DEPOT DIFFERENCES IN ADIPOSE TISSUE METABOLISM AND FUNCTION IN OBESE BLACK SOUTH AFRICAN WOMEN AND CHANGES IN RESPONSE TO AN EXERCISE TRAINING INTERVENTION

By

Pamela Arielle NONO NANKAM

This thesis is presented for the degree of

DOCTOR OF PHILOSOPHY

In the Department of Human Biology

UNIVERSITY OF CAPE TOWN

SOUTH AFRICA

February 2020

UCT Research Unit for Exercise Science and Sports Medicine

Sports Science Institute of South Africa

Boundary Road, Newlands, 7725

South Africa

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

LIST OF SUPPLEMENTARY TABLES AND FIGURES

DECLARATION

I, ***Pamela Arielle Nono Nankam***, hereby declare that the work on which this thesis is based is my original work except where acknowledgements indicate otherwise.

Neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce for research either the whole or any portion of the contents in any manner whatsoever.

Signature:

Signed by candidate

Date: 07 February 2020

RELATIVE CONTRIBUTION TO THIS THESIS

I describe below my role and contribution to the realization of this thesis, by chapters.

Chapter 3:

I drafted the research hypothesis. I analyzed and interpreted the data and drafted the whole chapter.

Chapter 4:

I drafted the research hypothesis and was responsible for selecting the inflammatory markers and genes to be analysed. I analysed serum samples for the determination of systemic inflammatory markers and performed the adipose tissue gene expression analysis. I performed the statistical analyses, interpreted the data and drafted the whole chapter.

Chapter 5:

I drafted the research hypothesis and was responsible for selecting the oxidative markers and genes to be analysed. I analysed the serum samples for the determination of systemic oxidative stress markers and performed the adipose tissue gene and protein expression analyses. I analyzed and interpreted the data and drafted the whole chapter.

Chapter 6:

I drafted the research hypothesis. I obtained assistance with the sample and statistical analysis. I interpreted the data and drafted the main body of the chapter.

PERSONAL FUNDING

• National Research Foundation (NRF) of South Africa: NRF-TWAS Renaissance Doctoral Fellowships

• Deutscher Akademischer Austauschdienst/ German Academic Exchange Service (DAAD): Research Grants-Short-Term Grants

• Deutsche Forschungsgemeinschaft (SFB1052)

• University of Cape Town

ACKNOWLEDGMENTS

"*When you meet somebody that has achieved what you want to achieve, that person knows what you do not know. Learn from them*!" A wise man said.

I would like to acknowledge and express my gratitude to several people without whom this thesis would have simply not been possible.

Firstly, **Julia Goedecke**, my Supervisor: I could not have a better supervisor than you. Your support has been inexpressible. I admire your great personality and leadership. Thank you for your patience and for believing in me when there was no reason to do so. Thank you for encouraging me, always reassuring me and challenging me to do better. I have learned so much from you, and I hope to always stay at your feet to continue learning wherever I will be. I apologize for the thousand drafts in each chapter. I thank you so much for the quick feedback and for helping me to make sense of my thoughts. You have been and will always be my "Star", my role model and an example to follow.

Amy Mendham, my co-supervisor: Your input and support were priceless in the accomplishment of this thesis. You have invested so much in this project. Thank you for the crucial role you have played in my PhD journey. The road has sometimes been bumpy, but you always kept your professionalism and helped me anytime, making sure that I was kept on track. I would also like to thank you for your patience and support. I appreciate it!

Deshnie Keswell: Thank you for your supervision at the very beginning of this project, and for your initiation to the gene expression and other laboratory techniques. Thank you for your kindness and your advice during the tough period of my early-PhD journey.

Andre Pascal Kengne: This opportunity would have certainly not been possible if you did not believe in me and help me to find my way to the University of Cape Town and particularly to my amazing supervisor. I will always be grateful for the day you clicked on that mail and forward it to Julia. I hope that I have made you proud.

Telesphore Benoit Nguelefack and **Elvine Pami Nguelefack-Mbuyo:** Thank you for the central role you have played by indicating me the direction to take. You have always been supportive, believing in me and I hope I could make you proud.

Nandipha Sinyanya, **Ntombekhaya Zoneleni**, for your undeniable assistance in participants' recruitment and translation, **Keitumetse Smouse** for directing the exercise training intervention and **Hendriena Victor** for your assistance in biochemical analyses during the study. I am also very grateful to all women that participated in this study, to the field workers, technicians and laboratory staff of the Division of Exercise Science and Sports Medicine at the University of Cape Town, and the Department of Endocrinology at the University of Leipzig.

Paul van Jaarsveld: For supervising in the measurement of fatty acids in the blood and adipose tissue samples and for the inputs and edits in the writing process of Chapter 3 and Chapter 4 of this thesis; and to **Johanna van Wyk** for the sample analyses of fatty acid composition and for teaching me the method. Thank you for your kindness.

Tommy Olsson: Thank you for your guidance and your kind encouragement, as well as your warm welcome and generosity in Sweden.

Elin Chorell: Thank you for your valuable help with a part of the statistical analyses in Chapter 3. Thank you for your ideas in this project and for always making our stay in Sweden a pleasant moment.

Matthias Blüher: Your doors have always been open for me during my research stay in Leipzig-Germany despite your busy schedule. Thank you for your valuable ideas in this project and the financial support provided. Your sense of leadership added to your kindness will mark me for my future career in research. Thank you for your guidance, for all your encouragement and for the favor which I believe I did not deserve. I expressly thank you for creating opportunities for me.

Stephanie Kehr: For your utmost needed help in the statistical analyses of the microarray data and for always be available for discussion of the output. It was my pleasure to work with you.

My colleagues on the large study of which this PhD thesis was part, **Melony F. deSmidt**, **Louise Clamp** and **Lindo Phiri**: For your invaluable role in the data collection, especially in anthropometry and body composition, dietary data, liver fat measurement, and insulin sensitivity parameters. Thank you for the team spirit and encouragement along the way. You

always had the perfect words in all circumstances although we were all in the same boat. I am privileged to have been part of this high-quality squad, which “make the bag feel lighter to carry along the way”. Thank you!

Asanda Mtintsilana: I hope I got this right! Thank you for being there for me throughout this, sometimes, painful way. For our beautiful Sundays’ hiking, our afternoon run, for taking me to the Gym to stress out and all the beautiful moment spent together. You are special to me and I am glad to have met you on this interesting journey. Looking forward to our final trip!

My very special dad, my coach, and leader, **Eyidi Ngando Alphonse**: Although far away from you, I woke up every morning with your voice in my head, telling me, among others, that this is nothing more than I can handle. Thank you for your words of wisdom which helped me to stay disciplined and focused on the goal. This will have never been the same without your presence in my life, without your love, care and of course correction! Thank you. I love you so much!

My Family: my lovely parents “**Monsieur et Madame Nankam**”, a big thank you for your continuous sacrifice and support. To my sisters, my brothers and my biggest family **CENA**, I am so grateful to have you all! Although I knew sometimes you didn’t understand why on earth this would take “so long”, or exactly what it is that I was doing, you believed in me, sometimes far more than I. Thank you!

To all **my friends**, thank you for the undescriptive motivation, support, and words of encouragement during these years. Each of you has been, in a way or another, very helpful in the accomplishment of this thesis, especially my friends encountered in South Africa, and those in Germany. Thank you for making me feel at “home” and make my stay pleasant in these respective countries.

Lastly, I would like to sincerely thank all the persons not cited here that have in any way contributed to the achievement of this thesis.

LIST OF ABBREVIATIONS

AA: Arachidonic acid

ACC: Acetyl Co-A carboxylase

ACLY: ATP-citrate lyase

AI: Agglomeration index

ALA: Alpha-linolenic acid

ANOVA: Analysis of variance

aSAT: Abdominal subcutaneous adipose tissue

AT: Adipose tissue

ATGL: Adipose triglyceride lipase

AUC: Area under the curve

BAT: Brown adipose tissue

BMI: Body mass index

BMP: Bone morphogenic protein

C/EBP: CCAAT enhancer-binding protein

CAT: Catalase

CGI-58: Comparative gene identification-58

CRP: C-reactive protein

CSF-1: Colony-stimulating factor-1

CVD: Cardiovascular diseases

D5D: Delta-5 desaturase

D6D: Delta-6 desaturase

D9D: Delta-9 desaturase

DAG: Diacylglyceride

DEGs: Differentially expressed genes

DGLA: Dihomo gamma-linolenic acid

DNL: de novo lipogenesis

DXA: Dual-energy-X-ray absorptiometry

ECM: Extracellular matrix composition

EI: Energy intake

EPA: Eicosapentaenoic acid

ER: endoplasmic reticulum

ER*α*: Oestrogen receptor alpha

FA: Fatty acids

FADS: Fatty acid desaturase

FAMEs: Fatty acid methyl esters

FASN: Fatty acid synthase

FATP1: Fatty acid transport protein-1

FC: Fold change

FDA: Federal Drug Administration

FDR: False discovery rate

FFA: Free fatty acids

FSIGT: Frequently sampled intravenous glucose tolerance test

FTH1: Ferritin heavy chain

GLUT4: glucose-transporter protein 4

GO: Gene ontology

GPx: Glutathione peroxidase

gSAT: gluteal subcutaneous adipose tissue

GSEA: Gene Set Enrichment Analysis

GWAS: Genome-wide association studies

H_2O_2: Hydrogen peroxide

HC: Hip circumference

HIF: Hypoxia-inducible factor-1a

HMW: High molecular weight

HOMA-IR: Homeostasis model assessment of insulin resistance

HsCRP: High- sensitivity C-reactive protein

HSL: Hormone-sensitive lipase

IL: Interleukin

INFγ: Interferon-gamma

IR: Insulin resistance

IRS: Insulin receptor

JNK: Jun N-terminal kinase

Kcal: Kilocalories

Kg: Kilograms

KJ: Kilojoules

LA: Linoleic acid

LC-PUFA: Long-chain polyunsaturated fatty acid

LFC: Log2 fold change

LPL: Lipoprotein lipase

MAG: Monoacylglyceride

MAPK: Mitogen-activated protein kinase

MCP1: Monocyte chemoattractant protein 1

MetS: Metabolic syndrome

MGL: Monoacylglyceride lipase

MHO: Metabolically healthy obesity

MIF: Macrophage migration inhibitory factor

MJ: Megajoule

MMP-9: Matrix metalloproteinase-9

MRI: Magnetic resonance imaging

MUO: Metabolically unhealthy obesity

NADPH: Nicotinamide adenine dinucleotide phosphate

NCDs: Non-communicable diseases

NFkB: Nuclear factor-kappa B

NOS3: Nitric oxide synthase 3

O_2^-: Superoxide

OH^-: Hydroxyl radical

OPLS: Orthogonal partial least squares of analyses

ORAC: Oxygen radical capacity absorbance

PA: Physical activity

PAI-1: Plasminogen activator inhibitor-1

PCA: Principal component analysis

PDE3B: Phosphodiesterase 3B

PHLs: Phospholipids

PiP3k: Phosphoinositol-3-kinase

PKA: Protein kinase A

PLIN1: Perilipin 1

PPARγ: Proliferator-activated receptor gamma

RBP4: Retinol binding protein-4

RNS: Reactive nitrogen species

ROS: Reactive oxygen species

RT-PCR: Real-time polymerase chain reaction

RYGB: Roux-en-Y gastric bypass

SA: South Africa

SAA: Serum amyloid A

SANHANES: South African National Health and Nutrition Examination Survey

SAT: Subcutaneous adipose tissue

SCD1: Stearoyl-CoA desaturase 1

SD: standard deviation

SES: Socio-economic status

S_I: Insulin sensitivity

SMG1: Serine/threonine-protein kinase

SNPs: Single nucleotide polymorphism

SOD: Superoxide dismutase

SSA: Sub-Sahara Africa

SVF: Stromal vascular fraction

T2D: Type 2 diabetes

TBARS: Thiobarbituric reactive acid substances

TCA: Tricarboxylic acid

TG: Triglycerides

TGFβ: Transforming growth factor-beta

TLC: Thin-layer chromatography

TNFα: Tumor necrosis factor-alpha

TPL: Total phospholipids

UPR: Unfolded protein response

VAT: Visceral adipose tissue

VLDL: Very low-density lipoprotein

VLED: Very low-calorie diets

VSG: Vertical sleeve gastrectomy

WAT: White adipose tissue

WC: Waist circumference

WHO: World health organization

WHR: Waist-to-hip ratio

ABSTRACT

Black South African (SA) women are disproportionally affected by obesity and insulin resistance, which have been associated with depot-specific alterations in adipose tissue function. This thesis aimed to evaluate the differences in fatty acid (FA) composition and gene expression between abdominal (aSAT) and gluteal subcutaneous adipose tissue (gSAT), and the changes in response to exercise training in relation to body composition, hepatic fat, inflammatory and oxidative stress markers, and insulin sensitivity (S_I) in obese black SA women.

This research evaluated the i) FA composition of aSAT and gSAT, and red blood cell total phospholipids (RBC-TPL) and their associations with body composition, hepatic fat and S_I, ii) changes in these FA profiles in response to exercise training and the relationship with changes in systemic inflammation, hepatic fat and S_I; iii) effects of exercise training on systemic markers and SAT gene expression of inflammation and oxidative stress; and iv) regional differences in transcriptome profiles of aSAT and gSAT pre- and post-exercise training.

Forty-five *IsiXhosa* women (30-40kg/m^2, 20-35 years) were randomized into control (n=22) or exercise groups (n=23; 12-week aerobic-resistance training, 40-60 min/session, 4 days/week). Pre- and post-intervention measurements included: anthropometry, body composition, cardiorespiratory fitness, dietary intake, S_I, hepatic fat, systemic markers and SAT gene expression of adipokines, inflammation and oxidative stress, RBC-TPL and SAT fatty acids profiles, and untargeted SAT gene expression analyses.

The main findings showed differences in the circulating (RBC-TPL) and stored (SAT) FA composition, also reflected in different associations between these FA profiles, centralization of body fat and S_I. Moreover, the changes in FA composition in response to exercise training were depot-specific, with the changes in RBC-TPL correlating with a decrease in systemic inflammation and hepatic fat. Exercise training alleviated systemic oxidative stress and induced increased gSAT inflammatory genes, reflecting SAT remodeling. These changes coincided with a reduction in gynoid fat and were not associated with increased S_I. Furthermore, there were unique depot-specific gene expression signatures relating to embryonic development at baseline and more diverse functional-related processes at post-training. This generated novel candidate genes potentially implicated in the relationship between body fat distribution and metabolic status in obese black SA women.

CHAPTER ONE

LITERATURE REVIEW

1.1.NON-COMMUNICABLE DISEASES AND DETERMINANTS

1.1.1. Prevalence of NCDs, T2D and metabolic risk factors

Non-communicable diseases (NCDs) refer to chronic "non-transmissible" diseases, contrary to infectious diseases, and are currently causing more deaths than all other causes combined worldwide (1). In 2012, the four major NCDs (cardiovascular diseases (CVD), cancers, chronic respiratory disease, and diabetes) caused more than 82% of deaths in the world, with nearly three-quarters of these deaths occurring in low- and middle-income countries (1). Among NCDs, type 2 diabetes (T2D) is the 4th leading cause of death and was estimated in 2017 to affect 451 million adults worldwide, with projections of 693 million cases by 2045 (2). T2D disproportionately affects low- and middle-income countries (3). Although sub-Saharan Africa (SSA) currently has the smallest number of people with T2D globally, this region has the highest projected rate of increase in the T2D population over the next 25 years (2). Indeed, the prevalence of T2D is estimated to increase from 14.2 million in 2015 to 34.2 million by 2040 (2). Within SSA, South Africa (SA) has the highest prevalence of T2D in adults (estimated at 5.5 (3.2-10.6)%; age-standardized) (2), which has significantly increased over the past 20 years, especially in black African population (4). Moreover, high rates of T2D in SA are reported in women, with 14.7% compared to 11.3% in men (4). This high prevalence of T2D in African women is compounded by an increasing prevalence of obesity and low levels of physical activity (PA) (1, 5). Indeed, the prevalence of obesity within SA is high and is associated with NDCs, in particular, accounting for 87 % of all T2D attributable risks (6).

1.1.2. Prevalence of obesity

Overweight and obesity are mostly defined by body mass index (BMI) values between 25 and 29.9 kg/m^2, and ≥30 kg/m^2, respectively (7). Although BMI does not directly measure the amount of adiposity, it has been associated with reduced life expectancy, mainly due to increased risk for NCDs including T2D, hypertension, dyslipidemia, CVD and many types of cancer (7, 8). Obesity is causing approximately 3.4 million deaths per year with a global prevalence of 9% (1). Obesity is the major attributable risk factor for T2D and is increasing in developing countries, with women having higher estimates than men (9, 10). Indeed, in Africa, the number of women affected by obesity is estimated at 15% compared to 6% of men (1, 4). Southern Africa is most affected by obesity (37%) (9), with a prevalence continuously increasing over the years (11), followed by the Western (11.9%), Eastern (8.8%) and Central regions (8.5%) (9). Moreover, SA women have the highest prevalence of overweight (25%) and obesity (40%) in SSA (11), with the highest prevalence among black SA women (42%) (9).

This is concerning, considering that the global burden of disease study estimated obesity to be the second leading risk factor for early death and disability in SA (12).

The high prevalence of obesity in black SA women, who are historically disadvantaged, has been associated with low socio-economic status (SES) (13). Low socio-economic communities in SA have insufficient economic and community resources, poor health care systems and education (14). However, SA is undergoing rapid socio-economic transition and urbanisation, which affects nutritional and lifestyles behaviours (5, 14-16). These changes are key driving factors for the increasing prevalence of obesity and are a major concern for governments in the prevention and management of the rising burden of metabolic diseases.

The prevalence of NCDs in developing countries has been increasing and is largely attributed to changes in lifestyles associated with globalisation and urbanisation (1). Urbanisation is the process of shifting from rural to urban life and the lifestyle changes this enforces. Although it has improved access to health care and the control of infectious diseases, urbanisation has also introduced several unexpected negative impacts on the health status of populations in low/middle-income countries (17). Indeed, urbanisation is strongly related to increased obesity rates, which is mediated by unhealthy diets and physical inactivity (**Figure 1.1**) (18). Still under rapid industrialisation and economic transition, SA is the scene of major demographic changes with rural-to-urban area migration, resulting in modifications in dietary and lifestyle behaviours (17).

The shift from manual labour to industrialised agriculture has resulted in increased production of processed and energy-dense foods. This is followed by an increase in energy intake, one of the main risk factors for obesity in both children and adults (1). Indeed, within SA the nutrition transition is characterized by a high total and saturated fat intake, high refined carbohydrate and added sugar intake, and low intake of fibre, fruits, vegetables, and micronutrients (19). The resultant poor access to healthy foods and high intakes of unhealthy food has been associated with the greater risk for obesity, T2D and other NCDs (11, 18).

Together with the nutritional transition, physical inactivity strongly increases the burden of obesity (1, 20). Indeed, urban development increased the rate of occupation-related physical inactivity (e.g. administrative and services jobs sectors) (21). Urbanisation has also led to a reduction of green spaces, progressively replaced with densely populated buildings and infrastructure, reducing available outdoor spaces and recreational lands appropriate for leisure and physical activities (22). Furthermore, proximity and connectivity resulting from developed transport networks encourage sedentary life (18). In SA and particularly among black African

women, the lack of access to affordable healthy food choices, together with decreased opportunities of PA in urban areas is the main factor implicated in the increased prevalence of obesity (**Figure 1.1**) (23).

1.1.3. Obesity as a major determinant for T2D risk

Obesity is the major determinant of T2D. Indeed, the risk of T2D increases with the extent and duration of obesity, and in SA, 87% of T2D incidence is attributable to elevated BMI (6). However, it is well established that it is not merely the increase in total adiposity that drives the increased risk for T2D, but rather, the distribution of body fat is more closely associated with insulin resistance (IR) and T2D. The level of IR varies with ethnicity, with black African women, irrespective of the geographic location, being more insulin resistant than their white European counterparts (24-26). In black African women, IR is associated with hyperinsulinemia due to a greater insulin response, as well as reduced hepatic insulin clearance than their white European counterparts (24, 26). Notably, ethnic differences in insulin sensitivity and response between women of African ancestry and European women were independent of total body fat accumulation (24, 27), but the relationship with regional fat distribution differed by ethnicity. In European women, centralization of body fat specifically increased visceral adipose tissue (VAT) has been identified as the major determinant of reduced insulin sensitivity (28); while greater peripheral (gluteal-femoral) subcutaneous adipose tissue (SAT) deposition was associated with improved insulin sensitivity in these women (29). Despite lower insulin sensitivity, black African women have less VAT and more abdominal and gluteo-femoral SAT than European women (30-34). Both abdominal (28) and gluteal SAT (35) has been negatively associated with insulin sensitivity in black SA women, and have been proposed as a central risk factor for IR and T2D in women of African ancestry (36, 37).

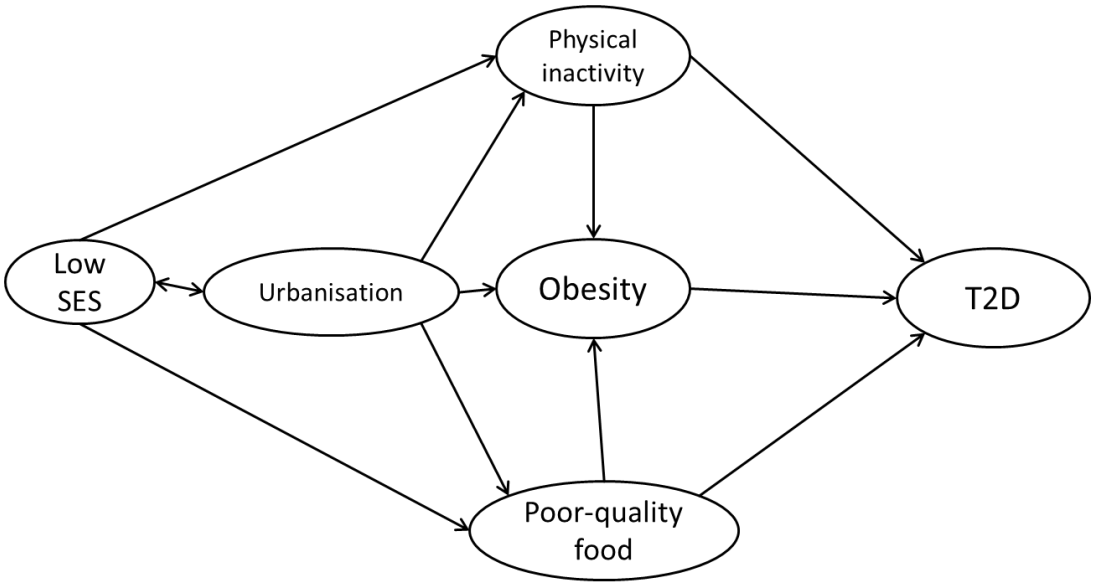


Figure 1.1. Representation of the relationships between urbanisation, lifestyle changes, obesity and type 2 diabetes (T2D) in a South African context.

In summary, obesity and its co-morbidities negatively affect the lives of many South Africans and represent a major health burden, contributing to the increasing cost of health care. This epidemic disproportionately affects women and individuals from lower SES communities. Environmental and lifestyle modifications resulting from modernisation and urbanisation have contributed to an exponential increase in obesity prevalence, with more than 65% of black SA women classified as overweight or obese (11). Concomitantly, T2D prevalence has significantly increased within SA over the past 20 years, particularly in black African urban-dwelling populations, with higher prevalence among women compared to men (4, 11). Black African women present with a phenotype of low insulin sensitivity and hyperinsulinemia, despite relatively low levels of VAT and high abdominal and gluteal SAT depots (27, 36, 37). However, the mechanisms underlying the increased risk for IR and T2D in women of African ancestry are not known. Therefore, this thesis focused on obesity and body fat distribution in the development of IR, as the major risk factors of T2D in black SA women.

1.2. DETERMINANTS OF ADIPOSE TISSUE DISTRIBUTION

1.2.1. Adipose tissue biology and distribution

Obesity is characterized by an excessive accumulation of triglycerides (TG) in adipose tissue (AT) from a continuous positive energy balance exceeding energy expenditure. Although obesity is a risk factor for IR, T2D, and CVD, obesity itself does not always lead to these comorbidities (38). The distribution of AT throughout the human body is an independent

contributor to the development of obesity-associated metabolic disorders (39, 40). AT is categorised in two major types, white AT (WAT) and brown AT (BAT), which are differentiated by their location, cellular composition and structure, vascularization and metabolic function.

BAT is composed of brown adipocytes with highly enriched mitochondria and multilocular lipids droplets. It is functionally characterized by its ability to uncouple mitochondrial respiration from ATP synthesis via uncoupling protein 1, allowing for enhanced free fatty acid (FFA) oxidation and heat production (41). Although BAT has been mostly described in rodents, this tissue has now been proven to exist and to be metabolically active in adult humans (42, 43). These have led to the concept that targeting an increased activity of BAT might provide a therapeutic strategy to improve insulin sensitivity in humans, given that BAT mass and activity is reduced in obese and diabetic patients (44, 45). However, although few studies have described BAT characteristics in humans, the molecular mechanisms demonstrating the beneficial effects of this tissue in adults, especially during obesity, has not been elucidated. Therefore, the relevance of this tissue in human obesity and related metabolic complications remains questionable. Contrary to BAT, WAT is quantitatively the most important fat pad in the human body (46). Because WAT is the main tissue involved in obesity-associated metabolic disorders, it will be the focus of this thesis, and the term AT will refer to WAT throughout.

AT mass varies across individuals and can represent 5-60% of total body weight or more in morbid obesity (46). This endocrine organ is further divided into several sub-types, mainly grouped by their location in different sites of the human body. SAT represents 80-90% of total fat tissue (46). SAT is principally distributed in the abdominal, subscapular (upper back), gluteal and femoral regions (46-48). Another significant sub-type of AT, VAT is distributed in the abdominal cavity around internal organs. VAT represents 10-20% of total body fat in men and 5-10% in women and includes the omental depot, the mesenteric depot around the intestines, and retroperitoneal depot around the kidney (46). WAT also infiltrates some organs including the liver, skeletal muscles (intramyocellular and intermuscular fat), heart (epicardial fat), and pancreas where it is termed ectopic fat and may serve specialized functions related to these neighbouring tissues (49, 50). AT heterogeneity is not only reflected by its differential location, but also by its cellular composition.

AT is composed of different cell types including endothelial cells, pericytes, immune cells such as macrophages, neutrophils, lymphocytes and T-cells, pluripotent stem cells,

preadipocytes, and mature adipocytes (51). Adipocytes constitute approximately 90% of AT total volume (46). These cells contain unilocular lipid droplets where TG are stored, occupying about 95% of their volume and determining their size (20-200 μm) (46). In addition to their principal role in TG storage, adipocytes can secrete bioactive molecules (adipokines or adipocytokines) (52). These adipokines may act as a signal via autocrine, paracrine or endocrine pathways to exert different metabolic effects on other cells and organs (e.g. brain, liver, muscle), making AT a highly active endocrine organ (38). However, the endocrine function of AT is not only exerted via the production of adipokines by adipocytes. Rather, more than 90% of adipocytokines released from AT are produced by non-adipose cells (53). Immune cells play a central role in AT biology, especially during AT expansion and/or reduction (54). Amongst AT immune cells, macrophages are the most abundant (up to 50%) and functionally dominant cell type. These cells accumulate during AT expansion and predominantly secrete inflammatory cytokines in obesity (52). Notably, the phenotype of macrophages varies with the physiological state of AT. Activated M2 macrophages can release anti-inflammatory cytokines, contributing to tissue homeostasis and repair (55). Conversely, M1 macrophages, differentiated from blood monocytes, predominantly release pro-inflammatory cytokines and sustain a chronic low-grade inflammatory state and impair insulin signalling. While M1 macrophages are highly expressed in obesity, M2 macrophages are predominantly found in AT of lean individuals (56). Macrophages are also involved in preadipocyte differentiation, adipogenesis, and angiogenesis, which are all necessary for “healthy” expansion of AT (52, 57).

AT depots differ in their cellular composition (cell types and abundance) and function and are known to be independently associated with metabolic diseases in obesity (46). Gynoid or “pear-shape” obesity refers to AT accumulation in the lower-body, preferentially in the gluteo-femoral region. Gynoid obesity is typically found in women, in contrast to android or “apple-shape” obesity, characterized by the accumulation of AT in upper-body areas (abdominal subcutaneous and visceral), mostly found in men (52). While android or central fat accumulation has been associated with greater risk for developing metabolic diseases, lower-body fat (gluteo-femoral) appears to have positive effects on metabolic health (39, 58, 59). The mechanisms underlying these depot-specific associations with metabolic risk are not fully understood. However, several factors such as age, gender, total body fat content, energy balance, ethnicity, and genetic factors have been proposed to play a role in the fat distribution pattern (38, 60, 61).

1.2.2. Determinants of body fat distribution

Body fat distribution is a major contributor to the development of obesity-associated metabolic risks, independently of total fat mass (38-40). Women generally possess higher total adiposity that is predominantly distributed subcutaneously in the gluteo-femoral area in pre-menopausal women, while men accumulate more fat in the abdominal regions (39, 46, 62). This difference in fat distribution between men and women has been principally attributed to the difference in sex hormones, shown to modulate AT depot-specific accumulation (39). Oestrogens can be produced locally from AT, mediated by aromatase (63). Oestrogen regulates depot-specific AT accumulation preferentially in gluteo-femoral, rather than visceral regions by modulating lipolysis and lipogenesis through oestrogen receptors (ERα) (39). In contrast, testosterone was shown to inhibit fat storage in the gluteo-femoral area (64, 65).

Alterations in endocrine signalling may also contribute to changes in fat distribution pattern, as observed in hyperandrogenism (66) or patients with Cushing's syndrome (60). These observations underline the significant role of gender-associated to sex hormones and age in body composition and fat distribution patterns in humans.

Obesity status and regional fat distribution vary by ethnicity (67). Europeans generally present with lower obesity rates than other ethnic groups, while Africans have a higher total body fat percentage (68, 69) mostly distributed in SAT, with relatively lower VAT than other groups (34, 70, 71). Interestingly, SA black women have also been shown with higher gluteal SAT (gSAT) and less VAT compared to their white counterparts (32, 33). These ethnic-differences in fat distribution have been attributed to differences in anthropometric, metabolic and behavioural mechanisms between ethnicities (69). Further, the interplay between genetic and environmental factors most likely determine these ethnic-specific differences in obesity rates and body fat distribution (32).

Obesity is a complex condition that is determined by genetic and environmental factors. The heritability of BMI, based on families and twin genetic studies, showed a large range of variability, estimated to be between 40-70 % (39). One of the pioneering studies exploring the influence of genetic predisposition to fat distribution comes from the study by Bouchard *et al.* on identical twins (72). They showed that within-pair similarity was particularly evident for differences in regional fat distribution and VAT accumulation, with significantly greater variance between pairs than within pairs (72). This study and others suggested that fat distribution is strongly controlled by both genetic and environmental factors (72-74). Moreover, recent genome-wide transcriptional analyses comparing SAT and VAT identified hundreds of

genes as differentially expressed between these depots (39, 52, 61, 75). Further evidence supports the role of genetic background in fat distribution. These studies demonstrated that waist-hip ratio (WHR), a surrogate measure that differentiates gluteo-femoral from central fat distribution, is a heritable trait estimated to contribute to up to 60% of inter-individual variation in body fat distribution, independent of BMI (76, 77). Based on these findings, the genetic architecture underlying fat distribution has been suggested to influence mechanisms related to AT development and metabolism (39). Therefore, AT accumulation follows a specific individual pattern during modifications of fat mass quantity, with preferential subcutaneous or visceral depot expansion/reduction largely determined by their heritable potential (39, 52).

Notably, gene expression studies showed that developmental genes are differentially expressed in various fat compartments and might also be involved in the regulation of fat distribution and obesity-related metabolic traits (52, 61, 78, 79). In the same line, *in vitro* experiments showed unique gene expression signatures in adipocytes from different depots, which were conserved after numerous cell differentiation cycles, suggesting that the depot-specific metabolic phenotype is intrinsic and programmed in the developmental/early life stage (80-82). Despite extensive research on the genetic influence of obesity and fat distribution, there seems to have no consensus on the exact mechanisms by which this influence occurs. Besides, although genome-wide association studies (GWAS) approach has identified more than 50 loci associated with BMI, WHR, body fat percentage and extreme obesity (83), most of these studies were conducted primarily in cohorts of European ancestry populations, with very few studies investigating the genetics of body fat distribution in individuals of African ancestry. One of the largest studies on Africans used a meta-analysis of GWAS of waist circumference (WC) and WHR, adjusted and unadjusted for BMI, in up to 33,591 and 27,350 individuals, respectively (84). This study has identified two loci associated with body fat distribution independently of generalized adiposity in African ancestry individuals, in addition to confirming 6 loci previously identified in populations of European ancestry (84). Ancestral genetic background, therefore, contributes to ethnic differences in body composition, total fat accumulation, and distribution above and beyond the effects of ethnic classification (85). However, no genetic studies on body fat distribution have been conducted on populations from Southern Africa, except for a recent study in n=1926 black South Africans assessing whether European body composition-associated gene loci (11 loci evaluated) played a similar role in this population (86). Although single nucleotide polymorphism (SNPs) in several gene loci were similarly associated with phenotypes linked to fat mass percentage and WHR in this African cohort, in-depth genomic studies in larger African cohorts is needed. Such studies may reveal novel SNPs

for body composition and adiposity, which will provide greater insight into the etiology of obesity in populations from Southern Africa (86).

1.3. ADIPOSE TISSUE METABOLISM AND FUNCTION

AT is a heterogeneous tissue with a complex structure (47). This heterogeneity is not only reflected by the different locations of this tissue, but also in its metabolism and function. Indeed, AT depots differ in their cellular composition, micro-vasculature, innervation, metabolism, extracellular matrix (ECM) composition, as well as secretory profile (46). This section firstly provides an overview of the general AT metabolism and function under normal physiological conditions, followed by the mechanisms underlying the impairment of AT function in the obese state and finally describes depot-specific AT metabolism and function, as well as the depot-specific associations with metabolic risk factors.

1.3.1. Adipose tissue metabolism and function under normal physiological conditions

The primary function of AT is the storage of TG during calorie intake and the release of fatty acid (FA) in periods of energy demand. SAT is the largest adipose depot and the most important in terms of its quantitative contribution to lipid storage/release and endocrine function (87). The principal contributors to lipid storage/release from AT are adipocytes, although they only represent one-third of the tissue cells, in addition to fibroblasts, macrophages, monocytes, stromal cells and preadipocytes (51).

1.3.1.1. Adipogenesis

Mature adipocytes are formed from precursor cells and preadipocytes through adipogenesis. This process is required for healthy AT development and function. AT development and expansion can occur via the increase in adipocytes number referred to as hyperplasia, the increase in cell size named as adipocytes hypertrophy, or both (88). Adipocyte hypertrophy is characterized by the presence of large, lipid-laden adipocytes in AT (89, 90). Of note, this increase in adipocyte size might simply be the response to the need to sequester available excess lipid, rather than a dysfunctional mechanism *per se*.

Adipogenesis is mediated by several hormones and transcriptional factors and is dependent on body energy status and storage needs (91). The inability of an individual to increase the number of adipocytes is associated with the development of metabolic diseases (92).

During adipogenesis, precursor cells are first committed to the adipocyte lineage and this is controlled by the expression of the bone morphogenic protein (BMP)-4 (93). Subsequently, pre-adipocytes undergo clonal expansion, multiplication, and early and terminal differentiation to mature adipocytes. These steps are controlled by CCAAT enhancer-binding protein (C/EBP)-β/δ, followed by the activation and expression of C/EBPα and peroxisome proliferator-activated receptor-gamma (PPARγ) (94, 95). PPARγ is one of the most important transcription factors for the maturation of preadipocytes to adipocytes. Low or dysregulated expression of PPARγ has been associated with impaired mitochondrial function and abnormal fat accumulation in non-AT organs (96, 97). Conversely, increased expression of PPARγ enhances AT storage capacity by inducing the recruitment of new adipocytes, which has been associated with an improvement in whole-body insulin sensitivity (98, 99). The release of FFAs from large adipocytes can also stimulate the differentiation of nearby preadipocytes to increase the lipid storage capacity of AT (100).

1.3.1.2. Adipose tissue lipogenesis

Lipid accumulation or storage in AT is governed by lipogenesis and incorporates the process of uptake, synthesis and storage of TG from non-esterified fatty acids (NEFA; also called FFAs), carbohydrates and/or other energy sources (94). This process, as well as lipolysis (the mobilization of this energy source as FFA), is highly regulated by genetic, nutritional, hormonal, and paracrine factors (101).

In the postprandial state, the majority of FFAs are delivered to adipocytes either through the breakdown of circulating TG (from chylomicrons or VLDL), via direct uptake of circulating FFAs or after endogenous synthesis (**Figure 1.2**). Fatty acid synthesis or *de novo* lipogenesis (DNL) in the adipocyte plays a minor role in fat deposition in human AT under normal physiological conditions (101, 102). However, adipocyte DNL may also be an important source of endogenous FAs, playing a key role in maintaining systemic metabolic homeostasis (103).

Chylomicron- or VLDL-containing TGs are hydrolysed into FFAs by insulin-stimulated action of LPL within vascular endothelium in AT (104). Released FFAs enter adipocytes through FA transporters such as CD36 and FA transport protein-1 (FATP1) (105, 106). Meanwhile, insulin also stimulates adipocyte glucose uptake, which drives DNL in the cytoplasm (**Figure 1.2**). FAs from these two sources are esterified using glycerol 3-phosphate derived from glucose to form TG that is stored in lipid droplets (103). The esterification of FAs is mainly controlled by acyl-coenzyme A diacylglycerol acyltransferase (DGAT) catalysing the final step of TG synthesis (101, 107) and thereby playing a crucial role in lipid deposition in

adipocytes (108, 109). DGAT also catalyses the synthesis of TGs from diacylglycerides in adipocytes (107). DGAT2 is widely expressed in hepatocytes and adipocytes and over-expression results in large TG droplets (110).

The rate of glucose uptake into adipocytes is essential for TG storage (46). Moreover, glucose molecules provide glycerol for esterifying FA directly taken up from the circulation (111). The transport of glucose into adipocytes is exerted via the glucose-transporter protein 4 (GLUT4), expressed under the stimulation and signalling cascades consecutive to the reaction of insulin and its receptor (IRS). Glucose derived from dietary carbohydrates stimulates the release of insulin from the pancreas, which in turn stimulates glucose uptake into adipocytes (**Figure 1.2**). Subsequently, glucose undergoes glycolysis and enters the tricarboxylic acid (TCA) cycle in the mitochondria to produce citrate. Citrate is subsequently transported into the cytosol and releases acetyl-CoA by ATP-citrate lyase (ACLY). Acetyl-CoA (substrate for DNL) is successively converted to malonyl-CoA by acetyl-CoA carboxylases 1 (ACC1) and palmitate by fatty acid synthase (FASN) (103, 111). Palmitate further undergoes elongation and desaturation reactions to generate FAs such as stearic acid, palmitoleic acid, and oleic acid (103). Insulin also promotes FA esterification through multiple mechanisms including activation of LPL, induction of translocation of FATP and upregulation of related gene expression in adipocytes (111). Moreover, insulin stimulates the expression of the lipogenic gene sterol regulatory element-binding protein 1 (SREBP1) that controls the expression of genes required for FA, TG and phospholipid synthesis (112). In addition to SREBP1, carbohydrate response element-binding protein (ChREBP), promotes DNL gene expression and has been shown to modulate both lipid and glucose metabolism in AT and consequently influence whole-body insulin sensitivity (113, 114). Taken together, AT is a fuel reservoir that plays a vital role in buffering FA fluxes and regulating clearance of plasma TGs to protect peripheral organs from lipotoxicity and therefore, prevent ectopic fat accumulation, IR and metabolic syndrome (115). This concept of lipotoxicity arose from observations of an impaired AT function in insulin-resistant states, in the excess fat accumulation with an impaired buffering FA flux, or in the pathological reduction/loss of AT mass as observed in lipodystrophy (115). In either case, a dysregulated ability of AT to buffer excessive FA flux will inevitably lead to increased lipid exposure and deposition in other tissues, providing a well-accepted link between obesity and cardiometabolic complications. AT lipid storage capacity is therefore essential for the maintenance of whole-body insulin sensitivity (103).

1.3.1.3. Adipose tissue lipolysis

Contrary to lipogenesis, lipolysis is the catabolic process involving the breakdown of TGs stored in adipocytes to release FFAs and glycerol for energy metabolism or re-esterification in the AT (116-118). In periods of energy demand, such as during fasting or physical exercise, adipocytes mobilize stored fat to fulfil the energy needs of other organs (**Figure 1.2**). Each molecule of TG is broken down into three molecules of FA and one molecule of glycerol. Glycerol and FA molecules are subsequently transported in the blood and can infiltrate into muscle, liver and other organs, thereby modulating whole-body energy balance (**Figure 1.2**) (115). Glycerol can be used for hepatic gluconeogenesis and FAs can be oxidized in muscles and liver or used for TG in the liver, according to energy needs (46, 103, 111, 119). FFAs are important sources of energy in peripheral tissues (87). The availability of FFAs and TG in non-adipose tissues is dependent on the lipolytic activity of the AT (87). Adipose triglyceride lipase (ATGL) is the first enzyme implicated in the hydrolysis of the stored TG into diacylglyceride (DAG). Hormone-sensitive lipase (HSL) cleaves DAG into monoacylglycerol (MAG), followed by the conversion of MAG to glycerol and FA. This last step is catalysed by the monoacylglyceride lipase (MGL) (103, 111). While ATGL mostly acts in the basal or non-stimulated lipolysis, HSL is necessary for maximal catecholamine-stimulated lipolysis (64, 120).

In the basal/non-stimulated state, adipocytes are protected from lipases by coating proteins surrounding the lipid droplet on the cytosolic surface (121). In humans, the most expressed lipid droplet-coating protein is perilipin 1 (PLIN1), the major substrate for cAMP-dependent protein kinase A (PKA) (122). PLIN1 promotes TG storage during basal conditions by restricting access of cytosolic lipases to the lipid droplet. On another hand, this protein facilitates maximal lipolysis during increased energy demand by its phosphorylation via PKA and stimulation of HSL (122). Therefore, perilipin regulates both basal and stimulated lipolysis. AT lipolysis can be stimulated by glucagon or catecholamines (effect on β-adrenergic receptor) and via the cAMP pathways, leading to PKA phosphorylation and phosphorylation of PLIN1 (123). Of note, PLIN1 can also be phosphorylated via cGMP-dependent pathways by the activation of protein kinase G, stimulated by natriuretic peptides (124). PLIN1 sequesters ATGL co-activator, comparative gene identification-58 (CGI-58) in basal conditions. This key regulatory protein (CGI-58) is very important for the activation of ATGL. Upon PLIN1 phosphorylation, CGI-58 is released and interacts with ATGL to allow full activation of this lipase (123). Also, PLIN1 phosphorylation is responsible for the activation, translocation and

docking of HSL to the adipocyte lipid droplet, inducing lipolysis (123). Conversely, the reaction of insulin with its receptor in insulin sensitive AT is followed by consecutive activations of phosphoinositol-3-kinase (PiP3k), PKB/Akt and phosphodiesterase 3B (PDE3B), which converts cAMP (necessary for PKA phosphorylation) into 5'-AMP and inhibits lipolysis (123).

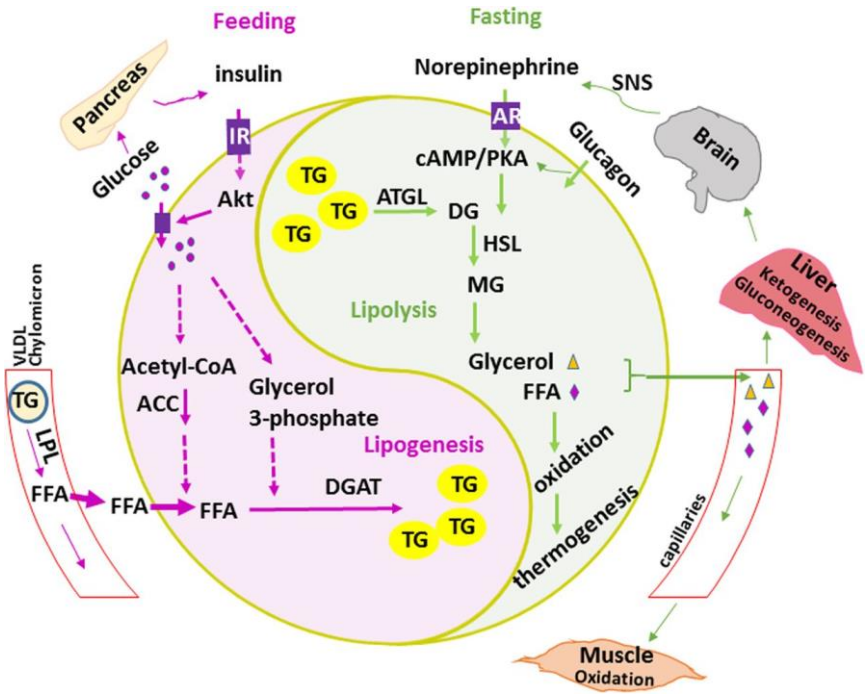


Figure 1.2. Adipose tissue lipogenesis and lipolysis.

Lipogenic and lipolytic pathways are inter-related and the maintenance of a regulatory balance between these pathways is essential for energy homeostasis and insulin sensitivity. Disruption in the equilibrium between fat storage and mobilization can lead to pathological AT expansion, function, and development of insulin resistance. AR: adrenergic receptor; cAMP: cyclic adenosine monophosphate; IR: insulin receptor; PKA: protein kinase A (111).

In addition to cell requirements in periods of nutrient scarcity and high energy expenditure, adipocytes can undergo lipolysis when their storage capacity is exceeded (124, 125). Under normal physiological conditions, lipogenesis and lipolysis are tightly co-ordinated and regulated by signals or endogenous stimuli (e.g. hormones, neurotransmitters…) from peripheral tissues and the central nervous system consecutive to the energy status (126). The lipid content in AT is controlled by a dynamic balance between these pathways, which is critical to maintaining systemic energy homeostasis and insulin sensitivity. Progressive and continuous energy surplus deposited in AT through the lipogenic pathway results in increased lipid droplet size and enlarged adipocytes (111), AT expansion and subsequent obesity (127). This can disrupt the balance between lipogenesis and lipolysis as observed under pathological conditions

such as during the early stages of IR (101). The following increase in unrestrained lipolysis results in excessive FFA spillover into the circulation. Consequently, this contributes to higher postprandial FFA plasma concentrations reaching peripheral tissues (128), leading to lipotoxicity and IR (129). Therefore, positive energy balance and dysregulated equilibrium between fat storage and mobilization are central factors in the pathogenesis of several metabolic characteristics of obesity-associated IR (101).

1.3.1.4. Adipose tissue endocrine function and adipokine production

In addition to its role in the maintenance of energy homeostasis as a passive fuel reservoir, AT is an active secretory organ known to produce and secrete hundreds of molecules acting via autocrine, paracrine or endocrine signalling (46, 103, 111). although the function of many of these active biomolecules is not yet fully understood, they can modulate systemic metabolism and inflammation (130). Indeed, these bioactive factors secreted from AT into the circulation relay information to other metabolically active organs such as muscle, liver, pancreas, and brain via endocrine mechanisms (131-133). These molecules are grouped in different categories depending on their origin (or secretory cells) and secretory pathways. Adipokines are secreted by adipocytes and include, among others, leptin, adiponectin, resistin, chemerin, serum amyloid A (SAA) and retinol-binding protein 4 (RBP-4) (111, 134). Leptin (135) and adiponectin (136) are the main adipocyte hormones and play a central role in the regulation of energy homeostasis (137). Cytokines such as omentin, visfatin, resistin, apelin, plasminogen activator inhibitor 1 (PAI-1), monocyte chemoattractant protein 1 (MCP-1), tumor necrosis factor-alpha (TNFα), macrophage migration inhibitory factor (MIF) and interleukins (e.g IL-1, IL-6, IL-8) are secreted mainly by adipose-resident immune and endothelial cells of the stromal vascular fraction (SVF) (134). Moreover, AT secretes lipokines such as palmitoleate and fatty acid esters of hydroxyl fatty acids (FAHFAs) that regulate systemic glucose and lipid metabolism (103).

Adipokines or adipocytokines are involved in numerous metabolic pathways, contributing to the regulation of appetite control, energy expenditure and activity, fat distribution, adipocyte metabolism and function, regulation of adipogenesis, migration of immune cell into AT and inflammation (tissue and systemic) (**Figure 1.3**) (88). Importantly, adipocytokines influence β-cell function and insulin sensitivity by regulating energy metabolism in insulin-sensitive tissues (e.g. liver, muscle and AT) (**Figure 1.3**) (88, 89). Adipocytokines are mainly mediated by binding to their receptors on the membrane of target cells and trigger intracellular signaling pathways (134). These molecules are relevant clinical biomarkers in obesity and associated metabolic dysfunction such as detrimental fat distribution

and function, ectopic fat deposition, impairment of insulin sensitivity, systemic and tissue inflammation (89).

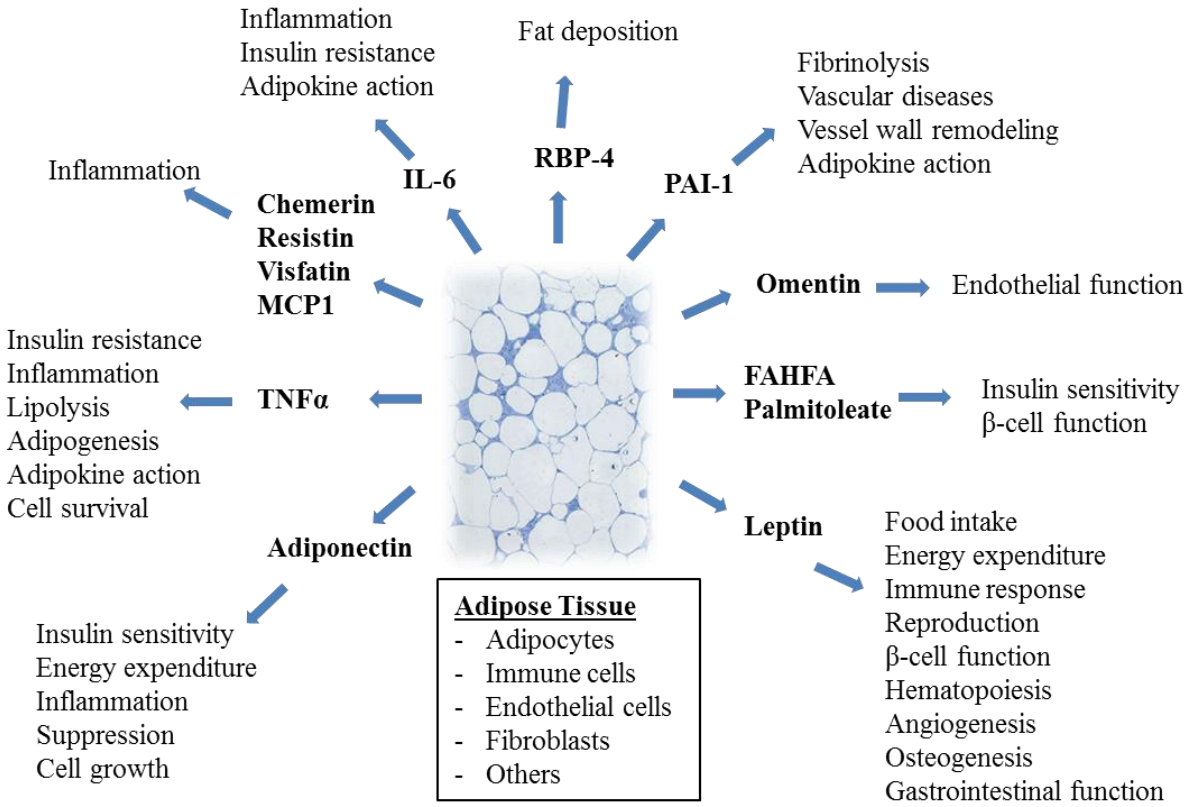


Figure 1.3. Example of physiological effects of adipocytokines

Adipocytokines (and lipokines) act on peripheral tissues via autocrine, paracrine and endocrine signaling pathways by regulation of adipogenesis, adipocyte metabolism, inflammation, insulin sensitivity, energy expenditure and activity, cardiovascular function, behaviour and cell growth. IL-6, interleukin 6; MCP1, monocyte chemoattractant protein 1; PAI-1, plasminogen activator inhibitor 1; TNFα, tumor necrosis factor-alpha; FAHFA: fatty acid esters of hydroxyl fatty acids; RBP-4: retinol-binding protein 4. Adapted from (89, 111).

1.3.2. Adipose tissue metabolism and function in the obese state

Obesity is characterized by an increased volume of AT, which is the major supplier of FFA to other organs in the post-absorptive state. Excess lipid storage in adipocytes during positive energy intake and the consequent increased basal lipolysis results in higher levels of circulating FFAs, involved in the development of low-grade inflammation and IR (134). The adaptation of AT to different physiologic conditions is therefore susceptible to alter whole-body metabolism (138).

1.3.2.1. Adipose tissue expandability

Although SAT is the largest adipose depot for lipid storage, this tissue has a limited expansion capacity (the so-called "tipping point"), depending on the ability of cells to enlarge and/or to recruit new cells (139). AT expansion limit varies between individuals and is dependent on the level of total body adiposity and AT depot (139). Consequently, this threshold limit may be reached in a non-obese range in certain individuals and may not be reached at all even during morbid obesity in other individuals (139). Once the tipping point is reached, the SAT is now unable to efficiently expand to serve as an energy buffer. The continuous calorie intake is followed by the redirection of excess lipids to VAT and ectopic fat depots (134). This pathological expansion, referred to as the AT expandability, is one of the most accepted hypotheses in the development of obesity-associated IR.

SAT has been referred to as a metabolic reservoir where lipids are sequestrated, thereby protecting other tissues against excess lipids or ectopic fat deposition (127). The impairment of SAT function and the metabolic disorders associated with obesity may result from AT impaired storage ability (127). In this condition, continuous calorie/energy intake favours the accumulation of fat ectopically (e.g. liver, heart, muscle) and contributes to hepatic and peripheral IR (140, 141). This is mostly observed in metabolically unhealthy obese (MUO) individuals (127). In contrast, a subcategory of morbidly obese individuals has been shown with a functional SAT expansion resulting in the maintenance of insulin-sensitive, healthy obese phenotype referred to as metabolically healthy obesity (MHO), although this term has been heavily debated and remains questionable (142, 143). VAT accumulation has been associated with the impairment of SAT function in healthy individuals in response to lipid overfeeding (141). This supports the hypothesis that the incapacity of SAT to further expand contributes to VAT accumulation and its adverse metabolic effects. The main described events characterizing the occurrence of AT dysfunction after an impaired expansion capacity have been proposed to consist of impaired adipogenesis, adipocyte hypertrophy, increased hypoxia, changes in the ECM resulting in fibrosis, increased immune cell infiltration into AT and inflammation, AT stresses, altered lipid metabolism, ectopic fat accumulation and modifications in AT transcriptomic profile changes (61, 134).

