accelerometers will be used to provide a more accurate assessment of the intensity of your activity, and Actipals provide more information on sedentary time. You should wear the monitors at all times, except when swimming, bathing, showering and sleeping. At the same time that you wear the monitors (week -1, 4, 8 and 12), we will request that you record your dietary intake for three days (2 week days and 1 weekend day) as explained in the first session. Attendance at each session, and the compliance to the exercise training will be monitored by the biokineticist.

What are the risks and discomforts of this study?

Insulin test – a measure of insulin secretion and insulin sensitivity:

There are no appreciable risks for this test, other than those associated with routine blood sampling. All procedures will be supervised and carried out by a medical doctor and appropriately trained medical personnel using sterile techniques to minimise any risks of infection. These tests are used routinely in research to accurately determine insulin secretion and insulin sensitivity. A maximum of 200 ml of blood will be drawn

during the entire study, which is less than half that drawn during standard blood donation.

Body composition:
The only risk associated with the DXA scan is exposure to radiation. However, the radiation exposure with a DXA scan is less than half that of a chest x-ray (11.3 microSieverts).

Physical activity:
There are no risks or side-effects from wearing the physical activity monitors.

Magnetic resonance spectroscopy (MRS) scan
You will not experience any pain or discomfort when having the MRS scan, and there are no known harmful long-term effects of the magnetic fields used in this study. When the scanner takes the pictures, the bed may shake, and you will hear loud banging noises. You will be given earplugs or headphones to protect your ears. Also, some people feel nervous in a small closed space, such as when they are in the scanner. You will be able to see out of the scanner at all times, and we will not start until you tell us that you are comfortable. You will be able to stop at any time by squeezing a ball that you will hold in one hand and can talk to us using an intercom that is built into the scanner. If you have any of the following conditions, you may not have an MRS scan: implanted medical devices such as aneurysm clips in the brain, heart pacemakers, and cochlear (inner ear) implants; lead-based tattoos; or pieces of metal close to or in an important organ (such as the eye); claustrophobia or fear of being confined in a small space.

12-week exercise/control intervention
The training program is designed for exercise progression to ensure minimal physical discomfort and associated soreness. In the unlikely event that you sustain an injury due to the exercise intervention, you will be referred to the appropriate clinic for treatment at no expense to yourself. However, you might experience some physical discomfort and associated muscle soreness following the first session (and in some cases the first week) of training. All efforts will be made to ensure a suitable training environment, including safe setting up and provision of equipment, sufficient lighting, suitable temperature, suitable ventilation, and convenient access to toilets, etc. In the unlikely event that medical assistance is required a mobile phone and automated external defibrillator (AED) will be present at all exercise sessions with a biokineticist that has all required certificates and training for using this device.

Are there any benefits to you for being in the study?
You will receive your own results, including body composition (weight, height, waist circumference, muscle and fat mass and bone density), blood pressure, risk for diabetes, physical fitness and dietary analysis, with some recommendations made by a dietician on how to adapt your dietary intake to improve your health. If you are

in the control group, after the completion of the study, you will have the opportunity to participate in the same exercise training as the exercise group. This is completely voluntary. Following the training (if you were in the exercise or control group), you will receive guidelines and recommendations on how to continue your exercise training. If you have any abnormal results, you will be given a referral letter and directed to the appropriate health practitioner or local clinic.

What will happen when the study is over?

As mentioned above, when the testing of all the women has been completed, you will receive your individual results and the provisional findings of the study will be presented. Once the final analyses have been completed, the final results of the study will be shared with you. This information will assist in our understanding of the effect of exercising training on the risk for diabetes, and therefore help us to prevent and/or manage the problem of diabetes in South African women.

Will any of your blood samples be stored and used for research in the future?

The researchers will ask your permission to store your blood samples for future research. All samples will be kept in a freezer in a secure facility with access limited to research personnel. Future research analyses will be based on new research that we are at present not aware of, but may be important in our understanding of the risk for type 2 diabetes. Any research done on your blood in the future must be approved by the Faculty of Health Sciences Research Ethics Committee at the University of Cape Town that is set up to determine that the research is done according to accepted standards. You will not be penalized in any way for not allowing the use of your blood for future research. If you decide not to donate blood for future research, it will be destroyed on completion of this trial. Strict confidentiality of results will be maintained.