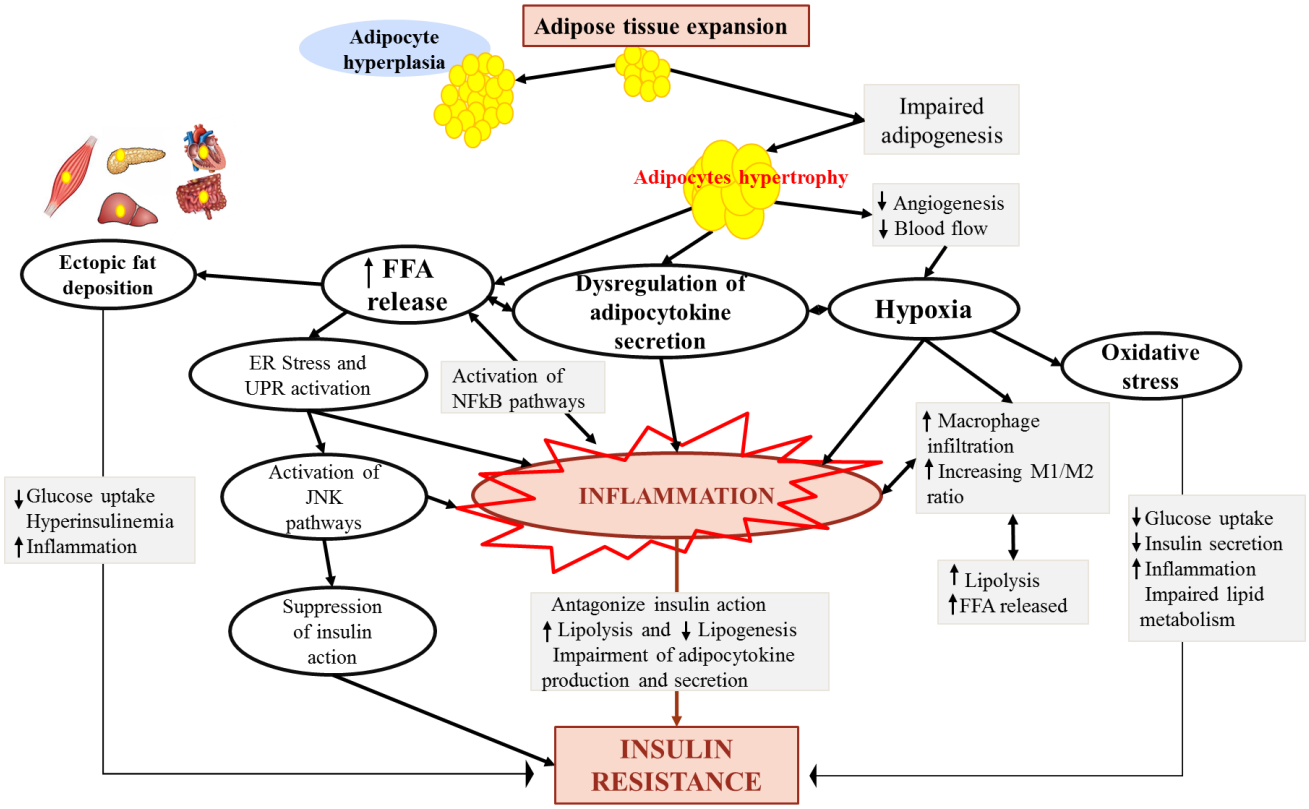


Figure 1.4. Adipose tissue expandability hypothesis: proposed mechanisms whereby hypertrophic AT expansion is linked to IR.

The most accepted factors implicated in the impairment of AT function during excess fat accumulation are the impairment of adipogenesis and adipocyte hypertrophy, followed by increased FFA release and ectopic fat deposition, dysregulation of adipocytokine secretion, increased cellular hypoxia and AT stresses such as ER and oxidative stress. These mechanisms all contribute to the establishment of a pro-inflammatory state in AT, interfering with the insulin signalling pathway and leading to peripheral and systemic insulin resistance. ER: endoplasmic reticulum; FFA: free fatty acids; UPR: unfolded protein response; JNK: Jun N-terminal kinase; NFkB: nuclear factor-kappa B.

1.3.2.2. Impaired adipogenesis, adipocytes hypertrophy and angiogenesis in the obese state

While the expansion of AT through hyperplasia has been shown in insulin-sensitive AT, an increase in cell size has mostly been associated with detrimental or pathologic development as described in insulin-resistant AT. An impaired ability of precursor cells to enter adipogenesis or adipocyte development process may be the upstream factor leading to AT dysfunction and involved in the development of obesity-related systemic IR and inflammation in humans (61, 134, 144) (**Figure 1.4**). Indeed, decreased levels of adipogenic genes have been associated with systemic IR in obese individuals (131, 145) and related to AT inflammation (146). However, pro-inflammatory signals released from enlarging adipocytes have been proposed to favour

excess fat storage by stimulating adipogenesis and/or terminal differentiation of preadipocytes (147). Therefore, besides restricting accommodation of excess fat in the mature adipocytes, impaired adipogenesis may build up the inflammatory response of the hypertrophic adipocytes (61). Consistently, defective adipogenic potential has been positively associated with adipose secretion of IL-6 and IR in obese individuals (148). Impaired adipogenesis, therefore, restrains AT expansion and favours adipocyte hypertrophy and adipose/systemic inflammation, inducing abnormal glucose regulation and IR (61, 139).

A study evaluating the relationship between adipocytes size and insulin sensitivity showed that hypertrophic adipocytes were found in patients with T2D and prediabetic individuals and were an independent marker of IR in SAT from prediabetic patients (149). Furthermore, increased adipocyte volume in obese individuals correlated with impairment in whole-body insulin sensitivity and higher systemic inflammatory and oxidative stress status and increased AT macrophage number (143). Decreased adipogenesis along with adipocyte hypertrophy might not be followed by adequate angiogenesis, setting up a local hypoxic environment, which might cause adipocyte apoptosis and necrosis. Indeed, AT expansion requires suitable vascularization and angiogenesis (150, 151). For instance, SAT angiogenic capacity decreases with increasing BMI and is associated with IR, suggesting that impaired angiogenesis in SAT contributes to obesity-related metabolic complications (144). During AT hypertrophy, oxygen level supply may decrease, possibly due to a decreased or insufficient angiogenesis for the level of lipogenesis, and a decreased blood flow, creating a hypoxic state in AT and macrophage infiltration (**Figure 1.4**) (152).

1.3.2.3. Adipose tissue hypoxia

Hypoxia in expanding AT is a suggested factor triggering stresses and inflammatory processes within the tissue and subsequently impaired AT function, although the mechanisms are not yet well established (88). It has been proposed that, if AT expansion occurs with insufficient angiogenesis, groups of adipocytes can become distant from the vasculature creating an insufficient amount of oxygen in the AT micro-environment (88, 153). The impairment of AT function could therefore be a specific consequence of relative hypoxia observed in obese individuals (152, 153). For instance, it has been shown that hypoxia (experimental) can stimulate adipocytokines secretion (e.g. angiopoietin-like protein 4, IL-6, leptin and macrophage migration inhibitory factor (MIF-1)) and induce oxidative and endoplasmic reticulum (ER) stress, potential additional pathways of impairment of AT function by low O_2 tension in the tissue (153).

1.3.2.4. Immune cell infiltration and adipose tissue inflammation

Several efforts have been made to identify triggers of AT inflammation subsequently involved in obesity-associated development of IR. The expansion of AT is associated with increased infiltration of pro-inflammatory immune cells initiating chronic low-grade inflammation (steady, low-level inflammation throughout the body) (154). Indeed, during adipocyte hypertrophy, higher amounts of pro-inflammatory adipocytokines and FFAs and are released from AT (52, 139). Saturated FAs serve as ligands for toll-like receptor 4 (TLR4), thereby inducing inflammatory reactions in AT through activation of nuclear factor-kappa B (NFkB) pathways (52, 139, 155). For instance, palmitate induces increases in TNFα production from macrophages (156). On another hand, elevated production of pro-inflammatory cytokines induces lipolysis, increases the release of more FFAs and inflammatory changes in adipocytes, creating a vicious circle between lipolysis and inflammation (156). Therefore, FFAs are significant adipocyte-derived paracrine mediators of inflammation in AT (156). The resultant effects could be the downregulation of cellular insulin signalling, activation and migration of monocytes, recruitment of additional macrophages through MCP1, propagation of inflammation by interleukins and TNFα, and impairment of tissue matrix remodelling through matrix metalloproteinase-9 (MMP-9) (139). These inflammatory mediators and metabolic pathways may further limit the recruitment and maturation of new adipocytes resulting in the impairment of AT function or AT dysfunction (88), playing a critical role in the development of obesity-associated IR (134, 157-159). Moreover, macrophages-derived cytokines influence local and systemic inflammation (88, 154). Obesity and inflammation are therefore tightly interconnected in the pathogenesis of IR and T2D (160).

Both animal and human studies showed that in the obese state, AT is the target of immune cell infiltration, especially macrophages (161). The number of macrophages infiltrating into AT has been shown to increase with BMI, body fat mass and adipocyte hypertrophy, and decrease upon weight loss (162, 163). The death of hypertrophic adipocytes may also induce the infiltration and activation of macrophages into AT where they aggregate in ‘crown-like structures’ around the damaged cells (164). These immune cells may be attracted from the circulation by chemoattractant proteins such as MCP-1, chemerin, progranulin and colony-stimulating factor-1 (CSF-1) (88, 162, 163). Once infiltrated into AT, macrophages are activated by FFAs released from hypertrophic adipocytes and produce elevated amounts of inflammatory cytokines (156). The infiltrated macrophages therefore contribute to increase inflammatory state in AT and enhance circulating levels of pro-inflammatory cytokines (88).

Dysregulation in the production or secretion of adipocytokines may cause AT dysfunction, due to their inflammatory properties, proposed as the mechanism by which adipocytokines induce IR in obese state (134, 165). A large number of AT-derived bioactive factors has been recently identified, although their specific function, molecular targets, intracellular signal transduction, and potential clinical relevance in the treatment of obesity and metabolic diseases remain to be elucidated (134). Nevertheless, the majority of adipocytokines (mostly pro-inflammatory) are elevated in obesity and correlate with total fat mass, body fat distribution and insulin sensitivity (88). Conversely, the production of anti-inflammatory adipocytokines decreases in the obese state, consecutive to the shift in macrophages population, from M2-type (anti-inflammatory) to M1-type (pro-inflammatory) and increase of M1/M2 ratio (89). Therefore, via their significant endocrine effects on target tissues like brain, liver, muscle, blood vessels, heart, and pancreatic β-cells, adipocytokines represent important determinant in metabolic dysfunction associated with excessive during fat accumulation (89, 134).

1.3.2.5. Adipose Tissue stresses

Overfeeding and obesity can cause a variety of stresses on AT such as metabolic (from increased metabolic fluxes and availability of glucose and lipids), oxidative and ER stress (88, 89, 166). Indeed, adipocyte hypertrophy and impaired subcutaneous expandability may induce AT stresses by the activation of stress sensing pathways leading to failure in cellular function and contributing to obesity-associated metabolic disorders (134). Among obesity-related stress pathways, kinases are committed in the transmission of stress responses, including the stress-activated protein kinases p38 mitogen-activated protein kinase (MAPK) and Jun N-terminal kinase (JNK) (88). JNK has been involved in the establishment of obesity-induced IR and inflammation (88). Indeed, JNK1-dependent secretion of the inflammatory cytokine IL-6 by AT causes increased expression of liver SOCS3, a protein that induces hepatic IR (167). This suggests that activation of stress signalling pathways in AT may also cause a shift toward the secretion of proinflammatory adipocytokines.

Obesity is also associated with ER stress, reflecting functional overload of ER in response to increased protein synthesis or secretion requirements and/or reduced elimination of misfolded proteins (88, 166). Proteins are folded and assembled by chaperones in the ER under normal conditions (88). However, in a hypoxic environment or with the over-supply of nutrients, the unfolded protein response (UPR) cascade can be activated (166, 168). Activation of the UPR in AT leads to transcriptional induction of genes involved in the assembly, folding, modification, and degradation of proteins to relieve ER stress (168), increase JNK, IKKβ

activity and pro-inflammatory cytokine expression (such as IL-6, TNFα and MCP1) (168). Notably, FFAs can trigger ER stress in adipocytes (**Figure 1.4**) (168), supporting that with increasing nutrient flux – including increased serum concentrations of FFAs and fat accumulation, AT is exposed to several stresses capable of generating a pro-inflammatory state and impairment of AT function in obesity. Besides the kinase-induced stress responses, and nutrient overflow causing ER stress, oxidative stress also occurs in AT. This has been evidenced by increased levels of oxidized proteins in AT of obese mice (169). Moreover, AT of insulin-resistant and insulin-sensitive morbidly obese individuals could be differentiated by increased oxidative stress in the former (143, 170).

1.3.2.6. Oxidative stress

The increase in cell size following progressive fat accumulation in AT, the impaired angiogenesis and resulting decreased vasculature, as well as the lack of appropriate ECM remodelling may result in the reduction of oxygen supply to adipocytes. These events proposed to set up local hypoxia may contribute to inflammation and oxidative stress within AT (**Figure 1.4**) (134). Oxidative stress has been demonstrated in obese individuals with elevated markers of reactive oxygen species (ROS) such as urinary 8-isoprostanes, thiobarbituric reactive acid substances (TBARS), and lipid hydroperoxides, as well as reduced antioxidant defence system (171). Oxidative stress is associated with systemic inflammation (171), impairment of glucose uptake in adipocytes (172), and decreased insulin secretion from pancreatic β-cells (173), supporting its critical role in the pathogenesis of IR. Increased oxidative stress in accumulated fat has also been associated with dysregulation of adipocytokines secretion and IR observed in obese individuals (174). Moreover, obesity-induced inflammation is associated with increased systemic and AT oxidative stress (171, 174). It is therefore valuable to understand the mechanism underlying the association between obesity-induced oxidative stress and IR.

Oxidative stress generally refers to an imbalance between the production of pro-oxidant substances (ROS) and antioxidant defences (such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT)), resulting in cellular damage (171). ROS is generated during cellular metabolism when the chemical reduction of oxygen forms unstable free radicals, characterized by an unpaired electron (e.g. including superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-)) (175). Under normal conditions, these oxidizing agents are essential for physiological functions such as gene expression, cellular growth and infection defence (171, 175). Their physiological levels in cells are conserved by the action of antioxidants from enzymatic (e.g. SOD, GPx and reductase, CAT) or non-enzymatic sources

(ascorbate, carotenoids, phenolic compounds, proline, polyamines) (171). Through the inhibition of ROS formation and action, or by repairing ROS-damaged cells, antioxidants are important to prevent oxidizing damage of ROS (171). However, long-term exposure to elevated ROS reduces insulin sensitivity and impairs glucose and lipid metabolism (174).

Systemic oxidative stress has been closely associated with excess fat accumulation and BMI (176). AT is the major source of elevated circulating ROS in the obese state. Indeed, lipid accumulation in adipocytes leads to the generation of ROS, principally through the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (174, 177). Leptin, which plays a key role in mediating pro-inflammatory state in obese individuals, has been proposed to induce oxidative stress by stimulation of mitochondrial oxidation of FAs (178). Leptin can also induce AT oxidative stress through increasing pro-inflammatory cytokines secretion (179). High levels of leptin have been associated with increased production of pro-inflammatory cytokines such as TNF-α, a potent activator for activation of NADPH oxidase, resulting in the formation of ROS (180).

As previously reported in several studies, macrophages infiltrate obese AT and play a vital role in regulating obese AT inflammation by the ability to shift adipocyte secretory profile to a pro-inflammatory condition (T-helper 1 subtype) (181). Macrophages can produce ROS (e.g. O_2^-, H_2O_2 and OH^-), which also provides positive feedback to upregulate T-helper 1 cell activation and further sustain a pro-inflammatory state (182). Moreover, macrophage-induced ROS production increases the activity of NADPH oxidase in obese AT, which subsequently induces a dysregulated expression of inflammatory adipocytokines, including adiponectin, PAI-1, IL-6, and MCP1 and the decreased production of antioxidant enzymes (174, 177). On another hand, elevated ROS can upregulate the mRNA expression of NADPH oxidase, creating a vicious circle and resulting in increased oxidative stress in AT and the circulation (174). Indeed, ROS and by-products of lipid peroxidation such as TBARS have also shown to increase the expression of MCP-1, resulting in attraction, infiltration and activation of macrophages into AT and inflammation (174). Moreover, prolonged exposure of adipocytes to ROS results in impairment of insulin-induced activation of PI3-kinase and Akt, insulin-stimulated lipogenesis, glucose uptake, and GLUT4 translocation to the plasma membrane (172, 183). ROS also suppressed the mRNA expression of lipogenic genes, such as FASN and SREBP-1c (174).

Oxidative stress was further shown to suppress PPARγ mRNA expression in adipocytes (174), and consequently, is implicated in the impairment of adipogenesis and AT expansion. Furthermore, PPARγ positively regulates the transcription of the adiponectin gene via PPARγ-

responsive element in the promoter (184). Therefore, downregulation of adiponectin expression in obese subjects may be partially attributed to the decreased gene expression and a smaller amount of nuclear PPARγ under conditions of oxidative stress (174). Additionally, elevated FFAs resulting from impaired lipolysis and lipogenesis in hypertrophic adipocytes can activate NADPH oxidase and induce ROS production in AT (174). These findings suggest that increased ROS production during lipid accumulation may dysregulate adipocytokine production, impair further lipid storage and favour ectopic lipid deposition, ultimately leading to IR.

In summary, increased pro-inflammatory response and immune cell infiltration in obese AT promote the formation of ROS and increase oxidative stress. This may lead to dysregulated production of adipocytokines, which exacerbates AT inflammation. Further, increased ROS production in AT leads to elevated systemic oxidative stress and potentially instigates whole-body IR by affecting other peripheral organs' function. Therefore, oxidative stress could be a useful target in the development of therapies to reduce obesity-associated metabolic dysfunction. The increase in endogenous activity of antioxidant enzymes can reduce the incidence of oxidative stress and associated metabolic disorders by regulating or delaying the production of ROS (185, 186). In this regard, antioxidant treatment improves insulin function in people living with diabetes (187). The inhibition of NADPH oxidase has also been proposed to improve the dysregulation of adipocytokines and insulin sensitivity via restoration of normal ROS production in obese adipocytes (174).

1.3.2.7. Ectopic fat deposition

Ectopic fat deposition contributes to low-grade inflammation and IR. Indeed, ectopic deposition of lipids may result in polarization of macrophages, stimulate changes in adipokine secretion and induce inflammation (87). Lipid accumulation in non-adipose organs is indicative of an oversupply of FFAs more than what can be oxidised (e.g. skeletal muscle) and/or secreted (e.g. liver, visceral area) (**Figure 1.4**). An increase in the delivery and storage of FFAs in skeletal muscle impairs the activity of glucose transporter (GLUT 4) and decreased glucose uptake, resulting in a decrease of peripheral insulin sensitivity (46, 188). Accumulation of TGs in the liver promotes steatohepatitis and non-alcoholic fatty liver disease. This results in hepatic IR by decreasing glucose uptake and increased glucose production (188). Furthermore, while FFA accumulation in the heart can result in diastolic dysfunction (188), FFAs increase pancreatic insulin secretion and systemic basal insulin concentrations leading to hyperinsulinemia, impairment of glucose-stimulated insulin secretion and IR (46).

1.3.3. Genetic factors implicated in AT dysfunction

The failure of appropriate AT expansion and fat storage leading to overflow of lipids as ectopic fat has been associated with the absence of "favourable adiposity alleles", predisposing these individuals to poor AT expandability and leading to obesity-related complications. This is usually the case in obese individuals characterized by greater VAT and lower SAT, contrary to carriers of the "favourable adiposity alleles" characterized by a fat distribution directed towards subcutaneous, rather than VAT expansion (189).

Genetically programmed developmental differences in adipocytes and their precursors in different regions of the body are thought to have a fundamental role in obesity and fat distribution (134). Genetic variants of several previously unknown genes associated with fat distribution have been reported from GWAS (76). However, the function of these genes and the mechanisms underlying their association with detrimental fat distribution and impairment of AT function are not fully understood (88). GWAS suggested that body fat distribution is associated with variations in genes involved in pattern formation during embryonic development (190). To understand the mechanisms implicated in the impairment of AT in the course of obesity, the specificity in metabolism and function of each AT depot, and the depot-specific gene expression signatures need to be considered. Indeed, pre-adipocytes from SAT and VAT are thought to be derived from different precursors programmed through epigenetic modulation (81, 82). In addition, different precursors seem to be responsible for specific adipose depot development, which confers distinctive biological features and may participate in determining differences in function and gene expression (e.g. VAT and SAT (reviewed in (46) and/or between aSAT and gSAT (reviewed in (78, 79)). The next section provides some differences in adipose-depots biology, metabolism and function as well as the causal factors of differential associations with obesity-related metabolic risk factors.

1.3.4. Depot-specific differences in adipose tissue function and associated metabolic risk factors

Adipose depots present differences in several metabolic and functional pathways, which may explain their differential associations with metabolic risk in the obese state. AT development is preceded by capillary network growth. Endothelial cells contribute to the stimulation of preadipocyte differentiation (191). SAT has a higher capacity to expand its capillary network, and higher angiogenic growth capacity compared to VAT (144). Preadipocyte proportion and subtypes with different proliferative capacities also differ between fat depots (192). The predominant adipogenesis type (hypertrophy vs hyperplasia) determines

the size and number of fat cells in different depots and contributes to the predisposition to obesity-related metabolic disorders (52). VAT contains fewer adipogenic precursors, with lower proliferation and differentiation capacities (193), smaller adipocytes (46), lower number of pre-adipocytes (192) and a higher number of stromal cells compared to SAT (194). However, in obese individuals, stromal cells from the SAT region proliferated faster than those from VAT depots (194).

Differences in secretory profiles have also been shown between VAT and SAT, with a higher expression of pro-inflammatory cytokines in the former (including TNF-α and PAI-1) while levels of adiponectin, leptin and IL-10 are higher in SAT (46, 195). Moreover, it has been reported by several research groups that VAT is a major contributor to metabolic risk (139, 196), whereas SAT may have a protective role (195, 197). The first proposed explanation of the association of VAT with metabolic disorders was the portal vein hypothesis, based on the anatomical position of this depot in proximity to the liver. Hypertrophic adipocytes in VAT are less sensitive to the anabolic action of insulin and are characterized by a hyperlipolytic state, thus producing larger amounts of circulating FFAs (61). According to the portal vein hypothesis, these high levels of FFAs, directly enter the liver through the portal circulation and lead to increased lipid synthesis and gluconeogenesis (198). This can result in impaired hepatic insulin sensitivity, increased hepatic glucose production, fasting hyperglycemia and hyperlipidemia, hepatic steatosis and ultimately IR (46, 196). Further, excess FFAs deposition in skeletal muscle can inhibit glucose uptake and lead to peripheral IR (46, 188). However, if VAT was the sole contributor to metabolic risk, it should be the major source of systemic FFA pool. In contrast, only a small portion of total body fat (7%-8% in women) is located in the intra-abdominal region and contributes to only 15% of the total systemic FFAs, whereas the majority of FFA is stored in SAT (199). This observation raised doubts on the exclusive contribution of VAT to the development of IR. Therefore, although VAT is considered a unique pathogenic depot that confers risk beyond its contribution to overall adiposity, SAT volume and quality also seem to be involved in the incidence of metabolic risk factors (200).

The adverse effects of excessive upper-body adiposity have often been attributed to VAT, and the role of aSAT in the regulation of metabolic health has been less acknowledged until recently (128). Abdominal SAT shares some morphological and functional similarities to VAT (201). The expansion of this depot may therefore have a negative impact on effective fat storage (128). Abdominal SAT volume is approximately five times larger than VAT volume at the same level (L4-L5 level) (139) and this depot (aSAT) is the major source of systemic FFA flux,

greater than the VAT depot and the lower-body SAT (190). Moreover, aSAT inflammation has been associated with the partitioning of fat towards the VAT and liver and altered β-cell function, independent of total adiposity (202). Therefore, both SAT and VAT may contribute to ectopic lipid deposition and associated complications. Each AT depot has a unique pattern of FA storage, suggesting distinctive regulation and intracellular processing of FA (107). Abdominal adiposity (including aSAT and VAT depots) is the primary site for immediate storage of diet-derived fat (128). The differences in the rate of lipid accumulation between AT depots been proposed to be influenced by DGAT activity. This enzyme can create a favourable gradient for uptake and storage of circulating FAs by modulating adipocyte size (107). Furthermore, regional differences in direct FFA storage is dependent on LPL activity and was shown to be more elevated in VAT compared to aSAT in women (46, 203). Moreover, although VAT and aSAT have a high lipid turnover rate, VAT is more metabolically active (fat storage and release) compared to aSAT (46, 195).

In contrast to VAT and aSAT, gSAT is characterized by reduced lipid turnover, and a high capacity to accommodate lipids undergoing redistribution, consisting of recirculated FAs from VLDL or directly from the FFA pool (204). The uptake of diet-derived FAs occurs less efficiently in gSAT than in aSAT, which is balanced by fewer FA release from gSAT in the post-absorptive state or during adrenergic lipolytic stimuli (128). Indeed, evidence showed that gSAT adipocytes are less sensitive to factors stimulating lipolysis and have a lower rate of lipolysis than in aSAT (205). Indeed, adipocytes from aSAT are more sensitive to β-adrenergic agonists and gSAT adipocytes are more responsive to the anti-lipolytic effect of α-2 adrenergic agonist, with lower lipolytic responses to mixed agonist (46). *In vivo* studies also showed greater lipolytic activity in aSAT with higher that FFA delivery to the periphery compared to gSAT (46). Moreover, during weight loss, the reduction of gSAT occurs slower than aSAT, suggesting and elevated retention capacity of FA in gSAT (128). Besides FFA levels, the expression of pro-inflammatory cytokines in aSAT like in VAT, as well as the number of infiltrated macrophages, was shown to be higher than in gSAT (206). Furthermore, a population-based study found that aSAT is negatively and gSAT is positively correlated with serum adiponectin concentrations (207). Adiponectin is critical for AT expansion and its dominant secretion also corresponds with the adipogenic potential of gSAT (52). Indeed, upper and lower-body AT respond differently to increasing demands for fat storage, underlying differences in expansion capacity between aSAT and gSAT. Whilst aSAT expands through a hypertrophic response (more fat in each adipocyte), gSAT shows a proliferative or hyperplastic response (new fat deposited in new adipocytes) (208).

Oxidative stress also seems to play a role in the high cardio-metabolic risk associated with VAT accumulation. This adipose depot has been strongly associated with urinary 8-isoprostanes (a marker of oxidative stress) and MCP-1 compared to SAT (209). In addition, higher android rather than gynoid fat correlates positively with systemic oxidative stress, independent of cardiovascular risk factors and inflammation (177). Further, a reduction in android fat mass, but not gynoid fat or BMI was associated with decreases in oxidative stress after 1 year of follow-up (177). A recent study on SAT depot-specific proteome in overweight and obese women showed a higher expression of the antioxidant enzyme SOD1 and lower expression of nitric oxide production related enzymes in gSAT compared to aSAT, supporting differences in the level of oxidative stress in SAT depots, with higher oxidative stress profile in aSAT (210).

Taken together, AT depots have distinct influences on metabolic health, with central fat accumulation (VAT and aSAT) associated with a detrimental metabolic profile, mostly attributed to VAT, which has been associated with metabolic syndrome even in non-obese individuals (211). Conversely, individuals with greater gSAT mass have been found with a more favourable metabolic profile (52, 139). In addition to their functional differences and distinctive associations with metabolic risk, epigenetic differences between fat depots (SAT vs VAT and aSAT vs gSAT) have been shown (46, 78). These data are mainly derived from European studies and may not be directly attributed to depot-specific SAT associations with metabolic risks in Africans. Indeed, different ethnic groups have distinct patterns of body fat distribution, conferring differential associations with metabolic risks (36, 37). Therefore, the metabolic effects of AT depots in the development of IR and T2D may vary in women of different ethnicities (32).

1.3.5. Ethnic-specific differences in metabolic risk factors

Different ethnic groups are distinctively susceptible to metabolic diseases, which may be partly explained by differential patterns of body fat distribution. For instance, African Americans have a higher risk for abdominal fat-related chronic conditions such as CVD and IR than non-Hispanic white women (69). Conversely, leg fat indices tend to negatively correlate with the risk of IR, regardless of ethnicity (195). Contrary to Europeans, fat accumulates mostly in SAT with relatively less VAT in African women (34, 70, 71). These last have a weaker association between VAT and parameters of metabolic syndrome (e.g. blood pressure, TG, high-density lipoprotein cholesterol and total cholesterol concentrations) than European descendants (212). In contrast, aSAT was negatively correlated with insulin sensitivity in

African-American women, but not in European women (25). Interestingly, black and white SA women present with different patterns of body fat distribution which have been associated, at least in part, with differences in cardio-metabolic risks between these ethnic groups (33). In this regard, centralization of body fat, including an increase in both VAT and aSAT are important risk factors for IR and T2D in both black and white (25, 32, 37).

Paradoxically, although black SA women have shown greater SAT accumulation in the gluteo-femoral region and less VAT for a similar BMI (31-33), they have higher levels of IR than their white counterparts (24). Only a few studies have investigated the AT biology and function in black SA women, despite their higher metabolic risks. Impairment in SAT function, especially in the gSAT, has been proposed as a possible mechanism explaining the higher incidence of IR in obese black SA women (35). Indeed, gSAT in obese black SA women is characterized by reduced expandability capacity with decreased adipogenic and lipogenic gene expression, associated with decreased insulin sensitivity (35). Further, black African women had larger adipocyte size compared to their white counterparts (213), which has been shown to correlate with insulin levels in postmenopausal African American women, but not white American women (36). Moreover, larger median gluteal adipocyte size and a greater proportion of large gluteal adipocytes were shown in black SA women, while white women had a greater proportion of small cells (36).

Furthermore, elevated expression of genes involved in hypoxia and ECM remodelling (HIF-1, collagen type V a1 and collagen VI a1) and reduced vascular endothelial growth factor-a expression have been shown in gSAT of black SA women, which correlated with decreased insulin sensitivity (214). The association between SAT mass and lower insulin sensitivity in black women may therefore be driven by increased hypoxia, which in turn induces a fibrotic and inflammatory response, leading to IR. To address the implication of SAT inflammation in this association, it was further shown that pre-menopausal black SA women had higher expression of the chemokine C–C motif ligand and its receptor; higher expression of the macrophage marker CD68, and greater expression of the cytokines TNFα, MIF and CSF-1 compared to their white counterparts, independent of VAT, adiposity and age (213). Strikingly, the higher SAT inflammatory gene expression profile of African women only explained 20% of the variation in insulin sensitivity, in comparison to 56% in white women (213). This suggested that despite the Africans displaying a higher gSAT inflammatory response (213), and carrying genetic variants associated with higher inflammation (215), factors other than SAT inflammation might be stronger contributors to insulin sensitivity in these women.

Ethnic differences in insulin sensitivity may also be related to increased oxidative stress. Indeed, high levels of hypoxia may increase oxidative stress in African women (36), which has been associated with increased FFAs and reduced insulin sensitivity in African Americans, but not European or Americans (216). However, there is a lack of studies examining the association between oxidative stress in AT and IR in black SA women. More studies are required to understand the mechanisms implicating obesity-induced oxidative stress in the development of IR in black SA women.

Another important mediator of obesity-related IR may be the increased release of FFAs from SAT, resulting in the accumulation of lipids and their intermediates in ectopic sites, which disrupt metabolic processes and reduce insulin sensitivity in these tissues (188). Although black African women have lower hepatic lipid deposition compared to their white counterparts (217, 218), there is evidence to suggest that obese African women are more sensitive to the effects of ectopic lipid deposition and VAT on hepatic insulin sensitivity than white SA women (32, 37, 217). In addition, it has been shown that the activity of the lipogenic enzyme stearoyl-CoA desaturase 1 (SCD1), the principal enzyme involved in the desaturation of palmitic acid (16:0) to palmitoleic acid (16:1n-7), is reduced in obese African American women compared to their white counterparts (219). Therefore, lipid and FA metabolism in AT may be implicated in the higher metabolic risk in African women, but it is not known whether SAT FA metabolism and FA profile can explain the impairment in insulin sensitivity in obese black SA women. Mechanistic studies are required to explore the differential function and metabolism of aSAT and gSAT in obese black African women, where both SAT depots have been associated with risk for IR and T2D (32, 35, 37).

1.4. FATTY ACID METABOLISM IN OBESITY

FA composition of stored TG during positive energy balance may exert positive and/or detrimental metabolic effects on insulin signalling pathways within AT tissue and peripheral organs (220, 221). Indeed, FAs are important receptor agonists that can regulate AT function by modulating gene expression (222), lipid metabolism (155), trigger transcriptional profiles associated with adipogenesis and inflammation (223), potentially contributing to IR in individuals with obesity (156, 224). Moreover, changes in FA profiles in the circulation or AT has been reported in obese individuals and proposed to significantly affect their metabolism and the risk of IR and T2D (225). The implication of biologically active FAs stored and released by AT in the development of metabolic control underlies the need to give special attention on

these molecules in a context of metabolic control and prevention of comorbidities in obese individuals.

1.4.1. Fatty acid classes and metabolism

FAs are essential components of almost all lipids and constitute structural elements of phospholipids (PHLs) and glycolipids building biological membranes. These molecules are also the principal component of TGs, which accumulate in AT and serve as an energy source (226). FAs are a diverse group of compounds differing in chain length, degree of saturation and metabolic effects. They may originate from dietary intake, from endogenous synthesis during DNL or from AT release (225). According to their degree of unsaturation (presence, number and position of carbon-carbon double bonds), FAs are generally grouped into three main classes.

Saturated fatty acids (SFA) are mainly derived from animal and dairy products and can also be synthesized endogenously (225). This class is generally considered to exert unfavourable effects and to induce numerous diseases, principally related to inflammation (225). High dietary intake of SFAs results in increased concentration of their metabolites, which have been shown to stimulate the synthesis of inflammatory cytokines in various tissues (227). The dietary influence on the concentration of FAs has been shown by a strong correlation between dietary intake of SFAs and the degree of PHL saturation and the proportion of SFAs within serum PHLs (228). However, some studies have suggested that membrane PHLs and circulating SFA contents derive from endogenous synthesis (229).

Mono-unsaturated fatty acids (MUFA) are an FA class defined by the presence of one double bond in the carbon chain. These FAs are abundant in vegetable oils and can be actively synthesized in human liver and AT from their SFA precursors (e.g. palmitic and stearic acids) by desaturation (mainly regulated by SCD1). MUFAs form the main substrates for TGs, PHLs and cholesteryl ester synthesis and are the major FAs in VAT and SAT, and consequently play a role in the pathogenesis of obesity and associated T2D (230).

Poly-unsaturated fatty acids (PUFA), are characterized by the presence of more than one double bonds in the carbon chain, and mainly derived from vegetable oils and marine products (231). This FA class is further subdivided into two groups according to the position of the final double bond, that is, n-3 and n-6 PUFAs (231). Long-chain PUFAs (LC-PUFAs) derived from a series of desaturations, elongations and β-oxidation of their main precursor, i.e. alpha-linolenic acid (ALA; 18:3 n-3) and linoleic acid (LA; (18:2 n-6) respectively, referred as essential fatty acids. Essentials FAs are FAs that cannot be synthesised from DNL and can only

be obtained from the diet. N-3 PUFAs and n-6 PUFAs compete for the same desaturase enzymes in their respective metabolic pathways. However, they generally exert opposite metabolic effects, with n-3 PUFAs considered to exhibit anti-inflammatory properties, whereas n-6 PUFAs are thought to promote inflammation (225). Elevated n-6 PUFAs and a high ratio of n-6/n-3 have also been associated with the development of inflammatory diseases and obesity (232, 233). Conversely, n-3 PUFAs have been shown to contribute to the improvement of metabolic profile such as an improvement in lipid metabolism in obese humans (234) and bodyweight reduction in mice (235).

Apart from their dietary origin, FAs are synthesized endogenously principally in human liver and AT. Although endogenous synthesis of FAs is minor under normal physiological conditions (such as in lean individuals), it represents a major determinant of circulating lipid profiles in obesity, and especially in individuals with high-carbohydrate diets (236). Glucose is the main substrate in this pathway and is metabolized via glycolysis to produce pyruvate and acetyl-CoA, forming substrates for FA synthesis. FASN is the principal enzyme involved in the synthesis of FAs and its main product is palmitic acid (225). Palmitic acid can be subsequently activated by acyl-CoA synthase and can also subject to desaturation and elongation. In human AT (and liver), the main enzyme responsible for desaturation of FAs is delta-9 desaturase (D9D) or SCD1(225). This enzyme mainly desaturates palmitic (16:0) and stearic acids (18:0) to produce palmitoleic acid (16:1n-7) and oleic acid (18:1n-7), which have been identified as the major constituents of FAs in stored adipose TGs (237). Therefore, the activity of SCD1 is a determinant of MUFAs content in blood and AT (237, 238). SCD1 has been proposed to contribute to the development of obesity and IR. Indeed, pharmacological inhibition of this enzyme may attenuate the course of obesity-related disorders (236-238). Moreover, SCD1-knockout mice exhibited an enhanced muscular uptake of glucose and increased FAs oxidation in the liver, showing that SCD1 deficiency may prevent the development of diabetes and obesity (239, 240).

In addition to SCD1, other desaturases, delta-6 desaturase (D6D) and delta-5 desaturase (D5D), are involved in the metabolism of FA, essentially in the PUFAs synthesis pathways. Together with elongases, D5D and D6D convert the essential dietary FAs ALA and LA into their corresponding LC-PUFA products eicosapentaenoic acid (EPA; 20:5n-3) and arachidonic acid (AA; 20:4n-6) (241). Dysregulation in the activities of these enzymes, specifically an increase of D6D and decrease of D5D are independent predictors of metabolic syndrome (242). Moreover, a higher BMI correlates with lower D5D activity (243) and enhanced activity of

D6D has been associated with dysregulations in PUFAs metabolism in obese individuals (244). Notably, PUFAs released from membrane PHLs can also be metabolized by cyclooxygenases, lipoxygenases and cytochromes, forming oxylipins (including prostaglandins, thromboxane and resolvins) (245), which can be transformed to various peroxidation products, if exposed to elevated oxidative stress (246).

1.4.2. Fatty acid profile in blood and adipose tissue in obesity

FA metabolism in AT is central to the maintenance of lipid homeostasis, particularly in obese individuals that are characterised by excessive AT accumulation. However, if the AT tipping point is reached or exceeded, one of the early malfunctions occurring is the dysregulation of lipid metabolism characterized by elevated lipolysis (basal and/or stimulated), as well as a reduction of lipogenesis, consecutive to impaired adipogenesis (134). The immediate effect of this incapacity to sequester more lipids into adipocytes is the increase of circulating saturated FFAs, the alteration of blood FA profiles, the lipotoxicity and the impairment of whole-body metabolism (247). The composition of FAs in the blood is reflective of both dietary fat intake and their endogenous synthesis/AT release. However, it is difficult to unravel the source of FA composition in the circulation without having information on dietary fat intake. This information can only be obtained if accurate and precise measurements of usual dietary fat intake are available. Dietary fat intake from self-reported data has shown to be highly biased due to the under-reporting of food consumption (especially in overweight individuals), alteration of usual diet during the recording period (influenced by the intervention), interviewer bias, incorrect portion size estimations, coding and computational errors (248). Additionally, the unavailability of exact food match and incomplete food composition in databases (especially regarding the exact values for individual FAs) have been described as limitations of self-reported dietary intake (248). Therefore, there is great interest in using tissue and blood FA composition as biological markers of fat intake to improve dietary assessments and understand the relationships between diet, metabolism and diseases.

FA in plasma (or serum) and erythrocyte or red blood cell (RBC) membranes are used as biomarkers of dietary fat intake. While the FA composition of plasma and serum reflects fat intake over the past weeks, RBC PHLs FA profile reflects the dietary intake over 2-3 months and has therefore been proposed as the preferential indicator of an individual's dietary FA intake (231, 249). These cells can preserve their membrane FA composition with lower diet-induced fluctuations than plasma or serum FA profile (80, 250, 251). Furthermore, the FA profile of AT is considered the gold standard for the representation of dietary FA in the context

of obesity research, due to its slow lipid turnover in weight stable individuals (225, 248), with a half-life (t1/2) of lipids estimated to be 1-2 years (248). Moreover, the total FA composition of AT is typically measured in TG, which constitutes 99% of TG in adult humans (252). The FA composition of SAT depots has been shown to differ between gSAT and aSAT, with higher contents of SFA and lower MUFA in aSAT than gSAT (252, 253). These differences have also been shown between SAT and VAT with higher proportions of MUFA and lower SFA in SAT compared to VAT, while no obvious differences were reported for PUFA between these depots or within SAT depots (253, 254). The correlation between serum FFA profile and FA composition of AT (255) supports the major contribution of AT-FA to circulating FFA pools, consecutive to higher TG hydrolysis. Therefore, alterations of lipid metabolism pathways in obese AT can be reflected by substantial modification in circulating lipid profile and increased risk for oxidative stress, inflammation, diabetes and CVD in obese subjects.

Obesity has been associated with substantial quantitative and qualitative alterations in the serum/plasma FA profile (232, 243). Moreover, greater circulating saturated FFA concentrations result in FA incorporation into membranes PHLs (225). Therefore, not only the FA composition of plasma/serum but also FA profiles of RBC total phospholipids (RBC-TPL) are altered in obesity (225). These alterations are characterized by increased SFA and n-6 PUFA (e.g. 20:3n-6, 20:4n-6, 22:4n-6 and 22:5n-6) contents with lower proportions of MUFA and n-3 PUFAs (225). The FA composition of PHLs membranes determines not only their physical properties but also their biochemical properties (248). For instance, FAs of membrane PHLs are implicated in several fundamental regulatory processes (e.g. gene expression, signal transduction and synthesis of lipid or lipid-derived messengers) (138, 256) and were demonstrated to be involved in the development of IR and metabolic syndrome (225). PHLs are also precursors of intracellular messenger molecules and are very sensitive or susceptible to the detrimental effects of ROS generated in excessive amounts by cells in an obese micro-environment (257). The FA profile of RBC-TPL has also been strongly associated with the liver FA profile and therefore is proposed as a marker of dysfunctional lipid metabolism in the liver of obese patients, due to the restricted accessibility of human liver as a biological sample in research (258). Alterations in circulating and tissue FA profile in obesity could also result from dysregulation of desaturase enzyme activity. For instance, the increased activity of D6D may be responsible for the impairment of LC-PUFAs metabolism in serum, RBC and AT (244). The changes in desaturases activities and their association with the incidence of T2D may derive from genetic variation in the expression of genes regulating these enzymes (259).

1.4.3. Genetic background and ethnicity: Implications in the relationship between fatty acids and metabolic disorders

The influence of genotype and ethnicity on FA composition in AT and blood has not been extensively studied. However, differences in AT FA composition have been shown between black and white men, with lower MUFA (16:1n-7) and higher SFA (18:0) in gSAT and VAT of black African compared to white men (260). These differences were suggested to result from higher elongase activity in black Africans, as opposed to higher desaturase activity in their white counterparts (260). However, ethnic-specific differences in FA composition could also be explained by differences in the dietary intake between ethnic groups (261, 262). For instance, black African women consume higher proportions of n-6 PUFA and lower SFA compared to their white counterparts (32, 263). Genetic factors may also mediate the ethnic differences in FA metabolism. A strong genetic influence on food choices, post-absorptive metabolic processes and FA incorporation in cell membranes, independently of diet have been reported (264). Besides, differences in AA levels and fatty acid desaturase (FADS) gene variants have been found between African Americans and European Americans (265). The genotype G-allele (rs174537) associated with higher circulating levels of AA was higher in African Americans compared to their Europeans counterparts, resulting from an increased D5D enzymatic conversion of dihomo-γ-linolenic acid (DGLA) to AA in individuals of African ancestry (265, 266). Therefore, apart from differences in the pattern of dietary intake, differences in FA metabolism may also be attributed to genetic mechanisms, which may contribute to health disparities between ethnic groups with greater risk observed in black African populations. The regulation of FA metabolism and composition represents a valuable aspect to consider in the development of multidisciplinary strategies of treatment of obesity. Obesity and its associated metabolic complications currently constitute a tangible challenge for future generations, especially with the continuous urbanization-related development in African countries. Therefore, lifestyle modifications (or “re-modification” aiming to “regain” the habits before the emergence of metabolic era associated with urbanisation and civilisation) represent a fundamental aspect in the management of obesity and related disorders.

1.5. MANAGEMENT OF OBESITY AND METABOLIC RISKS

Obesity is now considered a disease entity, with multi-factorial etiology, including the involvement of genetic, epigenetic, environmental, social and behavioural components (267). Most cases of obesity result from the interaction between these factors, rather than due to a single causal factor, and the progression and severity of this condition varies over time and among individuals (268). Consequently, the management of obesity and related comorbidities is extremely challenging among societies. The increasing burden of obesity and the consequences at individual and government level has led to the development of numerous weight-loss therapies ranging from behavioural, pharmacological, and surgical, to lifestyle interventions aiming to prevent and slow down its exponential progression.

1.5.1. Pharmacological, surgical and dietary interventions

1.5.1.1. Pharmacological treatment

Pharmacotherapy is generally indicated for patients with BMI ≥ 30 kg/m^2 and those with a BMI ≥ 27 kg/m^2 with at least one obesity-related comorbidity (e.g. T2D, hypertension, hyperlipidemia), and mostly as an adjunct to behavioural counselling (269). The five medications currently approved by the Federal Drug Administration (FDA) for long-term obesity treatment include orlistat, Lorcaserin (Belviq), Phentermine-topiramate extended-release (Qsymia), Naltrexone-bupropion extended-release (Contrave) and higher dose liraglutide 3.0 mg (Saxenda) (270, 271). However, the benefits of medication may be lost if the treatment is discontinued. Patients responding to the treatment (defined as at least 5% weight loss after 3 months of treatment), should therefore continue the medication, with goals of continuous weight loss and maintenance of lost weight (269). Although many intermediate cardiovascular risk factors are improved by the use of these medications, long-term cardiovascular and neuro-cognitive safety has not yet been established (270, 271).

1.5.1.2. Surgical treatment

Bariatric surgery is indicated for patients with morbid obesity (BMI ≥ 40 kg/m^2) or those with a BMI ≥35 kg/m^2 with at least one obesity-related comorbidity, and who have failed conservative treatment (269, 272). The most common bariatric surgeries are vertical sleeve gastrectomy (VSG) and Roux-en-Y gastric bypass (RYGB)(270). Surgical treatment of obesity seems the most effective long-term weight reduction therapy (271). This method has provided notable data of weight reduction of up to 30% accompanied with the improvement of several parameters of the metabolic syndrome (T2D, hypertension, hyperlipidemia), as well as improvements of long-term mortality (270). However, most of the weight loss after surgery

occurs in the first-year post-surgery, follow by a weight plateau or regain (271). Moreover, bariatric surgery comprises the most adverse safety profile, primarily because of perioperative morbidity and mortality (271). Notably, this method is also limited, especially in low- and middle-income countries by the lack of accessibility in public hospital system, the lack of well experienced medical teams and equipped structures, the high costs related to this procedure as well as lack of long-term follow-up systems. Nevertheless, bariatric surgery and to a lesser extent pharmacological treatment, overall seem to be promising long term intervention strategies by both resulting in weight reduction and improvement of obesity-associated cardio-metabolic risks (270). It is, however, important to consider the cost-effectiveness of these therapies given the global obesity epidemic, and the fact that obesity is a chronic disease requiring long-term management, especially in low-middle income countries characterized by a low socio-economic status. Therefore, lifestyle interventions and counselling, which are also critical during pharmacotherapy and post-surgery remain the basis of treatment of obesity and prevention of metabolic alterations.

1.5.1.3. Dietary intervention

Lifestyle factors such as 'unhealthy' and/or excess food intake and increased sedentary lifestyle and physical inactivity are particularly important factors to consider in weight maintenance. Globalisation has increased the availability of food, including food of high energy but low nutritional value, high in unhealthy fats, sugar and salt, which are usually affordable and easily accessible even in impoverished communities (11). The creation of an energy deficit by addressing caloric intake and energy expenditure is among the more efficient alternatives to manage obesity (273). Dietary interventions such as calorie restriction aim for an energy deficit of 2-4 MJ per day (approximately 500-1000 Kcal) to achieve a steady rate of weight loss of 0.5-1 kg per week (271). This moderate and progressive weight loss has been shown to improve metabolic function in different tissues and contributes to dose-dependent changes in the main AT biological pathways (268). Very-low calories diets (VLCD) are the most intensive dietary intervention in obesity management, with the replacement of usual dietary intake with nutritionally complete commercial products, providing a total of 450-800 Kcal (1855-3297 kJ) per day (271). Although this may result in a weight loss of approximately 10-15% over 3 months, a substantial weight regain is envisaged, particularly in the absence of a maintenance program (272). Several studies have examined the effects of dietary interventions on cardio-metabolic risks (e.g. reviewed in (274-276)) in obese individuals. However, this is out of the scope of this thesis. The hierarchical approach to the treatment of obesity does not only

emphasise the importance of sustained dietary interventions but also importantly address energy expenditure by increasing PA as a central aspect in obesity management.

1.5.2. Physical activity and exercise training in the management of obesity and insulin resistance

Physical inactivity is one of the 10 leading risk factors for mortality worldwide and a major contributor to the rising prevalence of obesity, although other factors such as excess energy intake, genetic predisposition and environmental factors are also involved (1). Physical inactivity and low cardiorespiratory fitness have been suggested to be higher risks factor than obesity itself in the development of NCDs (277). The global prevalence of physical inactivity is about 23% in adults, with women being less active (27%) than men (20%) (1). Among populations of African ancestry, particularly in women, the lack of PA is a leading cause of the rapid increase in obesity rates (278). Therefore, PA is one of the prevailing strategies against the progression of obesity. In SA, approximately 40% of men and more than 50% of women are considered insufficiently physically active based on WHO guidelines (1). However, these guidelines are questionable in developing countries, where most women meet the guidelines but have very poor cardiorespiratory fitness, are obese and at high risk of metabolic syndrome (279). Low cardiorespiratory fitness is a risk factor for all-cause mortality (280).

PA is defined as "any bodily movement produced by skeletal muscles that require energy expenditure" (1). Regular PA is a key determinant of energy expenditure and is fundamental for energy balance and weight control during excess calories supply (278), influencing body composition and substrate oxidation and metabolism (281). Interestingly, the use of PA in the prevention or treatment of obesity is not only adequate for adults but is also beneficial for children. Accordingly, an inverse association between PA levels and fat mass has been reported in SA children (281). PA may, therefore, benefit from early-life which is a critical period for both the prevention and the elimination of cardio-metabolic risk factors (282).

Moderate aerobic PA with at least 30 minutes brisk walking five times a week (≥150 minutes/week) has been shown to achieve an energy deficit and reduce T2D risk (273). Furthermore, higher levels of activity (200-300 minutes/week) are recommended over the long term in order to prevent weight regain (273). Strength and resistance exercise training involving major muscle groups 2–3 times per week may prevent chronic disease, extend healthy lifespan in adults, and improve metabolism by stimulating muscle mass development (270, 283). It is proposed that 1-2 sets of 8 to 12 repetitions per set, with an intensity greater than 80% of 1-repetition maximum (1RM; the maximum load that can be lifted once only throughout a

complete range of motion), with 8 to 10 exercises per session and 2-3 sessions per week, are optimal for maximising the beneficial health effects of increased strength and muscle mass (284, 285).

Exercise training is a principal component of PA and has been shown to exert positive effects on several organs and tissues by the accumulative response from each training bouts (286). Aerobic and resistance exercise training increases cardiorespiratory fitness, and reduce fasting blood glucose, Hb1Ac and increase insulin sensitivity in obese individuals (287-289). Indeed, the exercise-induced improvements in whole-body glucose metabolism and insulin sensitivity have been well established to be exerted via its predominant action on skeletal muscle (290-292). However, long-term exercise training may also induce beneficial changes in metabolic status, such as improvement of systemic glucose homeostasis, via modifications in AT metabolism and function, above and beyond the exercise-induced reduction in fat mass (292, 293). Indeed, it has been extensively shown that exercise training from as little as 8 weeks up to 9 months is effective in improving body composition by decreasing of body weight, WC, BMI and body fat mass in obese women of different ethnicities (283, 294-299). Besides, reductions in abdominal and visceral fat percentages were observed in obese women after 12 weeks of moderate-intensity combined aerobic and resistance training, and these changes were greater than those observed in response to resistance or aerobic training alone (295, 299). Although aerobic and resistance exercises interact to have a greater effect than either type alone, the mechanisms involved are unclear and further investigations are necessary. Nevertheless, executed alone or in combination, these types of exercise training result in increased maximum oxygen uptake (VO_{2max}) (299, 300). Improved cardiorespiratory fitness has been associated with decreased risk of disease and death (299). Furthermore, obese individuals with higher levels of cardiorespiratory fitness have reduced mortality rates compared to their sedentary normal-weight counterparts (300). Interventions targeting the improvement of cardiorespiratory fitness are therefore of high relevance, especially in the context of black SA women in which low cardiorespiratory fitness has been identified as a significant determinant of IR (301).

Exercise training-related reductions in SAT mass have also been shown to be higher in combined (aerobic and resistance) exercise training than in aerobic training alone in obese women of different ethnicities (287-289, 302). Although exercise-induced weight loss is relatively lower than the other methods of weight reduction, as little as 3% sustained bodyweight loss may result in improvements in glycemic control, lipid profile, blood pressure, IR and risk of T2D (267, 273). This highlights the role of AT changes in the improvement of

metabolic health in obese individuals. Indeed, exercise training modulates lipids metabolism by stimulating AT lipolysis and decreasing lipogenesis. This results in FFA release into the circulation and their oxidation in muscle or utilisation by other tissues (303-305). The increase in AT lipolysis has been shown in humans and animal models by increasing ATGL, as well as HSL and MGL activities (303, 304). Exercise-induced AT lipolysis has been proposed to be regulated by epinephrine, signalling PKA activation via cAMP, followed by the phosphorylation of PLIN1 and activation of ATGL to initiate lipolysis (306). Moreover, upon PLIN1phosphorylation HSL translocates to the lipid droplet and degrades DAG to MAG with the subsequent production of FA and glycerol by MGL (306). Therefore, exercise training may stimulate lipolytic activity within AT, contributing to the more efficient reduction of AT mass and/or prevent accumulation thereof. In contrast, prolonged exercise training may reduce lipogenesis by decreasing the activity of LPL and FA uptake (305). The changes in adipocyte metabolism in response to exercise training are therefore associated with modifications of FA metabolism and composition in AT (305). Indeed, the reduction of FA uptake results in the improvement of mitochondrial function, upregulation of enzymes involved in the metabolism of PUFA such as FADS1 and elongase 5 (138, 305, 307), and may consequently alter circulating FA composition (138, 225, 308). Moreover, exercise training has been shown to decrease AT synthesis of MUFA (especially 18:1) by decreasing FA desaturase SCD1 activity (138). Although these findings demonstrated that exercise training induces changes in the expression of enzymes involved in lipid metabolism, data on the exercise-induced changes in the activity of other lipogenic enzymes are inconclusive (305). Additionally, studies investigating the effects of exercise training on FA composition in human AT and circulating FA profiles are limited, especially in African populations.

Another notable effect of exercise training on AT mass is the reduction of adipocytes size (298). Hypertrophy of adipocytes is known to influence adipokines expression in the obese state (309). Therefore, the effect of exercise training on dysregulated expression of adipokines and AT inflammation has been suggested to largely depend on a decrease in adipocyte size (298). The resultant decreased expression of pro-inflammatory cytokines from the AT contributes to the attenuation of systemic inflammation (310). Indeed, exercise training (aerobic, resistance and combined types) has been extensively shown to mitigate inflammation in human and animal models, with or without a significant change in body composition (reviewed in (298)). For instance, exercise training has been shown to reduce circulating levels of CRP, leptin, TNFα, IL-6, IFN-γ (311-314); and increase adiponectin levels in circulation and in AT of obese humans (reviewed in (298)). The upregulation of adiponectin expression in AT was associated

with downregulation of TNF-α gene expression (298, 315). Recent studies in rats also showed decreased expression and circulating concentrations of adipocytokines such as apelin and resistin in response to exercise training, resulting in improvements in IR (305). Altogether, these findings suggest that long-term exercise training may improve the profile of adipokines released from the AT, systemic inflammation and thus, may be beneficial for health. However, data on the improvement of the pro-inflammatory state in AT remain conflicting (305, 315). For example, a study in obese women showed no effect of 12 weeks of exercise training on SAT adiponectin expression (316), and the expression of IL-6 in the AT did not change in another study after 3 months of dynamic strength training in obese men (317). These disparities could be due to the differences in experimental design, participants, exercise intensity, or exercise duration (298). Therefore, the effects of exercise training on AT function may vary depending on the participants, highlighting the importance of selecting the adequate exercise type/intensity for each population. Studies of the effects of exercise training on obese individuals in African regions are rare and more investigations in this population are needed.

Exercise training has also been reported to improve oxidative stress in obese individuals, characterised by the reduction of urinary markers of oxidative stress (e.g. 8-hydroxy-2-deoxyguanosine and 8-isoprostanes) (318) and the reduction of circulating ROS (294). Besides, the reduction of oxidative stress in AT has been described as one of the unique effects of exercise training in addition to fat mass reduction (315). Given that oxidative stress is closely related to the dysregulation of adipocytokines expression in AT, reducing AT oxidative stress may be essential in the prevention of obesity and related metabolic conditions. In animal models, a decrease in lipid peroxidation in AT was shown concomitantly with a reduction of TNFα and MPC1 after exercise training (315, 319). Also, the phosphorylation of ERK, which is activated by oxidative stress and is involved in the expression of MCP1, is diminished after exercise training (319). The improvement of AT oxidative stress has been proposed to be exerted by the antioxidative effect of long-term exercise training (315). Indeed, SOD and catalase (content, expression and activity) increased in AT of mice and rats after exercise training, at the expense of NADPH oxidase and MCP1 (315, 319). Notably, this evidence derives from animal studies, and further research to confirm these changes in obese humans is warranted. Yet, the reduction of AT oxidative stress is a proposed pathway in the exercise-incused improvement of inflammation (319). Altogether, these different effects of exercise training on AT function might contribute to the reported exercise-induced improvement of whole-body insulin sensitivity (320).

It is important to note that exercise-induced metabolic effects are dependent upon many factors including the model or type of exercise (e.g. cycling, jogging, swimming), the intensity (percentage of VO_{2max}), and the duration (total time exercising at a given percentage of VO_{2max}) of the exercise training program (171). The characteristics of the individuals involved (e.g. fitness or training levels, gender, and clinical disease status) constitute another major determinant of the response to exercise training (171). The effect of exercise training therefore differs between ethnicities (278). In addition to higher disease risk, African women have lower rates of exercise training and structured leisure time PA compared to women from European ancestry (278, 321). Moreover, resting energy expenditure and fat oxidation and VO_{2max} are lower in women of African ancestry than European women (278). These differences may partly explain the higher risk of metabolic diseases in the former, as aerobic fitness is inversely associated with AT accumulation and body fatness (322, 323).

The South African National Health and Nutrition Examination Survey (SANHANES) data from 2013 show that 66% of males and only 38% of females (18 to 24 years old) were considered fit (11). SA women have a very poor cardiorespiratory fitness (301) and high prevalence of obesity and metabolic syndrome despite meeting the PA guidelines from the WHO (279). Indeed, meeting these guidelines is not associated with reduced obesity or body fat, or improved cardio-metabolic outcomes in these women (279). This could be explained by the low intensity of PA in black SA women, who mostly participate in light-intensity PA (mainly by walking for transport), with almost no vigorous/high-intensity PA (301, 324). Low cardiorespiratory fitness has been associated with greater adiposity (325), higher VAT and with lower insulin sensitivity, independent of fat mass and PA in a cohort of young black SA women (301). Another study showed that increase cardiorespiratory fitness over 1 year was associated with reduced levels of fasting plasma glucose and fasting serum insulin at follow-up (326). Low cardiorespiratory fitness levels strongly predict the incidence cardio-metabolic syndrome, independent of BMI (327).

Moreover, the rate of sedentary behaviour is very high in young black SA women with up to 8-9h of sedentary time per day (>50% waking hours) (324). Further, sedentary behaviour inversely correlated with cardiorespiratory fitness (301). Indeed, independent of PA, the greater the amount of time spent in sedentary behaviours, the higher the BMI and greater the risk of cardio-metabolic disease (328, 329). High levels of sedentary behaviour and low fitness may therefore result in excess adiposity and increased BMI, which in turn further increases sedentary time (324). This high sedentary time and low amounts of vigorous-intensity activity, in

combination with a high prevalence of overweight and obesity; and the relationships with fitness levels have been proposed as the principal factors responsible for the high risk of cardio-metabolic diseases in young black SA women (324). Therefore, it is essential to promote increased moderate-to-high intensity PA and exercise training programs among this population, to reduce obesity-associated metabolic risk. Such interventions may allow improvements in cardiorespiratory fitness, and therefore have positive health benefits by maintaining favourable adiposity levels and reducing VAT (279). However, few to no studies have investigated the effects of a structured exercise training program on obesity-associated metabolic risks in women from Southern Africa. The implementation of these programs is necessary and essential to reduce this high metabolic risk among SA women.

1.6. SUMMARY AND RATIONALE FOR THE THESIS

Modernisation and urbanisation have led to environmental and lifestyle changes, contributing to increasing obesity prevalence within SA over the past 20 years, particularly in populations of African ancestry, with higher prevalence among women compared to men (1, 4). The high and rising prevalence of obesity in black SA women is associated with increased risks for T2D and other NCDs (1, 4, 9). Black SA women present with greater IR than their white counterparts for the same level of adiposity, underlying ethnic-specific associations between obesity and cardio-metabolic risks in populations living in the same country, but compounded with a high difference in SES (24, 27). Furthermore, body fat distribution is more important than total body adiposity in the development of obesity-associated complications. In this regards, central fat accumulation (VAT and aSAT) is associated with increased risk of metabolic diseases, whereas peripheral fat, especially distributed in gSAT is beneficial, exerting a protective role against the development of obesity-associated disorders (28, 29, 35, 36). Strikingly, black and white SA women also differ in body fat distribution patterns, which could explain their distinct cardio-metabolic risks. Unexpectedly, black SA women are at higher risk for metabolic diseases although they have less VAT and more gSAT than white SA women. Adipose depot-specific associations with IR and T2D risks therefore vary with ethnicity. In obese black SA women, specific changes in SAT function during progressive fat accumulation are proposed to explain the positive associations between this fat depot and impairment of insulin sensitivity (36, 37). However, the specific pathways driving these associations between SAT function and increased metabolic risk in black SA women are not know and need to be investigated. This will enable early identification of the risks and progression of these metabolic conditions in young women of African ancestry.

The increased body fat mass characterizing obese state negatively affects whole-body metabolism. These metabolic effects of fat accumulation vary with AT location independently of the size of the depot and are exerted partly via increased FFA release and the production of adipocytokines (132, 134). According to AT expandability hypothesis, the pathological expansion of AT is characterised by adipocyte hypertrophy, resulting from impairment of adipogenesis, followed by the reduction/insufficient angiogenesis to compensate for the AT expansion, hypoxia, macrophage infiltration and activation, and inflammation (134). Moreover, adipogenic and lipogenic capacities are further down-regulated by mechanisms mediated by higher levels of inflammation (148). This originates a vicious circle between inflammation and lipolysis, ultimately leading to ectopic fat deposition, systemic low-grade inflammation and peripheral IR (134, 139, 155, 157-159). A small study in SA has shown decreased adipogenic genes and higher expression of genes involved in hypoxia and inflammation in gSAT of black SA women (213, 214), suggesting that SAT function might be impaired in these women. Higher hypoxia may equally increase the levels of oxidative stress (36, 134). Indeed, not only high rates of lipolysis but also oxidative stress in obese AT is associated with the increased release and circulating concentrations of FFAs, contributing to impairment of insulin sensitivity in Africans (183). However, the association between oxidative stress in AT and IR has not been extensively studied in black SA women. FFA concentrations are also dependent on dietary fat intake which differs between ethnicities, with high intake of SFA and n-6 PUFAs in black women (32, 263). Concomitantly, high conversion rates of DGLA to AA have been shown in African, proposed to be mediated by genetic factors (265, 266). Therefore, FA metabolism and composition in AT and in the circulation is another potential pathway linking obesity to IR and T2D in black women.

Obesity and its associated metabolic diseases represent a significant economic burden for individuals and government due to its related comorbidities and costs (1). Lifestyle interventions, particularly an increase in PA remains the cornerstone amongst the management strategies for obesity and its associated cardio-metabolic risks (278, 281). Exercise training as a structured intervention method is therefore advantageous, especially in low- and middle-income countries and low socio-economic communities, as it requires less economic resources (than other methods such as pharmacotherapy or bariatric surgery) and can be used in younger groups (children and adolescents). Moreover, this type of intervention may improve health-related quality of life by improving physical fitness (330), which is associated with all-cause mortality worldwide (277, 280). Exercise training has been demonstrated to reduce IR and T2D risk (331-333) and prevent the progression from impaired glucose tolerance to T2D (334).

These beneficial effects of exercise training may be exerted via changes in the metabolism of AT (292, 293). Sustained exercise training is currently proposed as one of the most effective methods to improve body composition and correct obesity-associated IR by increasing energy expenditure (270). Moreover, an appropriate combination of aerobic and resistance exercise has been shown as most effective for obese women with a high risk of disease (283). However, given the low PA levels, poor cardiorespiratory fitness and high rates of overweight and obesity in black SA women, intervention should target to increase the intensity of PA (from light to moderate-high intensity) and reduce sedentary behaviour (324). In addition to increasing the level and intensities of PA in black SA women, structured exercise training interventions should most importantly focus on increasing cardiorespiratory fitness, which has been shown to reduce the obesity-associated risk for IR and T2D (301). Intriguingly, the effects of such interventions on the cardio-metabolic status of black SA women have not been investigated. Specifically, no studies have examined the effects of exercise training on SAT FA metabolism, oxidative stress and inflammation and consequently on whole-body insulin sensitivity in black SA women who have low cardiorespiratory fitness, high levels of obesity and a very distinctive phenotype.

1.7. AIMS AND OBJECTIVES

The overall aim of this thesis was to: i) investigate the relationship between depot-specific SAT lipid metabolism and the circulating FA profile and body composition and insulin sensitivity in sedentary obese black SA women; ii) examine the depot-specific changes in SAT metabolism and function in response to 12 weeks of supervised combined aerobic and resistance training; and iii) explore the relationship between these changes and improvements in metabolic status. This aim was achieved by completing four studies presented as separate chapters and described below:

Chapter 3: *Circulating and subcutaneous adipose tissue fatty acid composition in obese black South African women: relationship with body composition and insulin sensitivity*

Aim: Evaluate the circulating and SAT FA composition in obese black SA women and explore the relationships with body composition and insulin sensitivity.

Objectives:

1. Compare the FA composition of RBC-TPL, aSAT and gSAT,

2. Examine the relationship between the FA composition of each depot and VAT/SAT ratio (as a measure of centralisation of body fat) and insulin sensitivity in these women.

Hypothesis: FA profile of RBC membrane may reflect the FA metabolism in SAT depots and the relationship between FAs and central fat distribution and insulin sensitivity does not variate according to their storage site and/or the blood compartment or fraction where they occur.

Chapter 4: *Exercise training alters red blood cell fatty acid desaturase activity and adipose tissue fatty acid composition in obese black South African women*

Aim: Evaluate the effect of 12-week combined resistance and aerobic exercise training without dietary modification on RBC-TPL and SAT FA composition and desaturase activity in obese and previously sedentary black SA women, and explore the relationships with changes in insulin sensitivity.

Objectives:

1. Evaluate the changes in cardiorespiratory fitness, body composition and insulin sensitivity in response to exercise training.

2. Evaluate the changes in RBC-TPL, aSAT and gSAT FA composition and desaturase activities in response to exercise training.

3. Assess the changes in the expression of genes involved in adipogenesis, lipid metabolism and insulin signalling in aSAT and gSAT after the 12-week exercise training.

4. Examine the association between these changes in RBC-TPL and SAT depots and changes in systemic inflammatory markers, liver fat and insulin sensitivity.

Hypothesis: Twelve-weeks of supervised exercise training will alter the FA profile of RBC-TPL, and SAT FA and lipid metabolism and these changes will contribute to the improvement in insulin sensitivity in obese black SA women.

Chapter 5: *Changes in systemic and subcutaneous adipose tissue inflammation and oxidative stress in response to exercise training in obese black South African women*

Aim: Assess the effects of 12-week exercise training on systemic and SAT depot-specific inflammatory and oxidative stress status, and explore the relationship with the exercise-induced improvement of insulin sensitivity in obese black SA women.

Objectives:

1. Compare baseline's inflammatory and oxidative stress profiles between aSAT and gSAT.

2. Examine the changes in systemic oxidative stress markers in response to the 12-week intervention.

3. Evaluate the effects of exercise training on SAT depot-specific gene (inflammatory and oxidative stress markers) and protein expression (inflammatory markers).

4. Study the associations between the changes in systemic and SAT depot-specific inflammatory and oxidative stress status and changes in body composition and insulin sensitivity.

Hypothesis: Abdominal SAT will have higher inflammatory and oxidative stress profiles than gSAT and exercise training will induce depot-specific changes in the expression of genes and proteins involved in these pathways. Moreover, exercise training will reduce systemic oxidative stress in obese black SA women, and these systemic and depot-specific changes in inflammation and oxidative stress will contribute to the improvement in insulin sensitivity in these women.

Chapter 6: *Distinct abdominal and gluteal adipose tissue transcriptome signatures are altered by exercise training in obese black South African women*

Aim: Evaluate the regional differences in gene expression profiles between aSAT and gSAT in obese black SA women at baseline and after the exercise training intervention.

Objectives:

1. Compare whole-genome profiles between aSAT and gSAT pre- and post-exercise training.

2. Evaluate the changes in transcriptomic profiles within each depot (aSAT vs gSAT) in response to the 12-week exercise training program.

3. Identify the biological pathways represented by the differentially expressed genes between and within SAT depots.

Hypothesis: SAT depots have different developmental origins, and therefore possess distinct transcriptome signatures, driving their distinctive functional responses to external stimuli such as exercise training.

CHAPTER TWO

METHODS AND TECHNIQUES

2.1. Ethical Approval

Approval was obtained from the Human Research Ethics Committee of the Faculty of Health Sciences at the University of Cape Town (HREC REF: 827/2016) and registered to the Pan African Clinical Trial Registry (trial registration: PACTR201711002789113). The study was performed following the principles of the Declaration of Helsinki (1964, amended last in Fortaleza Brazil, 2013), ICH Good Clinical Practice (GCP), and the laws of South Africa. All participants provided verbal and written informed consent before their involvement in any aspect of the study.

2.2. Participants and Study design

Participants were recruited from universities, churches and community groups in Cape Town, SA. Participants were included in the study if they were i) of *isiXhosa* ancestry (both parents); ii) weight stable (i.e. <5% change from maximum weight for the last 6 months); iii) sedentary (i.e. not participating in exercise training (>1 session of >20 min per week) within the last 12 months); iv) not having known metabolic or inflammatory diseases (e.g. HIV, tuberculosis, active hepatitis, or rheumatoid arthritis); v) not taking any medications; vi) non-smoker; vii) not pregnant or lactating; viii) on injectable contraception (depot medroxyprogesterone acetate, 400 mg); ix) no orthopedic or medical problems that may prevent the participation in exercise training program; x) no surgical procedures within the last 6 months.

Before their inclusion in the study, the volunteers completed screening which included measurements of weight and height for the calculation of BMI, blood pressure measurements 3 times at 1-min intervals using an automated blood pressure monitor (Omron 711, Omron Health Care, Hamburg, Germany). In addition, the screening procedure included the determination of glucose, hemoglobin, HbA1c as well as HIV screening from venous blood samples. Counselling was provided pre- and post-HIV test by a trained counsellor and a referral was made to appropriate HIV clinics for participants diagnosed HIV-positive. Moreover, participants completed a physical activity readiness questionnaire (PARQ) and a questionnaire about their PA habits, contraception method, ancestry origin, smoking status and history, current medication and clinical condition.

Forty-five obese black SA women (based on the Xhosa ancestry of both parents) aged between 20-35 years, with a BMI of 30-40 kg/m^2 were randomized into control (n=22) and experimental (exercise n=23) groups. Ten participants did not complete the intervention

(dropout: n=7 in control and n=3 in exercise group) due to time commitments or loss to follow-up. Consequently, the final number of participants was 15 in control and 20 in the exercise group (**Figure 2.1**). Specific numbers are given in each chapter, were different to the number indicated in **Figure 2.1**. The detailed design of the study and the participants' baseline characteristics have been previously published (335).

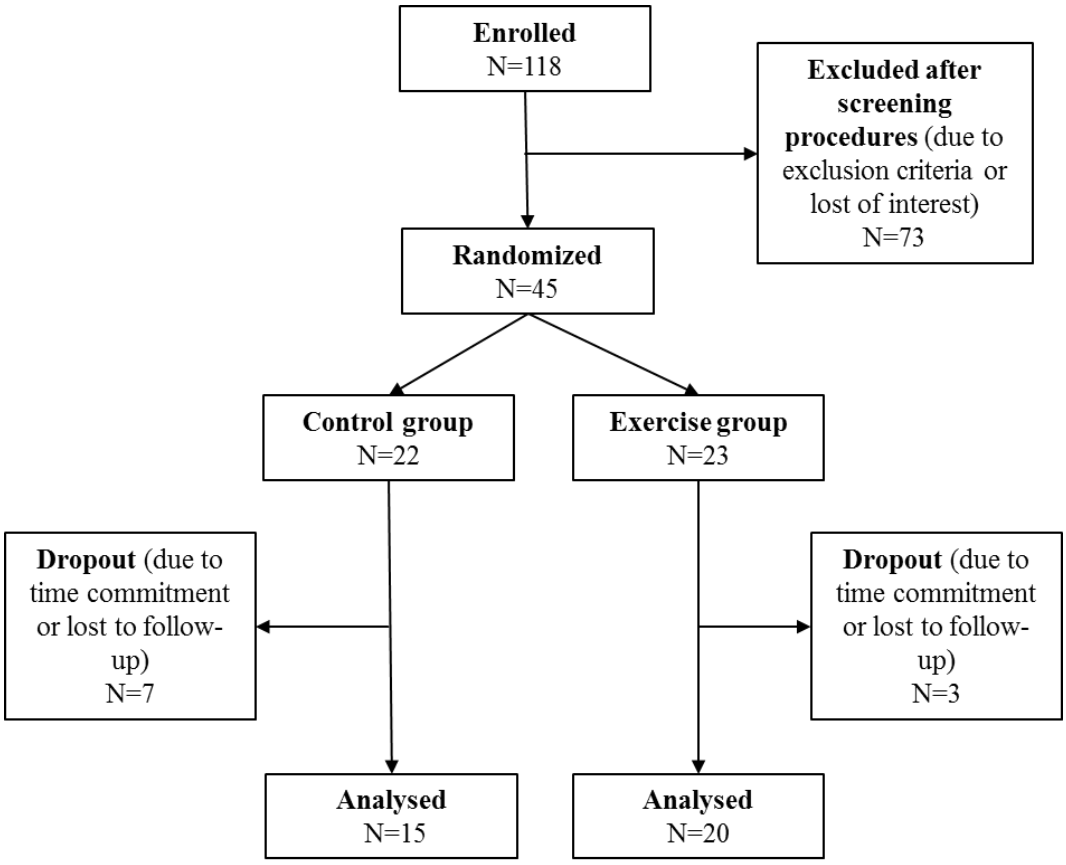


Figure 2.1. Diagram representing participants' enrolment, randomization and analysis. As reported in (335).

2.3. Study Intervention

The exercise training intervention consisted of 12 weeks of supervised combined aerobic and resistance training progressing from 40 to 60 min, 4 days per week by a trained facilitator. This training type was based on previous studies showing that combined aerobic and resistance training affected AT mass, glucose metabolism, insulin sensitivity and cardiorespiratory capacity to a greater extent than aerobic training alone (287, 288, 301). The frequency, duration and intensity of the exercise intervention were based on the recommendations from the British

Association of Sport and Exercise Sciences (BASES) (336). BASES recommends that 'those with increased risk for T2D may benefit in particular from going beyond the levels of activity recommended for "all healthy adults", to around 300 min or more of moderate-intensity aerobic activity per week, or around 150 min or more of vigorous-intensity aerobic activity per week, or equivalent combinations of moderate- and vigorous-intensity activity' (336). Using accelerometry, it was previously shown that black women from the same community perform on average 31 min of moderate-intensity exercise per day, but do not perform a vigorous-intensity activity (301). Most of the activity was performed as walking for travel with very little leisure time activity, and the women accumulated on average 10,459 steps per day (301). Therefore the exercise intervention aimed to increase moderate and vigorous-intensity exercise to achieve improvements in cardiorespiratory fitness.

Aerobic exercises included dance, running, skipping, and stepping that were performed at a moderate-vigorous intensity (75%-80% peak heart rate, HR_{peak}). Resistance exercises consisted of participants using their body weight and progressed to the use of equipment (e.g. bands and light free weights). These exercises mainly included squats, lunges, bicep curls, push-ups and shoulder press with a prescribed intensity of 60% to 70% HR_{peak}. Attendance was recorded at each training session, and a heart rate monitor (Polar A300, Kempele, Finland) was worn by participants to ensure the prescribed exercise intensity was maintained throughout the 12 weeks.

The control group was instructed to maintain their normal daily physical activity patterns, and not start any exercise training program which was verified through monthly monitoring using accelerometry (ActivPAL3c, PAL Technologies Ltd, Glasgow, UK). Accelerometry provides an accurate assessment of the intensity of activity. Both groups were instructed to maintain their usual dietary intake, which was evaluated every four weeks by a food frequency questionnaire, as previously described (337), to monitor any changes in dietary patterns. Before and after the 12-week intervention, anthropometry, body composition, liver fat content and cardiorespiratory fitness were measured. Blood and SAT samples were collected for the evaluation of insulin sensitivity and the analyses of FA composition, inflammatory and oxidative stress markers as well as gene and protein expression, as described below.

2.4. Pre and post-intervention testing procedures

2.4.1. Body composition, body fat distribution and liver fat content

Basic measures of anthropometry included weight (in light-weight clothing without shoes), height, waist circumference (WC; at the level of umbilicus) and hip circumference (HC;

largest gluteal area). The whole-body composition was measured by dual-energy x-ray absorptiometry (DXA; Discovery-W, software version 12.7.3.7; Hologic, Bedford, MA) according to standard procedures. Regional body fat distribution (gynoid and android fat mass, VAT and SAT) was characterized as previously described (338, 339). The DXA measure of VAT has been shown to perform as well as a clinical read of VAT from a computerized tomography scan (340).

After a standardized meal (Energy: 2553 kJ), magnetic resonance imaging (MRI) was used to determine VAT and SAT volumes and liver fat content using a 3 Tesla whole-body human MRI scanner (MAGNETOM Skyra, Siemens Medical Solutions, Erlangen, Germany). A MATLAB algorithm separated the water and fat signals to create a fat fraction map calculated as the fat signal over the sum of the water and fat signals. A region of interest (ROI) was drawn on 7 consecutive slices in the right lobe of the liver using the following software OsiriX software (Pixmeo SARL, Geneva, Switzerland). Methods were adapted from (341).

2.4.2. Cardiorespiratory Fitness

Cardiorespiratory fitness was determined by measuring peak oxygen consumption (VO_{2peak}) using a walking treadmill-based (C, Quasar LE500CE, HP Cosmos, Nussdorf-Traunstein, Germany) graded exercise test with an increasing gradient (2% every minute until 16%), followed by alternate increased speed (0.5 km/h) and gradient (1%) until volitional exhaustion. Walking was chosen because the participants of the study were not accustomed to cycling or treadmill running exercise. Pulmonary gas exchange was measured to calculate VO_{2peak} using a metabolic gas analysis system (CPET, Cosmed, Rome Italy).

2.4.3. Fasting blood samples and frequently sampled intravenous glucose tolerance

Before and following the 12-week intervention, blood samples were collected after an overnight fast (10-12 h) and at least 72 h after the last exercise training session for subsequent biochemical analysis. Participants stayed overnight at the laboratory and were given a standardized evening meal at 8 PM and then fasted overnight. The standardized meal contained 2,456 kJ energy (E), 21 g protein (14% E), 49 g carbohydrate (33% E), and 32 g fat (48% E). At 7 AM, fasting blood samples were collected for the determination of adipocytokines, inflammatory and oxidative stress markers, and RBC-TPL FA composition.

After fasting blood collection, participants underwent a frequently sampled intravenous glucose tolerance test (FSIGT) to measure insulin sensitivity (S_I) calculated using Bergman's minimal model of glucose kinetics (335, 342). Baseline blood samples were collected (at -5 and

-1 min) prior to intravenous administration of glucose (50% dextrose; 11.4 g/m^2 body surface area) over 60 seconds. After 20 min, human insulin (0.02 U/kg; NovoRapid, Novo Nordisk) was infused over 5 min (HK400 Hawkmed Syringe Pump, Shenzhen Hawk Medical Instrument Co., Shenzhen, China) and samples were subsequently collected for the determination of plasma glucose and serum insulin concentrations (serial blood collection 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, and 240 min) (335). Fasting IR was estimated using the homeostasis model assessment of IR (HOMA-IR) (343).

Although the gold standard measurement of insulin sensitivity is the euglycemic hyperinsulinemic clamp, insulin secretion cannot be directly evaluated from this technique (344). The FSIGT method was chosen for this study as it provides a measure of both insulin sensitivity and secretion. Black SA women hyper-secrete insulin and it is not clear whether this is a cause or a consequence of insulin resistance. FSIGT allows the measurement of glucose, insulin and more often also C-peptide during the highly dynamic phase that immediately follows the glucose injection. Therefore, this technique quantifies the ability of insulin to enhance glucose uptake by insulin-dependent tissues and to inhibit liver glucose production (344).

2.4.4. Diet intake monitoring

Participants completed a self-reported 7-day food frequency questionnaire (FFQ) before and after the exercise training program for the estimation of their dietary intake. No dietary recommendations were given to the participants and they were instructed to maintain their habitual dietary intake. Energy and macronutrient intake were analyzed as previously described (337).

2.4.5. Biochemical analyses

Plasma glucose concentrations were determined using a colorimetric assay (Randox, Gauteng, South Africa) and serum insulin and high-sensitivity C-reactive protein (CRP) were measured using immunochemiluminometric assays (IMMULITE 1000 immunoassay system, Siemens Healthcare, Midrand, South Africa). Inflammatory cytokines (IL-6, IL1-Rα, IL-8, IL-10, IL-15, MCP-1, IFNγ, TNFα were measured using Milliplex MAP MAG Human Cytokine kit (Merck, Johannesburg, South Africa) and xMAP technology (Luminex, Austin, Texas) according to the manufacturer's instruction. The serum concentration of IL-1Rα, IL-6, IL-10 and IL-15 were below the detectable range and were excluded from the analysis. This resulted

from purely technical reason due to the low sensitivity of the Milliplex kit available from the manufacturer at the time of the study. Serum concentrations of leptin and high molecular weight (HMW) adiponectin were analysed using commercially available ELISA kits according to the manufacturer's instructions (EMD Millipore Corporation, St Charles, Missouri, USA). Systemic oxidative stress was evaluated in the serum by measuring concentrations of TBARS, the total antioxidant capacity of serum samples (oxygen radical capacity absorbance; ORAC), SOD and CAT activities via enzymatic assays (345) using a microplate data acquisition program (Synergy HT, Gen5 2.01; Biotek Instruments, Inc; Vermont, USA) (see detailed protocol in appendix).

2.4.6. Adipose Tissue Gene and Protein Expressions

Adipose Tissue collection

Participants underwent fat biopsies for SAT sample collection after 4-6 h of fasting and at least 72 h after the last exercise training session for the subsequent determination of fatty acid composition, gene and protein expression.

Abdominal samples were collected from the area around the umbilicus and gluteal samples were obtained from the right upper outer quadrant by mini-liposuction (213). After local anaesthesia with Lignocaine hydrochloride (2%, Intramed, Port Elizabeth, South Africa), 200 mL of normal saline with 20 mL 2% Lignocaine was infused using an infiltration cannula (Lamis 14 ga x 15 cm, Byron Medical Inc., Tucson, AZ, USA). An aspiration cannula (Coleman, 12 ga x 15 cm, Byron Medical Inc.) attached to a 10 mL syringe was used to aspirate fat. Approximately 2 - 3 cm^3 of fat was extracted from each site, washed with normal saline until no blood was visible, immediately frozen in liquid nitrogen (N_2) and stored at −80°C until further analyses.

Determination of RBC-TPL and adipose tissue fatty acid composition

RBC-TPL FA composition in gluteal and abdominal SAT samples was evaluated using pairwise analyses that ensured samples of a participant were analyzed in the same batch on the same day. Aliquots (300 µL) of saline (0.9% NaCl in distilled water) washed RBCs and 100 mg portions of saline washed adipose tissue (335) were subjected to total lipid extraction with chloroform (amylene stabilised):methanol (2:1; vol:vol; containing 0.01% butylated hydroxytoluene) by using a modification of the method of Folch et al (346, 347). Thin-layer chromatography (TLC) was applied to isolate the TPL fraction in the RBC total lipid extract (347). The TLC-isolated RBC-TPL fractions and a small aliquot of the AT total lipid extracts

were *trans*-methylated using methanol:sulphuric acid (95:5; vol:vol) at 70 °C for 2 h to yield fatty acid methyl esters (FAMEs) which, after cooling, were extracted with water and *n*-hexane. The organic layer containing the FAMEs was evaporated, redissolved in a small volume of *n*-hexane and analysed by gas-liquid chromatography on a Finnigan Focus Gas Chromatograph equipped with flame ionisation detector (Thermo Electron Corporation, Austin, TX, USA) and a 30m capillary column of 0.32 mm internal diameter; BPX70 0.25 µm (SGE International Pty Ltd, Victoria, Australia) as described (347). The sample FAMEs were identified by comparison of the retention times with those of a standard FAME mixture (27 FAMEs; Nu-Chek Prep Inc., MN, USA). The relative percentage of a FAME was calculated by taking the area under the curve (AUC) of a given FAME as a percentage of the total area count of all the FAMEs identified in the sample (%, wt:wt). The mixed standard contained saturated (12:0, 14:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0) mono-unsaturated (16:1n-7, 18:1n-7, 18:1n-9, 20:1n-9, 22:1n-9, 24:1n-9) and poly-unsaturated (18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:2n-6, 22:4n-6, 22:5n-6, 18:3n-3, 20:3n-3, 20:5n-3, 22:5n-3, 22:6n-3) FAMEs. Product-to-precursor FA ratios of the samples were used as a proxy to reflect enzyme activity: 18:3n-6/18:2n-6 (GLA/LA) and 20:4n-6/20:3n-6 (AA/DGLA) ratios were used to estimate D6D and D5D activities, respectively (348, 349). SCD1 activity was estimated by the ratios of 16:1n-7/16:0 (for SCD1-16) and 18:1n-9/18:0 (for SCD1-18) (350).

RNA extraction and Real-Time PCR

Total RNA was extracted from SAT samples using RNeasy Mini lipid kit (Qiagen Ltd, Germantown, MD, USA). The yield and purity were determined spectrophotometrically using a microplate data acquisition program (Synergy HT, Gen5 2.01; Biotek Instruments, Inc; Vermont, USA) and the RNA integrity was checked using 1% agarose gel electrophoresis. The extracted RNA (1.2 µg) was reverse transcribed to cDNA using High-Capacity cDNA Reverse Transcription Kit with RNase inhibitors (Applied Biosystems Foster City, CA, USA). Real-Time PCR (RT-PCR) was performed in triplicate for each sample using Applied Biosystems QuantStudioTM^3 Real-Time PCR system with predesigned Taqman assays from Applied Biosystems (Warrington, UK). A standard curve was constructed for each primer-probe set using a serial dilution of cDNA pooled from all samples. The evaluated genes were selected based on the physiological pathways involved in adipogenesis (*PPARγ*), lipid metabolism (*LPL, DGAT2, PLIN1, ATGL*), insulin signalling (*IRS1, GLUT4*, serine/threonine-protein kinase; *SMG1*), inflammation (*adiponectin, leptin, MIF, MCP1, IL-10, TLR4, NFκB1* and *TNFα*) and oxidative stress (nitric oxide synthase 3 (*NOS3*), *CAT, SOD1*). The amplification plots showing

the level of expression of each gene (with cycle threshold (Ct) values) are presented in the appendix section. These genes were specifically chosen because of their extensive documented association with alteration of AT function and IR during obesity and were therefore quantified in both aSAT and gSAT (TaqMan gene IDs in **Supplementary Table 2.1**).

Endogenous ("housekeeping") genes were evaluated by comparing ribosomal protein lateral stalk subunit PO (*RPLPo*) and low-density lipoprotein receptor-related protein 10 (*LRP10*) against the full study cohort using the NormFinder algorithm (v0.953, Denmark). *RPLPo* was identified as the most stable gene and its mRNA level did not differ within and between aSAT and gSAT, and pre- vs post-training. The expression levels of the target genes were therefore normalized to *RPLPo* as the endogenous control and presented as the ratio of abundance of the gene of interest: abundance of *RPLPo*.

Protein extraction and western blot analysis

The evaluation of protein expression was determined in both SAT depots on a sub-sample of participants (n=10). Within the exercise group, five participants with the greatest responsiveness to the exercise training, based on a slightly decreased BMI, and increased VO_{2peak} and S_I were selected. In contrast, five participants with slightly increased BMI over the intervention period were selected in the control group. SAT samples (100 mg) were homogenized in 200 µl RIPA buffer with protease inhibitor cocktail 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (Roche Diagnostic GmbH, Germany) and centrifuged at 9500 g, 4°C for 15 min. The protein concentration was determined in the supernatant using a Bradford protein assay kit. The lysate (20 µg of total protein per lane) was separated using precast gels (4-15%, Mini-PROTEAN®TGX™, BIORAD, USA) and transferred to a polyvinylidene fluoride membrane (Millipore) for semi-dry transfer blotting (Biometra Fastblot™, Germany). The expression of adiponectin (AF1065, R&D Systems), leptin (Ab16227, abcam), IL-10 (PA1354, Booster Biological), TNFα (TNF706+p/T2, enquire Bioreagents), MIF (AF-289-PB, R&D Systems), MCP1 (MA5-17040, ThermoFisher scientific), TLR4 (76B357.1, ThermoFisher scientific), and NFkBp65 (#8242, Cell Signaling, most abundant protein and transcriptional activators of NFkB1 signalling pathway) were measured and normalized to beta-actin (A5060, Sigma). The expression of beta-actin was not different between depots and was not affected by the exercise training. These proteins were selected to correspond to the evaluated inflammatory genes described above. Integrated optical densities of the immune-reactive protein bands were measured and quantified using G: Box Chemi-XX9 GeneSys® version 1.6.4.0 and GeneTool® version 4.3.9.0, respectively. The

images were captured within the linear range and signal exposure was automatically corrected to ensure appropriate analysis and quantification of the generated data. The background was equally subtracted from the bands before normalization and plotting.

Array data extraction and analysis

Another set of purified RNA samples (from aSAT and gSAT) were analyzed before and after exercise training using Illumina Expression BeadChip (Epicentre Biotechnologies, Madison, WI, USA) on a total number of 12 participants. These participants' samples were selected and included in this analysis, based on their greatest response, in terms of improvement of body composition after the exercise training intervention. Although the weight loss was not the main outcome of this study, these participants were specifically selected to maximize the responses within SAT depots to exercise training. One array per tissue sample, for each SAT depot and for each time point (i.e. 4 arrays per participant x 12 participants) was used for the gene expression profiling (total of 24 arrays for gSAT samples and 24 arrays for aSAT samples). Each total RNA aliquot of 250 ng was ethanol precipitated with GlycoBlue (Invitrogen) as the carrier and dissolved at a concentration of 100-150 ng/µl before probe synthesis using the TargetAmp™- Nano Labeling Kit for Illumina Expression BeadChip. 750 ng of cRNA were hybridized to Human HT-12 v4 Expression BeadChips (Illumina, San Diego, CA, USA) and scanned on the Illumina iScan instrument according to the manufacturer's specifications. Raw expression intensities of 47,323 probes were extracted using Illumina GenomeStudio (Version 1.9.0) and the normalization, background correction and analysis were executed using R statistic software (version 3.6.1).

2.4.7. Statistical analyses

All data were expressed as mean ± standard deviation (SD) or median interquartile range (25^{th}-75^{th} percentile) depending on the normality of quantitative variables. Normality was tested using Shapiro-Wilks test and skewed data were transformed before analysis. The data were transformed if p values from the normality test were lower than 0.05 and transformations were performed after checking the best-normalized option, which was mostly log-transformation. The equality of variance of the groups was checked using *Levene's test* and there was homogeneity of variances. Subsequently, the following statistical analyses specific to each chapter were performed:

Chapter 3: *Circulating and subcutaneous adipose tissue fatty acid composition in obese black South African women: relationship with body composition and insulin sensitivity*

The comparison of FA composition between RBC, aSAT and gSAT was performed using a one-way repeated measures ANOVA. The relationship between the FA profiles in each tissue type and VAT/SAT ratio and S_I were explored using multivariate analysis. Firstly, the data set was inspected by a principal component analysis (PCA) to detect groupings, trends and outliers. Secondly, the associations between tissue-specific FA profiles and VAT/SAT ratio and S_I were explored using orthogonal partial least squares of analyses (OPLS). All models were validated based on ANOVA of the cross-validated OPLS scores (CV-ANOVA) for significance testing (351). All OPLS-analyses were performed using SIMCA v.16. Metabolites were considered significant when fulfilling the statistical significance criteria using post-hoc linear regression on loadings calculated from the validated OPLS-models on a 95% confidence level (352).

Chapter 4: *Exercise training alters red blood cells fatty acid desaturase activity and adipose tissue fatty acid composition in obese black South African women*

Changes in cardiorespiratory fitness, body composition, insulin sensitivity (including fasting insulin, fasting glucose and HOMA2-IR), FA composition and gene expression between the groups over time were analyzed using two-way ANOVA with repeated measures, followed by a post-hoc analysis when $p<0.1$ for interaction effect or $p<0.05$ for time or group effects. Pearson´s correlations were used to evaluate the associations between the changes in RBC-TPL and SAT FA composition and SAT gene expression, systemic inflammatory markers, liver fat and S_I. Analyses were performed using STATA statistical software 13.1 (StataCorp, College Station, Texas 77845 USA) and significance levels were set at $p<0.05$.

Chapter 5: *Changes in systemic and subcutaneous adipose tissue inflammation and oxidative stress in response to exercise training in obese black South African women*

The difference between the groups in response to exercise training (for inflammatory and oxidative stress markers at systemic, gene and protein expression levels) was analyzed using a two-way analysis of variance (ANOVA) with repeated measures on transformed data, and post-hoc analysis was conducted when $p<0.1$ for interaction effect or $p<0.05$ for time or group effects. A paired *t-test* was used to compare gene and protein expression between the two depots at baseline. Pearson´s correlations were used to evaluate the association between the changes in systemic and SAT inflammatory and oxidative stress markers, body composition, S_I and HOMA-IR. Significance levels were set at $p<0.05$ and the analyses were performed using Stata Software version 13.1 (StataCorp, College Station, Texas 77845 USA).

Chapter 6: *Distinct abdominal and gluteal adipose tissue transcriptome signatures are altered by exercise training in obese black South African women*

Changes in cardiorespiratory fitness and body composition in response to exercise training were analyzed using a paired *t-test*. Significance levels were set at $p<0.05$ and the analyses were performed using Stata Software version 13.1 (StataCorp, College Station, Texas 77845 USA).

Data quality control and differential gene expression analysis were subsequently performed. For these analyses, gene expression profiles of aSAT and gSAT were processed from the raw data obtained from Illumina GenomeStudio following the protocol of Ritchie at al. (353). After background correction, normalization, and $\log_2$ transformation (with neqc function), the probes annotated as "No match" or "Bad" were removed from the analysis. However, before removing, the 53 genes annotated as 'Bad" but with high average expression intensities (>12) were further investigated. Forty-five were repetitive Alu-elements, and the remaining were ribosomal proteins, EEF1A1 (eukaryotic translation elongation factor), and FTH1 (Ferritin heavy chain) all of which were encoded at multiple genomic loci, making further analysis questionable.

The filtered expression data were then used for differential expression analysis of the genes across the two depots, separately for each time points. A linear model was fitted, and the moderated t-statistics, moderated F-statistics, and log-odds were computed for the pairwise contrasts by empirical Bayes from the limma package. This comprised correction for multiple testing (false discovery rate; FDR). Differential expression was investigated between aSAT and gSAT at baseline and post-training as well as comparison within each fat depot before and after exercise training. Genes were identified as differentially expressed when differences in the expression intensities were equal or higher than 33% (i.e. fold change (FC) ≤ 0.67 for lower/downregulated genes and FC ≥ 1.5 for higher/upregulated genes) or an absolute $\log_2$ fold change ($|LFC|$) ≥ 0.58, with a p-value ≤ 0.05.

A Gene Set Enrichment Analysis (GSEA) was performed to determine the enriched biological processes and functional pathways represented by the identified differentially expressed genes (DEGs) using STRING (354). Afterwards, the number of expected genes in a random set of the same size was compared to the number of observed DEGs assigned to a specifically enriched gene ontology (GO)-term.

2.5. Ethical aspects of the study

There were no appreciable risks associated with the methods chosen for this study other than those associated with routine blood sampling for the measure of insulin sensitivity. In this test, the procedures were supervised and carried out by a medical doctor and appropriately trained medical personnel using sterile techniques to minimise any risks of infection. A maximum of 200 ml of blood was drawn during the entire study, which is less than half that drawn during standard blood donation.

The methods used to measure body composition and fat distribution (DXA and MRI scans) were not associated with any significant or harmful risk. Although the fat biopsies were performed under local anaesthetic, this procedure represented a challenge for the participants due to the discomfort during and after the biopsies (e.g. local stinging after the anaesthetic, small bruising, temporary loss of feeling in the area around the biopsy site, itching). However, these were generally resolved after a short while and there were no adverse events reported.

The training program was designed for exercise progression to ensure minimal physical discomfort and associated soreness. This was supervised by a trained biokineticist, who monitored each participant's progress (using heart rate data) and adjusted the sessions accordingly to ensure adequate improvement in cardiorespiratory fitness throughout the 12-week program. All efforts were 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, and necessary assistance in case of need was provided during and after the training sessions. The participants assigned to the control group were offered the opportunity to undergo the same 12-week exercise training program as the exercise group, and participation was totally voluntary.

The participants of this study received their individual results after completion, including body composition (weight, height, waist circumference and fat mass), blood pressure, risk for diabetes, physical fitness and dietary analysis, with some recommendations made by a dietician on how to adapt their dietary intake to improve their health. Additionally, the participants were given guidelines and recommendations on how to continue exercise training in their daily life.

CHAPTER THREE

CIRCULATING AND SUBCUTANEOUS ADIPOSE TISSUE FATTY ACID COMPOSITION IN OBESE BLACK SOUTH AFRICAN WOMEN: RELATIONSHIP WITH BODY COMPOSITION AND INSULIN SENSITIVITY

3.1. INTRODUCTION

During positive energy balance, adipocyte number and/or size increase to accommodate the accumulation of TGs into adipocytes, expanding the AT mass (102). This has been described as a physiological mechanism protecting other tissues against lipotoxicity (355). However, excess TG storage in SAT may cause the impairment of tissue metabolism and function (134). The impairment in SAT lipid metabolism may critically influence systemic FFA concentrations (356). Indeed, increased basal and/or stimulated lipolysis characterizing obese SAT elevates circulating concentrations of FFA, which may interfere with glucose transport/uptake and phosphorylation activity, contributing to the weakening of peripheral insulin sensitivity (250, 357). Moreover, alterations of the composition of cell membrane PHLs in insulin-sensitive tissues (e.g. AT, liver and muscle), as observed in obesity, may change membrane fluidity and consequently, impair insulin signalling pathways, resulting in IR (358). On the other hand, FFAs released from SAT are redirected to ectopic regions including VAT (134, 247). Therefore, VAT accumulation, an independent risk factor for obesity-associated metabolic disorders, may be influenced by SAT lipid metabolism and circulating FA profiles (224).

High concentrations of FFA may not only derive from AT but also dietary fat intake. Plasma or serum FA composition is subject to great diet-FA-induced variations compared to the RBC membrane. RBCs can maintain their membrane FA composition for several weeks and are therefore a good biological material to study the implication of FA (both from dietary intake and/or AT lipid metabolism) in obesity-associated metabolic risk (250). In this regard, alterations RBC-TPL FA composition have been associated with IR (358). These changes in membrane composition could either derive from changes in dietary FA intake or changes in the endogenous synthesis of long-chain FA by desaturase enzymes (358). Δ9-desaturase or SCD1 desaturates palmitic (16:0) and stearic (18:0) acids into MUFAs palmitoleic (16:1n-7) and oleic (18:1n-9) acids, respectively (102). Additionally, D6D and D5D together with elongases convert the two essential dietary FAs, ALA (18:3n-3) and LA (18:2n-6), into their corresponding LC-PUFA products, EPA (20:5n-3) and AA (20:4n-6) (241). Dysregulation in the activity of these enzymes specifically increases of SCD1 and D6D, and a reduction of D5D as assessed in plasma, serum (242, 349, 359), RBC (360, 361) and AT (220, 349, 362) have been associated with obesity and related impairment of insulin sensitivity (358).

The mechanisms involved in the regulation of FA metabolism are specific to each tissue. Indeed, the rates of FA synthesis, uptake and release differ between gSAT and aSAT (47, 78,

79). These differences have been suggested to contribute to variations in FA composition (221, 305) and the differential associations of gSAT and aSAT with metabolic status (79). Additionally, desaturase activity and FA composition vary among different ethnicities, which may be explained by differences in lifestyle factors such as dietary intake (261, 262). When compared to their white counterparts, black African women have lower SFA intake and higher intake of n-6 PUFA, corresponding to their serum FA composition (32, 263). Genetic factors may also mediate these ethnic differences. African Americans carry the genotype associated with higher D5D activity and are consequently more efficient in the conversion of DGLA to AA than their white counterparts (265, 266). Further, the susceptibility to develop metabolic diseases varies in different ethnicities with African descendants at higher risk than European descendants (261). Notably, the differences in dietary intake between these ethnicities have also been associated with differences in basal insulin sensitivity and secretion (262), suggesting that the relationship between FA and markers of IR is ethnic-specific. Therefore, focusing on women of African ancestry, this study aimed to assess the FA composition of RBC-TPL, aSAT and gSAT, and their relationship with the centralisation of body fat (VAT/SAT ratio) and insulin sensitivity (S_I). The hypothesis was that the FA profile of RBC membranes which is affected by direct exchange with plasma FFA pools may reflect FA metabolism in AT, as circulating FFAs are mainly derived from SAT in individuals with obesity. Therefore, the relationship between FA composition and central fat distribution and insulin sensitivity would not variate according to the storage site and/or the blood compartment or fraction where they occur.

3.2. RESULTS

3.2.1. Participant characteristics and dietary macronutrient intake

Basic characteristics of the participants in this cross-sectional study are presented in **Table 3.1.** Self-reported daily dietary fat intake (**Table 3.2**) comprised 34 % of total energy (%E), of which 9.5 %E was from SFA, 11.4 %E from MUFA and 7.9 %E from PUFA.

Table 3.1. Basic characteristics of participants

Variables	Values (n=41)
Age (year)	23 (21 - 27)
Body composition	
BMI (kg/m^2)	33.9 ± 2.8
Waist circumference (cm)	103.8 ± 8.0
Hip circumference (cm)	116 (114 – 121)
WHR	0.9 (0.8 – 0.9)
Body FM (%)	50.4 ± 12.0
Android FM (%)	8.1 ± 1.1
Gynoid FM (%)	18.6 ± 2.0
VAT (cm^3)	900 ± 345
SAT (cm^3)	5454 ± 1547
VAT/SAT	0.2 ± 0.2
Insulin sensitivity	
S_I (mU/L)$^{-1}$min^{-1}	2.5 ± 1.4
Fasting glucose (mmol/L)	5.3 (4.5 – 5.8)
Fasting Insulin (µIU/mL)	13.1 (8.4 – 16.7)

Values are expressed as mean ± SD or median (25th – 75th percentile). BMI: body mass index; FM: fat-mass; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue; S_I: insulin sensitivity.

Table 3.2. Daily energy, macronutrient and fatty acid intake

Variables	Median (25^{th}-75^{th} percentile)
Energy (kJ)	12507 (9330-15374)
Total protein (g)	96.1 (67.5-119.9)
Protein (%E)	13.2 (12.2-14.1)
Total CHO (g)	372.7 (295.1-464.7)
CHO (%E)	53.1 (49.0-54.8)
Total Fat (g)	113.0 (71.5-131.4)
Fat (% E)	33.4 (30.1-36.5)
SFA (g)	31.4 (18.7-42.3)
SFA (%E)	9.5 (7.9-11.6)
MUFA (g)	36.0 (24.1-44.6)
MUFA (%E)	11.4 (10.1-12.5)
PUFA (g)	25.8 (18.0-32.5)
PUFA (%E)	7.9 (7.2-9.8)

Data presented as median (25^{th} and 75^{th} percentiles). kJ: kilojoules; CHO: carbohydrate; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

3.2.2. Tissue-specific fatty acid composition

The FA composition of RBC-TPL was 44% SFA, 16% MUFA and 40% PUFA, while gSAT was composed of 31% SFA, 43% MUFA and 26% PUFA and aSAT comprised 32% SFA, 42% MUFA and 26% PUFA (**Table 3.3**). Accordingly, total SFA and PUFA were higher, while total MUFA was lower in RBC-TPL compared to SAT depots ($p<0.001$). When comparing these FA classes between SAT depots, total SFA was higher and total MUFA was lower in aSAT than in gSAT ($p<0.01$), with no difference in total PUFA content ($p>0.05$).

When comparing individual FAs in RBC-TPL and SAT depots (**Table 3.3**), long-chain SFAs (18:0, 20:0, 22:0 and 24:0) were higher and the medium-chain SFA 14:0 was lower in RBC-TPL than in SAT depots ($p<0.001$). Notably, 16:0 and 18:0 were higher in aSAT compared to gSAT ($p<0.05$). Individual MUFAs (16:1n-7, 18:1n-7, 18:1n-9, 20:1n-9) were lower in RBC-TPL compared to SAT depots ($p<0.001$), and 16:1n-7 and 18:1n-7 were higher in gSAT than in aSAT ($p<0.01$). In contrast, n-3 PUFAs EPA (20:5n-3), DPA n-3 (22:5n-3), DHA (22:6n-3) and total n-3 FAs were higher in RBC-TPL than in SAT depots ($p<0.001$), with no significant difference between aSAT and gSAT ($p>0.05$). Likewise, n-6 PUFAs DGLA (20:3n-6), AA (20:4n-6), adrenic acid (22:4n-6), DPA n-6 (22:5n-6) and total n-6 PUFA were higher in RBC-TPL than in SAT depots ($p<0.001$), and only DGLA was higher in gSAT compared to aSAT ($p<0.05$). Conversely, LA (18:2n-6) and eicosadienoic acid (20:2n.6) were lower in RBC-TPL compared to SAT depots ($p<0.001$) but were not different between gSAT and aSAT ($p>0.05$). The estimated activities of D5D (20:4n-6/20:3n-6), D6D (18:3n-6/18:2n-6) and SCD1-18 (18:1n-9/18:0) were higher ($p<0.001$), and SCD1-16 (16:1n-7/16:0) was lower in RBC-TPL compared to SAT depots ($p<0.001$). Notably, both SCD1-16 and SCD1-18 were higher in gSAT compared to aSAT ($p<0.01$).

Table 3.3. Comparison of fatty acid composition (percentage) between red blood cell total phospholipids, gluteal and abdominal subcutaneous adipose tissue depots

	RBC-TPL	gSAT	aSAT	P VALUES
Saturated fatty acids (SFAs)				
14:0 (Myristic acid)	**0.24 ± 0.06** [a,b]	2.77 ± 0.62	2.89 ± 0.59	<0.001
16:0 (Palmitic acid)	21.88 ± 2.06	21.70 ± 1.12	**22.52 ± 1.11** [c]	0.024
18:0 (Stearic acid)	**16.31 ± 0.78** [a,b]	5.25 ± 1.53	**5.75 ± 1.49**[c]	<0.001
20:0 (Arachidic acid)	**0.35 ± 0.04** [a,b]	0.16 ± 0.06	0.16 ± 0.05	<0.001
22:0 (Behenic acid)	**1.39 ± 0.20** [a,b]	0.06 ±0.03	0.05 ± 0.01	<0.001
24:0 (Lignoceric acid)	**3.99 ± 0.64** [a,b]	0.05 ± 0.02	0.04 ± 0.01	<0.001
Total SFAs	**44.16 ± 1.68** [a,b]	30.76 ± 2.88	**32.23 ± 2.46** [c]	<0.001
Mono-unsaturated fatty acids (MUFAs)				
16:1n-7 (Palmitoleic acid)	**0.24 ± 0.07** [a,b]	**6.61 ± 2.09** [c]	5.93 ± 1.68	<0.001
18:1n-7 (cis-Vaccenic acid)	**1.11 ± 0.22** [a,b]	**3.00 ± 0.49** [c]	2.79 ± 0.43	<0.001
18:1n-9 (Oleic acid)	**10.70 ± 0.87** [a,b]	32.56 ± 0.92	32.49 ± 1.01	<0.001
20:1n-9 (Eicosenoic acid)	**0.18 ± 0.02** [a,b]	0.71 ± 0.10	0.69 ± 0.11	<0.001
Total n-7 MUFAs	**1.34 ± 0.27** [a,b]	**9.61 ± 2.50** [c]	8.71 ± 2.02	<0.001
Total n-9 MUFAs	**14.23 ± 0.89** [a,b]	33.31 ± 0.94	33.22 ± 1.04	<0.001
Total MUFAs	**15.57 ± 0.97** [a,b]	**42.92 ± 2.64** [c]	41.93 ± 2.25	<0.001
Poly-unsaturated fatty acids (PUFAs)				
20:5n-3 (EPA)	**0.47 ± 0.23** [a,b]	0.09 ± 0.04	0.08 ± 0.04	<0.001
22:5n-3 (DPA n-3)	**1.96 ± 0.31** [a,b]	0.27 ± 0.09	0.24 ± 0.08<	<0.001
22:6n-3 (DHA)	**4.45 ± 0.81** [a,b]	0.24 ± 0.08	0.21 ± 0.08<	<0.001
18:2n-6 (LA)	**11.71 ± 1.31** [a,b]	22.33 ± 1.64	22.15 ± 1.55	<0.001
20:2n-6 (Eicosadienoic acid)	**0.35 ± 0.04** [a,b]	0.50 ± 0.10	0.48 ± 0.12	<0.001
20:3n-6 (DGLA)	**1.53 ± 0.22** [a,b]	**0.43 ±0.12** [c]	0.37 ± 0.12	<0.001
20:4n-6 (AA)	**15.74 ± 1.19** [a,b]	0.73 ±0.18	0.67 ± 0.17	<0.001
22:4n-6 (Adrenic acid)	**3.37 ± 0.59** [a,b]	0.30 ± 0.09	0.27± 0.09	<0.001
22:5n-6 (DPA-n-6)	**0.68 ± 0.16** [a,b]	0.08 ± 0.04	0.08 ± 0.03	<0.001
Total n-3	**6.90 ± 1.87** [a,b]	1.76 ± 0.33	1.66 ± 0.32	<0.001
Total n-6	**33.38 ± 1.87** [a,b]	24.56 ± 1.73	24.18 ± 1.64	<0.001
Total PUFAs	**40.27 ± 1.77** [a,b]	26.32 ± 1.95	25.84 ± 1.80	<0.001
Estimated enzyme activities				
D5D	**10.57 ± 1.95** [a,b]	1.79 ± 0.43	1.90 ± 0.37	<0.001
D6D	**0.13 ± 0.02** [a,b]	0.019 ± 0.01	0.016 ± 0.01	<0.001
SCD1-16	**0.01 ± 0.00** [a,b]	***0.31 ± 0.11*** [c]	0.27 ± 0.08	<0.001
SCD1-18	**0.68 ± 0.01** [a,b]	**0.65 ± 0.30** [c]	0.53 ± 0.21	<0.001

*Fatty acids (FA) presented as relative percentages of total FAs (%, wt:wt) and mean ± SD (n=41). P values represent the differences in FA composition between the sites using one-way repeated measures ANOVA as follows: **a**: RBC vs gSAT; **b**: RBC vs aSAT and **c**: gSAT vs aSAT. LA: linoleic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; DGLA: dihomo-gamma-linolenic acid; AA: arachidonic acid; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; D5D: delta-5-desaturase index (20:4n-6/20:3n-6); D6D: delta-6-desaturase index (18:3n-6/18:2n-6); SCD1: stearoyl-CoA desaturase-1 (SCD1-16: 16:1n-7/16:0; SCD1-18: 18:1n-9/18:0).*

3.2.3. Relationship between fatty acid profiles and VAT/SAT ratio

The relationships between site-specific FA profiles and the VAT/SAT ratio were evaluated using multivariate analysis (**Figure 3.1**). In RBC-TPL, MUFAs (16:1n-7 and 18:1n-7, n-7 MUFA) and SCD1-16 were positively associated with the VAT/SAT ratio, while DGLA was negatively associated with the VAT/SAT ratio. Both SAT depots showed a similar pattern of association with the VAT/SAT ratio for SFA and PUFA. Indeed, lower SFAs (12:0, 14:0, 18:0, 20:0 and total SFA) and higher PUFAs (ALA, AA, DPA n-3, total PUFA, total n-6, total n-3) and SCD1-18 were associated with higher VAT/SAT ratio in both SAT depots. However, while vaccenic acid (18:1n-7), positively associated with VAT/SAT ratio in gSAT, oleic acid (18:1n-9) and total n-9 MUFA in aSAT inversely correlated with the VAT/SAT ratio.