Will you receive reimbursement for transport, time and inconvenience?

All transport required to get to the training and testing facilities will be arranged by the researchers and be at no cost to you. You will receive R30/day to cover their transport costs to the training venue in Khayelitsha (4 x training sessions per week for 12 weeks + 3 monthly monitoring visits). In addition, you will receive R60/day for transport to the testing facilities at UCT and Groote Schuur (R50 x 2 testing sessions). The transport money will be paid to you at the end of each session.

To compensate you for your time and inconvenience, you will be reimbursed R20/hr for the training session (48 hrs), and R50/hr for the testing sessions (18 hrs of testing and monitoring). Payment for time and inconvenience will be paid on a pro-rata basis at the end of the 12-week study period.

Who will see the information that is collected about you during the study?

Strict confidentiality of results will be maintained. Your name will be removed from all data, and you will be assigned a number, which will be used to identify data relating to you. All records will be kept in a locked room and in a secure computer database in the research unit. Your name will not be used in any publication of the results.

What if Something Goes Wrong?

The University of Cape Town (UCT) has insurance cover for the event that research-related injury or harm results from your participation in the trial. The insurer will pay all reasonable medical expenses in accordance with the South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI) in the event of an injury or side effect resulting directly from your participation in the trial. You will not be required to prove fault on the part of the University.

The University **will not be liable** for any loss, injuries and/or harm that you may sustain where the loss is caused by

- The use of unauthorised medicine or substances during the study
- Any injury that results from you not following the protocol requirements or the instructions that the study doctor may give you
- Any injury that arises from inadequate action or lack of action to deal adequately with a side effect or reaction to the intervention
- An injury that results from negligence on your part

By agreeing to participate in this study, you do not give up your right to claim compensation for injury where you can prove negligence, in separate litigation. In particular, your right to pursue such a claim in a South African court in terms of South African law must be ensured. Note, however, that you will usually be requested to accept that payment made by the University under the SA GCP guideline 4.11 is in full settlement of the claim relating to the medical expenses.

An injury is considered trial-related if, and to the extent that, it is caused by study activities. You must notify the study doctor immediately of any side effects and/or injuries during the trial, whether they are research-related or other related complications.

UCT reserves the right not to provide compensation if, and to the extent that, your injury came about because you chose not to follow the instructions that you were given while you were taking part in the study. Your right in law to claim compensation for injury where you prove negligence is not affected. Copies of these guidelines are available on request.

Who do I contact if I have any questions about the study?

If you have any questions or you experience any problems during or after the tests, please contact Professor Julia Goedecke or Dr Amy Mendham:

Associate Professor Julia Goedecke (PhD)
Principal Investigator
Division of Exercise Science and Sports Medicine
Department of Human Biology
3rd Floor, Sports Science Institute of South Africa
Boundary Road, Newlands, 7725
Cape Town
Tel: 021-6504573 (w) 0828255616 (cell)
Email : julia.goedecke@uct.ac.za

Dr Amy Mendham (PhD
Project coordinator
Division of Exercise Science and Sports Medicine
Department of Human Biology
3rd Floor, Sports Science Institute of South Africa
Boundary Road, Newlands, 7725
Cape Town
Tel: 021-6504567(w), 0723879889 (cell)
Email : Amy.Mendham@uct.ac.za

Should you have any concerns about this study, you are also free to contact the head of the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee, Professor Marc Blockman.

Professor Marc Blockman
Head, Human Research Ethics Committee
Faculty of Health Sciences
Room E52-24 Groote Schuur Hospital Old Main Building, Observatory 7925
Telephone [021] 406 6338 .Facsimile [021] 406 6411

Subject code:

THE EFFECT OF EXERCISE TRAINING ON INSULIN SECRETION AND SENSITIVITY IN OBESE BLACK SOUTH AFRICAN WOMEN

Consent to participate in the study:

"I, _____, hereby give consent to participate in this research trial to be conducted by the Division of Exercise Science and Sports Medicine, within the Department of Human Biology at the University of Cape Town.