3.2.4. Relationship between fatty acid profiles and insulin sensitivity

The relationships between site-specific FA profiles and S_I (**Figure 3.2**) were evaluated using multivariate analysis. Higher RBC-TPL total SFA and 20:2n-6, and lower 22:4n-6 were associated with lower S_I. In gSAT, PUFAs (LA, DGLA, DPA n-3, total n-6, total PUFA), SCD1-18 and D6D were inversely correlated with S_I, while MUFAs (16:1n-7, total n-7 and total MUFA) were positively associated with S_I. Likewise, total MUFA in aSAT were positively associated with S_I, while individual PUFA (LA, eicosadienoic acid, DGLA, adrenic acid, DPA n-3, DHA, total n-6, total PUFA) and D6D were negatively associated with S_I.

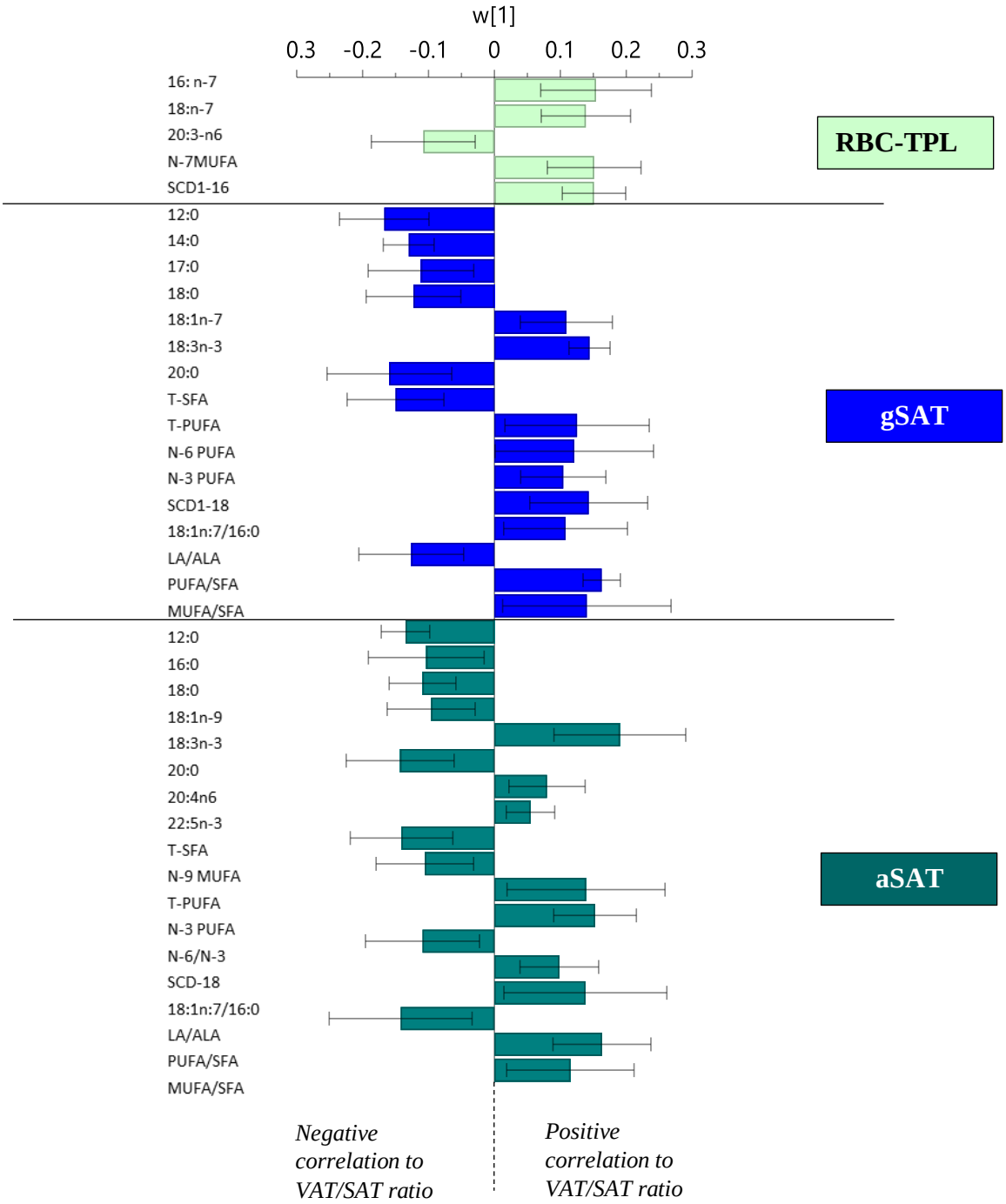


Figure 3.1. Associations between tissue-specific fatty acid composition and VAT/SAT ratio from multivariate models

Data represent the individual and grouped FA and FA ratios in each tissue type which significantly contributing to the multivariate model (CV-ANOVA P=0.02), with a 95% confidence interval (error bars). T-SFA: total saturated fatty acid; T-MUFA: total monounsaturated fatty acid; T-PUFA: total polyunsaturated fatty acid; LA: linoleic acid; ALA: alpha-linolenic acid; SCD1: stearoyl-CoA desaturase-1 (SCD1-16: 16:1n-7/16:0; SCD1-18: 18:1n-9/18:0); gSAT: gluteal subcutaneous adipose tissue; aSAT: abdominal subcutaneous adipose tissue.

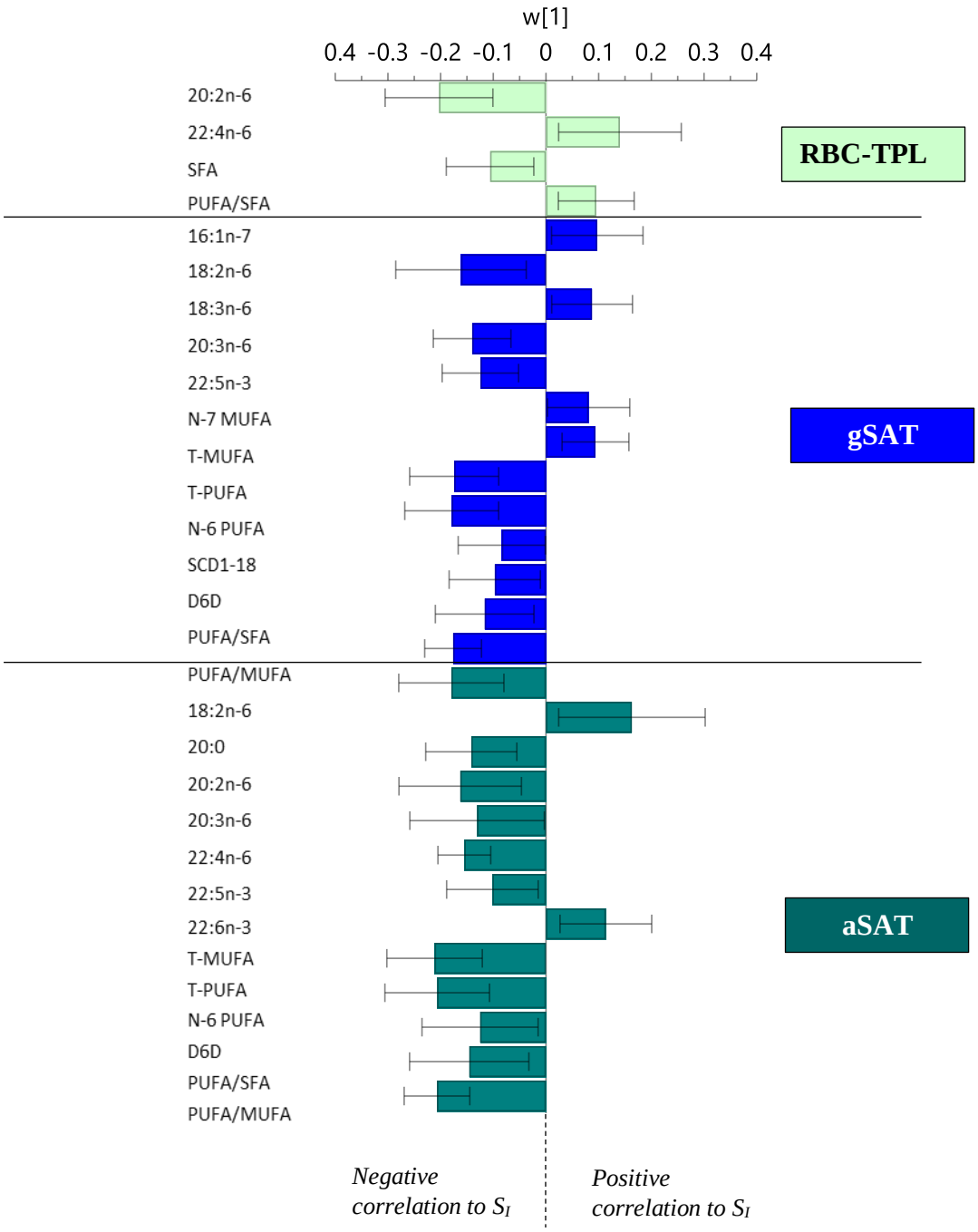


Figure 3.2. Associations between tissue-specific fatty acid composition and insulin sensitivity from multivariate models

Data represent the individual and grouped FA and FA ratios in each tissue type which significantly contributing to the multivariate model (CV-ANOVA P=0.03), with a 95% confidence interval (error bars). T-SFA: total saturated fatty acid; T-MUFA: total monounsaturated fatty acid; T-PUFA: total polyunsaturated fatty acid; LA: linoleic acid; ALA: alpha-linolenic acid; D6D: delta-6-desaturase index (18:3n-6/18:2n-6); SCD1: stearoyl-CoA desaturase-1 (SCD1-16: 16:1n-7/16:0; SCD1-18: 18:1n-9/18:0); gSAT: gluteal subcutaneous adipose tissue; aSAT: abdominal subcutaneous adipose tissue.

3.3. DISCUSSION

The novel finding of this study is that the FA composition of circulating phospholipids (RBC-TPL) and SAT are distinctly associated with the centralization of body fat and S_I in black South African women with obesity. Notably, the FA composition of RBC-TPL is different from TG FA profiles in SAT. Specifically, RBC-TPL contained higher proportions of SFAs and PUFAs and lower MUFAs than SAT. The higher content of SFA in RBC-TPL was not associated with the VAT/SAT ratio, but rather with lower S_I. Furthermore, the estimated activities of D5D, D6D and SCD1-18 were higher and SCD1-16 was lower in RBC-TPL compared to SAT depots. Interestingly, SAT depots had distinct FA composition, with higher SFAs (16:0, 18:0 and total SFA) and lower MUFAs (16:1n-7, 18:1n-7 and total MUFA), as well as lower SCD1-16 and SCD1-18 activity in aSAT compared to gSAT. Despite these differences, the associations with VAT/SAT ratio and S_I did not differ by SAT depot. Indeed, in both SAT depots, lower SFAs and higher PUFAs (n-3 and n-6) correlated with higher VAT/SAT ratio. Further, lower PUFAs (n-3 and n-6) and higher total MUFA correlated with higher S_I. Notably, SCD1-18 was positively associated with the VAT/SAT ratio and D6D inversely correlated with S_I in both SAT depots. These findings show that the association of FAs with metabolic status is dependent on the FA class and the blood compartment/ fraction where they occur or tissue type where they are stored.

The FA profile of cell membrane PHLs is modulated by the FA composition of dietary fat (363, 364). This is specifically true for PUFA intake (358). The present study showed high proportions of SFA and PUFA in RBC-TPL, which may indicate a high consumption of these FA types in obese black SA women. This is commensurate with previous research in this population (365). However, this was not supported by the self-reported dietary intake of the participants in this study, which reported lower SFA (9.5% of total energy intake, EI) and PUFA (8% EI) intake than MUFA intake (11% EI). It is important to note that under-reporting of dietary intake is well recognized, and may significantly influence investigations of nutrient pattern and association with disease (366). This makes the evaluation of FA profile in biological matrices a preferential method over self-reported dietary intake (248). Nevertheless, SFAs such as 16:0 can be synthesized endogenously from acetyl-CoA during DNL, which can be subsequently elongated (to 18:0) and desaturated to generate MUFAs (such as 16:1 and 18:1) (250, 358, 367). This pathway is elevated in obese individuals and under high-carbohydrate loads and excess energy intake (250), which is characteristic of the participants in the present study. Excess consumption of carbohydrates and high glycemic load results in increase

lipogenesis with the rapid synthesis of SFA, especially 16:0 and 18:0 in the circulation (232, 368). Accordingly, these were the most abundant SFAs in RBC-TPL in the present study (16:0 (22%) and 18:0 (16%)). However, RBC cannot undergo *de novo* PHL synthesis or FA desaturation, but rather renew their FA composition by direct exchange with plasma FFA pools (248). Therefore, high content of SFA and PUFA in RBC-TPL could derive from plasma FFAs, highly influenced by both dietary intake (especially for PUFA) and endogenous synthesis in other tissues such as AT and liver (especially for SFA). Further studies are required to elucidate the origin of these FA classes and proportions in RBC membranes in obese black SA women.

This study further reported lower MUFA contents in RBC-TPL compared to SAT depots. Although no previous studies have compared the FA composition between RBC-TPL and SAT depots, previous data showed high SFA and PUFA and lower MUFA levels in RBC-TPL of obese compared to lean individuals (369, 370). Concomitantly with higher SFA and lower MUFA, the present study showed lower desaturation rate (SCD1-16 activity) in RBC-TPL than in SAT depots. SCD1-16 is involved in the endogenous synthesis of MUFA from SFA and is mainly active in AT were it is very important for *de novo* synthesis and storage of excess energy as TGs (349). In contrast, in circulating PHLs, SCD1-18 is mostly modulated by dietary intake of 18:1n-9 (371). This potentially explains the higher activity of SCD1-18 in RBC-TPL, as the FA composition in this fraction mostly represents the short-term (2-3 months) dietary intake than the FA profile in SAT representing FA intake over a much longer-term (248).

MUFAs form the major class of FA in adipose TGs (372), mainly consisting of long-chain 16 and 18 carbons; and oleic acid has been reported as the major species in adipose TGs (225, 232, 248, 373, 374). Accordingly, oleic acid (18:1n-9) was the most abundant FA in SAT depots (32.5%) in the present study. Remarkably, MUFA content was lower in aSAT (with higher SFA) than gSAT in these women. This is similar to what has been previously found in European populations (248, 253). This difference in FA profiles might be attributed to the previously reported depot-specific differences in FA metabolism between SAT depots (including synthesis, uptake, release, deposition rate, mobilisation and endogenous synthesis) have been reported (47, 78, 79), suggested being dependent on the energy balance (253). The higher saturation of aSAT has also been linked to the physical properties of this depot (semi-solid) in relation to its anatomical role in the protection of abdominal organs (253). Conversely, the lower content of SFA in gSAT in the present study was complemented by the higher SCD1 activity and a higher percentage of MUFA (16:1n-7, 18:1n-7 and total MUFA). This suggests a higher desaturation rate and therefore, a preferential FA species accumulation in gSAT in

these women. However, PUFA content was similar between aSAT and gSAT, which is supported by previous studies (232, 248, 253, 254, 375). These data support the notion that PUFA content in SAT depots is dependent on dietary intake (with 18:2 representing the major dietary PUFA in SAT); and LC-PUFAs are also synthesized from essential FAs (18:2n-6 and 18:3n-3) that can only be obtained from food (232, 253). Collectively, these findings suggest comparable PUFA metabolism (deposition, mobilization and desaturation rates) between aSAT and gSAT, further supported by the similar estimated desaturase activities observed between these depots.

The alteration of circulating and AT FA composition in obese individuals can influence the physical properties of membranes (e.g. fluidity and permeability) and increase the susceptibility to metabolic diseases (369). Importantly, the metabolic effects of individual FAs are dependent on their respective class (100, 231). For instance, high contents of SFAs and low MUFAs increased cell membrane rigidity resulting in a reduced number of insulin receptors and binding affinity in insulin-sensitive cells (250, 358). The opposite effects have been shown for high MUFA and PUFA, which increase membrane fluidity, thereby explaining, at least in part, the different associations of FA classes with metabolic risks (358). Henceforth, the relationships between the FA profiles in RBC-TPL and SAT depots, centralisation of body fat and S_I were assessed in this study. Despite differential SFA and MUFA composition in SAT depots, there were no differences in the association of these FA classes with the centralisation of body fat and S_I. Indeed, lower individual and total SFAs in both SAT depots correlated with a higher VAT/SAT ratio. In contrast, higher total SFA in RBC-TPL, and not the SAT depots, was associated with lower S_I. These findings, similar to a previous study in black South Africans showing a positive association between plasma PHL SFAs and measures of adiposity and metabolic syndrome (374), add to the notion that circulating SFAs have an adverse effect on metabolic profile. Indeed, high intake of dietary SFA may significantly increase IR via alterations of desaturases activity (mainly D5D and D9D) of cell membrane PHLs and inhibition of insulin-stimulated Akt activation followed by the reduction in glucose uptake (358, 376).

In contrast to circulating SFA, the implication of PUFAs in the development of obesity-associated metabolic disorders is controversial. Increase in membrane unsaturation has been associated with higher S_I (358). For instance, Gunes et *al.* showed an association between membrane enrichment in n-3 PUFA and improvement of S_I in obese adolescents (250). Moreover, rats fed with diets rich in n-6 PUFA exhibited an increased insulin-stimulated

glucose uptake in adipocytes (377). In addition, plasma total n-3 PUFA was negatively associated with markers of obesity and metabolic syndrome in obese individuals (378, 379). However, other studies reported positive associations between n-6 PUFAs intake, obesity and metabolic syndrome (380, 381), as well as inverse associations between RBC total n-6 PUFAs and metabolic syndrome (382, 383). These discrepancies in the association between circulating PUFAs and metabolic status were also observed in the present study where RBC-TPL adrenic acid (22:4n-6) was positively and eicosadienoic acid (20:2n-6) was negatively correlated with S_I.

Further, both n-6 and n-3 PUFAs in SAT depots were negatively associated with S_I and positively correlated with VAT/SAT ratio in the present study. PUFAs regulate lipogenic gene expression during adipogenesis, hence increasing TG storage in SAT (384). However, n-6 and n-3 PUFAs are known to exert opposite effects on adipogenesis and lipid metabolism, with n-6 PUFAs involved in the terminal differentiation of preadipocytes to mature adipocytes; processes reported to be inhibited by n-3 PUFAs (231). Furthermore, while n-6 PUFAs have been shown to induce an increase of cellular TG content (via increased membrane permeability), n-3 PUFAs may reduce fat deposition in AT by suppressing lipogenic enzymes and increasing beta-oxidation (231). The current diverse associations between PUFA (n-3 and n-6) and SFA content in SAT with the centralisation of body fat and S_I accord with a previous study in a French population showing positive associations between n-6 PUFA content of SAT (abdominal) and central fat distribution (232). Moreover, studies in Swedish cohorts reported a positive association between individual n-3 PUFA (EPA and DHA) intake and body fat accumulation (385) and positive correlations between aSAT n-6 PUFA (20:3n-6 and 20:4n-6) content and HOMA-IR (386). Further, Iggman *et al.* showed strong positive correlations between that gSAT content of individual SFAs (12:0, 14:0 and 18:0) and LA with insulin sensitivity, while DHA negatively correlated with insulin sensitivity (387). Conversely, high SFA intake caused a higher deposition of VAT and total body fat, whereas increased dietary PUFA (mainly LA) reduced central fat accumulation (385). These data highlight the complexity in the relationship between FA classes (especially PUFAs) and obesity-associated metabolic diseases. The role of circulating and SAT FAs species in central fat accumulation and impairment of insulin sensitivity in different population therefore warrants further investigations. Nevertheless, the similar associations of n-6 and n-3 PUFAs and desaturases in both SAT depots suggest that the differential relationship between SAT depots and metabolic risks in obesity might not be driven by PUFA metabolism. Likewise, total MUFAs in both SAT depots were positively associated with S_I, whereas RBC-TPL MUFA contents were positively

associated with central fat accumulation in the present study. These findings suggest that high circulating MUFA could exert negative metabolic effects, which are negated once MUFAs are sequestrated into adipose depots, thereby preventing lipotoxicity and associated IR in peripheral organs. Accordingly, MUFA content of AT has been suggested to be protective against cardio-metabolic risks in overweight and obese individuals (232).

Another novel aspect of this study is the multivariate models, most significant for FA composition of SAT depots. This highlights a stronger influence of SAT FA metabolism over dietary FA intake (reflected by RBC-TPL), on the metabolic status in obese individuals, represented in this study by centralisation of body fat and insulin sensitivity. These findings support the contribution of excess SAT accumulation in the incidence of metabolic disorders (200) both as a major source of systemic FFAs and as a determinant of ectopic lipid deposition and whole-body insulin sensitivity (250, 357).

A limitation of this study is that the desaturase enzyme activities were estimated based on product to precursor FA ratios and may simply indicate a higher proportion of product than precursor in this tissue and not necessarily a higher desaturation level. Future studies should measure the direct activity of the main desaturases, as well as elongases involved in FA metabolism. In addition, the cross-sectional nature of these analyses prevents conclusions on cause and effect. The lack of detailed information on the dietary FA intake may also represent a limitation. Besides, only whole-body insulin sensitivity was measured using the FSIGT. Accordingly, changes in hepatic and muscle insulin sensitivity could not be determined, which would have been of particular interest. However, the strength of this research work is the robust measure of the FA composition of RBC-TPL and two main depots of SAT represent strengths of this research work. This method is regarded as more objective and accurate than estimations from dietary reporting in the investigation of the effects of these lipid molecules in the development of metabolic diseases (248, 366). Because this study only included obese SA women, prospective studies in a more representative population (including men, and normal-weight individuals) are required to gain a better understanding of the associations between RBC-TPL and SAT FA composition and central fat accumulation and development IR.

This was the first study that has comprehensively measured and compared the FA composition of RBC-TPL and two distinct SAT depots in humans with obesity; and evaluating the tissue-specific relationships between FA composition, desaturase activities and measures of central fat distribution and insulin sensitivity, making this research work original and novel. It was shown that the circulating (RBC-TPL) FA profile is distinctly different from the FA

composition of SAT, with higher SFAs and PUFAs and lower MUFAs in RBC-TPL compared to the SAT depots. Notably, differences were also observed within SAT depots with higher SFAs and lower MUFAs in aSAT compared to gSAT, but no differences in PUFA content. However, the differential SFA and MUFA contents between aSAT and gSAT was not reflected in the relationship with the VAT/SAT ratio and S_I. SAT depots, rather than RBC-TPL, were the major contributors to the variance in central fat accumulation and insulin sensitivity. This study showed for the first time that the FA composition of RBC-TPL and SAT is associated with metabolic risk in black SA women with obesity, and these associations are not only dependent on their class, but also on the tissue type and blood compartment or fraction in which they are distributed. Intervention studies such as exercise training are known to alter AT lipid and FA metabolism as well as circulating FA composition, which might contribute to the improvement of insulin sensitivity in obese individuals. Such interventions are required to elucidate the causal relationship between FA composition and metabolic profile, as well as the specific roles of the individual FAs and total FA groups in mediating obesity-associated impairment of insulin sensitivity.

CHAPTER FOUR

EXERCISE TRAINING ALTERS RED BLOOD CELLS FATTY ACID DESATURASE ACTIVITY AND ADIPOSE TISSUE FATTY ACID COMPOSITION IN OBESE BLACK SOUTH AFRICAN WOMEN

4.1. INTRODUCTION

The previous chapter of this thesis (Chapter 3) showed that SAT FA composition is the major contributor of the variance in central fat accumulation and insulin sensitivity in obese black SA women, rather than RBC-TPL FA profile. However, the differential FA content between aSAT and gSAT was not reflected in the relationship with the VAT/SAT ratio and S_I. This study was limited by its cross-sectional nature and hence an understanding of the causative link between FA composition in SAT depots and RBC-TPL and whole-body insulin sensitivity could not be gained. This was further investigated in the present chapter using exercise training as an intervention model.

Lifestyle interventions such as exercise training represent a key component in the management of obesity and related IR. Exercise training has been well established to improve whole-body glucose metabolism and insulin sensitivity via its predominant action on skeletal muscle (290-292). However, long term exercise training can induce beneficial adaptations in AT (292, 388). Indeed, exercise training can modify lipid metabolism and FA composition, independently of changes in nutrition (138). In this regard, exercise training can stimulate AT lipolysis, decrease lipogenesis and improve insulin sensitivity (290, 292, 303, 304). Exercise training can also alter FA synthesis, desaturation and elongation in AT (221, 305), as well as change the FA composition of RBC membranes (225). However, data on the effects of exercise training on FA metabolism in African populations are scarce. This is particularly relevant given the reported ethnic differences in desaturase activity and capacities of LC-PUFAs synthesis (265, 266). Evaluation of the impact of exercise training on these pathways and the association with insulin sensitivity can provide an understanding of the mechanisms linking FA metabolism to IR in specific ethnic groups. Indeed, obese black SA women are less insulin sensitive than their white counterparts despite having less VAT (30-34) and liver fat content (389). Moreover, reduced insulin sensitivity in obese state has been associated with systemic inflammation and partitioning of excess lipids to VAT and the liver due to elevated circulating FFA concentrations deriving from SAT impaired function (134, 139, 155). This study therefore represents a unique opportunity to investigate whether exercise-induced improvement in insulin sensitivity is associated with changes in SAT FA metabolism and therefore changes in circulating (reflected here in RBC-TPL) FA profile in obese black women. Specifically, the aim of this chapter was to evaluate i) the effect of exercise training, without dietary modification, on RBC-TPL and SAT FA composition and desaturase activity in obese and previously sedentary black SA

women; and ii) the associations with changes in SAT lipid metabolism, systemic inflammatory markers, liver fat and insulin sensitivity in respone to exercise training.

4.2. RESULTS

4.2.1. Compliance, participant characteristics and dietary macronutrient intake in response to the intervention

A total of 48 exercise training sessions were conducted, in which participants attended 79±13 (range: 52-100) % at a mean intensity of 79.7±4.0 (range: 71-85) % HRpeak.

A summary of participant characteristics at baseline and in response to the intervention is presented in **Table 4.1**. At baseline, the cardio-respiratory fitness, body composition and S_I did not differ between the groups. Following the intervention, VO_{2peak} increased in the exercise group and was unchanged in the control group ($p<0.01$ for group x time interaction). There were small but significant decreases in body weight, BMI, WC and gynoid fat (% of total fat mass; FM) in the exercise group ($p<0.01$). In contrast, body weight, BMI and WC ($p<0.01$), and SAT ($p<0.05$) volume increased in the control group. Total body FM (%), android FM (%) and VAT did not change in either group. Liver fat tended to decrease in the exercise group ($p=0.074$), with no changes in the control group ($p=0.356$). S_I increased in the exercise group and did not change in the control group ($p<0.05$ for group x time interaction). No changes in fasting glucose, insulin, HOMA2-IR and circulating inflammatory markers were observed in either group in response to the intervention.

Dietary energy, macronutrient and FA intake were not significantly different between the groups at baseline and did not change in response to the intervention (**Table 4.2**).

Table 4.1. Changes in anthropometry, cardiorespiratory fitness, insulin sensitivity and systemic inflammation in response to the 12-week exercise intervention in exercise and control groups

Variables	CONTROL (n=15)		EXERCISE (n=20)		P VALUES		
	Pre	Post	Pre	Post	Time	Group	Interaction
Cardiorespiratory fitness							
VO2peak (ml/min)	2099 ± 282	2032 ± 196	2077 ± 211	2278 ± 231$^{\#}$	0.130	0.100	**0.001**
VO2peak (ml/kg)	23.90 ± 2.97	22.98 ± 2.64	24.91 ± 2.24	27.58 ± 3.39$^{\#}$	0.078	**0.003**	**<0.001**
Body composition							
Weight (kg)	87.8 ± 2.5	88.8 ± 2.5 **	84.1 ± 2.2	83.3 ± 2.2**	0.787	0.187	**0.003**
BMI (kg/m²)	33.4 ± 0.7	33.8 ± 0.7**	34.1 ± 0.6	33.8± 0.6**	0.898	0.695	**0.003**
WC (cm)	103.4 ± 2.1	106.1 ± 2.1***	103.6± 1.8	100.4 ± 1.8**	0.700	0.331	**<0.001**
Body FM (%)	50.4 ± 0.9	50.6 ± 0.9	50.2 ± 0.8	50.1 ± 0.8	0.698	0.782	0.471
Android FM (%)	8.0 ± 0.3	7.9 ± 0.30	8.3 ± 0.3	8.1 ± 0.3	0.033	0.608	0.860
Gynoid FM (%)	19.4 ± 0.5	19.5 ± 0.5	18.5 ±0.4	18.2 ± 0.4$^{\#}$	0.139	0.095	**0.002**
VAT (cm^3)	931.1 ± 427.1	968.7 ±392.4	920.0 ±322.1	906.2 ± 346.9	0.266	0.619	0.196
SAT (cm^3)	5440.3 ± 1858.0	5532.8 ± 1890.0*	5489.3 ±1053.4	5444.7 ± 1260.7	0.640	0.823	**0.026**
Ectopic fat							
Liver fat %	4.72 (4.33-5.41)	4.54 (4.21-5.29)	5.23 (4.47-6.47)	4.84 (3.93-5.52)	0.679	0.921	0.069
Insulin sensitivity							
$S_I x10^{-4}(mU/L)^{-1}min^{-1}$	2.01 (1.29-3.24)	1.83 (1.65-2.64)	2.04 (1.20-2.77)	2.17 (1.45-3.69)*	0.145	0.422	**0.045**
Fasting glucose (mmol/L)	5.0 (±0.7)	5.1 (±0.8)	5.5 (±0.8)	5.1 (±1.0)	0.169	0.645	0.217
Fasting Insulin (µIU/mL)	13.0 (9.6-14.2)	12.9 (7.7-19.6)	14.8 (6.4-19.1)	12.6 (10.5-17.1)	0.811	0.977	0.773
HOMA-2IR	1.8 (1.4-2.2)	2.0 (1.1-2.9)	2.2 (1.0-2.9)	1.9 (1.4-2.6)	0.956	0.916	0.943
Circulating inflammatory markers							
hsCRP (mg/L)	2.5 (1.9-8.0)	4.02 (2.8-9.6)	5.0 (2.1-11.5)	4.6 (2.8-9.0)	0.151	0.575	0.225
TNFα (pg/mL)	7.0 (3.8-8.5)	6.4 (3.7-9.3)	4.1 (3.27-7.8)	4.6 (4.0-6.3)	0.816	0.461	0.515

MCP1 (pg/mL)	487.0 (334.9-537.7)	453.3 (356.6-608.3)	283.6 (237.5-370.3)	302.8 (235.4-431.1)	0.206	**0.042**	0.358
IL-8 (pg/mL)	3.0 (2.3-5.5)	4.0 (1.1-6.7)	2.3 (0.2-4.1)	2.3 (1.7-5.1)	0.859	0.382	0.377
Leptin (ng/mL)	67.1 (57.2-79.3)	75.7 (53.7-80.2)	62.5 (47.3-81.5)	63.4 (46.7-79.1)	0.900	0.305	0.613
Adiponectin (µg/mL)	3.9 (3.3-5.2)	3.6 (2.0-4.7)	3.3 (1.9-5.0)	2.1 (1.3-3.3)	<0.001	0.060	0.793

*Data presented as means ± SD for normally distributed variables and median (25^{th} - 75^{th} percentile) for skewed variables; *P<0.05, **P<0.01 and ***p<0.001 represent the difference between the groups after the intervention and $^{\#}$P<0.01 and $^{\#}$P<0.001 represents the difference post vs pre in the exercise group. BMI: body mass index; WC: waist circumference; FM: fat-mass; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue; VO_2max: maximal oxygen uptake, used as a measure of cardiorespiratory fitness; S_I: insulin sensitivity; hsCRP: high-sensitive C-reactive protein; HOMA2IR: homeostatic model assessment of insulin resistance; TNFα: tumor necrosis factor-alpha; IL-8: interleukin 8; MCP1: monocyte chemoattractant protein 1.*

Table 4.2. Daily energy, macronutrient and fat intake at baseline and after the 12-week exercise training intervention in both groups

Variables	CONTROL (n=15)		EXERCISE (n=20)		P VALUE
	Pre	Post	Pre	Post	Interaction
Energy (kJ)	12102 (7571-14377)	10743 (8479-12547)	13546 (10162-15580)	11597 (10542-14506)	0.359
Total protein (g)	74.8 (59.4-114-2)	73.8 (70.4-95.9)	101.1 (90.0-115.1)	89.7 (83.0-106.2)	0.418
Protein (%E)	13.8 (11.4-14.3)	13.6 (12.7-14.5)	13.2 (12.4-13.9)	12:8 (12:0-14:1)	0.346
Total CHO (g)	352.9 (246.3-435.4)	318.4 (271.9-411.6)	413.4 (312.3-484.8)	363.8 (286.0-429.4)	0.189
CHO (%E)	53.2 (49.1-56.4)	54.2 (49.2-55.9)	52.6 (49.8-54.2)	53.5 (50.4-56.1)	0.645
Total Fat (g)	113.0 (58.1-125.5)	85.7 (76.2-107.2)	115.5 (86.7-135.8)	100.5 (80.7-124.7)	0.241
Fat (% E)	34.0 (28.4-36.1)	32.2 (29.5-35.4)	33.3 (31.7-37.1)	31.5 (29.7-34.4)	0.288
SFA (g)	29.1 (18.8-40.6)	25.1 (21.7-38.6)	34.4 (22.9-44.5)	29.6 (23.7-37.7)	0.237
SFA (%E)	9.1 (4.1-11.8)	9.7 (8.2-11.3)	9.5 (7.9-11.3)	9.55 (8.0-10.2)	0.327
MUFA (g)	35.7 (19.2-41.6)	29.9 (23.5-35.3)	40.7 (31.1-51.6)	34.4 (28.4-3.6)	0.324
MUFA (%E)	11.2 (9.6-12.2)	11.5 (10.0-12.2)	11.1 (10.7-12.9)	11.2 (9.4-12.5)	0.172
PUFA (g)	26.6 (15.5-34.5)	18.5 (15.6-22.5)	25.8 (0.7-36.2)	22.6 (18.2-29.8)	0.479
PUFA (%E)	7.9 (7.2-10.1)	7.1 (6.4-8.4)	7.9 (7.2-9.6)	7.5 (6.6-8.2)	0.717

Data presented as median (25^{th} - 75^{th} percentiles). kJ: kilojoules; CHO: carbohydrate; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

4.1.1. Exercise training-induced changes in RBC-TPL fatty acid composition

At baseline, RBC-TPL FA composition was not different between groups and constituted of 44% SFA, 16% MUFA and 40% PUFA. In response to exercise training (**Table 4.3**), RBC-TPL total SFAs, individual and total MUFAs did not change and were not different between the groups. However, stearic acid (18:0) showed a significant group effect such that it was lower in the exercise group compared to the control group at post-training ($p=0.028$). Moreover, arachidic acid (20:0) decreased in the exercise group ($p=0.040$) and not in the control group ($p=0.525$) after the intervention period. In terms of PUFA, eicosadienoic acid (20:2n-6) decreased in both groups ($p=0.002$; time effect), whereas a trend was noted for an interaction effect ($p=0.069$) for DGLA (20:3n-6) such that it decreased in the exercise group ($p=0.019$) and not in the control group ($p=0.726$). Accordingly, the estimated enzyme activity of D5D (AA/DGLA) increased in the exercise group only ($p=0.021$), while D6D (GLA/LA) and SCD1-16 activities tended to be lower in the exercise compared to the control group at post-intervention ($p=0.070$ and $p=0.048$, respectively).

Table 4.3. RBC-TPL fatty acid composition (percentage) at baseline and in response to the 12-week exercise training intervention

Variables	CONTROL (n=15)		EXERCISE (n=20)		P VALUES		
	Pre	**Post**	**Pre**	**Post**	**Time**	**Group**	**Interaction**
Saturated fatty acids (SFAs)							
14:0 (Myristic acid)	0.21 (0.18-0.25)	0.24 (0.21-0.26)	0.23 (0.2-0.3)	0.25 (0.22-0.28)	0.807	0.354	0.333
16:0 (Palmitic acid)	22.00 (20.45-23.21)	21.33 (20.73-22.52)	21.31 (20.35-22.36)	21.31 (20.61-22.93)	0.560	0.663	0.790
18:0 (Stearic acid)	16.56 ± 0.91	16.82 ± 1.05	16.21 ± 0.75	16.11 ± 1.01	0.520	**0.080**	0.137
20:0 (Arachidic acid)	0.34 ± 0.04	0.34 ± 0.04	0.36 ± 0.05	0.33 ± 0.04	**0.069**	0.747	0.369
22:0 (Behenic acid)	1.33 ± 0.14	1.35 ± 0.18	1.42 ± 0.26	1.41 ± 0.18	0.897	0.219	0.594
24:0 (Lignoceric acid)	3.87 ± 0.58	4.02 ± 0.55	4.11 ± 0.80	4.19 ± 0.62	0.346	0.299	0.778
Total SFA	44.33 ± 1.82	44.38 ± 1.97	44.44 ± 1.81	44.21 ± 1.54	0.820	0.951	0.729
Mono-unsaturated fatty acids (MUFAs)							
16:1n-7 (Palmitoleic acid)	0.22 (0.19-0.3)	0.26 (0.20-0.30)	0.21 (0.19-0.27)	0.22 (0.18-0.27)	0.639	0.289	0.132
18:1n-7 (*cis*-Vaccenic acid)	1.13 (0.95-1.28)	1.06 (0.97-1.38)	1.05 (0.92-1.25)	1.11 (1.00-1.23)	0.277	0.360	0.588
18:1n-9 (Oleic acid)	10.84 (10.38-11.01)	10.95 (10.49-11.44)	10.31 (10.01-11.30)	10.59 (9.97-11.62)	0.765	0.539	0.777
20:1n-9 (Eicosenoic acid)	0.18 ± 0.03	0.18 ± 0.03	0.17 ± 0.02	0.18 ± 0.02	0.734	0.974	0.130
24:1n-9 (Nervonic acid)	3.27 (3.1-3.5)	3.53 (3.27-3.79)	3.63 (3.3-3.8)	3.68 (3.27-4.01)	**0.068**	0.423	0.987
Total n-7 MUFA	1.43 ± 0.36	1.46 ± 0.41	1.30 ± 0.24	1.32 ± 0.19	0.418	0.247	0.936
Total n-9 MUFA	14.39 (13.81-14.94)	14.53 (14.17-15.31)	14.23 (13.72-14.64)	14.47 (13.67-15.17)	**0.091**	0.768	0.818
Total MUFA	15.57 (15.03-16.23)	15.91 (15.47-16.47)	15.44 (15.05-15.82)	15.66 (15.08-16.48)	**0.036**	0.542	0.872
Poly-unsaturated fatty acids (PUFAs)							
20:5n-3 (EPA)	0.40 (0.32-0.47)	0.4 (0.33-0.50)	0.46 (0.36-0.53)	0.41 (0.37-0.44)	0.205	0.457	0.174
22:5n-3 (DPA n-3)	1.81 (1.66-2.06)	1.83 (1.75-1.96)	2. 00 (1.75-2.12)	1.9 (1.82-2.05)	0.548	0.531	0.998
22:6n-3 (DHA)	4.05 (3.78-4.56)	4.12 (3.80-4.50)	4.52 (3.98-507)	4.47 (4.16-4.96)	0.617	0.143	0.881
18:2n-6 (LA)	11.57 ± 1.59	11.36 ± 1.50	11.76 ± 1.55	11.57 ± 1.16	0.119	0.654	0.913
20:2n-6 (Eicosadienoic acid)	**0.35 ± 0.03**	**0.33 ± 0.04***	**0.36 ± 0.05**	**0.34 ± 0.04***	**0.002**	0.651	0.824

20:3n-6 (DGLA)	1.55 ± 0.26	1.56 ± 0.25	**1.54 ± 0.23**	**1.47 ± 0.18***	0.187	0.479	**0.069**
20:4n-6 (AA)	15.73 ± 0.98	15.68 ± 1.41	15.43 ± 1.29	15.66 ± 1.24	0.580	0.687	0.392
22:4n-6 (Adrenic acid)	3.44 ± 0.57	3.46 ± 0.64	3.24 ± 0.63	3.36 ± 0.42	0.282	0.408	0.455
Total PUFA	39.87 (38.35-41.15)	39.35 (38.56-41.23)	39.82 (38.76-41.26)	39.58 (38.72-41.55)	0.754	0.581	0.716
Total n-6	34.31 ± 1.71	33.06 ± 1.71	32.99 ± 2.09	33.07 ± 1.57	0.709	0.782	0.490
Total n-3	6.15 (5.83-6.74)	6.29 (6.05-6.95)	6.93 (6.27-7.52)	6.93 (6.49-7.15)	0.973	0.164	0.717
Estimated enzyme activities							
D5D	10.47 ± 2.04	10.34 ± 2.13	**10.32 ± 2.11***	**10.86 ± 1.83***	0.239	0.778	**0.057**
D6D	0.136 ± 0.020	**0.139 ± 0.019**	0.133 ± 0.022	**0.127 ± 0.017***	0.667	0.213	**0.086**
(SCD1)-16	0.010 (0.010-0.014)	**0.012 (0.010-0.014)**$^{\#}$	0.010 (0.010-0.013)	**0.010 (0.010-0.011)**$^{\#}$	0.455	0.300	**0.047**
(SCD1)-18	0.65 (0.65-0.65)	0.66 (0.64-0.66)	0.65 (0.64-0.68)	0.66 (0.64-0.68)	0.875	0.738	0.228

*Data presented as means ± SD for normally distributed variables and median (25^{th} - 75^{th} percentile) for skewed variables; *p<0.05 represents the difference between the groups in response to the intervention and $^{\#}$p<0.05 represents the difference between the groups at post-training. LA: linoleic acid; EPA: Eicosapentaenoic acid; DPA: Docosapentaenoic acid; DHA: Docosahexaenoic acid; DGLA: Dihomo-γ-linolenic acid; AA: Arachidonic acid; D5D: delta-5-desaturase index (20:4n-6/20:3n-6); D6D: delta-6-desaturase index (18:3n-6/18:2n-6); SCD1: Stearoyl-CoA desaturase-1 (SCD1-16: 16:1n-7/16:0; SCD1-18: 18:1n-9/18:0).*

4.2.2. Changes in the fatty acid composition of SAT depots in response to exercise training

The baseline FA composition of SAT depots was not different between the groups and consisted of 32% SFA, 42% MUFA and 26% PUFA in aSAT and 31% SFA, 43% MUFA and 26% PUFA in gSAT. **Table 4.4** and **Tables 4.5** present the changes in FA composition in SAT following the exercise training. Within the aSAT depot (**Table 4.4**), lauric (12:0) and myristic (14:0) acids decreased in the exercise group ($p=0.006$ and $p=0.001$, respectively) with no change in the control group ($p>0.05$). In contrast, the mono-unsaturated eicosenoic acid (20:1n-9) increased in the exercise group only ($p=0.003$), whereas oleic acid (18:1n-9) and total n-9 MUFA content increased in the control group ($p=0.035$ and $p=0.031$, respectively) and not in the exercise group ($p>0.05$). In terms of PUFA, eicosadienoic (20:2n-6) and adrenic (22:4n-6) acids increased in the exercise ($p=0.023$ and $p=0.018$, respectively) and not in the control group ($p>0.05$). The estimated enzyme activities (D5D, D6D and SCD1-16 and 18) did not change in the aSAT of either group in response to the exercise training intervention (**Table 4.4**).

Within the gSAT depot (**Table 4.5**), only GLA (18:3n-6) decreased in the exercise group ($p=0.007$) and not the control group ($p>0.05$). However, within the control group, total SFA increased ($p=0.046$), while palmitoleic acid (16:1n-7) and SCD1-16 decreased over the 12-week intervention period ($p=0.034$ and $p=0.023$, respectively).

Table 4.4. Fatty acid composition (percentage) of abdominal subcutaneous adipose tissue at baseline and in response to the 12-week exercise training intervention

Variables	CONTROL (n=15)		EXERCISE (n=20)		P VALUES		
	Pre	Post	Pre	Post	Time	Group	Interaction
Saturated fatty acids (SFAs)							
12:0 (Lauric acid)	0.36 (0.32-0.46)	0.40 (0.33-0.46)	**0.49 (0.38-0.62)****	**0.45 (0.37-0.56)****	0.251	0.085	**0.019**
14:0 (Myristic acid)	2.79 (2.44-3.17)	2.86 (2.51-3.07)	**3.08 (2.70-3.37)****	**2.94 (2.64-3.25)****	0.120	0.359	**0.004**
16:0 (Palmitic acid)	22.43 ± 1.31	22.60 ± 1.21	22.77 ± 1.08	22.64 ± 0.74	0.794	0.535	0.448
17:0 (Heptadecanoic acid)	0.38 (0.35-0.44)	0.36 (0.33-0.47)	0.39 (0.35-0.44)	0.40 (0.35-0.45)	0.989	0.742	0.965
18:0 (Stearic acid)	5.72 ± 1.66	5.54 ± 1.46	5.60 ± 1.01	5.77 ± 1.20	0.829	0.935	0.257
20:0 (Arachidic acid)	0.15 (0.12-0.18)	0.13 (0.11-0.18)	0.14 (0.13-0.18)	0.16 (0.13-0.18)	0.706	0.583	0.306
22:0 (Behenic acid)	0.05 (0,04-0,05)	0.05 (0.04-0.05)	0.04 (0.04-0.05)	0.04 (0.04-0.05)	0.706	0.932	0.186
Total SFA	31.94 ± 3.34	31.96 ± 2.95	32.52 ± 1.62	32.43 ± 1.63	0.742	0.538	0.683
Mono-unsaturated fatty acids (MUFAs)							
16:1n-7 (Palmitoleic acid)	6.11 ± 2.13	6.00 ± 1.91	5.64 ± 1.34	5.48 ± 1.44	0.248	0.361	0.709
18:1n-7 (*cis*-Vaccenic acid)	2.79 ± 0.57	2.84 ± 0.55	2.72 ± 0.35	2.84 ± 0.40	0.182	0.756	0.691
18:1n-9 (Oleic acid)	**32.66 (31.74-33.06)***	**32.84 (32.04-33.51)***	32.39 (32.04-32.92)	32.78 (32.05-33.70)	**0.012**	0.772	0.504
20:1n-9 (Eicosenoic acid)	0.67 ± 0.10	0.67 ± 0.12	**0.70 ± 0.13**	**0.76 ± 0.10****	**0.059**	0.135	**0.033**
Total n-7 MUFA	8.91 ± 7.65	8.84 ± 2.40	8.36 ± 1.55	8.32 ± 1.72	0.601	0.413	0.864
Total n-9 MUFA	**33.32 (32.46-33.78)***	**33.58 (32.72-34.27)***	33.07 (32.81-33.45)	33.51 (32.71-33.50)	**0.006**	0.645	0.664
Total MUFA	41.89 ± 3.05	42.27 ± 2.58	41.56 ± 1.47	41.85 ± 1.88	0.133	0.585	0.641
Poly-unsaturated fatty acids (PUFAs)							
18:3n-3 (ALA)	1.16 ± 0.26	1.15 ± 0.26	1.07 ± 0.18	1.04 ± 0.21	0.234	0.219	0.541
20:3n-3 (Eicosatrienoic acid)	0.05 (0.05-0.06)	0.05 (0.05-0.05)	0.04 (0.04-0.05)	0.05 (0.04-0.05)	0.804	0.513	0.217
20:5n-3 (EPA)	0.07 (0.04-0.11)	0.07 (0.04-0.08)	0.07 (0.06-0.10)	0.08 (0.06-0.09)	0.349	0.261	0.816
22:5n-3 (DPA n-3)	0.23 ± 0.09	0.23 ± 0.09	0.24 ± 0.08	0.26 ± 0.09	0.638	0.495	0.195

22:6n-3 (DHA)	0.20 ± 0.06	0.19 ± 0.06	0.23 ± 0.08	0.24 ± 0.08	0.822	0.215	0.436
18:2n-6 (LA)	22.42 ± 2.00	22.62 ± 2.20	22.24 ± 1.38	21.91 ± 1.15	0.823	0.544	0.179
18:3n-6 (GLA)	0.17 ± 0.03	0.16 ± 0.03	0.16 ± 0.03	0.15 ± 0.02	**0.053**	0.024	0.826
20:2n-6 (Eicosadienoic acid)	0.47 (0.36-0.57)	0.50 (0.36-0.55)	**0.44 (0.41-0.52)***	**0.49 (0.45-0.57)***	0.178	0.355	**0.090**
20:3n-6 (DGLA)	0.37 ± 0.16	0.37 ± 0.15	0.37 ± 0.10	0.39 ± 0.10	0.441	0.858	0.347
20:4n-6 (AA)	0.68 ± 0.23	0.66 ± 0.21	0.67 ± 0.12	0.70 ± 0.16	0.844	0.831	0.364
22:4n-6 (Adrenic acid)	0.27 ± 0.11	0.27 ± 0.11	**0.27 ± 0.07***	**0.30 ± 0.08***	0.262	0.745	**0.042**
22:5n-6 (DPA n-6)	0.07 ± 0.03	0.07 ± 0.02	0.08 ± 0.03	0.08 ± 0.03	0.411	0.230	0.564
Total PUFA	26.17 ± 2.45	26.28 ± 2.73	25.92 ± 1.46	25.71 ± 1.53	0.868	0.628	0.526
Total n-6	24.46 ± 2.21	24.61 ± 2.39	24.26 ± 1.36	24.06 ± 1.31	0.679	0.635	0.431
Total n-3	1.71 ± 0.37	1.67 ± 0.41	1.66 ± 0.29	1.65 ± 0.36	0.448	0.706	0.741
Estimated enzyme activities							
D5D	1.92 ± 0.44	1.89 ± 0.37	1.90 ± 0.40	1.84 ± 0.37	0.600	0.890	0.802
D6D	0.016 ± 0.006	0.016 ± 0.007	0.017 ± 0.005	0.018 ± 0.004	0.416	0.709	0.283
(SCD1)-16	0.28 ± 0.10	0.27 ± 0.10	0.25 ± 0.06	0.24 ± 0.047	0.167	0.471	0.528
(SCD1)-18	5.75 (4.93-7.51)	6.34 (4.75-7.89)	5.87 (5.28-6.69)	5.78 (4.78-6.40)	0.520	0.812	0.392

*Data presented as means ± SD for normally distributed variables and median (25^{th}-75^{th} percentile) for skewed variables; *p<0.05 and **p<0.01 represent the difference between the groups in response to the intervention. ALA: alpha-linolenic acid; LA: linoleic acid; EPA: Eicosapentaenoic acid; DPA: Docosapentaenoic acid; DHA: docosahexaenoic acid; GLA: γ-linolenic acid; DGLA: dihomo-γ-linolenic acid; AA: Arachidonic acid; D5D: delta-5-desaturase index (20:4n-6/20:3n-6); D6D: delta-6-desaturase index (18:3n-6/18:2n-6); SCD1: stearoyl-CoA desaturase-1 (SCD1-16: 16:1n-7/16:0; SCD1-18: 18:1n-9/18:0).*

Table 4.5. Fatty acid composition (percentage) of gluteal subcutaneous adipose tissue at baseline and in response to the 12-week exercise training intervention

Variables	CONTROL (n=15)		EXERCISE (n=20)		P VALUE		
	Pre	Post	Pre	Post	Time	Group	Interaction
Saturated fatty acids (SFAs)							
12:0 (Lauric acid)	0.38 (0.25-0.49)	0.33 (0.26-0.48)	0.47 (0.32-0.50)	0.44 (0.32-0.61)	0.443	0.141	0.946
14:0 (Myristic acid)	2.56 ± 0.84	2.69 ± 0.47	2.86 ± 0.47	2.84 ± 0.42	0.921	0.346	0.347
16:0 (Palmitic acid)	21.43 ± 1.35	21.80 ± 1.22	21.95 ± 1.07	22.18 ± 0.79	**0.069**	0.201	0.728
17:0 (Heptadecanoic acid)	0.35 (0.32-0.44)	0.36 (0.31-0.44)	0.42 (0.36-0.44)	0.39 (0.34-0.47)	0.773	0.184	0.283
18:0 (Stearic acid)	4.80 ± 1.59	5.15 ± 1.50	5.31 ± 1.23	5.48 ± 1.02	**0.077**	0.389	0.624
20:0 (Arachidic acid)	0.13 (0.10-0.18)	0.14 (0.12-0.17)	0.15 (0.12-0.19)	0.16 (0.13-0.18)	0.479	0.431	0.714
22:0 (Behenic acid)	0.05 (0.04-0.07)	0.04 (0.04-0.06)	0.05 (0.04-0.07)	0.05 (0.04-0.06)	0.236	0.661	0.357
Total SFA	**29.72 ± 3.55***	**30.58 ± 2.86***	31.19 ± 2.22	31.55 ± 1.71	**0.033**	0.166	0.366
Mono-unsaturated fatty acids (MUFAs)							
16:1n-7 (Palmitoleic acid)	**7.27 ± 2.30***	**6.64 ± 2.28***	6.20 ± 1.84	5.91 ± 1.45	**0.010**	0.175	0.533
18:1n-7 (*cis*-Vaccenic acid)	3.06 ± 0.61	3.01 ± 0.62	2.96 ± 0.41	2.88 ± 0.21	0.131	0.428	0.688
18:1n-9 (Oleic acid)	32.37 (32.02-33.25)	33.38 (32.54-33.31)	32.32 (31.70-33.01)	32.70 (32.28-33.28)	0.193	0.776	0.189
20:1n-9 (Eicosenoic acid)	0.69 ± 0.11	0.71 ±0.11	0.72 ± 0.10	0.75 ± 0.11	**0.098**	0.324	0.782
Total n-7 MUFA	10.34 ± 2.81	9.65 ± 2.82	9.16 ± 2.18	9.79 ± 1.67	**0.015**	0.181	0.677
Total n-9 MUFA	33.13 (32.82-33.82)	33.27 (32.24-33.03)	33.11 (32.67-33.74)	33.50 (33.04-34.13)	0.100	0.667	0.140
Total MUFA	43.59 ± 3.12	42.92 ± 3.19	42.41 ± 2.19	42.39 ± 1.75	0.101	0.282	0.310
Poly-unsaturated fatty acids (PUFAs)							
18:3n-3 (ALA)	1.17 (1.00-1.37)	1.21 (0.89-1.26)	1.09 (0.94-1.25)	1.07 (0.93-1.17)	**0.016**	0.161	0.712
20:3n-3 (Eicosatrienoic acid)	0.05 (0.04-0.05)	0.05 (0.05-0.06)	0.05 (0.04-0.06)	0.04 (0.04-0.05)	0.759	0.763	0.182
20:5n-3 (EPA)	0.08 (0.05-0.1)	0.06 (0.04-0.10)	0.09 (0.08-0.12)	0.08 (0.06-0.10)	**0.090**	**0.089**	0.361
22:5n-3 (DPA n-3)	0.26 ± 0.10	0.25 ± 0.10	0.27 ± 0.08	0.27 ± 0.08	0.308	0.705	0.916

22:6n-3 (DHA)	0.22 ± 0.08	0.21 ± 0.07	0.26 ± 0.08	0.24 ± 0.07	0.310	0.159	0.456
18:2n-6 (Linoleic acid)	22.72 ± 2.08	22.56 ± 2.08	22.34 ± 1.48	22.14 ± 1.25	0.298	0.600	0.461
18:3n-6 (GLA)	0.176 ± 0.035	0.170 ± 0.039	**0.171 ± 0.030****	**0.157 ± 0.028****	**0.012**	0.355	0.270
20:2n-6 (Eicosadienoic acid)	0.49 ± 0.13	0.50 ± 0.12	0.51 ± 0.10	0.51 ± 0.09	0.356	0.445	0.712
20:3n-6 (DGLA)	0.42 ± 0.16	0.43 ± 0.16	0.43 ± 0.11	0.43 ± 0.12	0.986	0.953	0.416
20:4n-6 (AA)	0.70 ± 0.21	0.71 ± 0.25	0.76 ± 0.16	0.73 ± 0.14	0.569	0.591	0.275
22:4n-6 (Adrenic acid)	0.29 ± 0.12	0.30 ± 0.12	0.31 ±0.07	0.30 ± 0.08	0.855	0.771	0.464
22:5n-6 (DPA n-6)	0.06 (0.05-0.09)	0.07 (0.05-0.09)	0.08 (0.06-0.10)	0.08 (0.06-0.10)	0.368	0.326	0.161
Total PUFA	26.68 ± 2.56	26.49 ± 2.53	26.41 ± 1.64	26.06 ± 1.45	**0.074**	0.690	0.999
Total n-6	24.88 ± 2.23	24.75 ± 2.25	24.64 ± 1.50	24.38 ± 1.29	0.181	0.715	0.904
Total n-3	1.80 ± 0.44	1.74 ± 0.39	1.76 ± 0.25	1.68 ± 0.29	**0.053**	0.661	0.771
Estimated enzyme activities							
D5D	1.73 (1.50-1.98)	1.63 (1.58-1.93)	1.77 (1.43-2.02)	1.70 (1.55-1.87)	0.275	0.668	0.636
D6D	0.019 ± 0.007	0.019 ± 0.007	0.019 ± 0.005	0.020 ± 0.005	0.856	0.807	0.432
(SCD1)-16	**0.36 (0.24-0.43)***	**0.30 (0.30-0.41)***	0.27 (0.23-0.34)	0.27 (0.22-0.31)	**0.012**	0.207	0.343
(SCD1)-18	7.12 (6.33-8.26)	6.83 (5.01-8.19)	6.21 (5.77-6.81)	6.00 (5.46-7.06)	**0.074**	0.235	0.509

*Data presented as means ± SD for normally distributed variables and median (25^{th}-75^{th} percentile) for skewed variables; *p<0.05 and **p<0.01 represent the difference between the groups in response to the intervention. ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; GLA: γ-linolenic acid; DGLA: dihomo-γ-linolenic acid; AA: arachidonic acid; D5D: delta-5-desaturase index (20:4n-6/20:3n-6); D6D: delta-6-desaturase index (18:3n-6/18:2n-6); SCD1: stearoyl-CoA desaturase-1 (SCD1-16: 16:1n-7/16:0; SCD1-18: 18:1n-9/18:0.*

4.2.3. Effect of exercise training on SAT gene expression

The expression of genes involved in SAT adipogenesis, lipid metabolism, insulin signalling and inflammation (**Table 4.6**) were evaluated in aSAT and gSAT before and after the 12-week intervention. *ATGL* mRNA content decreased in gSAT of both groups ($p=0.009$ in control and $p=0.024$ in exercise group) and in aSAT of the control group ($p=0.044$), whereas *GLUT4* mRNA content increased in aSAT of both groups ($p=0.047$ in control and $p=0.018$ in exercise). Moreover, there was a time effect for adiponectin mRNA in both SAT depots such that it decreased in both groups ($p<0.05$) although this effect could not be attributed to the exercise training intervention. The remaining evaluated genes (*IRS1*, *SCL2A4*, *SMG*, *LPL*, *PPARγ, PLIN1* and *DGAT2*) did not change in response to the exercise training intervention.

Table 4.6. Depot-specific adipose tissue gene expression before and after the 12-week exercise training intervention

Variables	CONTROL (n=15)		EXERCISE (n=20)		P VALUE		
	Pre	Post	Pre	Post	Time	Group	Interaction
Abdominal subcutaneous adipose tissue (aSAT)							
Adipogenesis							
PPARγ mRNA (AU)	0.97 ± 0.40	0.93 ± 0.40	1.11 ± 0.55	0.88 ± 0.49	0.096	0.767	0.313
Lipogenesis & lipolysis							
LPL mRNA (AU)	0.94 (0.71-1.24)	0.75 (0.61-0.89)	1.03 (0.80-1.31)	0.90 (0.58-1.16)	**0.082**	0.293	0.305
PLIN1 mRNA (AU)	2.04 (1.28-3.03)	1.57 (1.15-2.20)	1.73 (1.07-2.47)	1.65 (1.44-2.56)	0.302	0.756	0.297
ATGL mRNA (AU)	1.34 (0.82-1.76)	1.08 (0.84-1.37)	1.02 (0.78-1.74)	0.95 (0.59-1.15)	**0.027**[c]	0.594	0.414
DGAT2 mRNA (AU)	0.92 (0.71-1.66)	1.57 (0.84-2.20)	1.02 (0.39-2.37)	1.53 (0.94-2.97)	0.085	0.655	0.662
Insulin signalling							
Adiponectin mRNA (AU)	0.76 (0.53-0.98)	0.53 (0.49-0.66)	0.79 (0.44-1.01)	0.62 (0.42-0.78)	**0.020**	0.627	0.800
GLUT4 mRNA (AU)	1.18 (1.01-1.77)	1.95 (1.46-2.43)	1.39 (0.71-2.43)	2.33 (1.33-3.13)	**0.003**[b]	0.535	0.915
IRS1 mRNA (AU)	1.17 (0.93-1.86)	1.57 (0.87-1.80)	1.41 (0.96-2.10)	1.35 (1.01-1.98)	0.945	0.360	0.772
SMG1 mRNA (AU)	1.35 (1.05-1.67)	1.23 (1.02-1.73)	1.30 (0.97-1.70)	1.36 (1.06-2.08)	0.849	0.953	0.151
Gluteal subcutaneous adipose tissue (gSAT)							
Adipogenesis							
PPARγ mRNA (AU)	1.15 ± 0.46	0.99 ± 0.43	1.13 ± 0.41	1.13 ± 0.46	0.325	0.403	0.471
Lipogenesis & lipolysis							
LPL mRNA (AU)	1.34 (0.83-1.45)	1.06 (0.74-1.35)	1.04 (0.80-1.34)	0.32 (0.69-1.51)	0.095	0.964	0.529
PLIN1 mRNA (AU)	1.97 (1.32-2.37)	1.60 (1.32-2.42)	1.83 (1.44-2.39)	1.81 (1.04-2.48)	0.232	0.918	0.844
ATGL mRNA (AU)	1.53 (1.04-2.35)	1.20 (0.93-1.49)	1.40 (1.05-1.70)	1.02 (0.87-1.46)	**0.001**[b]	0.542	0.577
DGAT2 mRNA (AU)	1.53 (1.13-2.23)	1.23 (0.64-2.17)	1.31 (0.84-1.92)	1.21 (0.94-1.86)	0.599	0.574	0.795

Insulin signalling							
Adiponectin mRNA (AU)	0.84 (0.50-1.10)	0.52 (0.47-0.69)	0.69 (0.58-0.82)	0.64 (0.50-0.80)	**0.026**[c]	0.530	0.166
GLUT4 mRNA (AU)	1.41 (1.19-1.76)	1.62 (0.78-2.70)	1.42 (0.92-1.98)	1.92 (1.24-2.27)	0.258	0.996	0.772
IRS1 mRNA (AU)	1.08 (0.94-1.62)	1.21 (1.02-1.73)	1.31 (0.92-1.86)	1.30 (1.07-1.78)	0.439	0.405	0.626
SMG1 mRNA (AU)	1.63 (1.14-1.83)	1.23 (1.05-1.92)	1.37 (1.11-1.63)	1.48 (1.20-1.95)	0.895	0.836	0.322

Data presented as means ± SD for normally distributed variables and median (25^{th}-75^{th} percentile) for skewed variables. [c]P<0.05: difference pre vs post in the control group; [b]P<0.01: difference pre vs post in both groups. PPARγ: peroxisome proliferator-activated receptor gamma; LPL: lipoprotein lipase; PLIN1: perilipin 1; ATGL: adipose triglyceride lipase; DGAT2: diacylglycerol acyltransferase 2; GLUT4, glucose transporter 4; IRS1, insulin receptor substrate 1; SMG1: serine/threonine-protein kinase;

4.2.4. Correlations between changes in fatty acid composition and changes in metabolic parameters

The associations between the changes in individual FAs and estimated enzyme activities and the changes in systemic inflammatory markers are presented in **Figure 4.1**. As the associations between these changes did not differ by group (no interaction effect), the data for the exercise and control groups were combined. Lower RBC-TPL DGLA and higher D5D activity correlated with lower circulating leptin concentrations ($r=0.445$; $p=0.006$ and $r=-0.388$; $p=0.021$, respectively). In contrast, lower RBC-TPL D6D activity was associated with lower circulating TNFα concentrations ($r=0.395$; $p=0.025$). Notably, lower RBC-TPL SCD1-16 correlated with decreased liver fat ($r=0.439$, $p=0.009$).

There were no significant associations between the changes of the individual FA or estimated enzyme activities in both SAT depots and the changes in SAT lipid metabolism or insulin signalling genes. Moreover, the changes in FA composition in RBC-TPL and SAT were not associated with the improvement in S_I.

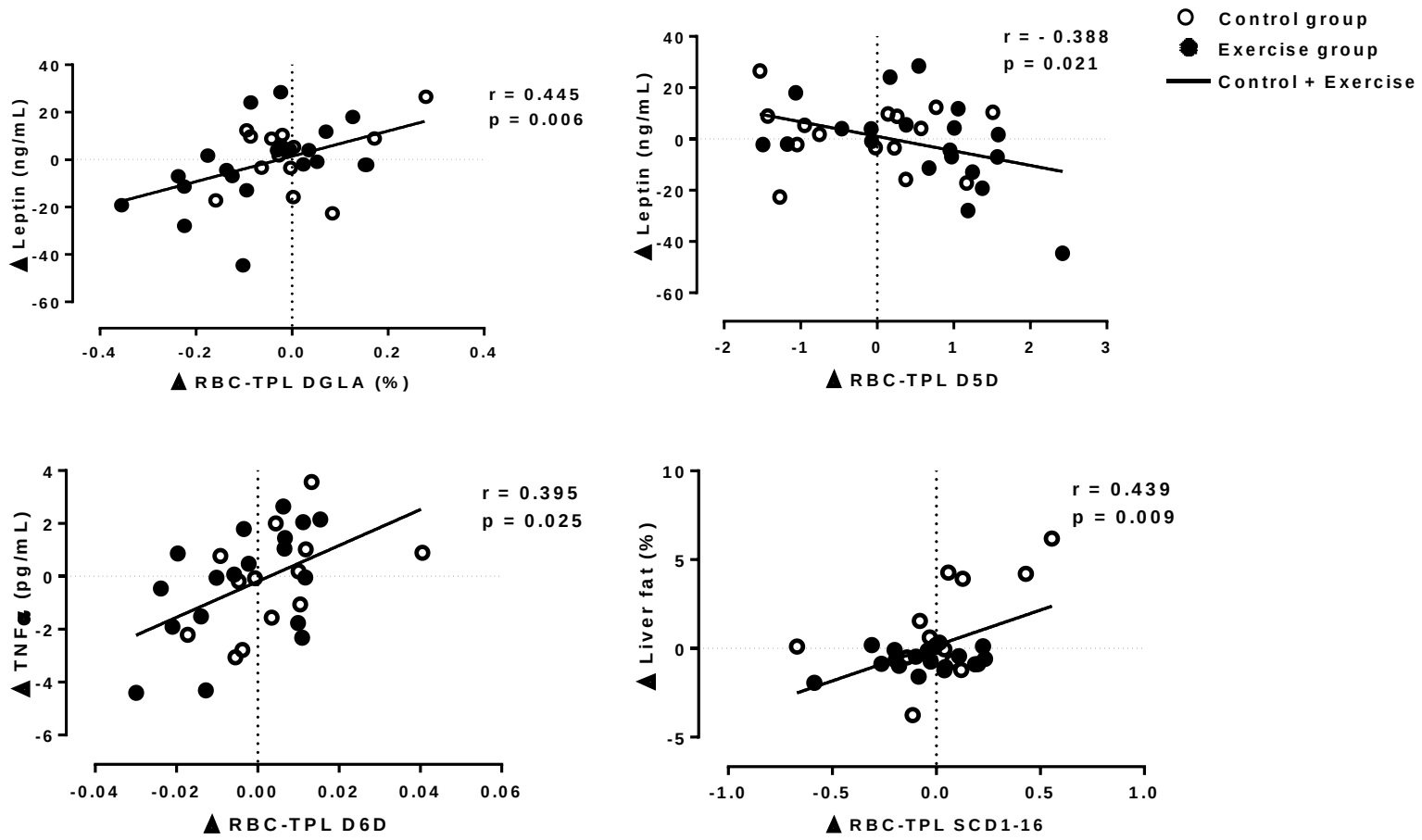


Figure 4.1. Correlations between changes in RBC fatty acids/estimated enzyme activities and changes of systemic inflammatory markers and liver fat in response to the exercise training intervention. *Pooled data from control (n=15) and exercise (n=20) presented as changes (Δ) from pre- to*

post-intervention. r and p values are from Pearson's pairwise correlations. DGLA: dihomo-γ-linolenic acid; D5D: delta-5-desaturase index (20:4n-6/20:3n6); D6D: delta-6-desaturase index (18:3n-6/18:2n-6), SCD1: stearoyl-CoA desaturase-1 (SCD1-16: 16:1n-7/16:0).

4.3. DISCUSSION

The novel findings of this randomized controlled trial were that 12 weeks of combined aerobic and resistance exercise training reduced RBC-TPL SCD1-16 and D6D (tendency), and increased D5D estimated activities, along with a reduction of DGLA (20:3n-6). These changes in estimated desaturase activities (and DGLA) were associated with decreased liver fat, as well as lower circulating leptin and TNFα concentrations. Within the SAT depots, exercise training induced a reduction of individual SFAs (12:0 and 14:0) and increased individual PUFAs (20:2n-6 and 22:4n-6) in aSAT, while in gSAT, only GLA (18:3n-6) decreased. In contrast, the expression of the genes involved in lipid metabolism and insulin signalling in both SAT depots did not change following this exercise training intervention.

The reduction in RBC-TPL SCD1-16 activity in response to the exercise intervention was accompanied by a reduction in individual RBC-TPL SFAs (18:0 and 20:0). The dietary intake of SFA and MUFA did not change in either group over the 12-week intervention period, suggesting that these changes were not mediated by dietary FA intake. Rather, SCD1 is a marker of DNL in the liver and AT, and is responsible for the conversion of SFA to MUFA as previously reported (390). Increased activity of this desaturase has been associated with obesity, inflammation, IR and metabolic syndrome (259, 362, 380). Conversely, SCD1-knockout mice are protected against the development of obesity, hypertriglyceridemia and IR (237, 239, 391). Moreover, the pharmacological inhibition of SCD1 in mice attenuated obesity-related hepatic steatosis (392). The reduction of SCD1-16 activity is therefore suggestive of a beneficial metabolic effect of exercise training on circulating FA metabolism. Notably, the reduction of SCD1-16 activity correlated with a reduction of liver fat in the present study. However, there was no association between the reduction of RBC-TPL SCD1-16 (or liver fat) and the improvement of S_I in response to exercise training. A previous study in black African individuals showed an inverse association between liver fat and insulin sensitivity (217), while others did not find this relationship (393). Moreover, although black African women have lower SCD1 activity than their white counterparts (389), they are less insulin sensitive (24). Hence, the lower activity of SCD1 in these women might explain their lower liver fat content as suggested by Chung *et al.* (389) but not the improvement in insulin sensitivity after the 12-week exercise training intervention.

In addition to the reduction of SCD1-16, there was a decrease of RBC-TPL DGLA and D6D (trend) and an increase of RBC-TPL D5D activity in response to this exercise training intervention, supporting our hypothesis of favourable changes in RBC FA metabolism induced

by exercise training. Plasma phospholipid D6D activity and DGLA content are higher in overweight and obese compared to lean individuals, which have been positively correlated with IR (244, 259, 359, 394). Moreover, circulating DGLA concentrations have consistently been shown to predict the risk of developing metabolic syndrome in obese individuals across four independent studies (reviewed in (390)) and identified as an independent determinant of IR in patients with T2D (394). In the present study, the decrease in RBC-TPL D6D activity and the consequent reduction in DGLA content were associated with lower systemic TNFα and leptin concentrations. DGLA can be metabolized by cyclooxygenases and lipoxygenases to produce 1-series prostaglandins (e.g. PGE_1) and 15-HETrE (15-(S)-hydroxy-8,11,13-eicosatrienoic acid), capable of suppressing inflammation (359, 395). Hence, in addition to the lower conversion rate of LA to DGLA from the reduced D6D activity, the decrease of DGLA could also stem from its use as a substrate for production of mild anti-inflammatory eicosanoids of the 1-series, to resolve the chronic low-grade pro-inflammatory state observed during obesity. On the other hand, the increased RBC-TPL D5D activity may also explain the reduction of DGLA by increased conversion rates of DGLA to AA. However, it is surprising that there was no increase in AA, especially given that African Americans are genetically predisposed for high conversion of medium-chain to long-chain PUFA (266), with stronger associations between *FADS* variants and increased AA compared to European Americans (265, 266). Nonetheless, the lack of increased AA after exercise training intuitively seems advantageous as this FA is a precursor of several potent bioactive eicosanoid products with pro-inflammatory properties (e.g. prostaglandins, thromboxane's and leukotrienes) (266). Further, the increased RBC-TPL D5D activity and decreased DGLA content were associated with decreased leptin concentrations in response to exercise training. Decreasing in D5D activity has been associated with high BMI, body fat and IR (244, 259, 359, 394). The findings of the present study suggest that the inverse association between RBC-TPL D5D activity and leptin reflect a protective, anti-inflammatory action of D5D on obesity-associated inflammation since leptin is a pro-inflammatory cytokine and an important regulator of body fatness (243). The lack of association between the changes in RBC-TPL desaturases and DGLA content and changes in S_I following this exercise training intervention suggest that the improvement of whole-body insulin sensitivity after exercise training is not mediated by the changes in circulating RBC FA metabolism.

Plasma FA composition can be modulated by AT lipid turnover, which is dependent on the lipolysis rate in response to exercise training (138). Moreover, exercise training can alter the exchange rate of phospholipid FA between plasma and RBC (396). The effects of exercise

training on SAT FA composition and gene expression were therefore investigated to clarify the mechanisms implicated in the alterations of RBC-TPL FA composition, and to ascertain whether the changes in SAT FA composition were associated with inflammatory markers, liver fat and/or insulin sensitivity. A reduction in individual SFAs (12:0 and 14:0) in aSAT was observed after exercise training, suggesting the use of these medium-chain FA as substrates for β-oxidation. Indeed, exercise training can activate the FA oxidation in adipocyte mitochondria to produce energy (305). The oxidation rate is determined by the FA chain type (saturated or unsaturated) and length, with the preferential oxidation of short to medium chains compared to long-chain FAs (397). Exercise training further increased percentages of individual polyunsaturated eicosadienoic (20:2n-6) and adrenic (22:4n-6) acids in aSAT, and decreased GLA (18:3n-6) in gSAT in the present study. These findings suggest an FA class- and tissue-specific effect of exercise training, as previously shown in other studies (221, 305). N-6 LC-PUFA content in the cell membrane has been reported to increase the number of insulin receptors, and enhances insulin binding and action, while SFAs decrease insulin binding and transport (398), and induce inflammation through NFκB/TLR4 related pathways (155). Therefore, the present study reports beneficial effects of exercise training via the reduction of individual SFAs and the increase of individual PUFAs in aSAT. These changes were not reflective of the changes in RBC-TPL FA composition, highlighting the tissue-specific effect of exercise training.

The modifications in SAT individual FAs could not be explained by changes in body fat mass, as there was only a small (but significant) change in body weight and no significant change in fat mass percentage or SAT volume in the exercise group. This suggests that the changes in SAT n-6 PUFA content are not mediated by the change in AT mass as previously shown by Sjögren *et al.* (221). These changes rather seem to reflect a direct effect of exercise training on SAT FA and lipid metabolism. However, there were no changes in the expression of selected genes involved in adipogenesis (*PPARγ*), lipogenesis (*DGAT2*, *LPL*, *PLIN1*, *GLUT4*), or lipolysis (*ATGL*), concomitantly with unaltered desaturase activities in both SAT depots after this intervention. The depot-specific changes of SAT FA composition were therefore unlikely due to alterations in the expression of these genes but could relate to changes in protein expression. It is also possible that these changes were the consequences of alterations in other pathways, as shown that lipolysis, inflammation and oxidative stress in AT are related and contributes to the impairment of AT function in obese state (52, 101, 111, 134, 139). This hypothesis requires further investigation. The participants of this study previously had a sedentary lifestyle, with very low cardiorespiratory fitness (VO_{2peak}) before participation in the

exercise training intervention. Thus, a longer duration of exercise training and/or higher intensity might be required to induce more profound changes in lipid and FA metabolism in SAT depots (138, 221). The lack of association between the changes in individual FAs in SAT depots and S_I suggests that the reported improvement in S_I in response to this intervention could have derived from modifications in other tissues such as skeletal muscle. Indeed, the FA composition of skeletal muscle has been associated with fasting insulin concentration and insulin sensitivity in normoglycemic individuals (398).

The strength of this randomised controlled exercise training study was the objective measurement of FA composition both in the blood (RBC) and in two major depots of SAT, in combination with detailed measures of systemic inflammatory markers, SAT gene expression, liver fat content and S_I. Moreover, the exclusion of potential confounders such as inflammatory-related diseases and medication during participant recruitment represent strengths of this research work. The reported dietary data were not sufficiently detailed for quantification of individual dietary FA composition, which may represent a limitation. However, it is unlikely that this would have influenced the outcomes as no dietary recommendations were given, but rather the participants were instructed to maintain their habitual dietary intake.

In conclusion, 12 weeks of exercise training altered RBC-TPL estimated desaturase enzyme activities by decreasing SCD1-16 and increasing D5D activity, along with a reduction of DGLA content and a trend for a reduction in D6D activity. These changes correlated with decreased liver fat content and lower circulating TNFα and leptin concentrations but were not associated with the improvement in S_I. Within the SAT depots, individual SFAs (lauric and myristic acids) decreased and individual n-6 PUFAs (eicosadienoic and adrenic acids) increased in aSAT, but these changes were not associated with changes in SAT lipid metabolism, liver fat or S_I. The mechanisms underlying the relationship between circulating FA profiles, desaturase activities, liver fat and systemic inflammation, as well as the clinical relevance of the changes in SAT individual FAs requires further investigation. Furthermore, the contribution of exercise-induced changes in AT metabolism and function in the improvement of S_I needs to be further elucidated. Of particular interest are the inflammatory and oxidative stress pathways, which have previously been associated with S_I, and importantly, are also subject to change during periods of increased energy demand/expenditure.

CHAPTER FIVE

CHANGES IN SYSTEMIC AND SUBCUTANEOUS ADIPOSE TISSUE INFLAMMATION AND OXIDATIVE STRESS IN RESPONSE TO EXERCISE TRAINING IN OBESE BLACK SOUTH AFRICAN WOMEN

Data from this chapter have been published in part in: Nono Nankam P.A., Mendham, A., De Smidt, M., Keswell, D., T. Olsson, Bluher, M., Goedecke, J. *Changes in systemic and subcutaneous adipose tissue inflammation and oxidative stress in response to exercise training in obese black African women.* J Physiol 598.3 (2020) pp 503–515.

5.1. INTRODUCTION

Obesity is associated with IR and the risk for developing T2D (134). One of the most accepted mechanisms linking obesity and IR is the pathological expansion of AT (134). During continuous energy intake exceeding energy expenditure, adipocytes progressively store excess lipids in the form of TGs, increasing their size and/or number. However, the pathophysiological expansion of AT is mainly exerted via an increase in cell size, which has been associated with alteration of immune cell populations and increase of pro-inflammatory processes (399). Activation of pro-inflammatory pathways may not only inhibit adipogenesis (400) but also stimulate ROS production (401). For instance, TNF-α can activate NADPH oxidase resulting in ROS production, and IFNγ is one of the most potent activators of macrophage-induced ROS production (171). On the other hand, elevated ROS concentrations can lead to IR via activation of inflammatory pathways such as NFkB and inhibitory phosphorylation of the insulin receptor (402-404). Notably, hypoxia associated with AT hypertrophy also increases macrophage infiltration, fibrosis and oxidative stress (405), resulting in cell damage, activation of stress signalling pathways, inflammatory processes and IR (401). Inflammation and oxidative stress are hence interrelated during obesity and are both suggested to be independent contributors to the development of IR (171, 406).

The distribution of AT is more important than total body fat mass to determine the individual obesity-related risk for cardio-metabolic diseases. Compared to SAT, VAT has the highest expression and secretion of pro-inflammatory cytokines and has been closely associated with IR (407). Depending on its location, the SAT also contributes to the development of oxidative and inflammatory phenotype (87, 408). Indeed, compared to peripheral SAT, abdominal SAT has a higher inflammatory profile and is negatively associated with insulin sensitivity (32, 408). In contrast, gluteo-femoral SAT is proposed to be "protective" against obesity-associated complications in obese individuals (408). Importantly, these relationships are modified by ethnicity (406). Black African women have more gluteo-femoral SAT and less VAT compared to their BMI- and age-matched white counterparts (30) but are less insulin sensitive (24). Linked to this, the gluteal AT depot of black African women has been shown to contain larger adipocytes (409) and higher expression of genes involved in hypoxia, fibrosis and inflammation (214), compared to white women. These findings suggest that the regulatory pathways underlying depot-specific AT metabolism and function and the associations with whole-body metabolism need to be investigated within specific ethnic groups. Further, studies using animal models have shown that AT from trained mice transplanted in untrained mice

improved their peripheral insulin sensitivity (87, 292), and the knockout of the insulin receptor in AT of mice (FIRKO mice) protected these animals against obesity-related glucose intolerance (410). Therefore, gluteal AT function might be altered in black African populations during excessive fat accumulation, potentially representing a link between obesity and impairment of insulin sensitivity in this ethnic group. Understanding the mechanisms underlying the causal molecular links between depot-specific AT function and obesity-associated IR in black African women is fundamental. This will be useful for future research focusing on targeted prevention and/or treatment strategies for obesity-associated complications in this ethnic group who is at particularly high risk for cardio-metabolic diseases (278).

In this chapter, exercise training was further used as a model to evaluate the link between inflammation and oxidative stress and insulin sensitivity in obese black SA women. Indeed, exercise training has been previously shown to attenuate AT inflammation by reducing the expression of pro-inflammatory adipocytokines (e.g. leptin, TNFα, IL-6, IFN-γ) and increasing adiponectin expression in AT (298). Moreover, although less extensively studied, exercise training has been reported to improve oxidative stress in the obese state by reducing circulating ROS levels (294), decreasing lipid peroxidation (315, 319) or by increasing antioxidant enzyme activity in AT (315). Further, findings from the previous chapter of this thesis (Chapter 4) showed an improvement of S_I in response to exercise training, but this was not associated with changes in circulating and SAT FA composition or SAT lipid metabolism. Whether the exercise-induced improvement in S_I is associated with changes in systemic and/or AT inflammation and oxidative stress in black SA women has not yet been investigated. Therefore, this study aimed to i) assess the effects of 12-week exercise training on systemic and depot-specific SAT (abdominal vs gluteal) inflammatory and oxidative stress status; and to ii) examine the relationship between these changes and the improvement of body composition and insulin sensitivity in obese black SA women. This study hypothesised that exercise training reduces systemic oxidative stress in obese black SA women and improves SAT inflammation and oxidative stress, which altogether contribute to the improvement of insulin sensitivity in these women.

5.2. RESULTS

5.2.1. Participant characteristics and dietary macronutrient intake

The adherence to this exercise training program as well as the participants' characteristics has been presented in Chapter 4 (**Table 4.1**). In brief, cardiorespiratory fitness and body composition were not different between the groups at baseline. Following the exercise training intervention, VO_{2peak} (ml/kg/min) increased in the exercise group only ($p<0.01$). There were small but significant decreases in body weight, BMI, WC and gynoid FM (%) in the exercise group ($p<0.01$). In contrast, body weight, BMI, WC ($p<0.01$) and SAT volume ($p<0.05$) increased in the control group. Liver fat tended to decrease in the exercise group ($p=0.074$), with no changes in the control group ($p=0.356$). Notably, S_I improved in the exercise compared to control group (median of 2.04 to 2.17 $x10^{-4}(mU/L)^{-1}min^{-1}$ vs. 2.01 to 1.83 $x10^{-4}(mU/L)^{-1}min^{-1}$, respectively; $p<0.05$ for interaction). No changes in circulating inflammatory markers were observed in both groups and dietary energy, macronutrient and FA intake were not significantly different between the groups and did not change in response to the intervention (as presented in **Table 4.2** of Chapter 4).

Protein expression in SAT depots was analysed in a sub-sample of participants in the present study. The characteristics of the sub-sample selected for this analysis are presented in **Table 5.1**. These participants had similar cardio-respiratory fitness, BMI and S_I between the groups at baseline. Following the intervention period, VO_{2peak} and S_I increased in the exercise group ($p<0.05$) with no significant changes in the control group ($p>0.05$, **Table 5.1**). Moreover, weight, BMI and SAT volume decreased in the exercise group ($p<0.01$) and increased in the control group ($p<0.05$). Gynoid fat and WC decreased in the exercise group ($p<0.05$) and was unchanged in the control group ($p>0.05$; **Table 5.1**). Notably, the basic characteristics of the sub-group selected were not different from the characteristics of the whole cohort presented in **Table 4.2**.

Table 5.1. Characteristics data (pre and post-exercise training) from the sub-sample selected for adipose tissue protein analysis.

Variables	EXERCISE (n=5)		CONTROL (n=5)		P VALUES		
	Pre	Post	Pre	Post	Time	Group	Interactions
Cardio-respiratory fitness							
VO_{2peak} (ml/min)	(2088.9 ± 271.7)	(2261.2 ± 286.9)	(2006.7 ± 268.7)	(2082.1 ± 195.6)	0.398	0.122	0.521
VO_{2peak} (ml/kg)	(26.3 ± 2.5)	(28.9 ± 2.8)*	(23.1 ± 2.9)	(23.4 ± 3.0)	0.104	**0.020**	0.181
Body composition							
Weight (kg)	(79.3 ± 8.7)	(77.7 ±9.1)**	(88.1 ± 15.2)	(90.4 ± 15.0)**	0.207	0.246	**<0.001**
BMI (kg/m²)	(33.1 ± 3.1)	(32.4 ± 3.3)**	(33.0 ± 3.0)	(33.9 ±3.1)**	0.740	0.427	**<0.001**
WC (cm)	(98.4 ± 5.4)	(95.6 ± 6.1)*	(102.0 ± 7.5)	(106.8 ± 7.5)	0.097	0.535	**0.043**
Body FM (%)	(50.3 ± 1.6)	(48.6 ± 1.8)	(51.6 ± 4.2)	(51.3 ± 4.2)	0.334	0.077	0.154
Android FM (%)	(8.3 ± 1.8)	(8.1 ± 1.8)	(7.9 ± 1.3)	(7.9 ± 1.6)	0.811	0.368	0.356
Gynoid FM (%)	(17.8 ± 2.3)	(17.4 ± 2.3)*	(18.7 ± 2.8)	(18.7 ± 2.8)	0.518	0.121	**0.024**
VAT (cm^3)	(1008.5 ± 500.0)	(959.8 ± 517.4)	(863.0 ± 389.0)	(1036.9 ± 300.5)	0.952	0.362	0.084
SAT (cm^3)	(5215.3 ± 686.2)	(4878.8 ± 687.4)*	(5886.1 ± 3148.5)	(6099.2 ± 2157.2)*	0.454	0.330	**0.002**
Insulin sensitivity							
$S_I x10^{-4}$(mU/L)$^{-1}$min^{-1}	1.2 (0.9-1.3)	2.3 (1.4-2.6)*	2.0 (1.3-3.1)	1.8 (1.6-3.1)	**0.040**	0.092	0.158

*Values are means ± SD for normally distributed variables and median (25^{th} - 75^{th} percentile) for non-normally distributed variables; *p<0.05 and **p<0.01 represent the difference post vs pre in each group. BMI: body mass index; WC: waist circumference; FM: fat-mass; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue; VO_2peak: maximal oxygen uptake, used as a measure of cardiorespiratory fitness; S_I: insulin sensitivity.*

5.2.2. Systemic oxidative stress markers in response to the intervention

Circulating markers of oxidative stress were altered in response to the exercise training and were unchanged in the control group (**Table 5.2**). Specifically, circulating TBARS concentrations decreased ($p<0.01$) while catalase activity increased ($p<0.05$) in the exercise group only. There was a tendency for SOD activity to increase ($p<0.1$) in the exercise group and there were no changes in total antioxidant capacity (ORAC) in both groups in response to the intervention.

Table 5.2. Changes in systemic oxidative stress markers in response to the 12-week exercise intervention in exercise and control groups

Variables	CONTROL (n=15)		EXERCISE (n=20)		P VALUES		
	Pre	Post	Pre	Post	Group	Time	Interaction
TBARS (µmol/L)	0.46 (0.42-0.49)	0.48 (0.44-0.51)	**0.49 (0.44-0.52)**	**0.46 (0.43-0.49)***	0.797	0.168	**0.003**
Catalase (UI/mg)	0.67 (0.27-1.97)	1.21 (0.55-1.85)	**0.80 (0.37-1.51)**	**1.04 (0.74-1.74)***	0.842	0.201	0.091
SOD (UI/mg)	0.17 (0.03-0.62)	0.21 (0.04-0.65)	**0.21 (0.14-0.52)**	**0.32 (0.17-0.48)#**	0.300	**0.049**	0.672
ORAC (nmol/L/mg)	823.3 (769.2-1080.8)	837.5 (779.1-1130.7)	919.3 (819.0-1171.2)	1012.5 (807.6-1146.9)	0.554	0.826	0.918

*Values are presented as median (interquartile range); **P<0.01, *P <0.05 and #P<0.1 represent the difference between post vs pre in the exercise group after the intervention (post-hoc). TBARS: thiobarbituric acid reactive substances; SOD: superoxide dismutase; ORAC: oxygen radical absorbance capacity.*

5.2.3. Gene and protein expression in subcutaneous adipose tissue

The expression of selected genes and protein levels were compared between the depots at baseline to evaluate depot-specific inflammatory and oxidative profiles (**Table 5.3**). *Leptin* mRNA content was higher in gSAT compared to aSAT ($p<0.01$) with no difference in *adiponectin* mRNA content. In terms of protein levels, MIF, MCP1, NFκBp65 and TNFα (detected at 12kDa, 25kDa, 65kDa and 25kDa respectively) were higher in aSAT than gSAT at baseline ($p<0.05$) without a difference in their mRNA content.

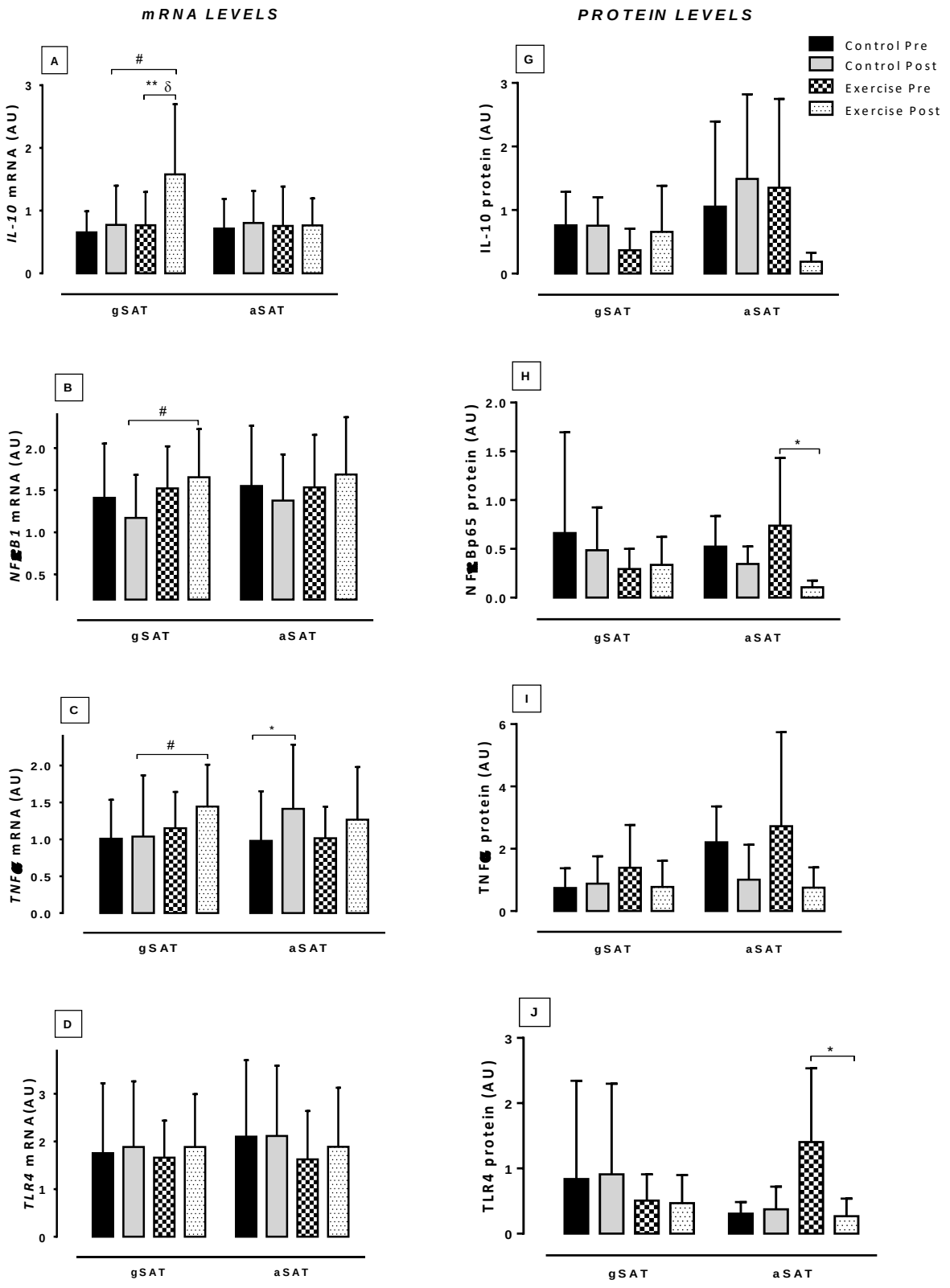
Table 5.3. Comparison of gene and protein expression between the gluteal (gSAT) and abdominal (aSAT) subcutaneous adipose tissue depots at baseline

		gSAT	aSAT	P value
Gene ID and definition				
ADIPOQ	Adiponectin	0.76 ± 0.31	0.79 ± 0.31	0.619
MCP1	Monocyte chemoattractant protein 1	1.16 ± 1.02	1.37 ± 1.25	0.216
IL-10	Interleukin 10	0.72 ± 0.43	0.73 ± 0.54	0.331
LEP	Leptin	**1.57 ± 0.97**	**1.22 ± 0.79**	**0.008****
MIF	Macrophage migration inhibitory factor	0.73 ± 0.25	0.74 ± 0.30	0.880
NFκB1	Nuclear factor kappa B	1.48 ± 0.53	1.56 ± 0.64	0.501
TLR4	Toll like receptor 4	1.78 ± 1.03	1.94 ± 1.25	0.385
TNFα	Tumor necrosis factor alpha	1.10 ± 0.49	1.04 ± 0.56	0.277
CAT	Catalase	1.78 ± 0.54	1.82 ± 0.62	0.896
NOS3	Nitric oxide synthase	1.49 ± 0.64	1.47 ± 0.86	0.243
SOD	Superoxide dismutase	2.01 ± 0.53	1.97 ± 0.84	0.337
Protein ID and definition				
ADIP	Adiponectin	3.46 ± 3.33	4.28 ± 2.82	0.536
MCP1	Monocyte chemoattractant protein 1	**0.87 ± 1.37**	**1.25 ± 1.06**	**0.019***
IL-10	Interleukin 10	0.59 ± 0.48	1.20 ± 1.28	0.722
LEP	Leptin	1.22 ± 0.82	2.17 ± 2.73	0.076
MIF	Macrophage migration inhibitory factor	**1.25 ± 0.84**	**2.83 ± 1.16**	**0.001***
NFκBp65	Nuclear factor kappa B p65	**0.50 ± 0.77**	**0.63 ± 0.51**	**0.018***
TLR4	Toll like receptor 4	0.69 ± 1.11	0.86 ± 0.95	0.773
TNFα	Tumor necrosis factor alpha	**1.03 ± 1.01**	**2.47 ± 2.13**	**0.001***

*Values are estimated means ± SD (n=41) and p values represent the t-test difference between the depots (*P<0.05 and **P<0.01).*

The changes in gene and protein expression in response to exercise training are presented in **Figure 5.1**. In gSAT, *IL-10* mRNA increased in the exercise group ($p<0.001$) but remained unchanged in the control group ($p=0.016$ for group x time interaction) and IL-10 protein level (detected at 25 kDa) did not change in both groups. There was a significant group effect for gSAT *NFκB1* and *TNFα* mRNA ($p=0.011$ and $p=0.025$ respectively), such that the expression of these genes was significantly higher in the exercise compared to the control group at 12 weeks ($p<0.01$). Of note, *TNFα* mRNA in aSAT tended to increase in both groups after the intervention ($p<0.05$ in control and $p<0.1$ in exercise group). In gSAT, the expression of none of the proteins changed following this intervention, whereas in aSAT, NFκBp65 (detected at 65kDa) and TLR4 (detected at 70kDa) proteins decreased in the exercise group ($p<0.05$, time effect), with no changes reported in the control group (**Figure 5.1**). Furthermore, gSAT *MIF* mRNA decreased in the control group only ($p=0.037$ for group x time interaction) and was significantly lower in this group compared to the exercise group at 12 weeks ($p=0.001$). MIF protein expression decreased in aSAT of both groups ($p<0.05$). *MCP1* mRNA content did not change in both depot and groups, whereas aSAT MCP1 protein tended to decrease in the exercise group ($p=0.087$, for time effect), with no changes in the control group (**Figure 5.1**).

Leptin protein (detected around 25kDa) and mRNA contents did not change in response to exercise training. Moreover, adiponectin mRNA (in both depots) tended to decrease in control group while the protein level (detected at 30 kDa) tended to decrease in aSAT of both groups ($p<0.05$ and $p<0.1$). The levels of *NOS3, SOD* and *catalase* mRNA were not different at baseline between the depots and did not change in response to the intervention (**Supplementary Figure 5.1**). The protein expression of these genes was not measured.


mRNA LEVELS
PROTEIN LEVELS
Control Pre
Control Post
Exercise Pre
Exercise Post
A
IL-10 mRNA (AU)
gSAT
aSAT
G
IL-10 protein (AU)
B
NFκBB1 mRNA (AU)
H
NFκBp65 protein (AU)
C
TNFα mRNA (AU)
I
TNFα protein (AU)
D
TLR4 mRNA(AU)
J
TLR4 protein (AU)

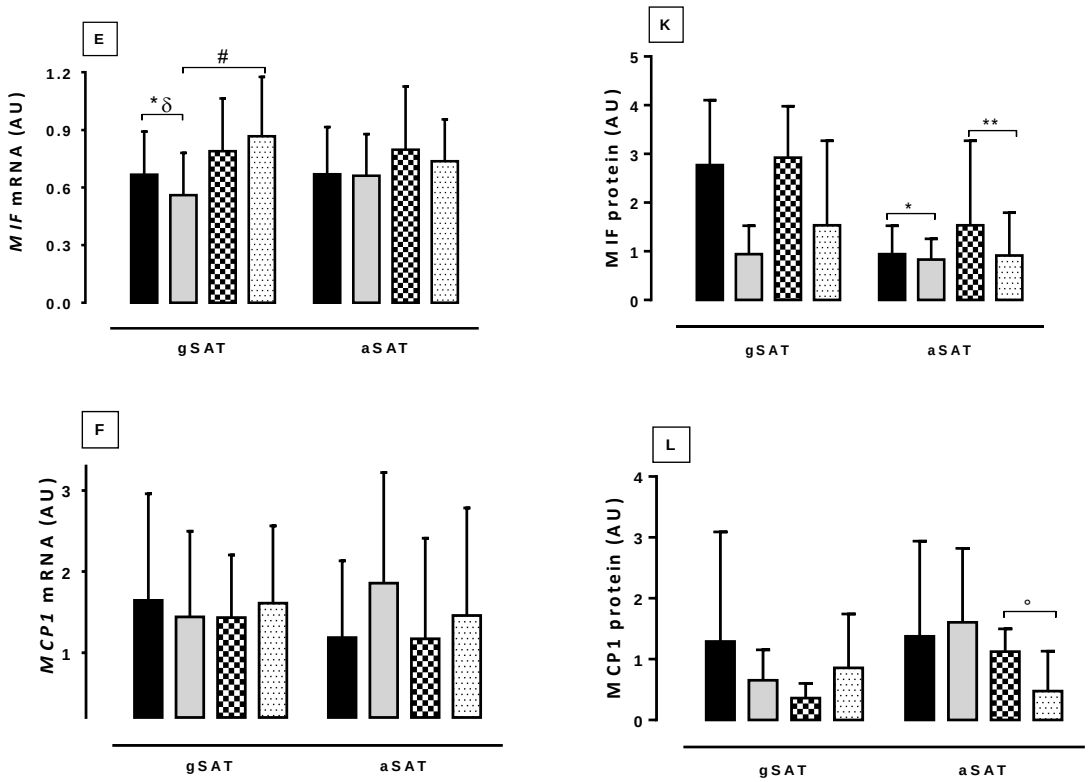


Figure 5.1. Effect of exercise training on gluteal (gSAT) and abdominal subcutaneous adipose tissue (aSAT) inflammatory gene (A-F) and protein (G-L) expression.

*°P<0.1; *P <0.05 and **P<0.01: pre vs post within groups following the intervention (time effect). $^{\delta}$P<0.05: interaction between groups throughout the intervention. $^{\#}$P < 0.01: differences between groups at post-intervention (ie. group effect at 12 weeks). Control group n=15, both depots and exercise group n=20 in aSAT and n=18 in gSAT at post-training due to biopsy interruption. IL-10: interleukin-10; MCP1: monocyte chemoattractant protein 1; MIF: Macrophage migration inhibitory factor; NFκB: nuclear factor kappa B; TLR4: toll-like receptor 4; TNFα: tumor necrosis factor-alpha.*

5.2.4. Correlations between changes of inflammatory markers and changes in body composition and insulin sensitivity/resistance over the intervention period

As the relationship between the changes in inflammatory and oxidative stress markers and changes in body composition and insulin sensitivity/resistance over the 12 weeks did not differ between groups (no interaction effect), the data from both groups were combined for the correlation analyses. An increase in gSAT MCP1 and TNFα mRNA was associated with a decrease in BMI (p<0.01; **Figure 5.2**), while an increase of gSAT IL-10 and MIF mRNA correlated with the reduction in gynoid FM (%) (p<0.05 and p<0.001 respectively; **Figure 5.2**). No significant associations were found between changes in aSAT gene expression and changes in body composition.

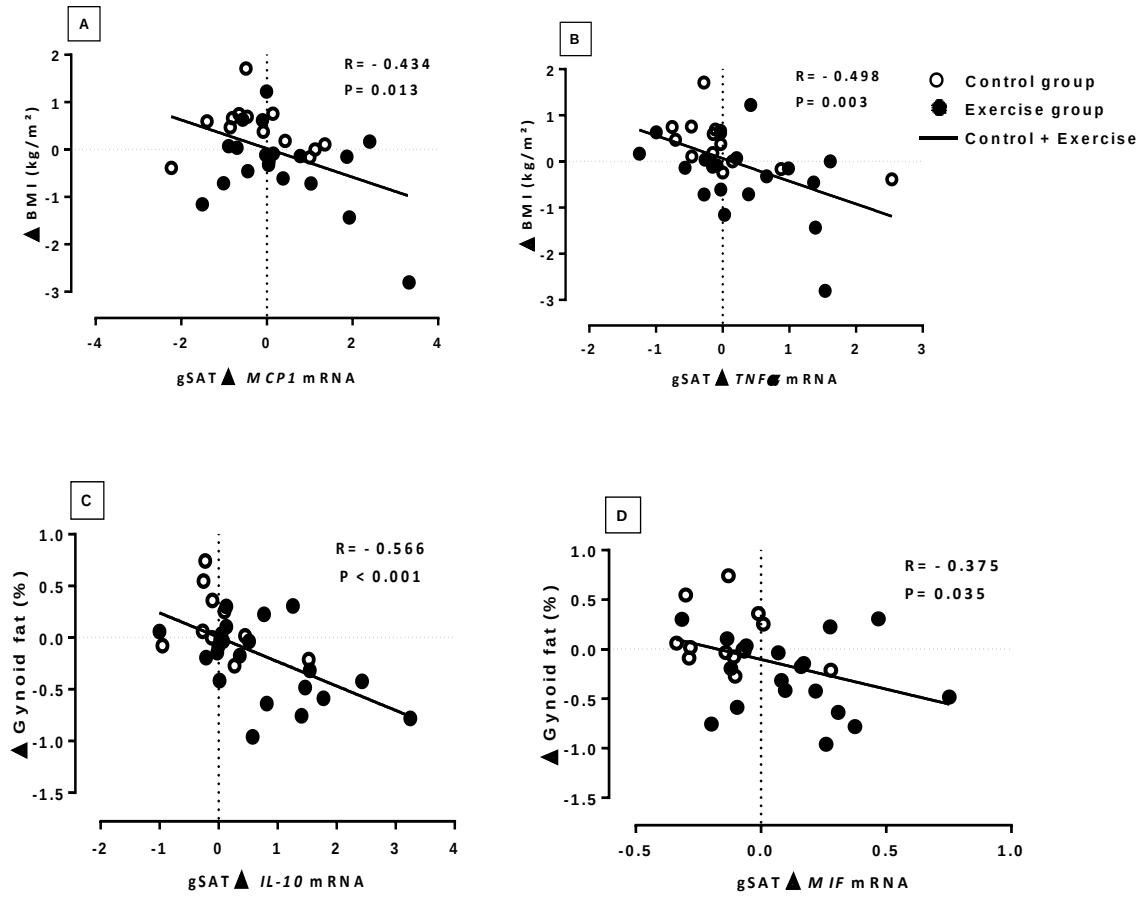


Figure 5.2. Correlations between changes in gluteal (gSAT) and abdominal (aSAT) subcutaneous mRNA expression and changes in body composition in response to the intervention. *Pooled data from control (n=15, both depots) and exercise (gSAT n=18) presented as changes (Δ) from pre- to post-intervention. R and P values are from Pearson's pairwise correlations.*

In terms of protein levels, these data can only be regarded as a pilot study due to the small sample size. Nevertheless, there was a tendency for the decrease in gynoid to be associated with decrease in aSAT MCP1 (r=0.843, p=0.004), NFκBp65 (r=0.616, p=0.077) and TLR4 (r=0.857, p=0.030).

The changes in systemic and SAT inflammatory markers (genes and proteins) were not associated with changes in S_I. However, the change of circulating TNFα and MCP1 concentrations were positively associated with changes of HOMA2IR (**Figure 5.3**), and this remained significant when adjusting for changes in BMI or gynoid FM (%) ($p<0.05$). Besides, changes of aSAT *IL-10* and *TLR4* mRNA content were positively associated with changes of HOMA2-IR (**Figure 5.3**), even after adjusting for changes in BMI (p=0.018 and p=0.014) or in gynoid FM (%) (p=0.009, p=0.011), respectively. Notably, changes in gSAT inflammatory markers were not associated with changes in HOMA2-IR over the 12-week intervention period.

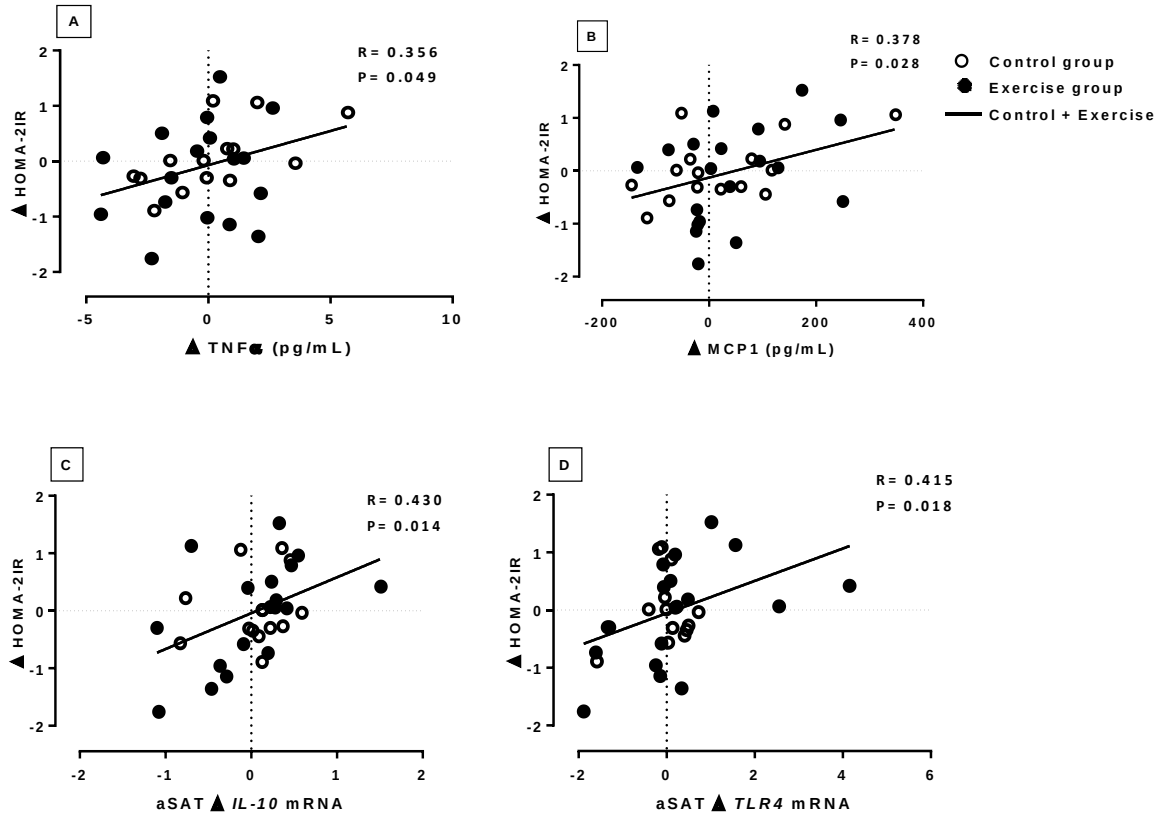


Figure 5.3. Correlations between changes in systemic inflammatory markers and abdominal subcutaneous adipose tissue (aSAT) mRNA expression and changes in insulin resistance (HOMA2-IR). *Pooled data from control (n=15) and exercise (n=20) presented as changes (Δ) from pre- to post-intervention. R and P values are from Pearson's pairwise correlations.*

5.2.5. Correlations between changes in oxidative stress and inflammatory genes

When exploring the associations between transcript levels of oxidative stress markers and inflammatory markers in the SAT depots, we found that gSAT levels of *MIF*, *NFκB1* and *TNFα* mRNA were positively correlated with gSAT *SOD1* (MIF r=0.289 p=0.114; NFκB1 r=0.459 p=0.010; TNFα r=0.422 p=0.018) and *catalase* (MIF r=0.399 p=0.026; NFκB1 r=0.568 p<0.001; TNFα r=0.537 p=0.002) mRNA content. There were no significant correlations between inflammatory and oxidative markers in aSAT, and no associations between SAT mRNA and circulating levels of systemic markers of inflammation and oxidative stress.

5.3. DISCUSSION

This is to the best of my knowledge, the first study in women of African ancestry that has evaluated changes in SAT depot-specific and systemic inflammatory and oxidative stress

markers in response to an exercise training intervention. As reported in the previous chapter on this thesis (Chapter 4), the 12 weeks of combined aerobic and resistance exercise training resulted in significant improvements in S_I, cardiorespiratory fitness and body composition, but did not reduce circulating markers of inflammation. In contrast, the present study showed that circulating TBARS concentrations, a by-product of lipid peroxidation by ROS decreased with a concomitant increase in circulating antioxidant enzyme activity, mainly catalase. Notably, exercise training increased gSAT *IL-10*, *TNFα*, *NFκB1* and *MIF* mRNA contents with a trend for aSAT MCP1, NFκBp65 and TLR4 protein levels to decrease. These changes did not correlate with the improvement in S_I but were rather associated with the reduction of gynoid fat mass. Moreover, changes in aSAT *IL-10* and *TLR4* mRNA were positively associated with changes in HOMA2-IR in response to the intervention and these were independent of changes in body composition.

The effect of exercise training on systemic inflammation in obese cohorts has been extensively studied but findings remain controversial. Some studies showed a reduction of systemic inflammatory markers after exercise training (311, 313), while others found no changes in these markers (411, 412). In the present study, there were no changes in circulating concentrations of leptin, adiponectin, CRP, TNFα, MCP1 and IL-8 after 12 weeks of exercise training. This may relate to the duration of the intervention and/or the limited effects that exercise training has on weight loss and body fat mass, with the small (≈1 kg) but significant decrease in body weight in the exercise group, whereas body weight, BMI and WC increased in the control group. Of note, the increased weight in the control group, although instructed to maintain their normal daily (physical activity and food intake) habits, is reflective of the typical changes in body weight expected in this group of young women (413). Contrary to the lack of changes in systemic inflammatory markers, Cordova et *al.* showed a reduction of cytokine levels (IFNγ, IL-6, TNFα) after 8 months of resistance training in obese women (311) and Trachta et *al* showed a decrease in circulating CRP with a decline in body fat of 4.1% after 3 months of aerobic exercise training (313). The differences in exercise duration and total fat mass reduction between these interventions and the present study could explain these discrepancies in changes in systemic inflammation in response to exercise training.