I understand that I will undergo preliminary testing to determine if I am eligible for the study. I understand that I will be randomly assigned to the exercise or the control group. If I am assigned to the exercise group, I understand that I will be required to complete 12 weeks of exercise training, consisting of 1 hour of exercise training 4 days/week by a trained facilitator in a central facility in Khayelitsha. If I am assigned to the control group, I understand that I will be required to continue with my normal daily living and not start a new exercise-training programme during the study period, but can be part of the same exercise-training programme once I have completed the study. I understand that irrespective of the group that I am assigned to, I will be required to complete 2 testing sessions before and at the end of the 12-week study. In addition, I understand that I will be required to visit the community centre every 4 weeks to have my weight, dietary intake and physical activity. I understand that the testing sessions will be performed at the Sports Science Institute and Groote Schuur Hospital and will include completion of a demographic and lifestyle questionnaire, the measurement of blood pressure, body composition including whole body scans and MRS scans of my calf muscle, pancreas and liver, blood glucose (sugar), inflammatory and hormone levels, physical fitness, as well as a test to measure insulin secretion and sensitivity.
I have read and have had explained to me the procedures described. I have had an opportunity to ask questions and my questions have been answered in a satisfactory way. I understand the nature of the trial and the risks and benefits associated with my participation and that I am free to withdraw from this study at any time.

I understand that all the information collected during the study will be treated with the strictest confidentiality and will only be used for scientific research purposes. All samples will be kept in a freezer in a secure facility with access limited to research personnel. All records will be kept in a locked room and in a secure computer database in the research unit. Your name will not be used in any publication of the results. I understand that for data verification and quality control purposes regulatory authorities and/or members of the University of Cape Town Faculty of Health

Sciences Human Research Ethics Committee may be allowed access to my personal data under conditions of strict confidentiality.

I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I consent voluntarily and understand that I have the right to withdraw my consent without this affecting the current research study or my medical care.

Print Name of Participant _____

Signature of Participant _____

Date _____

Subject code:

CONSENT FOR HIV TESTING:

<u>Additional consent to:</u> The effect of exercise training on insulin secretion and sensitivity in obese black South African women

I _____ agree to provide a blood sample for the purposes of <u>confidential</u> HIV testing. I understand that it is necessary for me to have this test to participate in the research study. If I test positive, I cannot participate in the study entitled “Mechanisms underlying insulin resistance in black South African women”, conducted by the Division of Exercise Science and Sports Medicine, within the Department of Human Biology at the University of Cape Town. I aware that I will receive pre and post-test counselling from a qualified HIV counsellor, and will be referred to an appropriate health care clinic if necessary. I understand the implications of performing the test.

I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I consent voluntarily and understand that I have the right to withdraw my consent without this affecting the current research study or my medical care.

Print Name of Participant _____

Signature of Participant _____

Date _____

Subject code:

CONSENT FOR STORAGE AND FUTURE USE OF UNUSED SAMPLES:

Additional consent to: The effect of exercise training on insulin secretion and sensitivity in obese black South African women

Information sheet:

We are seeking permission to store your unused blood samples for possible future in either our own research or collaborators' research studies. Permission to use these samples is in addition to the use of your samples for the current study. This is important as these analyses will be based on new research that we are at present not aware of, but may be important in our understanding of the risk for type 2 diabetes. Before the samples can be used for future research, approval by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee will be obtained. Please be aware that the samples will not be sold for profit.

When entering into the study, you will receive a unique code that will be used for sample and data analysis, which serves to maintain your confidentiality. When storing samples, you may choose that we keep the unique code on the sample so that we can link any new results to your existing data. If any clinically relevant information relating to this sample is found, we will inform you of the results. Alternatively, you can remove the identifying number, so that your information will not be linked to the sample and you will not be informed of any clinical results relating to the new analyses.

You may also refuse to allow future analyses of samples without being penalised, and your results relating to the current study will not be compromised in any way. If you refuse to allow future analyses of samples, your samples will be destroyed on completion of this trial. Furthermore, you may withdraw permission to use your samples at any time. If you wish to do this, please contact:

Associate Professor Julia Goedecke (PhD)
Principal Investigator
Division of Exercise Science and Sports Medicine
Department of Human Biology
3rd Floor, Sports Science Institute of South Africa
Boundary Road, Newlands, 7725
Cape Town
Tel: 021-6504573 (w) 0828255616 (cell)
Email : julia.goedecke@uct.ac.za

All information collected during the study will be treated with the strictest confidentiality and will only be used for scientific research purposes. All samples will be kept in a freezer in a secure facility with access limited to research personnel; all records will be kept in a locked room and in a secure computer database in the research unit. Your name will not be used in any publication of the results. For data verification and quality control purposes regulatory authorities and/or members of the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee may be allowed access to my personal data under conditions of strict confidentiality.