Inflammation and oxidative stress are interrelated during obesity, both contributing to the development of IR (171). While this study reported no changes in inflammatory markers, there was a decrease in TBARS and an increase in antioxidant enzyme activity, mainly catalase. Likewise, Oh et *al.* showed an increase of the glutathione peroxidase after 6 months of aerobic

training that was accompanied by a reduction of body weight (414). In contrast, 8 weeks of high-intensity exercise training without changes in body weight did not improve oxidative stress and inflammatory state in obese adolescents (415). Catalase and SOD represent the primary antioxidant protection against the harmful effects of ROS and contribute to lower oxidative stress and cell damage (416). The accumulation of modified "ROS-damaged" proteins can lead to IR by activating IκB via p38 mitogen-activated protein kinase (MAPK), followed by the activation of NFκB (403). Elevated ROS can also activate serine/threonine phosphorylation of insulin receptor substrate-1 (IRS1) leading to IR (403, 404). However, there were no associations between the changes in circulating TBARS levels, catalase and SOD activities and the improvement of S_I. The maintenance of a balanced oxidative stress status seems to reflect the beneficial effect of exercise training on body fat, but not a direct mechanism in the improvement of S_I in these women.

The distribution of body fat (abdominal vs. gluteo-femoral) is distinctively associated with metabolic risks, due to the different biological properties and metabolism of SAT depots (61). When comparing the gene expression between aSAT and gSAT at baseline, *leptin* mRNA level was higher in gSAT. Gluteal SAT has been shown to have larger adipocytes compared to aSAT in this population (36) and a strong positive correlation between cell size and *leptin* mRNA have been reported (309). These findings support the role of leptin as a signal of fat mass (and its effect on adipocytes metabolism). In contrast, MIF, MCP1, NFκBp65 and TNFα protein levels tended to be higher in aSAT compare to gSAT at baseline. This can be explained by the pronounced inflammatory profile previously reported in upper body fat compared to peripheral fat (61). Additionally, aSAT is a major source of FFA, which are a determinant link between obesity and adipose inflammation (190).

Surprisingly, the gene expression of the inflammatory markers in gSAT at 12 weeks (post-intervention) increased in response to this exercise training intervention, and this was associated with the reduction in gynoid fat mass, but not with the improvement in S_I. Noteworthy, the samples analysed in this study were collected 3 days after the last exercise training session to exclude the potential acute effects of the last bout of exercise training. The increase in inflammatory gene expression in the gSAT seems to be an adaptive response to fat mass reduction (417), potentially representing a remodelling process occurring within this tissue. Indeed, the AT inflammatory state is modulated during expansion and/or remodelling of this tissue (418). Additionally, recent evidence showed that inflammation in the adipocyte microenvironment is required for ECM remodelling and angiogenesis (147, 419). Fat mass

reduction, driven by an overall reduction of stored TGs consecutive to lipolysis, requires extensive remodelling of the tissue (420, 421). Exercise training has been shown to increase basal and/or stimulated adipocyte lipolysis (422, 423). The FFA released from adipocytes (from both basal and exercise-induced stimulated lipolysis) activate monocytes and create a paracrine loop between lipolysis and local inflammation (156), increasing the inflammatory state in the fat depot. The increased *IL-10* mRNA content in gSAT is supported by previous findings showing that the reduction of stored TGs in adipocytes is followed by an increased expression of lipolysis-associated M2 macrophage markers (424, 425). *IL-10* activates M2 macrophages for tissue repair and may also be a compensatory mechanism to cope with the increased inflammation. Although there was no change in the genes involved in lipid metabolism presented in Chapter 4, it cannot be concluded that SAT lipolysis was not enhanced after this exercise training intervention, as only *ATGL* and *PLIN1* mRNA expression were evaluated as markers of lipolysis. The changes could have therefore derived from the increase of other proteins such as HSL or MGL, and this is acknowledged as a limitation of this study. Increased inflammation can subsequently influence the oxidative stress state. Accordingly, there was a strong positive association between the expression of inflammatory genes (*MIF*, *NFκB1* and *TNFα* mRNA) and antioxidant enzymes (*catalase* and *SOD1* mRNA) in gSAT. Inflammatory markers such as TNFα can trigger the activation of NADPH oxidase and the production of ROS (171), followed by increased expression of antioxidant enzymes to buffer and maintain a "healthy" oxidative stress balance (426).

In contrast to the response in gSAT, aSAT inflammatory genes did not change after this intervention potentially explained by the unchanged android fat mass in the present study. This is in accordance with data provided by Lakhdar et *al.* who also did not find changes in aSAT inflammatory markers in response to 12 weeks of moderate exercise training, despite the expected improvements in body composition (411). However, there was a reduction trend for NFκBp65, TLR4 and MCP1 protein expression in the aSAT depot in response to exercise training, albeit in a sub-sample of participants. Khadir *et al.* also showed that 3 months of an aerobic exercise program decreased IL-6 and TNF-α protein expression in the aSAT of diabetic and non-diabetic obese patients (427) suggesting a beneficial effect of exercise training. In the present study, the decrease of inflammatory proteins in aSAT also tended to be associated with the reduction of gynoid fat, suggesting a favourable effect of lower-body fat loss following exercise training on abdominal inflammation. However, it is important to note that the protein analyses were undertaken in a small sub-sample of participants, which prevented me from making conclusive statements from these results. Nevertheless, I hypothesise that the reduction

in gynoid fat may reflect the "flexibility" of gSAT, providing additional storage space for future excess FFA in times of positive calorie balance. This would thereby limit redirection of excess FFA to upper-body central and ectopic depots and consequently, reduce inflammation associated with abdominal fat accumulation.

Another important finding of this study was that although no improvements in HOMA-2IR were observed, there was a positive association between changes in HOMA-2IR and changes in aSAT *IL-10* and *TLR4* mRNA content in response to the exercise training. In contrast to the whole-body S_I, estimated using the FSIGT, HOMA-2IR is based on fasting measures of insulin and glucose and is more representative of hepatic IR (428). Hence, this study showed a linear relationship between abdominal inflammation and estimated hepatic IR. However, to show an improvement in hepatic IR following exercise training, a more profound and sustained alteration of aSAT inflammatory profile, which may only be achieved by a pronounced reduction in abdominal fat mass, may be required (411). Furthermore, the concomitant absence of association between aSAT inflammatory profile and S_I is supported by a previous study where inflammation in this depot was weakly correlated with S_I (201).

The discrepancies found between mRNA and protein levels are supported by Wang's findings showing a weak correlation between mRNA and protein abundance (429). Indeed, RNA and protein molecules represent different steps of the cellular genetic information and are dynamically produced and degraded. These differential changes could be the result of features of the gene expression machinery at the transcriptional and translational levels, the speed of transportation as well as the degradation (RNA and protein) processes (reviewed in (429)).

This is the first study which robustly measured the effect of exercise training on systemic and depot-specific SAT inflammation and oxidative stress in obese women of African ancestry, which makes the findings unique, relevant and novel. However, some limitations should be mentioned. In addition to the analysis of protein content in only a sub-sample of participants (due to tissue availability and cost), NFkBp65 protein content and not the phosphorylation status was assessed, which is preferred measured as an indication of pro-inflammatory state. Moreover, the limited number of evaluated genes represents another limitation of this research work. Further studies exploring differences between SAT inflammatory markers at both the transcriptional and translational levels in response to exercise training in larger sample size is needed. This will help to gain a better understanding of the role of SAT inflammation and ROS in the exercise-induced changes in metabolic status. Notably, this study was restricted to young

black SA women and therefore, the findings cannot be directly translated to other groups of obese individuals with different phenotypes and/or age, sex, socioeconomic and health status.

In conclusion, 12-weeks of aerobic and resistance exercise training program improved systemic oxidative stress markers, increased inflammatory mRNA levels in gSAT and reduced pro-inflammatory protein levels in aSAT. These changes were associated with the reduction of gynoid fat but were not correlated with the improvement in whole-body insulin sensitivity in obese black SA women. Further investigations are required to evaluate other regulatory pathways in AT that could determine the beneficial effects of exercise training on S_I. This can be addressed by using a non-targeted analysis approach to explore the unrevealed pathways that may be affected by exercise training and that are associated with improved SAT function, as well as insulin sensitivity. Such studies have not yet been performed in an African population.

CHAPTER SIX

DISTINCT ABDOMINAL AND GLUTEAL ADIPOSE TISSUE TRANSCRIPTOME SIGNATURES ARE ALTERED BY EXERCISE TRAINING IN AFRICAN WOMEN WITH OBESITY

6.1. INTRODUCTION

Body fat distribution is an independent contributor to the development of obesity-associated metabolic diseases (38, 40). While central fat accumulation (both in visceral and abdominal subcutaneous areas) has been associated with greater risk for developing metabolic diseases, lower-body fat (gluteo-femoral) appears to have positive effects on metabolic health (39, 58, 59). The mechanisms underlying these depot-specific associations with metabolic risks are not fully understood. However, environmental and genetic factors could mediate differences in gene expression, metabolism and function between fat depots (430, 431).

The accumulation of AT during positive energy balance follows a specific individual pattern, with the preferential expansion of subcutaneous vs. visceral depot, largely determined by heritable factors (38). This specific response to energy intake could also be influenced by site-specific sets of developmental genes (81, 432). Numerous studies have reported an extensive number of differentially expressed genes (DEGs) between VAT and SAT (both abdominal and gluteal) (39, 61, 432, 433). This suggests different developmental lineage, potentially explaining depot-specific adipose metabolism and association with metabolic risk. However, whether the difference in embryonic origin exists within SAT depots remains elusive. Indeed, in addition to their functional differences and their distinctive associations with metabolic risk, evidence showed epigenetic differences between aSAT and gSAT (59, 78, 79, 432). However, these findings were mainly derived from studies in European populations and no data is available on the depot-specific SAT transcriptome profile exclusively in an African population. This highlights the need to study SAT signatures in Africans to understand the role of this tissue in the development of IR and T2D. Indeed, differential patterns of body fat distribution and association with IR have been found between obese black and white SA women (32, 34, 70). Moreover, there is evidence to suggest impaired adipogenesis and high hypoxic and fibrotic state in SAT of obese black SA women (214), suggesting that the gene expression profile of SAT depots is ethnic-specific and consequently, might play a role in the divergent metabolic profiles in African compared to European women.

It is known that exercise training affects several metabolic pathways in AT, which directly or indirectly contribute to the improvement of glucose homeostasis and insulin sensitivity (293). Findings from the previous chapters of this thesis (Chapter 4 and Chapter 5) showed SAT depot-specific adaptations to exercise training in obese black SA women. However, this was limited to a candidate gene approach, focusing on a small number of genes involved in inflammation, oxidative stress, lipid metabolism, adipogenesis and insulin signalling. The extent of these

changes on SAT transcriptome has not been investigated in African populations. The microarray technology represents a great instrument to identify and explore other potential pathways in SAT function that might be influencing peripheral or whole-body insulin sensitivity in these women at rest and after chronic exercise training. Therefore, using an unbiased microarray analysis approach, this study aimed to evaluate the regional differences in gene expression profiles between aSAT and gSAT in obese black SA women and tested the hypothesis that the gene expression pattern is altered in a depot-specific manner in response to 12-weeks of exercise training. These different transcriptome signatures might be driving their distinctive functional responses to external stimuli such as exercise training, potentially explaining the SAT depot-specific associations with higher metabolic risks in obese women of African ancestry.

6.2. RESULTS

6.2.1. Participants' characteristics

The basic characteristics of the participants involved in this study are presented in **Table 6.1** below. The women were on average 23 ± 3 years of age and had a BMI of 33.8 ± 2.6 kg/m². In response to exercise training, cardiorespiratory fitness (VO_{2peak}) increased ($p<0.05$), and weight, BMI, WC, waist/hip ratio (WHR) decreased. While total body fat (%) did not change, both android and gynoid fat mass (%) decreased ($p<0.05$), and there was a tendency for VAT and SAT volumes to decrease ($p<0.1$).

Table 6.1. Participant's characteristics before and after exercise training

Variables	Pre (n=12)	Post (n=12)	P Value
Cardiorespiratory fitness			
VO_{2peak} (ml/min)	2121 ± 217	2245 ± 204	**0.023**
VO_{2peak} (ml/kg/min)	25.9 ± 2.2	28.0 ± 2.6	**0.008**
Body composition			
Weight (kg)	82.2 ± 7.2	80.5 ± 7.5	**0.004**
BMI (kg/m²)	33.8 ± 2.6	33.1 ± 2.8	**0.004**
WC (cm)	102.0 ± 5.5	97.7 ± 5.42	**0.002**
WHR	0.90 ± 0.09	0.88 ± 0.06	**0.019**
Body FM (%)	49.7 ± 1.6	49.1 ± 1.5	0.205
Android FM (%)	8.4 ± 1.1	8.2 ± 1.2	**0.010**
Gynoid FM (%)	6.8 ± 1.0	6.6 ± 1.0	**0.033**
VAT (cm^3)	971 ± 330	909 ± 384	0.086
SAT (cm^3)	5451 ± 803	5266 ± 944	0.062

Data presented as means ± SD; p values represent the differences between baseline and post-training. VO_2peak, peak oxygen consumption, used as a measure of cardiorespiratory fitness; BMI: body mass index; WC: waist circumference; WHR: waist and hip ratio; FM: fat-mass; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue.

6.2.2. Differential gene expression profiles in subcutaneous adipose depots at baseline and in response to exercise training

The differences in gene expression profiles between aSAT and gSAT at baseline and after exercise training are presented in **Figure 6.1A** and **Figure 6.1B**, respectively. At baseline, only 15 genes were differentially expressed between aSAT and gSAT ($|LFC| \geq 0.58$; $p \leq 0.05$) with the expression of 13 genes being higher and 2 genes were lower in aSAT vs. gSAT (**Figure 6.1A; Table 6.2**). In contrast, a total of 318 DEGs were identified between the depots after exercise training (**Figure 6.1B**); with 166 genes higher and 152 genes lower in aSAT vs gSAT (**Table 6.3** for the top 10 DEGs; **Supplementary Table 6.1** for the complete list of DEGs). Notably, 11 of these genes overlapped between the SAT depots before and after exercise training (**Figure 6.1C**). The most prominent genes distinguishing the SAT depots at both time points (highlighted in bold in **Tables 6.2** and **Table 6.3**) were *DMRT2*, *DMRT3*, and *HOXA5*, with higher expression levels in aSAT compared to gSAT; as well as *CSN1S1*, with lower expression in aSAT vs gSAT. Several homeobox (HOX) genes had higher expression levels in aSAT than in gSAT at baseline or after exercise training (*HOXA3*, *HOXA5*, *HOXA9*, *HOXB5*, *HOXB8*, *HOPX*, *IRX2* and *IRX5*). The evaluation of the biological processes represented by the DEGs between the depots at both time points using STRING showed that they were mainly enriched for GO terms: embryonic development, anatomical structure and developmental processes at baseline (**Figure 6.2**), and more diverse functional-related processes at post-training (**Supplementary figure 6.1**).

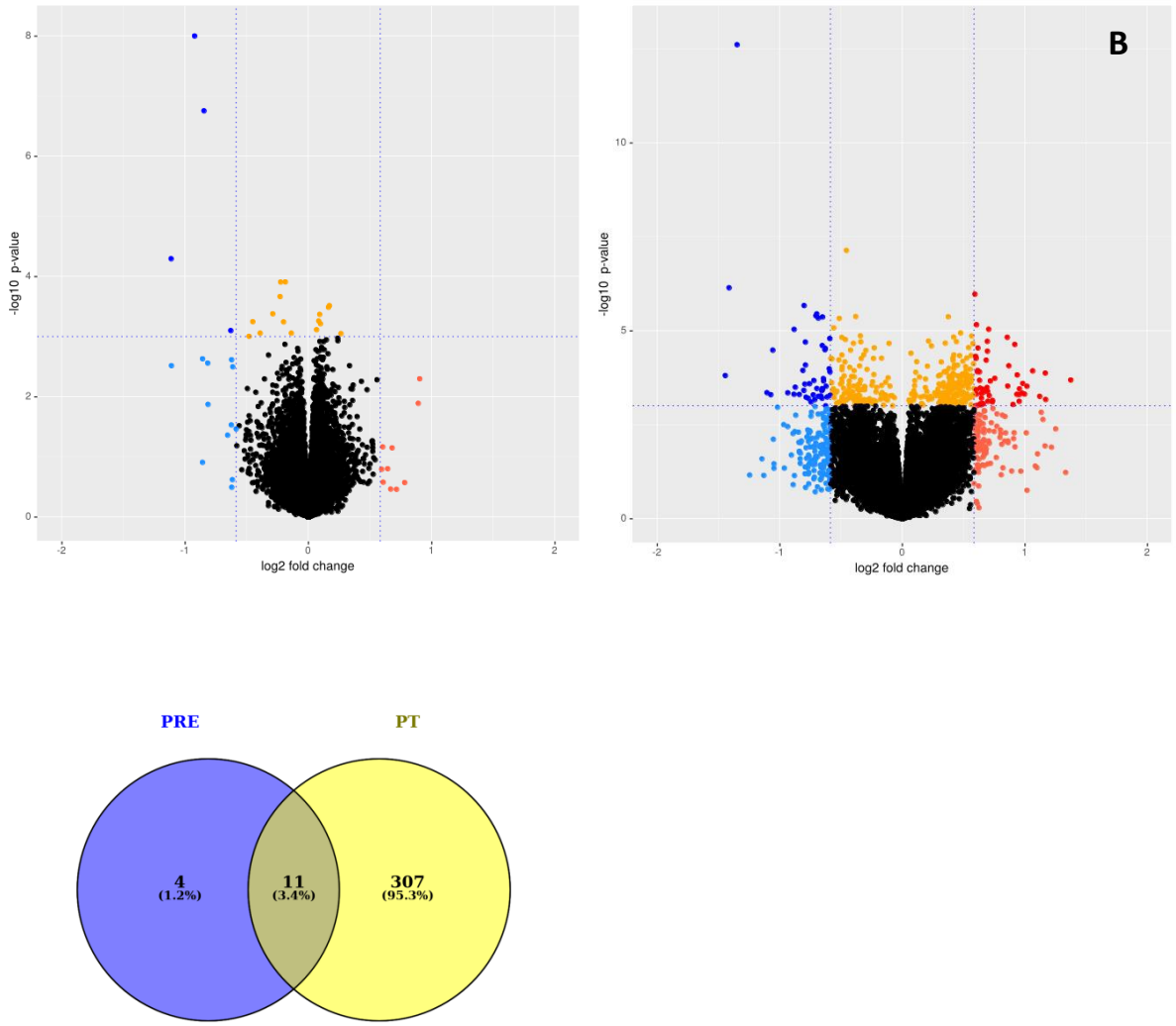


Figure 6.1. Comparison of abdominal SAT vs gluteal SAT, before (A) and after (B) exercise training, respectively.

The volcano plots highlight genes with p-value < 0.001 and fold change |LFC| > 0.58. Blue: higher gene expression in aSAT (LFC <-0.58); Red: lower gene expression in aSAT (LFC > 0.58); Orange: p-value < 0.001. The horizontal line corresponds to p < 0.001 and the vertical lines mark a |LFC| > 0.58. A: aSAT vs gSAT at baseline. B: aSAT vs gSAT after exercise training. C: Overlap of genes with changed expression (|LFC|>0.58) between aSAT and gSAT. PRE: difference between aSAT and gSAT pre-exercise training; PT: difference between aSAT and gSAT post-exercise training.

Table 6.2. List of all differentially expressed genes between SAT depots (up and down-regulated) before exercise training based on log2 fold change > 0.58

SYMBOL	SEARCH_KEY	logFC	DEFINITION
Higher in aSAT than gSAT			
DMRT2	**NM_181872.1**	**-1.11**	**Homo sapiens doublesex and mab-3 related transcription factor 2 (DMRT2), transcript variant 1, mRNA.**
HOXA5	**NM_019102.2**	**-1.11**	**Homo sapiens homeobox A5 (HOXA5), mRNA.**
DMRT3	**NM_021240.2**	**-0.92**	**Homo sapiens doublesex and mab-3 related transcription factor 3 (DMRT3), mRNA.**
RSPO3	NM_032784.3	-0.86	Homo sapiens R-spondin 3 homolog (Xenopus laevis) (RSPO3), mRNA.
IRX2	NM_033267.2	-0.85	Homo sapiens iroquois homeobox 2 (IRX2), mRNA.
C6	NM_000065.1	-0.82	Homo sapiens complement component 6 (C6), mRNA.
ALDH1A1	NM_000689.3	-0.81	Homo sapiens aldehyde dehydrogenase 1 family, member A1 (ALDH1A1), mRNA.
ALDH1A1	NM_000689.3	-0.66	Homo sapiens aldehyde dehydrogenase 1 family, member A1 (ALDH1A1), mRNA.
HOXB8	NM_024016.2	-0.63	Homo sapiens homeobox B8 (HOXB8), mRNA.
FGFBP2	NM_031950.2	-0.62	Homo sapiens fibroblast growth factor binding protein 2 (FGFBP2), mRNA.
IRX5	NM_005853.4	-0.62	Homo sapiens iroquois homeobox protein 5 (IRX5), mRNA.
LOC440928	XM_942885.1	-0.61	PREDICTED: Homo sapiens hypothetical LOC440928 (LOC440928), mRNA.
PPP1R1B	NM_181505.1	-0.59	Homo sapiens protein phosphatase 1, regulatory (inhibitor) subunit 1B (dopamine and cAMP regulated phosphoprotein, DARPP-32) (PPP1R1B), transcript variant 2, mRNA.
Lower in aSAT than gSAT			
CSN1S1	**NM_001890.1**	**0.90**	**Homo sapiens casein alpha s1 (CSN1S1), transcript variant 1, mRNA.**
CSN1S1	**NM_001025104.1**	**0.89**	**Homo sapiens casein alpha s1 (CSN1S1), transcript variant 1, mRNA.**

Table 6.3. Top 10 differentially expressed genes between SAT depots (up and down-regulated) after exercise training based on log2 fold change > 0.58

SYMBOL	SEARCH_KEY	logFC	DEFINITION
Higher in aSAT than gSAT			
HOXA5	**NM_019102.2**	**-1.44**	**Homo sapiens homeobox A5 (HOXA5), mRNA.**
DMRT2	**NM_181872.1**	**-1.41**	**Homo sapiens doublesex and mab-3 related transcription factor 2 (DMRT2), transcript variant 1, mRNA.**
DMRT3	**NM_021240.2**	**-1.35**	**Homo sapiens doublesex and mab-3 related transcription factor 3 (DMRT3), mRNA.**
MYL2	NM_000432.2	-1.15	Homo sapiens myosin, light chain 2, regulatory, cardiac, slow (MYL2), mRNA.
CDKN1B	NM_004064.2	-1.10	Homo sapiens cyclin-dependent kinase inhibitor 1B (p27, Kip1) (CDKN1B), mRNA.
MYH11	NM_002474.1	-1.07	Homo sapiens myosin, heavy chain 11, smooth muscle (MYH11), transcript variant SM1A, mRNA.
ANGPT2	NM_001118888.1	-1.06	Homo sapiens angiopoietin 2 (ANGPT2), transcript variant 3, mRNA.
LOC649841	XM_938906.1	-1.05	PREDICTED: Homo sapiens similar to protein immuno-reactive with anti-PTH polyclonal antibodies (LOC649841), mRNA.
PARM1	NM_015393.2	-1.05	Homo sapiens prostate androgen-regulated mucin-like protein 1 (PARM1), mRNA.
MYH7	NM_000257.1	-1.05	Homo sapiens myosin, heavy chain 7, cardiac muscle, beta (MYH7), mRNA.
Lower in aSAT than gSAT			
CSN1S1	**NM_001025104.1**	**1.37**	**Homo sapiens casein alpha s1 (CSN1S1), transcript variant 1, mRNA.**
SPP1	NM_001040058.1	1.25	Homo sapiens secreted phosphoprotein 1 (SPP1), transcript variant 1, mRNA.
LOC644936	NR_004845.1	1.22	Homo sapiens cytoplasmic beta-actin pseudogene (LOC644936), non-coding RNA.
IFI30	NM_006332.3	1.17	Homo sapiens interferon, gamma-inducible protein 30 (IFI30), mRNA.
LAPTM5	NM_006762.1	1.17	Homo sapiens lysosomal multispanning membrane protein 5 (LAPTM5), mRNA.
SPP1	NM_000582.2	1.16	Homo sapiens secreted phosphoprotein 1 (SPP1), transcript variant 2, mRNA.
FCGBP	NM_003890.1	1.15	Homo sapiens Fc fragment of IgG binding protein (FCGBP), mRNA.
TM4SF19	NM_138461.1	1.13	PREDICTED: Homo sapiens transmembrane 4 L six family member 19, transcript variant 2 (TM4SF19), mRNA.
CSN1S1	NM_001890.1	1.12	Homo sapiens casein alpha s1 (CSN1S1), transcript variant 1, mRNA.
FOSB	NM_006732.1	1.10	Homo sapiens FBJ murine osteosarcoma viral oncogene homolog B (FOSB), mRNA.

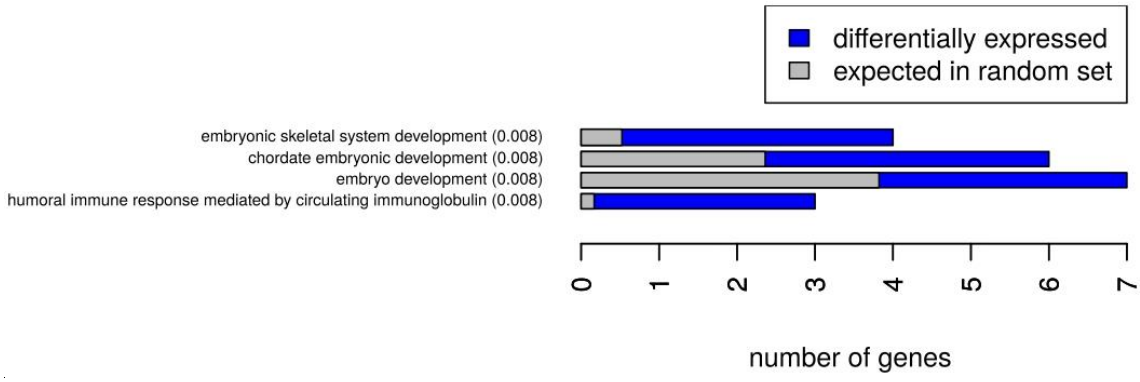


Figure 6.2. GO term enrichment of differentially expressed genes between abdominal and gluteal SAT at baseline.

6.2.3. Depot-specific responses of gluteal and abdominal subcutaneous adipose tissue to exercise training

To evaluate the depot-specific response to exercise training, the gene expression levels were compared in gSAT (**Figure 6.3A**) and aSAT **(Figure 6.3B)** pre and post-training, respectively.

In gSAT, 61 DEGs were identified ($|LFC| \geq 0.58$; $p \leq 0.05$) with 54 genes upregulated and 7 genes downregulated in response to exercise training (**Table 6.4; Supplementary Table 6.2**). In addition, the biological processes most affected by exercise training (**Figure 6.4**) were immune and inflammatory responses (e.g. *SPP1*, *CCL22*, *MMP9, CHI3L1*, *HP*), regulation of lipid metabolism (e.g. *APOE, PLTP, SPP1, C3, APOC1*, *SREBF1*) and regulation of plasma lipoprotein particle levels and remodelling (e.g. *APOC1*, *APOE, PLA2G7, PLTP, LIPA*).

Within aSAT, the expression of 77 genes changed in response to exercise training ($|LFC| \geq 0.58$; $p \leq 0.05$), with 55 genes upregulated and 22 genes downregulated (**Table 6.5; Supplementary Table 6.3**). Surprisingly, these genes mostly represented muscle-associated processes (e.g. *ACTA1, MYH7, MYL2, TCAP, CKM*) and immune response (e.g. *HP, COL1A1, CD3D, IL7R*) (**Figure 6.5**). However, *TWIST1*, a gene documented to play a role in obesity-associated inflammation and IR was down-regulated in aSAT after the exercise training (**Table 6.5**). Notably, only 3 genes had commonly altered expression levels in aSAT and gSAT after exercise training (*COL1A1, HP, CILP*) (**Figure 6.3C**).

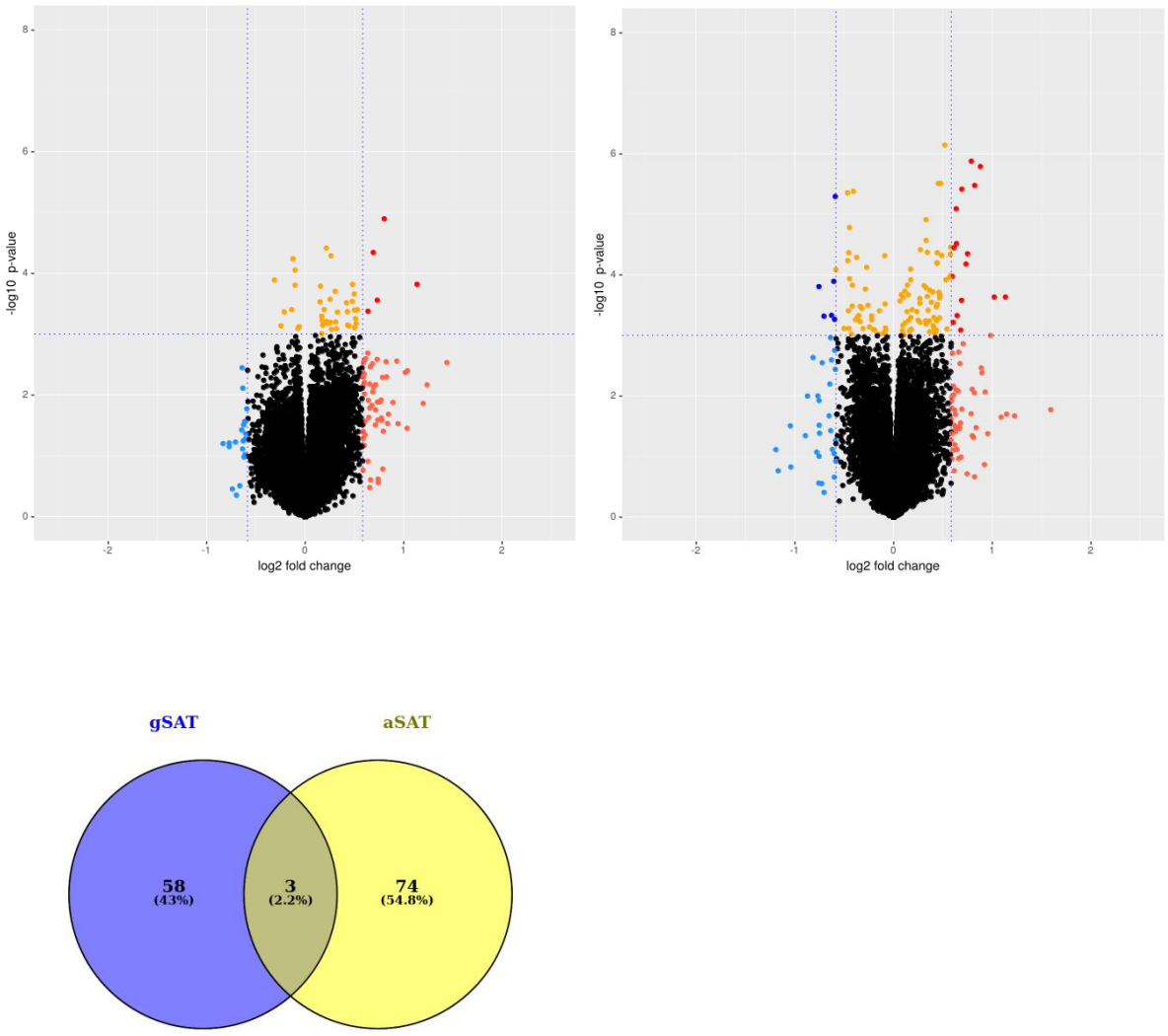


Figure 6.3. Comparison of gene expression changes induced by exercise training in gSAT (A) and aSAT (B), respectively.

The volcano plots highlight genes with p-value < 0.001 and fold change |LFC| > 0.58. Red: gene expression upregulated after exercise training (LFC > 0.58); Blue: gene expression downregulated after exercise training (LFC < -0.58); Orange: p-value < 0.001. The horizontal line corresponds to p < 0.001 and the vertical lines mark a |LFC| > 0.58. A: expression changes in gSAT after exercise. B: Gene expression changes in aSAT after exercise training. C: Overlapping genes with changed expression (|LFC| > 0.58) in both depots before and after exercise training.

Table 6.4. Differentially expressed genes in gluteal SAT in response to exercise training based on log2 fold change > 0.58 (Top 10 up-regulated genes and all down-regulated genes)

SYMBOL	SEARCH_KEY	logFC	DEFINITION
Up-regulated genes in gSAT after exercise training			
MMP9	NM_004994.2	-1.44	Homo sapiens matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase) (MMP9), mRNA.
SPP1	NM_001040058.1	-1.24	Homo sapiens secreted phosphoprotein 1 (SPP1), transcript variant 1, mRNA.
SPP1	NM_000582.2	-1.20	Homo sapiens secreted phosphoprotein 1 (SPP1), transcript variant 2, mRNA.
APOC1	NM_001645.3	-1.14	Homo sapiens apolipoprotein C-I (APOC1), mRNA.
ITGAX	NM_000887.3	-1.04	Homo sapiens integrin, alpha X (complement component 3 receptor 4 subunit) (ITGAX), mRNA.
TM4SF19	NM_138461.2	-1.04	Homo sapiens transmembrane 4 L six family member 19 (TM4SF19), mRNA.
IFI30	NM_006332.3	-1.02	Homo sapiens interferon, gamma-inducible protein 30 (IFI30), mRNA.
PLA2G7	NM_005084.2	-0.94	Homo sapiens phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma) (PLA2G7), mRNA.
LAPTM5	NM_006762.1	-0.93	Homo sapiens lysosomal multispanning membrane protein 5 (LAPTM5), mRNA.
CISH	NM_145071.1	-0.89	Homo sapiens cytokine-inducible SH2-containing protein (CISH), mRNA.
Down-regulated genes in gSAT after exercise training			
LOC651309	XM_942586.1	0.64	PREDICTED: Homo sapiens hypothetical protein LOC651309 (LOC651309), mRNA.
PCDH9	NM_020403.3	0.64	Homo sapiens protocadherin 9 (PCDH9), transcript variant 1, mRNA.
NTM	NM_016522.2	0.63	Homo sapiens neurotrimin (NTM), transcript variant 2, mRNA.
SLIT2	NM_004787.1	0.62	Homo sapiens slit homolog 2 (Drosophila) (SLIT2), mRNA.
NUTF2	NM_005796.1	0.61	Homo sapiens nuclear transport factor 2 (NUTF2), mRNA.
	Hs.99472	0.60	Homo sapiens mRNA; cDNA DKFZp564O0862 (from clone DKFZp564O0862)
FAM13A	NM_014883.2	0.59	Homo sapiens family with sequence similarity 13, member A (FAM13A), transcript variant 1, mRNA.

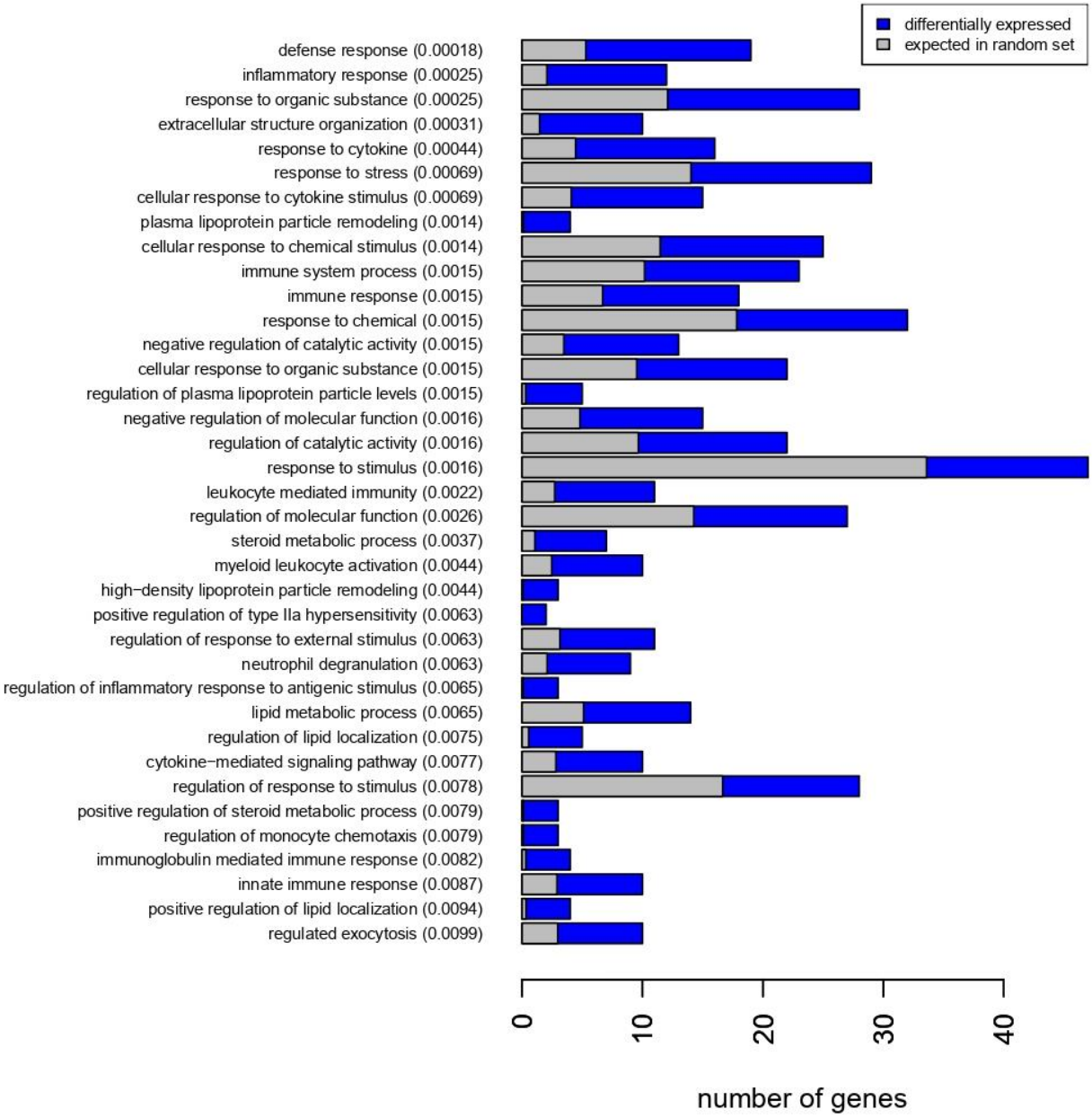


Figure 6.4. GO-term Enrichment of differentially expressed genes in gluteal SAT after exercise training

Table 6.5. Top 10 differentially expressed genes in abdominal SAT in response to exercise training based on log2 fold change > 0.58 (up and down-regulated)

SYMBOL	SEARCH_KEY	logFC	DEFINITION
Up-regulated genes in aSAT after exercise training			
ACTA1	NM_001100.3	-1.59	Homo sapiens actin, alpha 1, skeletal muscle (ACTA1), mRNA.
FOLR3	NM_000804.2	-1.23	Homo sapiens folate receptor 3 (gamma) (FOLR3), mRNA.
MYL2	NM_000432.2	-1.15	Homo sapiens myosin, light chain 2, regulatory, cardiac, slow (MYL2), mRNA.
CHI3L2	NM_004000.2	-1.13	Homo sapiens chitinase 3-like 2 (CHI3L2), transcript variant 1, mRNA.
MYH7	NM_000257.1	-1.09	Homo sapiens myosin, heavy chain 7, cardiac muscle, beta (MYH7), mRNA.
COL1A1	NM_000088.2	-1.02	Homo sapiens collagen, type I, alpha 1 (COL1A1), mRNA.
FLNC	NM_001458.2	-0.98	Homo sapiens filamin C, gamma (actin-binding protein 280) (FLNC), mRNA.
CKM	NM_001824.2	-0.96	Homo sapiens creatine kinase, muscle (CKM), mRNA.
LTB	NM_002341.1	-0.93	Homo sapiens lymphotoxin beta (TNF superfamily, member 3) (LTB), transcript variant 1, mRNA.
FNDC1	NM_032532.1	-0.90	Homo sapiens fibronectin type III domain containing 1 (FNDC1), mRNA.
Down-regulated genes in aSAT after exercise training			
FOS	NM_005252.2	1.05	Homo sapiens v-fos FBJ murine osteosarcoma viral oncogene homolog (FOS), mRNA.
FOSB	NM_006732.1	0.89	Homo sapiens FBJ murine osteosarcoma viral oncogene homolog B (FOSB), mRNA.
CSN1S1	NM_001025104.1	0.87	Homo sapiens casein alpha s1 (CSN1S1), transcript variant 1, mRNA.
MYOC	NM_000261.1	0.82	Homo sapiens myocilin, trabecular meshwork inducible glucocorticoid response (MYOC), mRNA.
TWIST1	NM_000474.3	0.77	Homo sapiens twist homolog 1 (Drosophila) (TWIST1), mRNA.
LOC643911	XR_042101.1	0.76	PREDICTED: Homo sapiens hCG1815491 (LOC643911), miscRNA.
CSN1S1	NM_001890.1	0.76	Homo sapiens casein alpha s1 (CSN1S1), transcript variant 1, mRNA.
MYOC	NM_000261.1	0.75	Homo sapiens myocilin, trabecular meshwork inducible glucocorticoid response (MYOC), mRNA.
THBS4	NM_003248.3	0.75	Homo sapiens thrombospondin 4 (THBS4), mRNA.
LOC643911	XM_931911.1	0.72	PREDICTED: Homo sapiens hypothetical LOC643911 (LOC643911), mRNA.

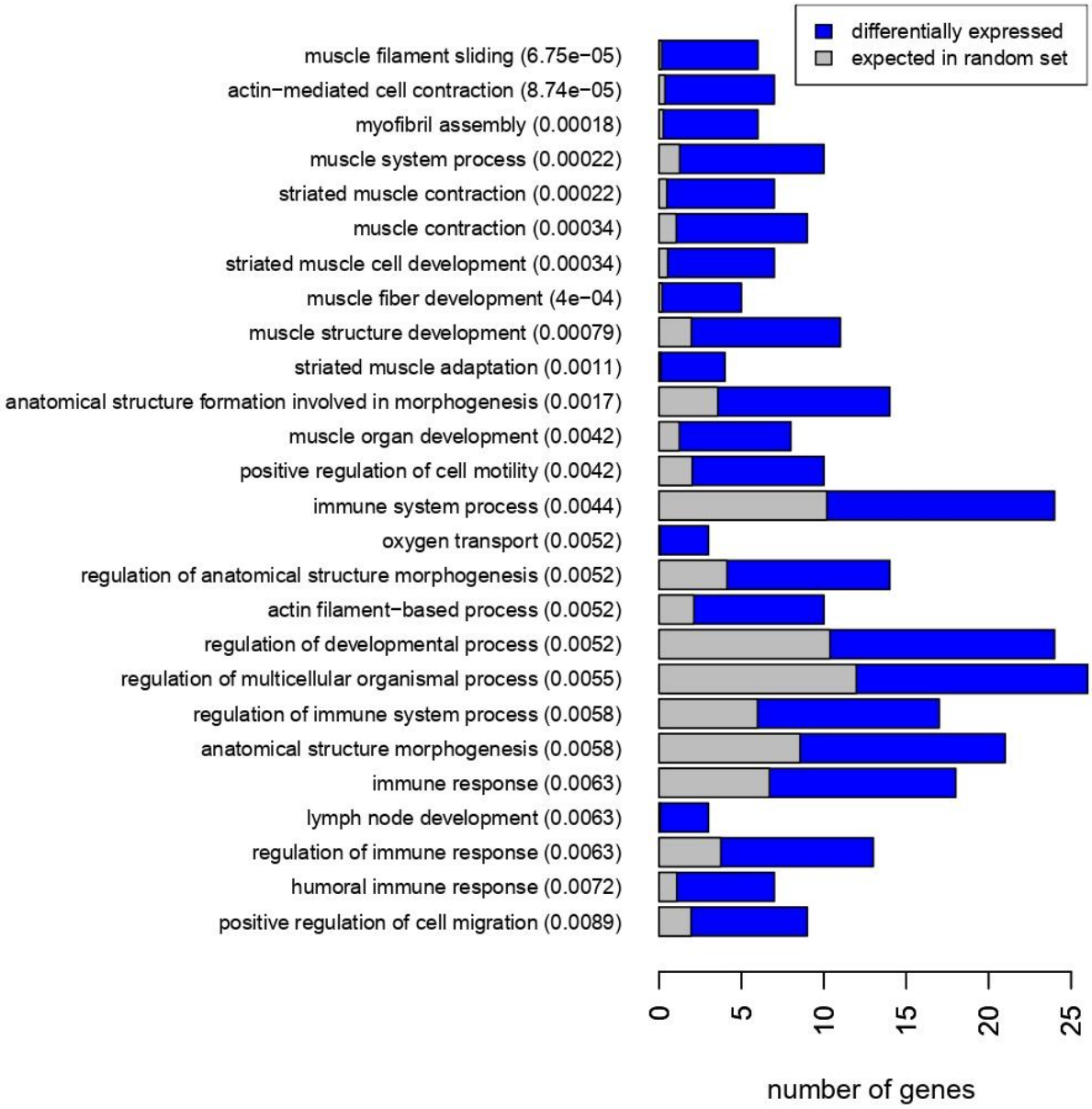


Figure 6.5. GO-term Enrichment of differentially expressed genes in abdominal SAT after exercise training

6.3. DISCUSSION

This is the first study that has investigated the regional differences in SAT transcriptome signatures in obese African women and showed differences in gene expression profiles between aSAT and gSAT at baseline and in response to exercise training. As a key result of this unbiased analysis, 15 DEGs were found between these fat depots at baseline, which were mainly associated with embryonic development (e.g. *HOXA5, DMRT2, HOXB8, IRX5, IRX2*) and regulation of anatomical structure morphogenesis (e.g. *DMRT2, DMRT3, HOXA5, RSPO3*). Depot-specific AT transcriptome signatures were strongly pronounced in response to the 12-weeks structured exercise training program, such that 318 genes were differentially expressed between the depots, with only three genes commonly changed in aSAT and gSAT. Interestingly, four developmental genes were identified as the most prominent DEGs between SAT depots (*DMRT2, DMRT3* and *HOXA5* higher, and *CSN1S1* lower expressed in aSAT compared to gSAT) that were unaffected by exercise training.

Adipose depots have been proposed to arise from different mesodermal layers during embryonic development, directed through epigenetic-programmed mechanisms (432, 434). Homeobox family (HOX) is among the most studied families of developmental genes in AT and has been widely identified as fundamental in controlling the body plan along the anterior-posterior axis (432, 435). HOX genes have also been suggested as determinants of early regional differentiation by directing the evolution and differentiation from mesoderm layers to different AT depots (78). Additionally, it has been previously shown in both mice and humans, that multiple HOX genes are involved in embryonic development and pattern specification (e.g. *HOXA5, HOXC8, HOXC9*), and play a role in obesity and body fat distribution, with potential functional differences between distinct AT depots (81). In the present study, the following HOX genes were found with higher expression levels in aSAT than in gSAT: *HOXA5* (at both time points), *IRX2, HOXB8 and IRX5* (at baseline), and *IRX2, HOPX, HOXA3, HOXB5* and *HOXA9* (after exercise training). Previous studies in Europeans also showed higher expression of *HOXA5* in aSAT compared to gSAT as well as higher expression of this gene in the stromal fraction of aSAT (78) and VAT (81, 430). Furthermore, high levels of *HOXA5* have been closely related to BMI and body fat distribution (72, 78, 81, 84, 430). In addition, several homeobox genes were showed to be upregulated in aSAT and VAT (e.g. *HOXA3, HOXA9, HOXB5, HOXB8, IRX5, IRX2* and *HOPX*) (59, 78, 79, 81, 431, 436), suggesting a similar developmental origin of aSAT and VAT, rather than aSAT and gSAT (432). Notably, *IRX* genes (Iroquois homeobox) are potentially involved in the mechanisms underlying the genetic

association between *FTO* and obesity (437). The causal variant (*rs1421085*) of this association eliminates *IRX3* and *IRX5* repression, leading to a cell-autonomous shift from browning and thermogenesis of white adipocytes to lipid storage, increased fat stores and body-weight gain (437). Higher expression of *IRX* genes in aSAT could therefore be one of the causal factors in the detrimental central fat accumulation (aSAT) in obese individuals. However, this should be further investigated in a cohort of African women characterized with a low central and high gynoid fat accumulation.

Unlike the homeobox family, DMRT (d*oublesex*- and *mab*-3-related transcription factor) family has been less studied in the context of obesity and body fat distribution. Mostly involved in sex differentiation and gonadal development during embryogenesis in a wide range of species (438-440), the expression of DMRT genes has also been reported in aSAT of individuals with obesity (440). Higher expression of *DMRT2* and *DMRT3* were found in aSAT vs gSAT at baseline and post-exercise training in the present study, similar to findings from Passaro *et al.* in healthy men at rest (432). Additionally, a recent study has identified *DMRT3* expression in omental vs aSAT as a novel marker for the development of IR (441). *DMRT2* and *DMRT3* might, therefore, be novel candidate genes involved in "unhealthy" central fat accumulation. However, their functional role in AT development and function remains to be elucidated.

This study showed a higher expression of *CSN1S1* in gSAT compared to aSAT at baseline and post-exercise training. Previously known as a functionally unreported adipose-specific gene, *CSN1S1* expression was recently found to be significantly higher expressed in SAT (abdominal) of obese compared to lean individuals (442). Moreover, this gene is downregulated in aSAT after weight loss in morbidly obese individuals (443). Increased *CSN1S1* is involved in immune/inflammatory responses (444) and may also play a key role in adipogenesis and lipid metabolism (442). Indeed, higher expression of *CSN1S1* has been shown in mature adipocytes compared to preadipocytes and its expression was high during adipose-derived stem cell differentiation (442). Based on these findings, higher expression of *CSN1S1* may be reflective of higher storage capacity in gSAT.

Taken together, the unique gene expression signatures of aSAT and gSAT suggest differences in developmental processes regulating AT distribution and expandability of distinct depots. These data add to the notion that there are intrinsic morphological and functional differences between central and lower-body fat (59, 78, 430). Interestingly, from the comparison of these depots at baseline and after exercise training, 18 of the DEGs were overlapping and were enriched for anatomical structure morphogenesis. These findings

therefore postulate that the expression of developmental genes is not affected by external/environmental stimuli (such as exercise training) once they have been set during the early life stage. Rather, these differences are maintained across the depots as previously shown in cell culture studies (78, 79, 81, 82). Moreover, developmental genes might be actively involved in AT function, distribution and remodelling (193). GWAS and meta-analyses on European individuals identified several genes within the WHR-associated loci with differential transcription between aSAT and gSAT (76). However, except for *RSPO3*, the DEGs emerging from the present study did not overlap with these previous reports (76, 445), most probably explained by the difference of ethnicities.

Given the lower number of DEGs between SAT depots at baseline (15 genes) compared to the number after exercise training (318 genes), I propose that these major depot-specific differences reflect the heterogeneous capacity of SAT to adapt to behavioural (or environmental) changes, such as exercise training. This hypothesis is supported by a recent study showing that fat depots (e.g. SAT, VAT, liver, perirenal) respond with a highly variable alteration in fat mass during a weight loss intervention (dietary and physical activity) (446). The depot-specific AT response to exercise training could provide insight on metabolic and signalling pathways implicating fat distribution in the development of metabolic disorders. The specific response of each SAT depot to exercise training was therefore investigated in the present study. The expression of different sets of genes (and associated biological processes) was altered in SAT depots in response to exercise training, with only six genes commonly changed in both depots. This suggests differential regulatory mechanisms involved in cellular and endocrine function in these depots. Specifically, the DEGs in gSAT were mostly enriched for immune and inflammatory responses, suggesting increased inflammation after exercise training as previously shown in Chapter 5 of this thesis. Exercise training has been shown to activate macrophage production of cytokines, leading to the modulation of monocyte chemotaxis, differentiation into macrophages and activation (447). In addition, adipocytes readily respond to exercise training by modulating the transcriptomic response of pro-inflammatory genes (447). Therefore, an increased inflammatory response is likely to originate both from adipocytes and AT immune cell signalling. In line with this, both cell types can display similar transcriptomic profiles especially when the macrophages engulf lipids (448). However, our experimental design did not allow dissecting the cell types and their contribution to whole AT transcriptome.

The increased inflammatory response seems to be contradictory, given the extensively reported association between AT inflammation and the development of metabolic diseases (161, 448, 449) and decreased AT inflammation after exercise training (450). However, elevated immune response and activation of inflammatory pathways have been shown in SAT after acute exercise sessions (447, 451); and no studies have investigated the specific adaptations in gSAT in response to a long-term exercise training in Africans. The upregulation of inflammatory-related response could reflect structural changes of AT and adaptations in the early phase of the training, which is downregulated once structural changes are established (447). However, the beneficial effect of exercise training on AT physiology is the product of continuous changes induced by repeated transient acute exercise bouts (286). Hence the observed upregulation of immune and inflammatory responses after the present exercise training program mirrors a cumulative response of single exercise bouts. Notably, the sample analyzed in the present study were collected at least 3 days after the last exercise training session to exclude the potential acute effects of the last bout of exercise training. Furthermore, the participants of this study have never completed structured exercise training before participating in this program, supporting the hypothesis that sustained inflammatory activation after 12-week exercise training is associated with AT reorganization and remodeling. Consistently, the identified DEGs in gSAT were also involved in biological processes regulating lipid metabolism and plasma lipoprotein particle levels and remodelling (e.g. *MMP9*, *SPP1*, *APOC1*, *ITGAX* and *PLA2G7*). Changes in metabolic homeostasis such as improved body composition or reduced adiposity can trigger immune and inflammatory processes (451). For instance, the stimulation of lipolysis and enhanced fat mobilization in AT after exercise training (452, 453) was shown to be promoted by enhanced production of pro-inflammatory cytokines, such as IL-6 (453, 454). Furthermore, pro-inflammatory signalling in adipocytes mediates angiogenesis and ECM remodelling (147, 419). Additionally, the overall reduction of stored TGs in response to exercise training has been shown to precede an extensive remodelling of AT (420, 455). On the other hand, FFA released and the reduction of stored TGs in adipocytes in negative energy balance (e.g. in response to fasting, weight loss or exercise training) not only increase the activation and migration of immune cells into AT but also increase the expression of lipolysis-associated M2 macrophage markers, which are involved in tissue repair and remodelling (424, 425). Therefore, in contrast to the negative effects assigned to inflammatory processes, the observed upregulation of inflammation in response to exercise training is suggested to reflect SAT adaptation and remodelling in these women.

Surprisingly, in aSAT, most of the DEGs were enriched for muscle-associated processes (e.g. *ACAT1, TCAP, MYL2, MYH7, MYBPC*). Similar muscle-related gene expression profiles were found for the first time in brown AT of high fat diet-induced obesity-resistant rats (e.g. *TNNT3*, *ACTA1*, *ACTN3*, *MYLPF*), but their functional role in AT has not been clarified (456). As whole AT was used in the present study, the possibility that these genes were expressed from non-adipocytes/non-immune cells cannot be excluded. Nevertheless, twist-related protein (*TWIST1)* was down-regulated in aSAT following this exercise intervention. *TWIST1* is a transcription factor first identified in *Drosophila melanogaster* as a gene regulating mesoderm development; and subsequently reported to be expressed in human AT, mostly from adipocytes, and also from other cells in the AT such as macrophages, endothelial and T cells (457). *TWIST1* silencing in adipocytes results in the down-regulation of the expression and secretion of inflammatory cytokines such as TNFα, IL-6 and MCP1 (457) and attenuates IR (458). Besides, these cytokines are well established to play a major role in promoting IR locally and peripherally via the effects on insulin signaling, and FA and adipokine release, as well as gene transcription (459). In the present study, the down-regulation of *TWIST1* in aSAT in response to exercise training is concomitant with the reduction in inflammatory proteins levels (NFκB, TLR4, MCP1) in this depot only and not in gSAT, as reported in Chapter 5 (**Figure 5.1**). These results suggest the beneficial role of exercise training in aSAT, which might have contributed to the improvement of insulin sensitivity reported in Chapter 4. It could therefore be interesting to further measure the mRNA expression of *TWIST1* and to evaluate the association with systemic inflammation and S_I in these women. Moreover, subsequent investigations on isolated cell fractions, or single-cell RNA sequencing approaches, as well as cell culture experiments are required to further elucidate the mechanistic link between exercise training and signature changes of aSAT.

It is important to note that these analyses were limited by the amount of material obtained from the SAT biopsies. Histological, as well as extensive proteome analyses could not be performed, and whether gene expression differences were translated at the protein level could not be concluded. Moreover, the lack of a normal-weight control as a comparison group prevented me from concluding as to whether these findings are relevant pathways related to obesity and exercise training rather than an adaptation to the obese state. As the detection of DEGs was based on statistical methods, further validation is needed to rule out the possibility of false positives. Nevertheless, records on AT transcriptome of African populations are rare and this is one of the first studies providing unique data on SAT depot-specific gene expression profiles and adaptation to exercise training in black African women with obesity. This research

work generated hypotheses about novel candidate genes potentially implicated in the relationship between body fat distribution, AT adaptations and metabolic status.

In conclusion, the current study showed differential gene expression profiles of SAT depots (gluteal and abdominal) in obese black SA women. While the differences at baseline suggest dissimilar developmental origin, exercise training unmasked a heterogeneous response of different SAT depots to a physical challenge. The specific functional and metabolic responses could be related to intrinsic differences in the expression of developmental genes set at an early-life stage in each adipose depot. These were represented by an upregulation of the immune response and inflammatory processes, lipid metabolism and tissue remodelling in gSAT and surprisingly, mainly muscle-related processes in aSAT. Importantly, four genes (*CSN1S1*, *DMRT2*, *DMRT3* and *HOXA5*) were identified as potential novel candidates of body fat distribution pattern in Africans, whose biological function and implication in the pathogenesis of obesity-associated metabolic diseases remain to be unravelled.

CHAPTER SEVEN

SUMMARY DISCUSSIONS AND CONCLUSIONS

7.1. Summary discussion

Body fat distribution rather than total adiposity is a major determinant of the development of metabolic diseases (38-40). Central body fat (VAT and aSAT) is associated with an increased risk for T2D, while lower-body fat (gSAT) is proposed to be protective against the development of obesity-associated metabolic disorders (39, 40, 58, 59). It is well established that the susceptibility to an adverse metabolic status varies with ethnicity, and for the same level of adiposity, African descendants are at greater risk for cardio-metabolic diseases than Europeans descendants (25, 33, 69, 217). These differences may be partly explained by the difference in the pattern of body fat distribution, and most importantly, the depot-specific AT function within each ethnic group. In black SA women, higher SAT is associated with lower insulin sensitivity (25, 32). Indeed, increased in AT mass may negatively affect systemic and whole-body metabolism via several mechanisms (52, 101, 111, 134, 139). Among these mechanisms, the increased release of FFAs in the circulation may contribute to the development of low-grade inflammation and affect the function of peripheral tissues (134, 155, 357). Moreover, SAT dysregulated production and secretion of adipocytokines, oxidative stress and ectopic fat deposition are also suggested to be mainly involved in the association between increased SAT mass and impairment of insulin sensitivity in the obese state (52, 101, 111, 134, 139, 155, 357). However, these associations have not been previously explored in African women, who present with a different phenotype to their European counterparts and at a higher risk for IR and T2D (31-33). This represents an area of interest which might help to decipher this higher prevalence of metabolic diseases in Africans.

Physical activity, including exercise training, is an essential component in the management of obesity and is inversely correlated with the incidence of NCDs (1, 277). Exercise training has been shown to improve glucose metabolism and whole-body insulin sensitivity (287-289). Although the favourable exercise-induced changes to the metabolic profile have been principally attributed to physiological adaptations within skeletal muscle, exercise training-induced improvements in insulin sensitivity in obese individuals may also be exerted by changes in AT mass and function (87, 292), and consequently changes in systemic metabolism. Indeed, exercise training may partly improve the metabolic risk via an increase in energy demand and the utilization of excess calories stored in the fat depots, resulting in the reduction of body fat accumulation. Exercise training therefore represents a suitable model to investigate the relationship between SAT function and metabolic risk in obese black SA women, which to my knowledge has not been previously undertaken. This thesis aimed to i)

explore the associations between circulating and depot-specific SAT (aSAT and gSAT) FA profile, SAT lipid metabolism and body composition and insulin sensitivity; ii) evaluate the depot-specific changes in SAT metabolism and function in response to 12 weeks of supervised combined aerobic and resistance training and iii) evaluate the relationship between these changes and modifications in metabolic risk in previously sedentary obese black SA women. The main findings are summarized below and are presented in **Table 7.1.**

Table 7.1. Summary, implication and novelty of each study and conclusion

Chapter	Summary/Highlights	Implications	Novelty
Three	• FA profile of RBC-TPL differed from the FA profile of SAT depots with higher proportions of SFAs and PUFAs and lower MUFA in RBC-TPL than SAT. Estimated activities of D5D, D6D and SCD1-18 were higher and SCD1-16 was lower in RBC-TPL compared to SAT depots. • Higher RBC-TPL SFA was associated with lower S_I but did not correlate with VAT/SAT. • The FA profile of aSAT and gSAT differed, with higher SFAs and lower MUFAs, SCD1-16 and SCD1-18 activity in aSAT compared to gSAT. • The associations between the FA profiles and VAT/SAT and S_I did not differ between SAT depots, despite the difference in FA profile. In both SAT depots, 1) Lower SFAs and higher n-3 and n-6 PUFAs correlated with higher VAT/SAT ratio; 2) Lower n-3 and n-6 PUFAs and higher total MUFA correlated with higher S_I; 3) Lower SCD1-18 was associated with lower VAT/SAT and higher D6D correlated to lower S_I.	These findings showed that the associations between the FA profiles and metabolic status in obese black SA women are not only dependent on the class of FA, but most importantly on the tissue type where they are stored.	This was the first study that has comprehensively measured and compared the FA composition of RBC-TPL and two distinct SAT depots in humans with obesity; and evaluating the tissue-specific relationships between FA composition, desaturase activities and measures of central fat distribution and insulin sensitivity.

Table 7.1 continued.

Chapter	Summary/Highlights	Implications	Novelty
Four	• Twelve-weeks of combined aerobic and resistance exercise training improved S_I, cardiorespiratory fitness and body composition. • Exercise training altered RBC-TPL FA composition and estimated desaturase activity by reducing SCD1-16 and D6D (tendency), and increasing D5D, along with a reduction of SFAs (18:0 and 20:0) and DGLA (20:3n-6). • These changes corresponded to a decrease in liver fat and circulating concentrations of leptin and TNFα. • Exercise training resulted in SAT depot-specific change in FA composition, mostly in aSAT. Within aSAT, individual SFAs 12:0 and 14:0 decreased and individual PUFAs 20:2n-6 and 22:4n-6 increased. Within gSAT only GLA (18:3n-6) decreased. • There was no association between these changes in SAT FAs and changes in liver fat, systemic inflammation or S_I. • Exercise training did not alter the expression of genes involved in lipid metabolism and insulin signalling in both SAT depots.	These findings suggested that exercise training stimulates tissue-specific changes in FA classes. Changes in RBC-TPL FA profiles were not reflective of the changes in FA profile and lipid metabolism in SAT. This study also showed that the exercise-induced improvement in S_I was not directly mediated by the changes in FA composition in the circulation and SAT, or by the changes in SAT lipid metabolism.	This was the first study evaluating the effects of exercise training on FA composition of RBC-TPL, aSAT and gSAT; however, these adaptations were not directly related to the exercise-induced improvements in S_I, but rather to lower systemic inflammation and lower liver fat accumulation.

Table 7.1 continued.

Chapter	Summary/Highlights	Implications	Novelty
Five	• The cross-sectional analysis comparing gene and protein expression between aSAT and gSAT showed that gSAT had a higher leptin mRNA expression, but the aSAT had a higher inflammatory protein expression than gSAT. • Exercise training improved systemic oxidative stress in obese black women (reduction in TBARs concentration and an increase in catalase activity) with no changes in circulating concentration of inflammatory markers. • Exercise training-induced changes in inflammatory mRNA levels were depot-specific, showing increased inflammatory gene expression (*IL-10*, *TNFα*, *NFκB1* and *MIF* mRNA) in gSAT, with no changes in aSAT. • In response to exercise training, inflammatory protein levels (MCP1, NFκBp65 and TLR4) decreased in aSAT only. • These changes in systemic (oxidative stress) and SAT depots (inflammatory markers) were associated with the reduction in gynoid fat, but not with the improvement in S_I.	This study showed that exercise training stimulated a depot-specific effect on the SAT inflammatory profile and importantly, suggested that the increase of inflammatory state in gSAT was reflective of tissue remodelling. This was consecutive to the reduction of gynoid fat but was not associated with the improvement in whole-body S_I in obese black SA women.	This was the first study in women of African ancestry evaluating changes in SAT depots and systemic inflammatory and oxidative stress markers in response to an exercise training intervention.

Table 7.1 continued.

Chapter	Summary/Highlights	Implications	Novelty
Six	• Transcriptomic profiles differed between aSAT and gSAT at baseline with 15 DEGs, which were mostly associated with embryonic development and anatomical structure morphogenesis. • Depot-specific differences in SAT transcriptome greatly increased with exercise training, with 318 DEGs, and only 3 genes (*COL1A1, HP* and *CILP*) commonly changed in aSAT and gSAT. • The most prominent DEGs between SAT depots were four developmental genes, with expression unaffected by the exercise training (*DMRT2, DMRT3* and *HOXA5* higher, and *CSN1S1,* for which the expressed was lower in aSAT compared to gSAT). • Exercise training resulted in changes in different sets of genes in the SAT depots. In gSAT DEGs were mostly enriched for immune and inflammatory responses, while aSAT DEGs reflected mostly muscle-associated processes.	This study demonstrated differential gene expression profiles of gluteal and abdominal SAT depots at baseline and after 12-weeks of exercise training. This study generated hypotheses on novel candidate genes potentially implicated in the relationship between body fat distribution, SAT function and metabolic status in black SA women with obesity.	Reports on the AT transcriptome of African populations are scarce. This is one of the first studies providing unique data on abdominal and gluteal SAT transcriptomic signatures and the depot-specific adaptation to an external stimulus such as exercise training in black African women with obesity.

FA: fatty acids; SAT: subcutaneous adipose tissue; SFA: saturated fatty acid; MUFA: mono-saturated fatty acid; PUFA: poly-saturated fatty acid; D5D: delta-5 desaturase; D6D: delta-6 desaturase; SCD1: stearoyl-CoA desaturase 1; SI: Insulin sensitivity; RBC-TPL: red blood cell total phospholipids aSAT: Abdominal subcutaneous adipose tissue; gSAT: gluteal subcutaneous adipose tissue; SA: South African; DGLA: dihomo gamma-linolenic acid; GLA: gamma-linolenic acid; TBARS: thiobarbituric reactive acid substances; IL-10: Interleukin 10; TNFα: tumor necrosis factor-alpha; NFkB: nuclear factor-kappa B; MIF: macrophage migration inhibitory factor; MCP1: monocyte chemoattractant protein 1; TLR4: toll-like receptor 4; DEGs: differentially expressed genes.

One of the principal characteristics of obesity is the excessive accumulation of TGs in AT depots, mainly SAT, which serves to protect other tissues against lipotoxicity. However, if the SAT expansion limit is reached, progressive energy intake results in the spillover of the excess lipids to non-adipose organs, including VAT (134). This limited storage capacity of the SAT is characterized by impaired lipogenesis, high lipolytic rates (basal and stimulated), and consequently increased FFA release into the bloodstream. FFAs interfere with glucose metabolism and phosphorylation activity in peripheral tissues, contributing to IR (250, 357). Further, this increased release of FFAs, in addition to high dietary fat intake, may alter circulating FA profiles. Alterations in the FA composition of RBC-TPL membrane have been associated with IR in obese individuals (358). Therefore, the dysregulation in SAT lipogenesis and lipolysis, as well as circulating and SAT FA composition might explain the higher susceptibility to metabolic risk in obese black SA women. No studies have investigated the association between both circulating FA profile and SAT lipid metabolism and metabolic risk in this population.

The first study of this thesis (Chapter 3) aimed to examine and compare the FA composition of RBC-TPL, aSAT and gSAT and to evaluate the tissue-specific associations of the differential FA profiles with VAT/SAT ratio and S_I in obese black SA women. The main findings were the distinct different FAs composition between circulating (RBC-TPL) and SAT. Specifically, SFA and PUFA contents were higher, and MUFA content lower in RBC-TPL compared to SAT depots. High dietary intake of PUFAs and endogenous synthesis of SFAs from energy sources such as carbohydrates may explain the high concentration of these FA classes in the circulation (365). This study further showed a tissue-specificity in FA composition within SAT depots, with higher SFAs and lower MUFA contents in aSAT compared to gSAT. Furthermore, this study showed that not only the metabolic effects of FAs vary between different FA classes, but more importantly these effects are dependent on the blood compartment/ fraction where they occur or tissue type where they are stored. Surprisingly, despite the differences in FA composition between SAT depots, these were similarly associated with VAT/SAT ratio and S_I. Specifically, lower SFAs content was inversely associated with higher VAT/SAT ratio, whereas higher total MUFA and lower PUFA were associated with higher S_I. Conversely, there were only a few associations between these variables and circulating FA profiles, with higher RBC-TPL SFA and 20:2n-6, and lower 22:4n-6 correlating with lower S_I. The variance in VAT/SAT ratio and S_I were mainly explained by the FA composition of SAT depots. This suggests a stronger influence of SAT lipid metabolism

and consequently SAT FA composition, than dietary FA intake (reflected by RBC-TPL) on the metabolic risk in obese black SA women.

Inflammation is one of the main factors linking obesity to increased risk for IR and T2D. In addition to ectopic fat deposition, increased basal lipolysis in the obese state and the resultant increase of FA in the circulation may induce systemic inflammation (155). In Chapter 3 of this thesis, the correlations between circulating individual PUFAs and S_I showed no consistent pattern. In addition, because of the cross-sectional design of this study, a causative relationship between SAT FA composition with VAT/SAT ratio and S_I could not be established. Since physical activity increases AT catabolism and can therefore modify SAT lipid and FA metabolism independently of dietary intake (138), an exercise training intervention was used as a model to further understand the tissue-specific metabolic effects of individuals FAs and desaturase activities on systemic inflammation, liver fat content and insulin sensitivity in these women. The changes in RBC-TPL and SAT FA composition and estimated desaturase activity in response to a 12-week (supervised) combined aerobic and resistance exercise training in obese and previously sedentary black SA women was therefore evaluated in Chapter 4 of this thesis. Moreover, the associations between these changes and alterations in SAT lipid metabolism, systemic inflammatory markers, liver fat and S_I were also explored.

Twelve weeks of supervised, combined aerobic and resistance training resulted in improvements in S_I, VO_{2peak} and body composition, including a reduction in body weight, BMI, WC and gynoid fat %. This study further showed FA class- and tissue-specific effects of exercise training, with decreased RBC-TPL SFAs (18:0 and 20:0), DGLA (20:3n-6), SCD1-16 and D6D, and increased in D5D activity. Remarkably, these changes were associated with decreased liver fat and lower circulating leptin and TNFα concentrations, and not with the improvement of S_I. Given the implication of ectopic fat deposition and systemic inflammation in the development of IR in obese state (61, 134), the changes in RBC-TPL associated with a reduction of liver fat content, circulating leptin and TNFα could represent an indirect mechanism by which exercise training improved S_I in these women. Conversely, the changes of individual FA levels mainly in aSAT with decreased SFAs (12:0 and 14:0) and increased PUFAs (20:2n-6 and 22:4n-6) did not correlate with the measured metabolic parameters, including S_I.

This is the first study showing positive effects of exercise training on the circulating FA profile, with decreased proportions of SFAs, and associations with an improved metabolic profile (lower inflammation and liver fat accumulation) in black SA women. The possibility

that these changes may have indirectly contributed to the reported improvement in S_I cannot be ruled out. However, the unaltered desaturase activities, unchanged expression of genes involved in adipogenesis, lipogenesis, lipolysis and insulin signalling in both SAT depots following this intervention suggests that these factors are not involved in the exercise-induced improvement in S_I, but further investigations are required. Indeed, given the hypothesis that there would be significant involvement of SAT function in the whole-body metabolic profile of obese individuals, what could be the mechanism/potential pathways linking SAT function with IR in obese black SA women?

To address this question, Chapter 5 of this thesis aimed to evaluate the effects of 12-week exercise training on systemic and aSAT vs gSAT inflammatory and oxidative status in black SA women. Indeed, unrestrained lipolysis is associated with increased inflammation in SAT and dysregulated secretion of adipo-cytokines into the circulation (134). Inflammation and oxidative stress are also interrelated during obesity and together, are major contributors to IR and development of T2D (171, 174). The main findings of this chapter were the improvement in systemic oxidative stress, characterized by a reduction of TBARS and an increase in catalase and SOD (tendency). Further, exercise training resulted in depot-specific changes in SAT inflammatory profile, characterized by an increase of inflammatory gene expression in gSAT and decrease trend of inflammatory protein expression in aSAT. Notably, these changes correlated with the reduction in gynoid fat % and not with the improvement in S_I. Similarly, a cross-sectional study in black SA women showed that high SAT (abdominal and gluteal) inflammatory gene expression did not explain the variance in S_I (213). However, the novel aspect of the finding from Chapter 5 of this thesis was the relationship between the depot-specific changes in SAT inflammation, the improvement of systemic oxidative stress and the reduction of gynoid fat.

This study showed for the first time in black African women that higher inflammation in gSAT could be reflective of remodelling of this tissue associated the decrease of fat mass, rather than increased inflammation described during pathological SAT expansion. This hypothesis is supported by data showing that fat mass reduction requires extensive tissue remodelling, and an adequate ECM and angiogenesis necessitates inflammation in the adipocyte microenvironment (147, 419-421). Therefore, I hypothesize that the reduction of gynoid fat in response to exercise training induced a positive metabolic profile in these women, characterized by lower systemic oxidative stress and lower inflammation in aSAT. Gynoid fat or lower-body fat accumulation is the preferential/major site of fat storage in women of African ancestry (31-

33). Therefore, I further hypothesize that the reduction of gynoid fat in response to exercise training may be interpreted as increasing the depot storage capacity, by making "extra room" to accommodate future TGs accumulation in periods of excess calorie intake and sedentary behaviour. This would consequently protect against the spillover of excess lipids to VAT (and other organs) and subsequently reduce or prevent inflammation in these tissues, as well as systemic oxidative stress. Therefore, although not directly associated with these changes, the exercise-induced improvement in S_I may be the indirect consequence of the reduction of gynoid fat mass and changes in systemic oxidative stress and SAT inflammatory status. However, this hypothesis should be verified in future studies. Nevertheless, these findings add to the concept of the protective nature of lower-body fat in obesity-associated metabolic disorders and highlight the need for further research exploring specific pathways in SAT function that may directly affect or regulate peripheral/whole-body insulin sensitivity. The identification of such pathways may be informative for future therapeutic targets in the management of obesity in this high-risk population in Africa.

The mechanisms underlying SAT depot-specific associations with metabolic risks are not fully understood. In addition to the evidence showing differences in metabolism (e.g. FA composition in Chapter 3 and Chapter 4) and function (e.g. inflammatory profile in Chapter 5), the differential metabolic effects of SAT depots may also be driven by distinctive gene expression profiles, which may be controlled by environmental and genetic factors (46, 78). Indeed, given the preferential expansion of AT depots largely determined by heritable factors (144), the specific response of AT to calorie intake and/or energy demand may be influenced by site-specific developmental processes. The embryonic origins of SAT depots remain under-studied and have not been investigated in black SA women. Understanding the developmental origins of this tissue may help to understand the differences in fat accumulation patterns in these women, as well as the specific response to external stimuli such as exercise training.

Therefore, Chapter 6 of this thesis used an untargeted gene expression analysis approach (unbiased microarray analysis) to explore the transcriptome signatures of SAT depots. This study aimed to differentiate aSAT and gSAT gene expression profiles in obese black SA women and to examine whether the gene expression patterns of these depots were also altered in a depot-specific manner by 12-week exercise training. This was the first study, to the best of my knowledge that has demonstrated differential gene expression profiles of aSAT and gSAT in non-stimulated (baseline) and stimulated (exercise training) states, using an untargeted analysis approach. The difference between the number of DEGs at baseline (15) and after the exercise

training intervention (318) was outstanding. These findings suggested that except for their differential developmental origin (showed here by the differences in sets of developmental genes mainly involved in embryonic development and anatomical structure at baseline), SAT depots are relatively similar in their functional pathways until an external stimulus (which could be comparable to environmental factors) is applied. These depots therefore exhibit highly divergent responses under different environmental/behavioural stimuli, illustrated in this study by exercise training. While the changes in aSAT transcriptome in response to exercise training requires further investigation in terms of the cell types involved, this study showed an upregulation of the immune response and inflammatory processes, lipid metabolism and tissue remodelling in gSAT, as per the findings of the previous chapter (Chapter 5). Another novel aspect of this study was the identification of four developmental genes, including higher expression of *DMRT2*, *DMRT3* and *HOXA5*, and lower expression of *CSN1S1* in aSAT compared to gSAT. I hypothesise that these genes may represent novel candidate genes in the body fat distribution pattern of black women of African ancestry, and could potentially be implicated in the development of IR. However, their biological function and implication in the pathogenesis of obesity-associated metabolic diseases remain to be revealed.

In summary, this thesis was the first research, to my knowledge, to investigate depot-specific SAT metabolism and function on systemic and whole-body metabolism, with the use of exercise training as an additional investigation model. This thesis explored depot-specific differences in SAT metabolism and function in obese black SA women at baseline and in response to exercise training, examining differential pathways. These pathways included FA composition, lipogenesis, lipolysis, adipogenesis, insulin signalling, oxidative stress and inflammatory profiles, as well as whole transcriptome signatures. Notably, SAT response to an external stimulus such as exercise training differs, with depot-specific modifications in FA composition, inflammatory profiles and transcriptomic signatures. However, the depot-specific differences in FA composition could not explain the differential association of SAT depots with insulin sensitivity/IR proposed in the literature. Moreover, the improvement in S_I with exercise training could not be accounted for by the changes in RBC-TPL and SAT FA composition, SAT inflammation or systemic oxidative stress. The reduction in gynoid fat in response to exercise training supported the proposed protective nature of gSAT over central body fat (aSAT) in these women, which was associated with an improved metabolic profile, including lower systemic oxidative stress and aSAT inflammatory profile. This thesis proposed that the protective nature of gSAT is exerted by its potential for increased storage capacity during periods of positive energy intake and sedentary lifestyle, thereby avoiding lipid spillover to

peripheral tissues. This is hypothesized to be a mechanism linking gSAT function to the improvement of S_I in response to long-term exercise training. However, it is more likely that the improvement in S_I in response to this intervention could have been derived from modifications in skeletal muscle physiology; for example, increased mitochondria oxidative capacity and FA oxidation, reduced inflammation or higher glucose uptake and insulin signalling. Further research is required to address this hypothesis.

The increased inflammatory profile in gSAT in response to exercise training reported in the Chapters 5 and 6 of this thesis might not be 'detrimental', as observed during the impairment of SAT expandability capacity during fat accumulation (61, 134). Rather, this thesis proposed that higher inflammatory profile after exercise training in black SA women is the response to the reduction in fat mass, specifically gSAT. It is therefore hypothesized that increased AT inflammation in the obese state (61, 134) may also be representative of the remodelling of this tissue to accommodate more fat. Noteworthy, most studies investigating the causality of inflammation in the development of obesity-associated IR have focused on central fat, especially VAT and aSAT, with very few studies focusing on gSAT function, particularly in African women. Further studies should therefore explore the inflammatory status of gSAT in the context of excess energy intake and its influence on whole-body insulin sensitivity, principally in African women, who accumulate a large amount of fat in this depot. Furthermore, GWAS or whole-genome profiling studies, have investigated the developmental origin of AT depots to understand the genetics of obesity and have identified several loci associated with BMI, body fat distribution and body fat percentage, as well as several genetic variants associated with the development of IR and T2D (39, 46, 52, 76, 190). Given that these studies were mostly conducted in European ancestry populations, this thesis is unique in that it reported for the first time, differences in transcriptomic signatures between aSAT and gSAT in women from African ancestry, with the identification of potential novel developmental genes. Future studies should explore these genes as candidate genes associated with body fat distribution and obesity-associated IR.

7.2. Strengths and limitations

Strengths of this thesis include the objective measurements of FA composition both in the circulation (RBC) and major AT reservoirs, detailed measures of systemic and depot-specific SAT oxidative stress and inflammatory markers, expression of genes involved in adipogenesis, lipolysis, lipogenesis and insulin signalling, as well as untargeted depot-specific gene expression profiling. In combination with these data, the measures of body fatness and

distribution, liver fat and whole-body insulin sensitivity, using state-of-the-art techniques, make the findings of this thesis novel and of clinical relevance in a context of obesity and associated metabolic risks in obese black SA women. Moreover, the exclusion of potential confounders such as inflammatory-related diseases and medication during participant recruitment represents additional strengths of this research work. African women have shown to be more insulin resistant than their white counterparts, despite their greater SAT accumulation mainly in the lower-body region, with less VAT. This makes the studied population unique, representing a major strength of this thesis. Notably, the specific effects of exercise training were evaluated in this thesis rather than the acute response of exercise, as measures of insulin sensitivity and biopsies were performed at least 72 hours after the last exercise session. Additionally, this exercise training intervention was supervised and well monitored by a trained exercise physiologist, who ensured that the prescribed exercise intensity was achieved by using heart rate monitoring and ratings of perceived exertion. The attendance was monitored throughout the 12 weeks, and this intervention had good overall participant adherence (range: 52-100 %), which is most likely because the exercise training program was based on prior focus group discussions in the same community (460).

Some limitations of this thesis should be stated. Firstly, the lack of different ethnic comparator group represents a major limitation of this research work. This makes it difficult to attribute the findings of this thesis specifically to black South African woman only. Moreover, this thesis did not include male participants. The data may therefore not be representative of the black SA population and cannot be directly translated to other groups of individuals with different phenotypes and/or age, sex, socioeconomic and health status. Moreover, the lack of a normal-weight control as a comparison group prevented conclusions as to whether these findings were specific pathways related to obesity and exercise training rather than an adaptation to the obese state. These results need to be verified in more representative samples of black Africans, including normal-weight groups. Besides, only whole-body insulin sensitivity was measured using the FSIGT. Accordingly, changes in hepatic and muscle insulin sensitivity could not be determined, which would have been of particular interest. However, the FSIGT method was chosen for this study as it provided a measure of both insulin sensitivity and secretion. Black SA women hyper-secrete insulin and it is not clear whether this is a cause or a consequence of insulin resistance. Hence this was a focus of the parent study.

The cross-sectional associations presented in Chapter 3 prevented me from concluding causality. For the determination of tissue-specific FA composition and metabolism in the Chapters 3 and 4, the desaturase enzyme activities were estimated based on product to precursor

FA ratios and may indicate the higher proportion of product than precursor in this tissue and not necessarily a higher desaturation level. Future studies may therefore measure the direct activity of the main desaturases as well as elongases involved in FA metabolism (e.g. FASN, ACC). The lack of detailed information on the dietary FA intake may also represent a limitation. However, the robust measure of the FA composition of RBC membrane and AT is regarded as more accurate than estimations from dietary self-reporting. Furthermore, the small sample of selected participants for the sub-study of protein analysis (due to tissue availability and related costs) in Chapter 5 represents a major limitation. It is important to note that the results from this analysis were presented as a pilot study monitoring the changes of the selected genes at the translational level in response to exercise training. Similarly, Chapter 5 was limited by the number of genes evaluated using a candidate genes approach. Unfortunately, due to limitations with tissue availability and cost, additional protein and mRNA analyses could not be completed. I acknowledge that there may be other genes and proteins involved in the evaluated pathways that were not measured. However, this issue was partly addressed in Chapter 6 of this thesis with the untargeted gene expression profiling of a sub-sample of women; although the small sample size also represents a limitation of this microarray analysis. Further, histological as well as extensive proteome analyses could not be performed, and it is difficult to conclude whether gene expression differences in the microarray analyses were translated at the protein level. The findings of this study need to be confirmed in a larger sample. As the detection of DEGs was based on statistical methods, further validation is needed to rule out the possibility of false positives.

7.3. Future research directions

Based on the results generated from this thesis, SAT metabolism and depot-specific changes in SAT function in response to exercise training do not directly explain the variations in whole-body insulin sensitivity in black SA women; although AT expansion has been generally associated with the development of IR and T2D in some individuals with obesity. Many questions therefore remained unanswered, particularly relating to the influence of SAT function on metabolic status and the main determinations of the exercise training-related improvement in S_I in black SA women. This underlies the complexity of obesity-associated IR in women of African ancestry. Further studies should focus on other organs/tissues that affect metabolic risk factors, such as skeletal muscle, liver or pancreas, with a specific focus on the effect of exercise training on ectopic fat deposition and function of these tissues, which may also contribute to disease risk. Furthermore, future research must unravel the molecular

mechanisms that direct the endogenous metabolism of circulating FA, the influence of RBC-TPL FA composition and desaturase activities on liver fat accumulation and systemic inflammation, as well as the clinical relevance of the changes in SAT FA composition in response to exercise training.

This thesis provided unique data on SAT depot-specific gene expression profiles and the adaptation to exercise training in black African women with obesity. This research work generated hypotheses about novel candidate genes potentially implicated in the relationship between body fat distribution, AT adaptation and metabolic status. Subsequent studies should investigate the biological function of these genes and explore their involvement in metabolic risk associated with body fat distribution in this population. Furthermore, future investigations on isolated cell fractions, or single-cell RNA sequencing approaches, as well as cell culture experiments should also be undertaken to further elucidate the mechanistic link between exercise training and changes of AT transcriptomic signatures.

The practical implication of these findings is that exercise training interventions provide an effective therapy for the management of IR and T2D risks in black SA women, with the improvement of cardiorespiratory fitness, body composition and insulin sensitivity. These women characterized with a poor cardiorespiratory fitness should be continuously encouraged to engage in moderate-to-high intensity PA. However, socio-economic issues related to adequate exercise practice in SA (e.g. lack of time and facilities, safety issues, cultural differences in body image and body size satisfaction or lack of community resources) are barriers to the uptake of PA (279, 460). Although the focus of this thesis was PA and the improvement of the metabolic risk in black SA women, this intervention did not extensively reduce obesity. Dietary interventions have been shown to stimulate body fat reduction and improvement of body composition to a greater extent than PA (270-272). Other studies should therefore focus on dietary interventions in this population, as more profound changes in body weight and body composition might have amplified the improvement of metabolic profile in these women. Finally, South Africans, both at the individual and governmental level, need to work together to improve conditions promoting sufficient physical activity and improve dietary quality, in order to tackle the epidemics of obesity and T2D in this country.

REFERENCES

1. WHO. GLOBAL STATUS REPORT on noncommunicable diseases 2014. "Attaining the nine global noncommunicable diseases targets; a shared responsibility"2014.

2. IDF Diabetes Atlas, 8th edition2017 (accessed 27 Decenber 2019).

3. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract. 2014;103(2):137-49.

4. Peer N, Steyn K, Lombard C, Lambert EV, Vythilingum B, Levitt NS. Rising diabetes prevalence among urban-dwelling black South Africans. PLoS One. 2012;7(9):e43336.

5. Peer N, Kengne AP, Motala AA, Mbanya JC. Diabetes in the Africa Region: an update. Diabetes Res Clin Pract. 2014;103(2):197-05.

6. Joubert J, Norman, R., Bradshaw, D., Goedecke, J. H., Steyn, N. P., Puoane, T. Estimating the burden of disease attributable to excess body weight in South Africa in 2000. South African Medical Journal. 2007;97(8):683-90.

7. Berrington de Gonzalez A, Hartge P, Cerhan JR, Flint AJ, Hannan L, MacInnis RJ, et al. Body-mass index and mortality among 1.46 million white adults. N Engl J Med. 2010;363(23):2211-119.

8. Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS. Prevalence of obesity, diabetes, and obesity-related health risk factors. JAMA. 2003;289(1):76-9.

9. Ng M, Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono C, Mullany, E. C., Biryukov, S., Abbafati, C., Abera, S. F., Abraham JP, Abu-Rmeileh NME, Achoki T, AlBuhairan FS, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. The Lancet. 2014;384(9945):766-81.

10. Hilawe EH, Yatsuya H, Kawaguchi L, Aoyama A. Differences by sex in the prevalence of diabetes mellitus, impaired fasting glycaemia and impaired glucose tolerance in sub-Saharan Africa: a systematic review and meta-analysis. Bull World Health Organ. 2013;91(9):671 - 82D.

11. Shisana O, Labadarios D, Rehle T, Simbayi L, Zuma K, Dhansay A, et al. South African National Health and Nutrition Examination Survey (SANHANES-1). Cape Town: HSRC Press; 2013.

12. Forouzanfar MH, Afshin A, Alexander LT, Anderson HR, Bhutta ZA, Biryukov S, et al. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. The Lancet. 2016;388(10053):1659-724.

13. Pheiffer C, Pillay-van Wyk V, Joubert JD, Levitt NS, Nglazi MD, Bradshaw SD. The prevalence of type 2 diabetes in South Africa: a systematic review protocol. BMJ Open. 2018;8:e021029.

14. Glezeva N, Chisale M, McDonald K, Ledwidge M, Gallagher J, Watson CJ. Diabetes and complications of the heart in Sub-Saharan Africa: An urgent need for improved awareness, diagnostics and management. Diabetes Res Clin Pract. 2018;137:10-9.

15. Vorster HH, Venter CS, Wissing MP, Margetts BM. The nutrition and health transition in the North West Province of South Africa: a review of the THUSA (Transition and Health during Urbanisation of South Africans) study. Public Health Nutr. 2005;8(5):480-90.

16. Steyn K, Kazenellenbogen JM, Lombard CJ, Bourne LT. Urbanisation and the risk for chronic diseases of lifestyle in the black population of the Cape Peninsula, South Africa. J Cardiovasc Risk. 1997;4:135-42.

17. Pisa PT, Pisa NM. Economic growth and obesity in South African adults: an ecological analysis between 1994 and 2014. The European Journal of Public Health. 2016;27(3):404-9.

18. Gassasse Z, Smith D, Finer S, Valentina GV. Association between urbanisation and type 2 diabetes: an ecological study. BMJ Glob Health. 2017;2:e000473.

19. Popkin BM. An overview on the nutrition transition and its health implications: the Bellagio meeting. Public Health Nutr. 2002;5(1A):93-103.

20. Assembly UNG. General Assembly on the comprehensive review and assessment of the progress achieved in the prevention and control of non-communicable diseases. 2014 17 July 2014.

21. van der Berg JD, Stehouwer CD, Bosma H, van der Velde JH, Willems PJ, Savelberg HH, et al. Associations of total amount and patterns of sedentary behaviour with type 2 diabetes and the metabolic syndrome: The Maastricht Study. Diabetologia. 2016;59(4):709-18.

22. den Braver NR, Lakerveld J, Rutters F, Schoonmade LJ, Brug J, Beulens JWJ. Built environmental characteristics and diabetes: a systematic review and meta-analysis. BMC Med. 2018;16(1):12.

23. Steyn NP, Nel JH, Parker W, Ayah R, Mbithe D. Urbanisation and the nutrition transition: a comparison of diet and weight status of South African and Kenyan women. Scand J Public Health. 2012;40(3):229-38.

24. Goedecke JH, Dave JA, Faulenbach MV, Utzschneider KM, Lambert EV, West S, et al. Insulin response in relation to insulin sensitivity: an appropriate beta-cell response in black South African women. Diabetes Care. 2009;32(5):860-5.

25. Lovejoy JC, de la Bretonne JA, Klemperer M, Tulley R. Abdominal fat distribution and metabolic risk factors: Effects of race. Metabolism. 1996;145(9).

26. Osei K, Schuster DP, Owusu SK, Amoah AG. Race and ethnicity determine serum insulin and C-peptide concentrations and hepatic insulin extraction and insulin clearance: comparative studies of three populations of West African ancestry and white Americans. Metab Clin Exp. 1997;46:53-8.

27. Van der Merwe MT, Crowther NJ, Schlaphoff GP, Gray IP, Joffe BI, Lönnroth PN. Evidence for insulin resistance in black women from South Africa. Int J Obes Relat Metab Disord 2000;24:1340-6.

28. Preis SR, Massaro, J. M., Robins, S. J., Hoffmann, U., Vasan, R. S., Irlbeck, T., Meigs, J. B., Sutherland, P., D'Agostino, R. B., Sr., O'Donnell, C. J., Fox, C. S. Abdominal subcutaneous and visceral adipose tissue and insulin resistance in the Framingham heart study. Obesity (Silver Spring). 2010;18(11):2191-8.

29. Shay CM, Carnethon, M. R., Church, T. R., Hankinson, A. L., Chan C, Jacobs, D. R., Jr., Lewis, C. E., Schreiner, P. J., Sternfeld, B., Sidney, S. Lower extremity fat mass is associated with insulin resistance in overweight and obese individuals: the CARDIA study. Obesity (Silver Spring). 2011;19(11):2248-53.

30. Rush EC, Goedecke JH, Jennings C, Micklesfield L, Dugas L, Lambert EV, et al. BMI, fat and muscle differences in urban women of five ethnicities from two countries. Int J Obes (Lond). 2007;31(8):1232-9.

31. Matsha TE, Ismail S, Speelman A, Hon GM, Davids S, Erasmus RT, et al. Visceral and subcutaneous adipose tissue association with metabolic syndrome and its components in a South African population. Clin Nutr ESPEN. 2019;32:76-1.

32. Goedecke JH, Levitt, N. S., Utzschneider, K. M., Faulenbach, M. V., Dave, J. A., West, S., Victor, H., Evans, J., Olsson, T., Walker, B. R., Seckl, J. R., Kahn, S. E. . Differential effects of abdominal adipose tissue distribution on insulin sensitivity in black and white South African women. Obesity (Silver Spring). 2009;17(8):1506-12.

33. Keswell D, Tootla, M., Goedecke, J. H. Associations between body fat distribution, insulin resistance and dyslipidaemia in black and white South African women. Cardiovasc J Afr. 2016;27(3):177-83.

34. Katzmarzyk PT, Bray GA, Greenway FL, Johnson WD, Newton RL, Jr., Ravussin E, et al. Racial differences in abdominal depot-specific adiposity in white and African American adults. Am J Clin Nutr. 2010;91(1):7-15.

35. Goedecke JH, Evans J, Keswell D, Stimson RH, Livingstone DE, Hayes P, et al. Reduced gluteal expression of adipogenic and lipogenic genes in Black South African women is associated with obesity-related insulin resistance. J Clin Endocrinol Metab. 2011;96(12):E2029-33.

36. Goedecke JH, Levitt, N. S., Evans, J., Ellman, N., Hume, D. J., Kotze, L., Tootla, M., Victor, H., Keswell, D. The role of adipose tissue in insulin resistance in women of African ancestry. J Obes. 2013;2013:952916.

37. Goedecke JH, Mtintsilana A, Dlamini SN, Kengne AP. Type 2 diabetes mellitus in African women. Diabetes Res Clin Pract. 2017;123:87-96.

38. Schleinitz D, Bottcher Y, Bluher M, Kovacs P. The genetics of fat distribution. Diabetologia. 2014;57(7):1276-86.

39. Frank AP, De Souza RS, Palmer BF, Clegg DJ. Determinants of body fat distribution in humans may provide insight about obesity-related health risks. Journal of Lipid Research. 2018.

40. InterAct C, Langenberg C, Sharp SJ, Schulze MB, Rolandsson O, Overvad K, et al. Long-term risk of incident type 2 diabetes and measures of overall and regional obesity: the EPIC-InterAct case-cohort study. PLoS Med. 2012;9(6):e1001230.

41. Cannon B, Nedergaard J. Brown Adipose Tissue: Function and Physiological Significance. Physiol Rev. 2004;84:277-359.

42. Ouellet V, Labbe SM, Blondin DP, Phoenix S, Guerin B, Haman F, et al. Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. J Clin Invest. 2012;122(2):545-52.

43. Scheele C, Larsen TJ, Nielsen S. Novel nuances of human brown fat. Adipocyte. 2014;3(1):54-7.

44. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, et al. Identification and importance of brown adipose tissue in adult humans. N Engl J Med. 2009;360(15):1509-17.

45. Santos RS, Frank AP, Fatima LA, Palmer BF, Oz OK, Clegg DJ. Activation of estrogen receptor alpha induces beiging of adipocytes. Mol Metab. 2018;18:51-9.

46. Lee MJ, Wu Y, Fried SK. Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. Mol Aspects Med. 2013;34(1):1-11.

47. Karastergiou K, Smith, S. R., Greenberg, A. S., Fried, S. K. Sex differences in human adipose tissues – the biology of pear shape. Biology of Sex Differences. 2012;3(13).

48. Ibrahim MM. Subcutaneous and visceral adipose tissue: structural and functional differences. Obes Rev. 2010;11(1):11-8.

49. Wronska A, Kmiec Z. Structural and biochemical characteristics of various white adipose tissue depots. Acta Physiol (Oxf). 2012;205(2):194-208.

50. Sacks HS, Fain JN. Human epicardial adipose tissue: a review. Am Heart J. 2007;153(6):907-17.

51. Lenz M, Arts ICW, Peeters RLM, de Kok TM, Ertaylan G. Adipose tissue in health and disease through the lens of its building blocks. Sci Rep. 2020;10(1):10433.

52. Pandžić JV, Grizelj D. Under the Surface of Subcutaneous Adipose Tissue Biology. Acta Dermatovenerologica Croatica. 2016;24(4):250-60.

53. Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology. 2004;145(5):2273-82.

54. Dalmas E, Tordjman J, Guerre-Millo M, Clement K. [Adipose tissue, a new playground for immune cells]. Med Sci (Paris). 2011;27(11):993-9.

55. Anderson EK, Gutierrez DA, Hasty AH. Adipose tissue recruitment of leukocytes. Curr Opin Lipidol. 2010;21(3):172-7.

56. Aron-Wisnewsky J, Tordjman J, Poitou C, Darakhshan F, Hugol D, Basdevant A, et al. Human adipose tissue macrophages: m1 and m2 cell surface markers in subcutaneous and omental depots and after weight loss. J Clin Endocrinol Metab. 2009;94(11):4619-23.

57. Bourlier V, Zakaroff-Girard A, Miranville A, De Barros S, Maumus M, Sengenes C, et al. Remodeling phenotype of human subcutaneous adipose tissue macrophages. Circulation. 2008;117(6):806-15.

58. Bluher M, Laufs U. New concepts for body shape-related cardiovascular risk: role of fat distribution and adipose tissue function. Eur Heart J. 2019;40(34):2856-8.

59. Fried SK, Lee MJ, Karastergiou K. Shaping Fat Distribution: New Insights into the Molecular Determinants of Depot- and Sex-Dependent Adipose Biology. Obesity. 2015;23:1345-52.

60. Wajchenberg BL. Subcutaneous and Visceral Adipose Tissue: Their Relation to the Metabolic Syndrome. Endocrine Reviews. 2000;21(6):697-738.

61. Lacobini C, Pugliese G, Fantauzz BC, Federici M, Menini S. Metabolically healthy versus metabolically unhealthy obesity. Metabolism Clinical and Experimental. 2019;92:51-60.

62. Geer EB, Shen W. Gender differences in insulin resistance, body composition, and energy balance. Gend Med. 2009;6(1).

63. Siiteri PK. Adipose tissue as a source of hormones. Am J Clin Nutr. 1987;45(1):277-82.

64. Ryden M, Jocken J, van Harmelen V, Dicker A, Hoffstedt J, Wiren M, et al. Comparative studies of the role of hormone-sensitive lipase and adipose triglyceride lipase in human fat cell lipolysis. Am J Physiol Endocrinol Metab. 2007;292(6):E1847-55.

65. Santosa S, Bush NC, Jensen MD. Acute Testosterone Deficiency Alters Adipose Tissue Fatty Acid Storage. J Clin Endocrinol Metab. 2017;102(8):3056-64.

66. Escobar-Morreale HF, San Millan JL. Abdominal adiposity and the polycystic ovary syndrome. Trends Endocrinol Metab. 2007;18(7):266-72.

67. McDowell MA, Fryar CD, Hirsch R, Ogden CL. Anthropometric reference data for children and adults: US population, 1999-2002. Adv Data 2005:1-5.

68. Wu CH, Heshka S, Wang J, Pierson RN, Jr., Heymsfield SB, Laferrere B, et al. Truncal fat in relation to total body fat: influences of age, sex, ethnicity and fatness. Int J Obes (Lond). 2007;31(9):1384-91.

69. Stults-Kolehmainen MA, Stanforth PR, Bartholomew JB. Fat in Android, Trunk, and Peripheral Regions Varies by Ethnicity and Race in College Aged Women. Obesity. 2012;20:660-5.

70. Carroll JF, Chiapa AL, Rodriquez M, Phelps DR, Cardarelli KM, Vishwanatha JK, et al. Visceral fat, waist circumference, and BMI: impact of race/ethnicity. Obesity (Silver Spring). 2008;16(3):600-7.

71. Hill JO, Sidney S, Lewis CE, Tolan K, Scherzinger AL, Stamm ER. Racial differences in amounts of visceral adipose tissue in young adults: the CARDIA (Coronary Artery Risk Development in Young Adults) study. Am J Clin Nutr. 1999;69:381-7.

72. Bouchard C, Tremblay A, Despres JP, Nadeau A, Lupien P, Theriault G, et al. The response to long-term overfeeding in identical twins. N Engl J Med. 1990;322:1477-82.

73. Peeters MW, Beunen GP, Maes HH, Loos R, Claessens AL, Vlietinck R, et al. Genetic and environmental determination of tracking in subcutaneous fat distribution during adolescence. Am J Clin Nutr. 2007;86:652-60.

74. Malis C, Rasmussen EL, Poulsen P, Petersen I, Christensen K, Beck-Nielsen H, et al. Total and regional fat distribution is strongly influenced by genetic factors in young and elderly twins. Obes Res. 2005;13:2139-45.

75. Vidal H. Gene expression in visceral and subcutaneous adipose tissues. Ann Med. 2001;33(3):547-55.

76. Heid IM, Jackson AU, Randall JC, Winkler TW, Qi L, Steinthorsdottir V, et al. Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. Nat Genet. 2010;42(11):949-60.

77. Souren NY, Paulussen AD, Loos RJ, Gielen M, Beunen G, Fagard R, et al. Anthropometry, carbohydrate and lipid metabolism in the East Flanders Prospective Twin Survey: heritabilities. Diabetologia. 2007;50(10):2107-16.

78. Karastergiou K, Fried, S. K., Xie, H., Lee, M. J., Divoux, A., Rosencrantz, M. A., Chang, R. J., Smith, S. R. Distinct developmental signatures of human abdominal and gluteal subcutaneous adipose tissue depots. J Clin Endocrinol Metab. 2013;98(1):362-71.

79. Karpe F, Pinnick, K. E. Biology of upper-body and lower-body adipose tissue--link to whole-body phenotypes. Nat Rev Endocrinol. 2015;11(2):90-100.

80. Smith JA, Martin DT, Telford RD, Ballas SK. Greater erythrocyte deformability in world-class endurance athletes. . Am J Physiol 1999;276 ((Heart Circ. Physiol. 45)):H2188–H93.

81. Gesta S, Blüher M, Yamamoto Y, Norris AW, Berndt J, Kralisch S, et al. Evidence for a role of developmental genes in the origin of obesity and body fat distribution. PNAS. 2006;103(17):6676-81.

82. Tchkonia T, Lenburg M, Thomou T, Giorgadze N, Frampton G, Pirtskhalava T, et al. Identification of depot-specific human fat cell progenitors through distinct expression profiles and developmental gene patterns. Am J Physiol Endocrinol Metab. 2007;292(1):E298-307.

83. Wellcome Trust Case Control C. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007;447(7145):661-78.

84. Liu C, Monda KL, Taylor KC, Lange L, Demerath EW, Palmas W, et al. Genome-wide association of body fat distribution in African ancestry populations suggests new loci. PLoS Genet. 2013;9(8):e1003681.

85. Cardel M, Higgins PB, Willig AL, Keita AD, Casazza K, Gower BA, et al. African genetic admixture is associated with body composition and fat distribution in a cross-sectional study of children. Int J Obes (Lond). 2011;35(1):60-5.

86. Sahibdeen V, Crowther NJ, Soodyall H, Hendry LM, Munthali RJ, Hazelhurst S, et al. Genetic variants in SEC16B are associated with body composition in black South Africans. Nutr Diabetes. 2018;8(1):43.

87. Smith U, Kahn BB. Adipose tissue regulates insulin sensitivity: role of adipogenesis, de novo lipogenesis and novel lipids. J Intern Med. 2016;280(5):465-75.

88. Blüher M. Adipose Tissue Dysfunction in Obesity. Exp Clin Endocrinol Diabetes. 2009;117:241-50.

89. Bluher M. Clinical relevance of adipokines. Diabetes Metab J. 2012;36(5):317-27.

90. Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, et al. Dynamics of fat cell turnover in humans. Nature. 2008;453(7196):783-7.

91. Farmer SR. Transcriptional control of adipocyte formation. Cell Metab. 2006;4(4):263-73.

92. Dubois SG, Heilbronn LK, Smith SR, Albu JB, Kelley DE, Ravussin E. Decreased expression of adipogenic genes in obese subjects with type 2 diabetes. Obesity (Silver Spring). 2006;14:1543e52.

93. Bowers RR, Kim JW, Otto TC, Lane MD. Stable stem cell commitment to the adipocyte lineage by inhibition of DNA methylation: role of the BMP-4 gene. Proc Natl Acad Sci USA. 2006;103:13022–7.

94. Vazquez-Vela ME, Torres N, Tovar AR. White adipose tissue as endocrine organ and its role in obesity. Arch Med Res. 2008;39(8):715-28.

95. Sul HS, Smas C, Mei B, Zhou L. Function of pref-1 as an inhibitor of adipocyte differentiation. Int J Obes Relat Metab Disord. 2000 24(4):15-9.

96. Slawik M, Vidal-Puig AJ. Lipotoxicity, overnutrition and energy metabolism in aging. Ageing Res Rev. 2006;5(2):144-64.

97. Tchkonia T, Pirtskhalava T, Thomou T, Cartwright MJ, Wise B, Karagiannides I, et al. Increased TNFalpha and CCAAT/enhancer-binding protein homologous protein with aging predispose preadipocytes to resist adipogenesis. Am J Physiol Endocrinol Metab. 2007;293(6):E1810-9.

98. Lee CH, Olson P, Evans RM. Minireview: lipid metabolism, metabolic diseases, and peroxisome proliferator-activated receptors. Endocrinology. 2003;144(6):2201-7.

99. Tontonoz P, Hu E, Spiegelman BM. Stimulation of Adipogenesis in Fibroblasts

by PPARy2, a Lipid-Activated Transcription Factor. Cell. 1994;79:1147-56.

100. Amri EZ, Ailhaud, G., Grimaldi, P.A. Fatty acids as signal transducing molecules: involvement in the differentiation of preadipose to adipose cells. Journal of Lipid Research. 1994;35:930-7.

101. Lewis GF, Carpentier A, Adeli K, Giacca A. Disordered Fat Storage and Mobilization in the Pathogenesis of Insulin Resistance and Type 2 Diabetes. Endocrine Reviews. 2002;23(2):201-29.

102. Roberts R, Hodson, L., Dennis, A. L., Neville, M. J., Humphreys, S. M., Harnden, K. E., Micklem, K. J., Frayn, K. N. Markers of de novo lipogenesis in adipose tissue: associations with small adipocytes and insulin sensitivity in humans. Diabetologia. 2009;52(5):882-90.

103. Song Z, Xiaoli AM, Yang F. Regulation and Metabolic Significance of De Novo Lipogenesis in Adipose Tissues. Nutrients. 2018;10(10).

104. Merkel M, Eckel RH, Goldberg IJ. Lipoprotein lipase: genetics, lipid uptake, and regulation. J Lipid Res. 2002;43(12):1997-2006.

105. Wu Q, Ortegon AM, Tsang B, Doege H, Feingold KR, Stahl A. FATP1 is an insulin-sensitive fatty acid transporter involved in diet-induced obesity. Mol Cell Biol. 2006;26(9):3455-67.

106. Endemann G, Stanton LW, Madden KS, Bryant CM, White RT, Protter AA. Cd36 is a receptor for oxidized low density lipoprotein. J Biol Chem 1993;268:11811-6.

107. Ratamess NA, Alvar BA, Evetoch TK, Housh TJ, Kibler WB, Kraemer WJ, et al. Progression models in resistance training for healthy adults. Med Sci Sports Exerc. 2009;41:687-708.

108. Smith SJ, Cases S, Jensen DR, Chen HC, Sande E, Tow B, et al. Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. Nature Genetics. 2000;27:87-90.

109. Harris CA, Haas JT, Streeper RS, Stone SJ, Kumari M, Yang K, et al. DGAT enzymes are required for triacylglycerol synthesis and lipid droplets in adipocytes. J Lipid Res. 2011;52(4):657-67.

110. Zammit VA, Buckett LK, Turnbull AV, Wure H, Proven A. Diacylglycerol acyltransferases: Potential roles as pharmacological targets. Pharmacol Ther. 2008;118(3):295-302.

111. Luo L, Liu M. Adipose tissue in control of metabolism. J Endocrinol. 2016;231(3):R77-R99.

112. Ferre P, Foufelle F. SREBP-1c transcription factor and lipid homeostasis: clinical perspective. Horm Res. 2007;68(2):72-82.

113. Eissing L, Scherer T, Todter K, Knippschild U, Greve JW, Buurman WA, et al. De novo lipogenesis in human fat and liver is linked to ChREBP-beta and metabolic health. Nat Commun. 2013;4:1528.

114. Herman MA, Peroni OD, Villoria J, Schon MR, Abumrad NA, Bluher M, et al. A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. Nature. 2012;484(7394):333-8.

115. Frayn KN. Adipose tissue as a buffer for daily lipid flux. Diabetologia. 2002;45(9):1201-10.

116. Zechner R, Strauss JG, Haemmerle G, Lass A, Zimmermann R. Lipolysis: pathway under construction. Current Opinion in Lipidology. 2005;16:333-40.

117. Carmen GY, Victor SM. Signalling mechanisms regulating lipolysis. Cell Signal. 2006;18(4):401-8.

118. Langin D. Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. Pharmacol Res. 2006;53(6):482-91.

119. Kuriyama H, Shimomura I, Kishida K, Kondo H, Furuyama N, Nishizawa H, et al. Coordinated regulation of fat-specific and liver-specific glycerol channels, aquaporin adipose and aquaporin 9. Diabetes. 2002;51:2915-21.

120. Langin D, Dicker A, Tavernier G, Hoffstedt J, Mairal A, Ryden M, et al. Adipocyte lipases and defect of lipolysis in human obesity. Diabetes. 2005;55:3190-7.

121. Brasaemle DL, Rubin B, Harten IA, Gruia-Gray J, Kimmel AR, Londos C. Perilipin A increases triacylglycerol storage by decreasing the rate of triacylglycerol hydrolysis. J Biol Chem. 2000;275(49):38486-93.

122. Brasaemle DL. Thematic review series: adipocyte biology. The perilipin family of structural lipid droplet proteins: stabilization of lipid droplets and control of lipolysis. J Lipid Res. 2007;48(12):2547-59.

123. Morigny P, Houssier M, Mouisel E, Langin D. Adipocyte lipolysis and insulin resistance. Biochimie. 2016;125:259-66.

124. Lafontan M, Langin D. Lipolysis and lipid mobilization in human adipose tissue. Prog Lipid Res. 2009;48(5):275-97.

125. Laurencikiene J, Skurk T, Kulyte A, Heden P, Astrom G, Sjolin E, et al. Regulation of lipolysis in small and large fat cells of the same subject. J Clin Endocrinol Metab. 2011;96(12):E2045-9.

126. Saponaro C, Gaggini M, Carli F, Gastaldelli A. The Subtle Balance between Lipolysis and Lipogenesis: A Critical Point in Metabolic Homeostasis. Nutrients. 2015;7(11):9453-74.

127. Tan CY, Vidal-Puig A. Adipose tissue expandability: the metabolic problems of obesity may arise from the inability to become more obese. Biochem Soc Trans. 2008;36(Pt 5):935-40.

128. Piché M, Vasan SK, Hodson L, Karpe F. Relevance of human fat distribution on lipid and lipoprotein metabolism and cardiovascular disease risk. Curr Opin Lipidol. 2018;29:285-92.