Certificate of Consent:

1) If any of the **BLOOD** that I have provided for this research project is unused or leftover when the project is completed (Tick **one** choice from each of the following boxes)
   - ☐ I wish my **blood** sample to be destroyed immediately.
   - ☐ I want my **blood** sample to be destroyed after ____ years.
   - ☐ I give permission for my **blood** sample to be stored indefinitely

   AND if my **blood** sample is to be stored:

☐ I give permission for my **blood** sample to be stored and used in future research but only on the same subject as the current research project: "The effect of exercise training on insulin secretion and sensitivity in obese black South African women".

- ☐ I give my permission for my **blood** sample to be stored and used in future research of any type, which has been properly approved
- ☐ I give permission for my **blood** sample to be stored and used in future research except for research about _____

AND

- ☐ I want my identity to be removed from my **blood** sample.
- ☐ I want my identity to be kept with my **blood** sample.

I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I consent voluntarily and understand that I have the right to withdraw my consent without this affecting the current research study or my medical care.

Print Name of Participant _____

Signature of Participant _____

Date _____

APPENDIX C: SCREENING QUESTIONNAIRE

Subject Code: _____ Date: _____ Doctor: _____

PRE-SCREENING: ANTHROPOMETRY & HISTORY

Age	
Height	
Weight	
BMI	
Has your weight changed over the past 6 months?	Specify any changes in kilos and/or dress size:
Waist circumference	
Hip circumference	
Do you currently take part in exercise training?	Type:_____ Time:_____ Duration:_____

ANCESTRY

Tribal or national background (e.g. Xhosa, Zulu, Dutch, English):

Ethnic Group Black/African _____ White _____

Father:_____	Grandfather: _____ Grandfather: _____
Mother:_____	Grandfather: _____ Grandfather: _____

CONTRACEPTION

Type of contraception_____

Name /duration :_____

Do you have a regular menstral cycle? YES NO

Start date of last menstral cycle:_____

Comments:_____

Subject Code: _____ Date: _____ Doctor: _____

HIV STATUS

Do you know your HIV status? YES NO

Are you willing to undergo a HIV test? YES NO

Result:_____

SMOKING

Do you **currently smoke** any tobacco products, such as cigarettes, cigars, or pipes? YES NO

Have you ever smoked in the past? YES NO

If yes,

When was your last cigarette?_____

How long did you smoke for?_____

MEDICATION

Do you use any medicine regularly or daily that a doctor or nurse has prescribed? YES NO

Specify name, duration and dosage of medication:_____

_____

_____

BLOOD GLUCOSE

Have you been told you have diabetes or high blood sugar levels? YES NO

Results of random blood test

Blood glucose:_____

HbA1c:_____

BLOOD PRESSURE

TIME:

1	2	3
Systolic pressure:_____	Systolic pressure:_____	Systolic pressure:_____
Diastolic pressure:_____	Diastolic pressure:_____	Diastolic pressure:_____
Pulse:	Pulse:	Pulse:

Subject Code: _____ Date: _____ Doctor: _____

CLINCAL CONDITIONS

Has a **doctor** or **nurse** or **health worker** at a **clinic** or at **hospital told you** that you had or have any of the following conditions.

	YES	NO	Don't Know	Comments
High blood pressure				
Heart attack or angina (chest pain)				
Stroke				
High cholesterol (fats in blood)				
Diabetes (blood sugar)				
Emphysema/Bronchitis				
Asthma				
Sore joints (i.e. arthritis, gout)				
Osteoporosis				
Epilepsy/fits				
Tuberculosis				Number episodes:_____ Medications:_____ Last TB episode:
Cancer				Type:_____ Treatment type/duration:_____
Surgical procedures in past 6 months (i.e. dental work)				Type:_____ Date:_____
Known allergies (i.e. Anaesthesia)				

Subject Code: _____ Date: _____ Doctor: _____

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Supported by: Health Canada Santé Canada

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