129. Kim JY, Nasr A, Tfayli H, Bacha F, Michaliszyn SF, Arslanian S. Increased lipolysis, diminished adipose tissue insulin sensitivity and impaired beta-cell function relative to adipose tissue insulin sensitivity in obese youth with impaired glucose tolerance (IGT). Diabetes 2017;66:3085-90.

130. Lehr S, Hartwig S, Sell H. Adipokines: a treasure trove for the discovery of biomarkers for metabolic disorders. Proteomics Clin Appl. 2012;6(1-2):91-101.

131. Maury E, Brichard SM. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. Mol Cell Endocrinol. 2010;314(1):1-16.

132. Scherer PE. Adipose tissue: from lipid storage compartment to endocrine organ. Diabetes. 2006;55(6):1537-45.

133. Parimisetty A, Dorsemans AC, Awada R, Ravanan P, Diotel N, Lefebvre d'Hellencourt C. Secret talk between adipose tissue and central nervous system via secreted factors-an emerging frontier in the neurodegenerative research. J Neuroinflammation. 2016;13(1):67.

134. Blüher M. Adipose tissue dysfunction contributes to obesity related metabolic diseases. Best Practice & Research Clinical Endocrinology & Metabolism. 2013;27:163-77.

135. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994;372(6505):425-32.

136. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes Journal of Biological Chemistry. 1995;270:26746-9.

137. Bluher M, Mantzoros CS. From leptin to other adipokines in health and disease: facts and expectations at the beginning of the 21st century. Metabolism. 2015;64(1):131-45.

138. Nikolaidis MG, Mougios, V. . Effects of Exercise on the Fatty-Acid Composition of Blood and Tissue Lipids. Sports Med Open. 2004;34(15):1051-76.

139. Patel P, Abate N. Body fat distribution and insulin resistance. Nutrients. 2013;5(6):2019-27.

140. Kabir M, Catalano KJ, Ananthnarayan S, Kim SP, Van Citters GW, Dea MK, et al. Molecular evidence supporting the portal theory: a causative link between visceral adiposity and hepatic insulin resistance. Am J Physiol Endocrinol Metab. 2005;288(2):E454-61.

141. Alligier M, Gabert L, Meugnier E, Lambert-Porcheron S, Chanseaume E, Pilleul F, et al. Visceral fat accumulation during lipid overfeeding is related to subcutaneous adipose tissue characteristics in healthy men. J Clin Endocrinol Metab. 2013;98(2):802-10.

142. Bluher M. Are there still healthy obese patients? Curr Opin Endocrinol Diabetes Obes. 2012;19(5):341-6.

143. Kloting N, Fasshauer M, Dietrich A, Kovacs P, Schon MR, Kern M, et al. Insulin-sensitive obesity. Am J Physiol Endocrinol Metab. 2010;299(3):E506-15.

144. Gealekman O, Guseva N, Hartigan C, Apotheker S, Gorgoglione M, Gurav K, et al. Depot-specific differences and insufficient subcutaneous adipose tissue angiogenesis in human obesity. Circulation. 2011;123(2):186-94.

145. McLaughlin T, Lamendola C, Coghlan N, Liu TC, Lerner K, Sherman A, et al. Subcutaneous adipose cell size and distribution: relationship to insulin resistance and body fat. Obesity (Silver Spring). 2014;22(3):673-80.

146. Pellegrinelli V, Carobbio S, Vidal-Puig A. Adipose tissue plasticity: how fat depots respond differently to pathophysiological cues. Diabetologia. 2016;59(6):1075-88.

147. Asterholm IW, C. T, Morley TS, Wang QA, Delgado-Lopez F, Wang ZV, et al. Adipocyte Inflammation Is Essential for Healthy Adipose Tissue Expansion and Remodeling. Cell Metabolism 2014;20:103-18.

148. Almuraikhy S, Kafienah W, Bashah M, Diboun I, Jaganjac M, Al-Khelaifi F, et al. Interleukin-6 induces impairment in human subcutaneous adipogenesis in obesity-associated insulin resistance. Diabetologia. 2016;59(11):2406-16.

149. Lundgren M, Svensson M, Lindmark S, Renstrom F, Ruge T, Eriksson JW. Fat cell enlargement is an independent marker of insulin resistance and 'hyperleptinaemia'. Diabetologia. 2007;50(3):625-33.

150. Bouloumie A, Lolmede K, Sengenes C, Galitzky J, Lafontan M. Angiogenesis in adipose tissue. Annales d'Endocrinologie (Paris). 2002;63:91-5.

151. Rupnick MA, Panigrahy D, Zhang CY, Dallabrida SM, Lowell BB, Langer R, et al. Adipose tissue mass can be regulated through the vasculature. Proceedings of the National Academy of Sciences U S A 2002;99:10730-5.

152. Pasarica M, Sereda OR, Redman LM, Albarado DC, Hymel DT, Roan LE, et al. Reduced adipose tissue oxygenation in human obesity: evidence for rarefaction, macrophage chemotaxis, and inflammation without an angiogenic response. Diabetes. 2009;58(3):718-25.

153. Trayhurn P. Hypoxia and adipose tissue function and dysfunction in obesity. Physiol Rev. 2013;93(1):1-21.

154. Sell H, Habich C, Eckel J. Adaptive immunity in obesity and insulin resistance. Nat Rev Endocrinol. 2012;8(12):709-16.

155. Suganami T, Tanimoto-Koyama K, Nishida J, Itoh M, Yuan X, Mizuarai S, et al. Role of the Toll-like receptor 4/NF-kappaB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. Arterioscler Thromb Vasc Biol. 2007;27(1):84-91.

156. Suganami T, Nishida J, Ogawa Y. A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor alpha. Arterioscler Thromb Vasc Biol. 2005;25(10):2062-8.

157. Rezaee F, Dashty M. Role of Adipose Tissue in Metabolic System Disorders. Journal of Diabetes & Metabolism. 2013;01(S13).

158. Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol. 2008;9(5):367-77.

159. Moreno-Indias I, Tinahones FJ. Impaired adipose tissue expandability and lipogenic capacities as ones of the main causes of metabolic disorders. J Diabetes Res. 2015;2015:970375.

160. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006;444(7121):860-7.

161. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. Journal of Clinical Investigation. 2003;112(12):1796-808.

162. Cancello R, Tordjman J, Poitou C, Guilhem G, Bouillot JL, Hugol D, et al. Increased infiltration of macrophages in omental adipose tissue is associated with marked hepatic lesions in morbid human obesity. Diabetes. 2006;55(6):1554-61.

163. Harman-Boehm I, Bluher M, Redel H, Sion-Vardy N, Ovadia S, Avinoach E, et al. Macrophage infiltration into omental versus subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity. J Clin Endocrinol Metab. 2007;92(6):2240-7.

164. Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res. 2005;46(11):2347-55.

165. Deng Y, Scherer PE. Adipokines as novel biomarkers and regulators of the metabolic syndrome. Ann N Y Acad Sci. 2010;1212:E1-E19.

166. Rudich A, Kanety H, Bashan N. Adipose stress-sensing kinases: linking obesity to malfunction. Trends Endocrinol Metab. 2007;18(8):291-9.

167. Sabio G, Das M, Mora A, Zhang Z, Jun JY, Ko HJ, et al. A stress signaling pathway in adipose tissue regulates hepatic insulin resistance. Science. 2008;322(5907):1539-43.

168. Eizirik DL, Cardozo AK, Cnop M. The role for endoplasmic reticulum stress in diabetes mellitus. Endocr Rev. 2008;29(1):42-61.

169. Palming J, Sjoholm K, Jernas M, Lystig TC, Gummesson A, Romeo S, et al. The expression of NAD(P)H:quinone oxidoreductase 1 is high in human adipose tissue, reduced by weight loss, and correlates with adiposity, insulin sensitivity, and markers of liver dysfunction. J Clin Endocrinol Metab. 2007;92(6):2346-52.

170. Xu XJ, Pories WJ, Dohm LG, Ruderman NB. What distinguishes adipose tissue of severely obese humans who are insulin sensitive and resistant? Curr Opin Lipidol. 2013;24(1):49-56.

171. Huang CJ, McAllister MJ, Slusher AL, Webb HE, Mock JT, Acevedo EO. Obesity-Related Oxidative Stress: the Impact of Physical Activity and Diet Manipulation. Sports Med Open. 2015;1(1):32.

172. Rudich A, Tirosh A, Potashnik R, Hemi R, Kanety H, Bashan N. Prolonged oxidative stress impairs insulin-induced GLUT4 translocation in 3T3-L1 adipocytes. Diabetes. 1998;47:1562-9.

173. Matsuoka T, Kajimoto Y, Watada H, Kaneto H, Kishimoto M, Umayahara Y, et al. Glycation-dependent, reactive oxygen species-mediated suppression of the insulin gene promoter activity in HIT cells. J Clin Invest. 1997;99:144-50.

174. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. Journal of Clinical Investigation. 2004;114(12):1752-61.

175. Montezano AC, Touyz RM. Reactive oxygen species and endothelial function--role of nitric oxide synthase uncoupling and Nox family nicotinamide adenine dinucleotide phosphate oxidases. Basic Clin Pharmacol Toxicol. 2012;110(1):87-94.

176. Keaney JF, Jr., Larson MG, Vasan RS, Wilson PW, Lipinska I, Corey D, et al. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. Arterioscler Thromb Vasc Biol. 2003;23(3):434-9.

177. Kelli HM, Corrigan FE, 3rd, Heinl RE, Dhindsa DS, Hammadah M, Samman-Tahhan A, et al. Relation of Changes in Body Fat Distribution to Oxidative Stress. Am J Cardiol. 2017;120(12):2289-93.

178. Yamagishi SI, Edelstein D, Du XL, Kaneda Y, Guzman M, Brownlee M. Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. J Biol Chem. 2001;276(27):25096-100.

179. Zhang H, Park Y, Wu J, Chen X, Lee S, Yang J, et al. Role of TNF-alpha in vascular dysfunction. Clin Sci (Lond). 2009;116(3):219-30.

180. Yan S, Zhang X, Zheng H, Hu D, Zhang Y, Guan Q, et al. Clematichinenoside inhibits VCAM-1 and ICAM-1 expression in TNF-alpha-treated endothelial cells via NADPH oxidase-dependent IkappaB kinase/NF-kappaB pathway. Free Radic Biol Med. 2015;78:190-201.

181. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. J Clin Invest. 2011;121(6):2111-7.

182. Murr C, Schroecksnadel K, Winkler C, Ledochowski M, Fuchs D. Antioxidants may increase the probability of developing allergic diseases and asthma. Med Hypotheses. 2005;64(5):973-7.

183. Fisher-Wellman K, Bloomer RJ. Acute exercise and oxidative stress: a 30 year history. Dyn Med. 2009;8:1.

184. Iwaki M, Matsuda M, Maeda N, Funahashi T, Matsuzawa Y, Makishima M, et al. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. Diabetes. 2003;52:1655-63.

185. Fukai T, Folz RJ, Landmesser U, Harrison DG. Extracellular superoxide dismutase and cardiovascular disease. Cardiovasc Res 2002;55:239-49.

186. Blankenberg S, Rupprecht HJ, Bickel C, Torzewski M, Hafner G, Tiret L, et al. Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. N Engl J Med. 2003;349:1605-513.

187. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev. 2002;23(5):599-622.

188. Snel M, Jonker JT, Schoones J, Lamb H, de Roos A, Pijl H, et al. Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions. Int J Endocrinol. 2012;2012:983814.

189. Vasan SK, Karpe F. Adipose tissue: Fat, yet fit. Nat Rev Endocrinol. 2016;12(7):375-6.

190. Tchkonia T, Thomou T, Zhu Y, Karagiannides I, Pothoulakis C, Jensen MD, et al. Mechanisms and metabolic implications of regional differences among fat depots. Cell Metab. 2013;17(5):644-56.

191. Nishimura S, Manabe I, Nagasaki M, Hosoya Y, Yamashita H, Fujita H, et al. Adipogenesis in obesity requires close interplay between differentiating adipocytes, stromal cells, and blood vessels. Diabetes. 2007;56(6):1517-26.

192. Tchkonia T, Tchoukalova YD, Giorgadze N, Pirtskhalava T, Karagiannides I, Forse RA, et al. Abundance of two human preadipocyte subtypes with distinct capacities for replication, adipogenesis, and apoptosis varies among fat depots. Am J Physiol Endocrinol Metab. 2005;288(1):E267-77.

193. Tchkonia T, Giorgadze N, Pirtskhalava T, Thomou T, DePonte M, Koo A, et al. Fat depot-specific characteristics are retained in strains derived from single human preadipocytes. Diabetes. 2006;55(9):2571-8.

194. Van Harmelen V, Rohrig K, Hauner H. Comparison of proliferation and differentiation capacity of human adipocyte precursor cells from the omental and subcutaneous adipose tissue depot of obese subjects. Metabolism. 2004;53(5):632-7.

195. Zhang X, Hu EA, Wu H, Malik V, Sun Q. Associations of Leg Fat Accumulation with Adiposity-Related Biological Factors and Risk of Metabolic Syndrome. Obesity (Silver Spring). 2013;21(4):824-30.

196. Matsuzawa Y, Shimomura I, Nakamura T, Keno Y, Kotani K, Tokunaga K. Pathophysiology and pathogenesis of visceral fat obesity. 1995, 2, . Obes Res 1995;2:187S-94S.

197. McLaughlin T, Lamendola C, Liu A, Abbasi F. Preferential fat deposition in subcutaneous versus visceral depots is associated with insulin sensitivity. J Clin Endocrinol Metab. 2011;96(11):E1756-60.

198. Bjorntorp P. "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. Arteriosclerosis. 1990;10:493-6.

199. Garg A. Regional adiposity and insulin resistance. J Clin Endocrinol Metab. 2004;89(9):4206-10.

200. Abraham TM, Pedley, A., Massaro, J. M., Hoffmann, U., Fox, C.S. Association between visceral and subcutaneous adipose depots and incident cardiovascular disease risk factors. Circulation. 2015;132(17):1639-47.

201. Marinou K, Hodson L, Vasan KS, Fielding BA, Banerjee R, Brismar K, et al. Structural and Functional Properties of Deep Abdominal Subcutaneous Adipose Tissue Explain Its Association With Insulin Resistance and Cardiovascular Risk in Men. Diabetes Care. 2014;37:821-9.

202. Le KA, Mahurkar S, Alderete TL, Hasson RE, Adam TC, Kim JS, et al. Subcutaneous adipose tissue macrophage infiltration is associated with hepatic and visceral fat deposition, hyperinsulinemia, and stimulation of NF-kappaB stress pathway. Diabetes. 2011;60(11):2802-9.

203. Koutsari C, Dumesic DA, Patterson BW, Votruba SB, Jensen MD. Plasma free fatty acid storage in subcutaneous and visceral adipose tissue in postabsorptive women. Diabetes. 2008;57(5):1186-94.

204. Shadid S, Koutsari C, Jensen MD. Direct free fatty acid uptake into human adipocytes in vivo: relation to body fat distribution. Diabetes. 2007;56(5):1369-75.

205. Arner E, Westermark PO, Spalding KL, Britton T, Ryden M, Frisen J, et al. Adipocyte turnover: relevance to human adipose tissue morphology. Diabetes. 2010;59(1):105-9.

206. Tordjman J, Divoux A, Prifti E, Poitou C, Pelloux V, Hugol D, et al. Structural and inflammatory heterogeneity in subcutaneous adipose tissue: relation with liver histopathology in morbid obesity. J Hepatol. 2012;56(5):1152-8.

207. Frederiksen L, Nielsen TL, Wraae K, Hagen C, Frystyk J, Flyvbjerg A, et al. Subcutaneous rather than visceral adipose tissue is associated with adiponectin levels and insulin resistance in young men. J Clin Endocrinol Metab. 2009;94(10):4010-5.

208. Tchoukalova YD, Votruba SB, Tchkonia T, Giorgadze N, Kirkland JL, Jensen MD. Regional differences in cellular mechanisms of adipose tissue gain with overfeeding. Proc Natl Acad Sci U S A. 2010;107(42):18226-31.

209. Pou KM, Massaro JM, Hoffmann U, Vasan RS, Maurovich-Horvat P, Larson MG, et al. Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: the Framingham Heart Study. Circulation. 2007;116(11):1234-41.

210. Vogel MAA, Wang P, Bouwman FG, Hoebers N, Blaak EE, Renes J, et al. A comparison between the abdominal and femoral adipose tissue proteome of overweight and obese women. Sci Rep. 2019;9(1):4202.

211. Goodpaster BH, Krishnaswami S, Harris TB, Katsiaras A, Kritchevsky SB, Simonsick EM, et al. Obesity, regional body fat distribution, and the metabolic syndrome in older men and women. Arch Intern Med. 2005;165:777-83.

212. Liu J, Hickson DA, Musani SK, Talegawkar SA, Carithers TC, Tucker KL, et al. Dietary Patterns, Abdominal Visceral Adipose Tissue, and Cardiometabolic Risk Factors in African Americans: The Jackson Heart Study. Obesity. 2012.

213. Evans J, Goedecke JH, I. S, Buren J, Alvehus M, C. B, et al. Depot- and ethnic-specific differences in the relationship between adipose tissue inflammation and insulin sensitivity. Clinical Endocrinology 2011;74:51-9.

214. Kotze-Horstmann LM, Keswell D, Adams K, Dlamini T, Goedecke JH. Hypoxia and extra-cellular matrix gene expression in adipose tissue associates with reduced insulin sensitivity in black South African women. Endocrine. 2016;55(1):144-52.

215. Ness RB, Haggerty CL, Harger G, Ferrell R. Differential distribution of allelic variants in cytokine genes among African Americans and White Americans. Am J Epidemiol. 2004;160:1033-8.

216. Fisher G, Alvarez JA, Ellis AC, Granger WM, Ovalle F, Man CD, et al. Race differences in the association of oxidative stress with insulin sensitivity in African- and European-American women. Obesity (Silver Spring). 2012;20(5):972-7.

217. Goedecke JH, Keswell D, Weinreich C, Fan J, Hauksson J, Victor H, et al. Ethnic differences in hepatic and systemic insulin sensitivity and their associated determinants in obese black and white South African women. Diabetologia. 2015;58(11):2647-52.

218. Guerrero R, Vega GL, Grundy SM, Browning JD. Ethnic differences in hepatic steatosis: an insulin resistance paradox? Hepatology. 2009;49(3):791-801.

219. Smith LM, Yao-Borengasser A, Starks T, Tripputi M, Kern PA, Rasouli N. Insulin resistance in African-American and Caucasian women: differences in lipotoxicity, adipokines, and gene expression in adipose tissue and muscle. J Clin Endocrinol Metab. 2010;95(9):4441-8.

220. Ralston JC, Matravadia S, Gaudio N, Holloway GP, Mutch DM. Polyunsaturated fatty acid regulation of adipocyte FADS1 and FADS2 expression and function. Obesity (Silver Spring). 2015;23(4):725-8.

221. Sjogren P, Sierra-Johnson J, Kallings LV, Cederholm T, Kolak M, Halldin M, et al. Functional changes in adipose tissue in a randomised controlled trial of physical activity. Lipids Health Dis. 2012;11:80.

222. Afman LA, Muller M. Human nutrigenomics of gene regulation by dietary fatty acids. Prog Lipid Res. 2012;51(1):63-70.

223. Shaw B, Lambert S, Wong MH, Ralston JC, Stryjecki C, Mutch DM. Individual saturated and monounsaturated fatty acids trigger distinct transcriptional networks in differentiated 3T3-L1 preadipocytes. J Nutrigenet Nutrigenomics. 2013;6(1):1-15.

224. Garaulet M, Hernandez-Morante JJ, Tebar FJ, Zamora S. Relation between degree of obesity and site-specific adipose tissue fatty acid composition in a Mediterranean population. Nutrition. 2011;27(2):170-6.

225. Mika A, Sledzinski, T. Alterations of specific lipid groups in serum of obese humans: a review. Obes Rev. 2017;18(2):247-72.

226. Yetukuri L, Katajamaa M, Medina-Gomez G, Seppanen-Laakso T, Vidal-Puig A, Oresic M. Bioinformatics strategies for lipidomics analysis: characterization of obesity related hepatic steatosis. BMC Syst Biol. 2007;1:12.

227. Tanti JF, Ceppo F, Jager J, Berthou F. Implication of inflammatory signaling pathways in obesity-induced insulin resistance. Front Endocrinol (Lausanne). 2012;3:181.

228. Kim J, Park J, Kim O, Ham B. Metabolic Profiling of Plasma in Overweight/Obese and Lean Menusing Ultra Performance Liquid Chromatography and Q-TOF MassSpectrometry (UPLC-Q-TOF MS. J Proteome Res. 2010;9:4368-75.

229. Matthan NR, Ooi EM, Van Horn L, Neuhouser ML, Woodman R, Lichtenstein AH. Plasma phospholipid fatty acid biomarkers of dietary fat quality and endogenous metabolism predict coronary heart disease risk: a nested case-control study within the Women's Health Initiative observational study. J Am Heart Assoc. 2014;3(4).

230. Sampath H, Ntambi JM. The role of stearoyl-CoA desaturase in obesity, insulin resistance, and inflammation. Ann N Y Acad Sci. 2011;1243:47-53.

231. Wang L, Manson, J. E., Rautiainen, S., Gaziano, J. M., Buring, J. E., Tsai, M. Y., Sesso, H. D. A prospective study of erythrocyte polyunsaturated fatty acid, weight gain, and risk of becoming overweight or obese in middle-aged and older women. Eur J Nutr. 2016;55(2):687-97.

232. Garaulet M, Pérez-Llamas, F., Pérez-Ayala, M., Martínez, P., Sánchez de Medina F, Tebar, F. J., Zamora, S. Site-specific differences in the fatty acid composition of abdominal adipose tissue in an obese population from a Mediterranean area: relation with dietary fatty acids, plasma lipid profile, serum insulin, and central obesity. Am J Clin Nutr. 2001;74:585-91.

233. Fernandes R, Beserra BT, Cunha RS, Hillesheim E, Camargo Cde Q, Pequito DC, et al. Relationship between acute phase proteins and serum fatty acid composition in morbidly obese patients. Dis Markers. 2013;35(2):105-12.

234. Itariu BK, Zeyda M, Hochbrugger EE, Neuhofer A, Prager G, Schindler K, et al. Long-chain n-3 PUFAs reduce adipose tissue and systemic inflammation in severely obese nondiabetic patients: a randomized controlled trial. Am J Clin Nutr. 2012;96(5):1137-49.

235. Sato A, Kawano H, Notsu T, Ohta M, Nakakuki M, Mizuguchi K, et al. Antiobesity effect of eicosapentaenoic acid in high-fat/high-sucrose diet-induced obesity: importance of hepatic lipogenesis. Diabetes. 2010;59(10):2495-504.

236. Ameer F, Scandiuzzi L, Hasnain S, Kalbacher H, Zaidi N. De novo lipogenesis in health and disease. Metabolism. 2014;63(7):895-902.

237. Dobrzyn P, Dobrzyn A, Miyazaki M, Cochen P, Asilmaz E, Hardie DG, et al. Stearoyl-CoA desaturase 1 deficiency increases fatty acid oxidation by activating AMP-activated protein kinase in liver. Proc Natl Acad Sci USA. 2003;101:6409–14.

238. Ntambi JM. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. J Lipid Res. 1999;40:1549–58.

239. Rahman SM, Dobrzyn A, Dobrzyn P, Lee S-H, Miyazaki M, Ntambi JM. Stearoyl-CoA desaturase 1 deficiency elevates insulin signaling components and down-regulates protein-tyrosine phosphatase 1B in muscle. Proc Natl Acad Sci USA. 2003;100:11110-5.

240. Dobrzyn P, Dobrzyn A, Miyazaki M, Cochen P, Asilmaz E, Grahame DH, et al. Stearoyl-CoA desaturase 1 deficiency increases fatty acid oxidation by activating AMP-activated protein kinase in liver. Proc Natl Acad Sci U S A. 2004;101:6409-14.

241. Merino DM, Ma DWL, Mutch DM. Genetic variation in lipid desaturases and its impact on the development of human disease. Lipids in Health and Disease. 2010;9:63.

242. Murakami K, Sasaki S, Takahashi Y, Uenishi K, Watanabe T, Kohri T, et al. Lower estimates of delta-5 desaturase and elongase activity are related to adverse profiles for several metabolic risk factors in young Japanese women. Nutr Res. 2008;28(12):816-24.

243. Pickens CA, Matsuo KH, Fenton JI. Relationship between Body Mass Index, C-Peptide, and Delta-5-Desaturase Enzyme Activity Estimates in Adult Males. PLoS One. 2016;11(3):e0149305.

244. Fekete K, Gyorei E, Lohner S, Verduci E, Agostoni C, Decsi T. Long-chain polyunsaturated fatty acid status in obesity: a systematic review and meta-analysis. Obes Rev. 2015;16(6):488-97.

245. Strassburg K, Esser D, Vreeken RJ, Hankemeier T, Muller M, van Duynhoven J, et al. Postprandial fatty acid specific changes in circulating oxylipins in lean and obese men after high-fat challenge tests. Mol Nutr Food Res. 2014;58(3):591-600.

246. Ilie M, Margin D. Trends in the Evaluation of Lipid Peroxidation Processes. Lipid Peroxidation2012.

247. Unger RH, Clark GO, Scherer PE, Orci L. Lipid homeostasis, lipotoxicity and the metabolic syndrome. Biochim Biophys Acta. 2010;1801(3):209-14.

248. Hodson L, Skeaff, C. M., Fielding, B. A. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Prog Lipid Res. 2008;47(5):348-80.

249. Skeaff CM, Hodson L, McKenzie JE. Dietary-induced changes in fatty acid composition of human plasma, platelet, and erythrocyte lipids follow a similar time course. J Nutr 2006;136:565-9.

250. Gunes O, Tascilar, E., Sertoglu, E., Tas, A., Serdar, M. A., Kaya, G., Kayadibi, H., Ozcan, O. Associations between erythrocyte membrane fatty acid compositions and insulin resistance in obese adolescents. Chem Phys Lipids. 2014;184:69-75.

251. T. K, S. T, S. A, S. O. Higher levels of erythrocyte membrane fluidity in sprinters and long-distance runners J Appl Physiol 1993;74(1):354-8.

252. Hirsch J, Farquhar JW, Ahrens JEH, Peterson ML, Stoffel W. Studies of adipose tissue in man. A microtechnic for sampling and analysis. Am J Clin Nutr. 1960;8:499-511.

253. Malcom GT, Bhattacharyya, A. K., Velez-Duran, M., Guzman, M. A., Oalmann, M. C., Strong, J. P. Fatty acid composition of adipose tissue in humans: differences between subcutaneous sites. Am J Clin Nutr. 1989;50(2):288-91.

254. Calder PC, Harvey, D. J., Pond, C. M., Newsholme, E. A. Site-specific differences in the fatty acid composition of human adipose tissue. Lipids. 1992;27:716-20.

255. Hellmuth C, Demmelmair H, Schmitt I, Peissner W, Bluher M, Koletzko B. Association between plasma nonesterified fatty acids species and adipose tissue fatty acid composition. PLoS One. 2013;8(10):e74927.

256. Helge JW, Ayre KJ, Hulbert AJ, Kiens B, Storlien LH. Muscle membranes, diet and exercise. Biochemical and Molecular Action of Nutrients. 1999:57-67.

257. Pamplona R. Membrane phospholipids, lipoxidative damage and molecular integrity: a causal role in aging and longevity. Biochim Biophys Acta. 2008;1777(10):1249-62.

258. Elizondo A, Araya J, Rodrigo R, Poniachik J, Csendes A, Maluenda F, et al. Polyunsaturated Fatty Acid Pattern in Liver and Erythrocyte Phospholipids from Obese Patients. Obesity 2007;15(1):24-31.

259. Kroger J, Schulze MB. Recent insights into the relation of Delta5 desaturase and Delta6 desaturase activity to the development of type 2 diabetes. Curr Opin Lipidol. 2012;23(1):4-10.

260. Bhattacharyya AK, Malcom GT, Guzman MA, Kokatnur MG, Oalmann MC, Strong JP. Differences in adipose tissue fatty acid composition between black and white men in New Orleans. Am J Clin Nutr. 1987;46:41-6.

261. Ralston JC, Zulyniak MA, Nielsen DE, Clarke S, Badawi A, El-Sohemy A, et al. Ethnic- and sex-specific associations between plasma fatty acids and markers of insulin resistance in healthy young adults. Nutrition & Metabolism. 2013;10:42.

262. Goff LM, Griffin, B. A., Lovegrove, J. A., Sanders. T.A., Jebb, S. A., Bluck, L. J., Frost, G. S. Ethnic differences in beta-cell function, dietary intake and expression of the metabolic syndrome among UK adults of South Asian, black African-Caribbean and white-European origin at high risk of metabolic syndrome. Diabetes & Vascular Disease Research. 2013;10(4):315-23.

263. Joffe YT, van der Merwe, L., Evans, J., Collins, M., Lambert, E. V., September, A. V., Goedecke, J. H. . Interleukin-6 Gene Polymorphisms, Dietary Fat Intake, Obesity and Serum Lipid Concentrations in Black and White South African Women. Nutrients. 2014;6:2436-65.

264. Kunesova M, Hainer V, Tvrzicka E, Phinney SD, Stich V, Parizkova J, et al. Assessment of dietary and genetic factors influencing serum and adipose fatty acid composition in obese female identical twins. Lipids. 2002;37:27-32.

265. Sergeant S, Hugenschmidt, C. E., Rudock, M. E., Ziegler, J. T., Ivester P, Ainsworth, H. C., Vaidya, D., Case, D., Langefeld, C. D., Freedman, B. I., Bowden, D. W., Mathias, R. A., Chiltonc, F. H. Differences in arachidonic acid levels and fatty acid desaturase (FADS) gene variants in African Americans and European Americans with diabetes or the metabolic syndrome. Br J Nutr 2011;107(4):547-55.

266. Mathias RA, Sergeant, S., Ruczinski, I., Torgerson, D. G., Hugenschmidt, C. E. . The impact of FADS genetic variants on ω6 polyunsaturated fatty acid metabolism in African Americans. BMC Genetics. 2011;12:50.

267. Peer N, Ganie YN. A weighty matter: Identification and management of overweight and obesity in adolescents. South African Medical Journal. 2016;106(7):662-5.

268. Longo M, Zatterale F, Naderi J, Parrillo L, Formisano P, Raciti GA, et al. Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. Int J Mol Sci. 2019;20(9).

269. Apovian CM, Aronne LJ, Bessesen DH, McDonnell ME, Murad MH, Pagotto U, et al. Pharmacological management of obesity: an endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2015;100(2):342-62.

270. Kahan S. Overweight and Obesity Management Strategies. Am J Manag Care. 2016;22:186-96.

271. Sweeting AN, Caterson ID. Approaches to obesity management. Intern Med J. 2017;47(7):734-9.

272. American Diabetes A. Obesity Management for the Treatment of Type 2 Diabetes. Diabetes Care. 2016;39 Suppl 1:S47-51.

273. Jensen MD, Ryan DH, Apovian CM, Ard JD, Comuzzie AG, Donato KA, et al. 2013 AHA/ACC/TOS Guideline for the Management of Overweight and Obesity in Adults. Circulation. 2014;129(25 suppl 2):S102-S38.

274. Botchlett R, Woo SL, Liu M, Pei Y, Guo X, Li H, et al. Nutritional approaches for managing obesity-associated metabolic diseases. J Endocrinol. 2017;233(3):R145-R71.

275. Kirkpatrick CF, Bolick JP, Kris-Etherton PM, Sikand G, Aspry KE, Soffer DE, et al. Review of current evidence and clinical recommendations on the effects of low-carbohydrate and very-low-carbohydrate (including ketogenic) diets for the management of body weight and other cardiometabolic risk factors: A scientific statement from the National Lipid Association Nutrition and Lifestyle Task Force. J Clin Lipidol. 2019;13(5):689-711.

276. Naude CE, Schoonees A, Senekal M, Young T, Garner P, Volmink J. Low carbohydrate versus isoenergetic balanced diets for reducing weight and cardiovascular risk: a systematic review and meta-analysis. PLoS One. 2014;9(7):e100652.

277. Ekelund U, Ward HA, Norat T, Luan J, May AM, Weiderpass E, et al. Physical activity and all-cause mortality across levels of overall and abdominal adiposity in European men and women: the European Prospective Investigation into Cancer and Nutrition Study (EPIC). Am J Clin Nutr. 2015;101(3):613-21.

278. Abraham PA, Kazman JB, Zeno SA, Deuster PA. Obesity and African Americans: Physiologic and Behavioral Pathways. ISRN Obesity. 2013:8.

279. Goedecke JH. Addressing the problem of obesity and associated cardiometabolic risk in black South African women - time for action! Glob Health Action. 2017;10(1):1366165.

280. Swift DL, Staiano AE, Johannsen NM, Lavie CJ, Earnest CP, Katzmarzyk PT, et al. Low cardiorespiratory fitness in African Americans: a health disparity risk factor? Sports Med. 2013;43(12):1301-13.

281. Goedecke JH, Jennings CL, Lambert EV. Chronic diseases of lifestyle in SA since 1995-2005. Cape Town, South Africa: South African Medical Research Council; 2006.

282. Ogunsina K, Dibaba DT, Akinyemiju T. Association between life-course socio-economic status and prevalence of cardio-metabolic risk ractors in five middle-income countries. J Glob Health. 2018;8(2):020405.

283. Jang SH, Paik IY, Ryu JH, Lee TH, Kim DE. Effects of aerobic and resistance exercises on circulating apelin-12 and apelin-36 concentrations in obese middle-aged women: a randomized controlled trial. BMC Womens Health. 2019;19(1):23.

284. Smith D, Bruce-Low S, Steele J, Fisher J. Evidence-Based Resistance Training Recommendations. Medicina Sportiva. 2011;15(3):147-62.

285. Strasser B, Schobersberger W. Evidence for resistance training as a treatment therapy in obesity. J Obes. 2011;2011.

286. Perry CG, Lally J, Holloway GP, Heigenhauser GJ, Bonen A, Spriet LL. Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle. J Physiol. 2010;588(Pt 23):4795-810.

287. Sanal E, Ardic F, Kirac S. Effects of aerobic or combined aerobic resistance exercise on body composition in overweight and obese adults: gender differences. A randomized intervention study. Eur J Phys Rehabil Med 2013;49:1-11.

288. Belay MA, Reddy RC, M. SB. The Effects of Combined Aerobic and Resistance Exercise Training on Obese Adults, Northwest Ethiopia. Research Journal of Recent Sciences. 2013;2(1):59-66.

289. Myers J, Kokkinos P, Nyelin E. Physical Activity, Cardiorespiratory Fitness, and the Metabolic Syndrome. Nutrients. 2019;11(7).

290. Colberg SR, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, et al. Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement executive summary. Diabetes Care. 2010;33(12):2692-6.

291. Ruth M. A PGC1–α–dependent myokine that drives brown–fat–like development of white fat and thermogenesis. Yearbook of Endocrinology. 2012;2012:114-6.

292. Stanford KI, Middelbeek RJW, Townsend KL, Lee M, Takahashi H, So K, et al. A Novel Role for Subcutaneous Adipose Tissue in Exercise-Induced Improvements in Glucose Homeostasis. Diabetes 2015;64:2002-14.

293. Min SY, Learnard H, Kant S, Gealikman O, Rojas-Rodriguez R, DeSouza T, et al. Exercise Rescues Gene Pathways Involved in Vascular Expansion and Promotes Functional Angiogenesis in Subcutaneous White Adipose Tissue. Int J Mol Sci. 2019;20(8).

294. Bianchi VE, Ribisl PM. Reactive Oxygen Species Response to Exercise Training and Weight Loss in Sedentary Overweight and Obese Female Adults. J Cardiopulm Rehabil Prev. 2015;35(4):263-7.

295. Ho SS, Dhaliwal SS, Hills AP, S. P. The effect of 12 weeks of aerobic, resistance or combination exercise training on cardiovascular risk factors in the overweight and obese in a randomized trial. BMC Public Health. 2012;12.

296. Choo J, Lee J, Cho JH, Burke LE, Sekikawa A, Jae SY. Effects of weight management by exercise modes on markers of subclinical atherosclerosis and cardiometabolic profile among women with abdominal obesity: a randomized controlled trial. BMC Cardiovasc Disord 2014;14.

297. Said MA, Abdelmoneem M, Almaqhawi A, Hamid Kotob AA, Alibrahim MC, Bougmiza I. Multidisciplinary approach to obesity: Aerobic or resistance physical exercise? J Exerc Sci Fit. 2018;16(3):118-23.

298. Sakurai T, Ogasawara J, Kizaki T, Sato S, Ishibashi Y, Takahashi M, et al. The effects of exercise training on obesity-induced dysregulated expression of adipokines in white adipose tissue. Int J Endocrinol. 2013;2013:801743.

299. Park DH, Ransone JW. Effects of submaximal exercise on high-density lipoprotein-cholesterol subfractions. Int J Sports Med. 2003;24(4):245-51.

300. Kokkinos PF, Fernhall B. Physical Activity and High Density Lipoprotein Cholesterol Levels. What is the Relationship? Sports Med. 1999;28(5).

301. Dickie K, Micklesfield LK, Chantler S, Lambert EV, Goedecke JH. Cardiorespiratory Fitness and Light-Intensity Physical Activity Are Independently Associated with Reduced Cardiovascular Disease Risk in Urban Black South African Women: A Cross-Sectional Study. Metab Syndr Relat Disord. 2016;14(1):23-32.

302. Cowan TE, Brennan AM, Stotz PJ, Clarke J, Lamarche B, Ross R. Separate Effects of Exercise Amount and Intensity on Adipose Tissue and Skeletal Muscle Mass in Adults with Abdominal Obesity. Obesity (Silver Spring). 2018;26(11):1696-703.

303. Woo J, Kang S. Diet change and exercise enhance protein expression of CREB, CRTC 2 and lipolitic enzymes in adipocytes of obese mice. Lipids Health Dis. 2016;15(1):147.

304. Petridou A, Chatzinikolaou A, Avloniti A, Jamurtas A, Loules G, Papassotiriou I, et al. Increased Triacylglycerol Lipase Activity in Adipose Tissue of Lean and Obese Men During Endurance Exercise. J Clin Endocrinol Metab. 2017;102(11):3945-52.

305. Mika A, Macaluso F, Barone R, Di Felice V, Sledzinski T. Effect of Exercise on Fatty Acid Metabolism and Adipokine Secretion in Adipose Tissue. Front Physiol. 2019;10:26.

306. Watt MJ, Steinberg GR. Regulation and function of triacylglycerol lipases in cellular metabolism. Biochem J. 2008;414(3):313-25.

307. Rocha-Rodrigues S, Rodriguez A, Becerril S, Ramirez B, Goncalves IO, Beleza J, et al. Physical exercise remodels visceral adipose tissue and mitochondrial lipid metabolism in rats fed a high-fat diet. Clin Exp Pharmacol Physiol. 2017;44(3):386-94.

308. Thorseng T, Witte DR, Vistisen D, Borch-Johnsen K, Bjerregaard P, Jørgensen ME. The association between n-3 fatty acids in erythrocyte membranes and insulin resistance: The inuit health in transition study. International Journal of Circumpolar Health. 2009;68(4):327-36.

309. Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between Adipocyte Size and Adipokine Expression and Secretion. Journal of Clinical Endocrinology & Metabolism. 2012;92(3):1023-33.

310. Gorgens SW, Eckardt K, Jensen J, Drevon CA, Eckel J. Exercise and Regulation of Adipokine and Myokine Production. Prog Mol Biol Transl Sci. 2015;135:313-36.

311. Cordova C, Lopes E, Silva FJ, Pires AS, Souza VC, Brito CJ, et al. Long-term resistance training is associated with reduced circulating levels of IL-6, IFN-gamma and TNF-alpha in elderly women. Neuroimmunomodulation. 2011;18(3):165-70.

312. Phillips MD, Patrizi RM, Cheek DJ, Wooten JS, Barbee JJ, Mitchell JB. Resistance training reduces subclinical inflammation in obese, postmenopausal women. Med Sci Sports Exerc. 2012;44(11):2099-110.

313. Trachta P, Kavalkova P, Touskova V, Cinkajzlova A, Lacinova MM, Zelinka T, et al. Three Months of Regular Aerobic Exercise in patients With Obesity Improve Systemic Subclinical Inflammation Without Major Influence on Blood Pressure and Endocrine Production of subcutaneous Fat. Physiological Research. 2014;63(2):299-308.

314. Lehnig AC, Stanford KI. Exercise-induced adaptations to white and brown adipose tissue. J Exp Biol. 2018;221:1-8.

315. Sakurai T, Ogasawara J, Shirato K, Izawa T, Oh-Ishi S, Ishibashi Y, et al. Exercise Training Attenuates the Dysregulated Expression of Adipokines and Oxidative Stress in White Adipose Tissue. Oxid Med Cell Longev. 2017;2017:9410954.

316. Polak J, Klimcakova E, Moro C, Viguerie N, Berlan M, Hejnova J, et al. Effect of aerobic training on plasma levels and subcutaneous abdominal adipose tissue gene expression of adiponectin, leptin, interleukin 6, and tumor necrosis factor alpha in obese women. Metabolism. 2006;55(10):1375-81.

317. Klimcakova E, Polak J, Moro C, Hejnova J, Majercik M, Viguerie N, et al. Dynamic strength training improves insulin sensitivity without altering plasma levels and gene expression of adipokines in subcutaneous adipose tissue in obese men. J Clin Endocrinol Metab. 2006;91(12):5107-12.

318. Samjoo IA, Safdar A, Hamadeh MJ, Raha S, Tarnopolsky MA. The effect of endurance exercise on both skeletal muscle and systemic oxidative stress in previously sedentary obese men. Nutr Diabetes. 2013;3:e88.

319. Sakurai T, Izawa T, Kizaki T, Ogasawara JE, Shirato K, Imaizumi K, et al. Exercise training decreases expression of inflammation-related adipokines through reduction of oxidative stress in rat white adipose tissue. Biochem Biophys Res Commun. 2009;379(2):605-9.

320. Dela F, Stallknecht B. Effect of physical training on insulin secretion and action in skeletal muscle and adipose tissue of first-degree relatives of type 2 diabetic patients. Am J Physiol Endocrinol Metab. 2010;299(1):E80-91.

321. Lee SH, Im EO. Ethnic differences in exercise and leisure time physical activity among midlife women. J Adv Nurs. 2010;66(4):814-27.

322. Willig AL, Hunter GR, Casazza K, Heimburger DC, Beasley TM, Fernandez JR. Body fat and racial genetic admixture are associated with aerobic fitness levels in a multiethnic pediatric population. Obesity (Silver Spring). 2011;19(11):2222-7.

323. Johnson MS, Figueroa-Colon R, Herd SL, Fields DA, Sun M, Hunter GR, et al. Aerobic fitness, not energy expenditure, influences subsequent increase in adiposity in black and white children. Pediatrics. 2000;106(4):e50.

324. Prioreschi A, Brage S, Westgate K, Norris SA, Micklesfield LK. Cardiorespiratory fitness levels and associations with physical activity and body composition in young South African adults from Soweto. BMC Public Health. 2017;17(1):301.

325. Cook I, Alberts M, Lambert EV. Development of a four-item physical activity index from information about subsistence living in rural African women: a descriptive, cross-sectional investigation. Int J Behav Nutr Phys Act. 2009;6:75.

326. Simmons RK, Griffin SJ, Steele R, Wareham NJ, Ekelund U, ProActive Research T. Increasing overall physical activity and aerobic fitness is associated with improvements in metabolic risk: cohort analysis of the ProActive trial. Diabetologia. 2008;51(5):787-94.

327. LaMonte MJ, Barlow CE, Jurca R, Kampert JB, Church TS, Blair SN. Cardiorespiratory fitness is inversely associated with the incidence of metabolic syndrome: a prospective study of men and women. Circulation. 2005;112(4):505-12.

328. Shephard RJ. Sitting Time and Mortality from All Causes, Cardiovascular Disease, and Cancer. Yearbook of Sports Medicine. 2010;2010:124-5.

329. Bankoski A, Harris TB, McClain JJ, Brychta RJ, Caserotti P, Chen KY, et al. Sedentary activity associated with metabolic syndrome independent of physical activity. Diabetes Care. 2011;34(2):497-503.

330. Oviedo GR, Tamulevicius N, Onagbiye SO, Phidza M, Sedumedi C, Cameron M, et al. Quality of life, physical activity and cardiorespiratory fitness in black African women: B-Healthy project. Qual Life Res. 2019:https://doi.org/10.1007/s11136-019-02368-6.

331. Bird SR, Hawley JA. Exercise and type 2 diabetes: new prescription for an old problem. Maturitas. 2012;72(4):311-6.

332. Hawley JA. Exercise as a therapeutic intervention for the prevention and treatment of insulin resistance. Diabetes Metab Res Rev. 2004;20(5):383-93.

333. Hawley JA, Lessard SJ. Exercise training-induced improvements in insulin action. Acta Physiol (Oxf). 2008;192(1):127-35.

334. Pan XR, Li GW, Hu YH, Wang J, Yang W, An Z, et al. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. Diabetes Care 1997;20:537-44.

335. Goedecke JH, Mendham, A. E., Clamp, L., Nono Nankam, P. A., Fortuin-de Smidt, M. C., Phiri, L., Micklesfield, L. K., Keswell, D., Woudberg, N. J., Lecour, S., Alhamud, A., Kaba, M., Lutomia, F. M., van Jaarsveld, P. J., de Villiers, A., Kahn, S. E., Chorell, E., Hauksson, J., Olsson, T. 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 Research Protocols. 2018;7(4).

336. O'Donovan G, Blazevich AJ, Boreham C, Cooper AR, Crank H, Ekelund U, et al. The ABC of Physical Activity for Health: a consensus statement from the British Association of Sport and Exercise Sciences. J Sports Sci. 2010;28(6):573-91.

337. Clamp LD, Mendham, A. E., Kroff, J., Goedecke, J. H. Higher baseline fat oxidation promotes gynoid fat mobilization in response to a 12 week exercise intervention in sedentary, obese black South African women. Appl Physiol Nutr Metab. 2019:https://doi.org/10.1139/apnm-2019-0460.

338. Goedecke JH, Micklesfield, L. K., Levitt, N. S., Lambert, E. V., West, S., Maartens, G., Dave, J. A. Effect of different antiretroviral drug regimens on body fat distribution of HIV-infected South African women. AIDS Res Hum Retroviruses. 2013;29(3):557-63.

339. Micklesfield LK, Goedecke, J. H., Punyanitya, M., Wilson, K. E., Kelly, T. L. . Dual-energy X-ray performs as well as clinical computed tomography for the measurement of visceral fat. Obesity (Silver Spring). 2012;20(5):1109-14.

340. Micklesfield LK, Goedecke JH, Punyanitya M, Wilson KE, Kelly TL. Dual-energy X-ray performs as well as clinical computed tomography for the measurement of visceral fat. Obesity (Silver Spring). 2012;20(5):1109-14.

341. Machann J, Bachmann OP, Brechtel K, Dahl DB, Wietek B, Klumpp B, et al. Lipid content in the musculature of the lower leg assessed by fat selective MRI: intra- and interindividual differences and correlation with anthropometric and metabolic data. J Magn Reson Imaging. 2003;17(3):350-7.

342. Bergman R, Ider, Y., Bowden, C., Cobelli, C. Quantitative estimation of insulin sensitivity. . Am J Physiol 1979;236(6):E667-E77.

343. Levy JC, Matthews DR, Hermans MP. Correct Homeostasis Model Assessment (HOMA) Evaluation uses the computer program. Diabetes Care. 1998;21:2191-2.

344. Ahrèn B, Pacini G. Importance of quantifying insulin secretion in relationto insulin sensitivity to accurately assess beta cell functionin clinical studies. European Journal of Endocrinology. 2004;150:97-104.

345. Maarman GJ. Melatonin as a novel cardioprotective therapz in pulmonary hypertension University of Cape Town; 2014.

346. Folch J, Lees, M., Sloane Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 1957:497-509.

347. Chimhashu T, Malan, L., Baumgartner, J., van Jaarsveld, P. J., Galetti V, Moretti, D., Smuts, C. M., Zimmermann, M. B. Sensitivity of fatty acid desaturation and elongation to plasma zinc concentration: a randomised controlled trial in Beninese children. British Journal of Nutrition. 2018;119:610-9.

348. Fan YY, Chapkin, R. S. . Importance of dietary γ-linolenic acid in human health and nutrition. J Nutr. 1998:1411-4.

349. Warensjo E, Rosell, M. Hellenius, M. L., Vessby, B., De Faire, U., Riserus, U. Associations between estimated fatty acid desaturase activities in serum lipids and adipose tissue in humans: links to obesity and insulin resistance. Lipids Health Dis. 2009;8:37.

350. Jump DB. Fatty acid regulation of hepatic lipid metabolism. Curr Opin Clin Nutr Metab Care. 2011;14(2):115-20.

351. Eriksson L, Trygg, J., Wold, S. CV-ANOVA for significance testing of PLS and OPLS® models. Journal of Chemometrics. 2008;22(11-12):594-600.

352. Efron B, Gong, G. A Leisurely Look at the Bootstrap, the Jackknife, and Cross-Validation. American Statistician. 1983;37(1).

353. Ritchie ME, Dunning MJ, Smith ML, Shi W, Lynch AG. BeadArray expression analysis using bioconductor. PLoS Comput Biol. 2011;7(12):e1002276.

354. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019;47(D1):D607-D13.

355. Unger RH, Scherer PE. Gluttony, Sloth and the Metabolic Syndrome: A roadmap to Lipotoxicity. Trends Endocrinol Metab. 2010;21(6):345-52.

356. Frayn KN. Visceral fat and insulin resistance--causative or correlative? Br J Nutr. 2000;83 Suppl 1:S71-7.

357. Roden M, Price, T. B., Perseghin, G., Petersen, K. F., Rothman, D. L., Cline, G. W., Shulman, G. I. Mechanism of free fatty acid induced insulin resistance in humans. J Clin Invest. 1996;97:2859-65.

358. Perona JS. Membrane lipid alterations in the metabolic syndrome and the role of dietary oils. Biochimica et Biophysica Acta. 2017;1859:1690-703.

359. Choi S, Jang HB, Park JY, Lee H, Kang J, Park K, et al. Associations Between Estimated Desaturase Activity and Insulin Resistance in Korean Boys Young Public Health Res Perspect. 2014;5(5):251-7.

360. Mahendran Y, Agren, J., Uusitupa, M., Cederberg, H., Vangipurapu, J., Stancakova, A., Schwab, U., Kuusisto, J., Laakso, M. Association of erythrocyte membrane fatty acids with changes in glycemia and risk of type 2 diabetes. Am J Clin Nutr. 2014;99(1):79-85.

361. Zhou YE, Kubow S, Dewailly E, Julien P, Egeland GM. Decreased activity of desaturase 5 in association with obesity and insulin resistance aggravates declining long-chain n-3 fatty acid status in Cree undergoing dietary transition. Br J Nutr. 2009;102(6):888-94.

362. Sjogren P, Sierra-Johnson, J., Gertow, K., Rosell, M., Vessby, B., de Faire, U., Hamsten, A., Hellenius, M. L., Fisher, R. M. Fatty acid desaturases in human adipose tissue: relationships between gene expression, desaturation indexes and insulin resistance. Diabetologia. 2008;51(2):328-35.

363. Perona JS, Vögler O, Sánchez-Domínguez JM, Montero E, Escribá PV, Ruiz-Gutierrez V. Consumption of virgin olive oil influences membrane lipid composition and regulates intracellular signaling in elderly adultswith type 2 diabetesmellitus. J Gerontol A Biol Sci Med Sci. 2007;62:256-63.

364. Guelzim N, Huneau JF, Mathé V, Tesseraud S, Mourot J, Simon N, et al. N-3 fatty acids improve body composition and insulin sensitivity during energy restriction in the rat. Prostaglandins Leukot Essent Fat Acids 2014;91:203-11.

365. MacIntyre U, Kruger, H., Venter, C., Vorster, H. Dietary intakes of an African population in different stages of transition in the North West Province, South Africa: the THUSA study. Nutr Res. 2002;22(3):239-56.

366. Markussen MS, Veierod, M. B., Ursin, G., Andersen, L. F. The effect of under-reporting of energy intake on dietary patterns and on the associations between dietary patterns and self-reported chronic disease in women aged 50-69 years. Br J Nutr. 2016;116(3):547-58.

367. Kroger J, Zietemann, V., Enzenbach, C., Weikert, C., Jansen, E. HJM., Döring, F., Joost, H., Boeing, H., Schulze, M. B. Erythrocyte membrane phospholipid fatty acids, desaturase activity, and dietary fatty acids in relation to risk of type 2 diabetes in the European prospective investigation into cancer and nutrition (EPIC)-potsdam study. Am J Clin Nutr 2011;93:127-42.

368. Gower BA, Alvarez, J. A., Bush, N. C., Hunter, G. R. Insulin sensitivity affects propensity to obesity in an ethnic-specific manner: results from two controlled weight loss intervention studies. Nutrition & Metabolism. 2013;10(3).

369. Gianmattia G, Ferreri, C., Marfella, R., Pournaras, D., Roux, C., del Genio, F., Paolo, L., Tolone, S., Docimo, L., Puca, A. A. Morbid Obesity is Associated to Altered Fatty Acid Profile of Erythrocyte Membranes. Journal of Diabetes & Metabolism. 2015;06(08).

370. Cordero A, González, J. E., Perona, J. S., Padilla L., Ferre A., , Hita O, Baeza, R., Barrilao, G., Rivas, G. Obesity and its relation with markers of inflammation and erythrocyte fatty acids in a group of overweight adolescents. Nutr Hosp. 2012;27:161-4.

371. Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, et al. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. Diabetologia. 2001;44(3):312-9.

372. Nakamura MT, Nara TY. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. Annu Rev Nutr. 2004;24:345-76.

373. Mika A, Kaska, L., Korczynska, J., Mirowska, A., Stepnowski, P., Proczko, M., Ratnicki-Sklucki, K., Goyke, E., Sledzinski, T. Visceral and subcutaneous adipose tissue stearoyl-CoA desaturase-1 mRNA levels and fatty acid desaturation index positively correlate with BMI in morbidly obese women. European Journal of Lipid Science and Technology. 2015;117(7):926-32.

374. Ojwang AA, Kruger, H. S., Zec, M., Ricci, C., Pieters, M., Kruger, I. M., Wentzel-Viljoen, E., Smuts, C. M. Plasma phospholipid fatty acid patterns are associated with adiposity and the metabolic syndrome in black South Africans: a cross-sectional study. Cardiovasc J Afr. 2019;30(4):228-38.

375. Phinney SD, Stern, J. S., Burke, K. E., Tang, A. B., Miller, G., Holman, R. T. Human subcutaneous adipose tissue shows site-specific differences in fatty acid composition. Am J Clin Nutr 1994;60:725-9.

376. Aoun M, Michel, F., Fouret, G., Schlernitzauer, A., Ollendorff, V., Wrutniak-Cabello, C., Cristol, J. P., Carbonneau MA, Coudray, C., Feillet-Coudray, C. A grape polyphenol extract modulates muscle membrane fatty acid composition and lipid metabolism in high-fat--high-sucrose diet-fed rats. Br J Nutr. 2011;106(4):491-501.

377. Fickova M, Hubert, P., Crémel, G., Leray, C. Dietary (n-3) and (n-6) polyunsaturated fatty acids rapidly modify fatty acid composition and insulin effects in rat adipocytes,. J Nutr. 1998;128:512-9.

378. Micallef M, Munro, I., Phang, M., Garg, M. Plasma n-3 Polyunsaturated Fatty Acids are negatively associated with obesity. Br J Nutr. 2009;102(9):1370-4.

379. Huang T, Bhulaidok, S., Cai, Z., Xu, T., Xu, F., Wahlqvist, M. L., Li, D. Plasma phospholipids n-3 polyunsaturated fatty acid is associated with metabolic syndrome. Mol Nutr Food Res. 2010;54(11):1628-35.

380. Aglago EK, Biessy, C., Torres-Mejia, G., Angeles-Llerenas, A., Gunter, M. J., Romieu, I., Chajes, V. Association between serum phospholipid fatty acid levels and adiposity in Mexican women. J Lipid Res. 2017;58(7):1462-70.

381. Mayneris-Perxachs J, Guerendiain, M., Castellote, A. I., Estruch, R., Covas, M. I., Fito, M., Salas-Salvado, J., Martinez-Gonzalez, M. A., Aros, F., Lamuela-Raventos, R. M., Lopez-Sabater, M. C., for, Predimed Study Investigators. Plasma fatty acid composition, estimated desaturase activities, and their relation with the metabolic syndrome in a population at high risk of cardiovascular disease. Clin Nutr. 2014;33(1):90-7.

382. Kabagambe EK, Tsai, M. Y., Hopkins, P. N., Ordovas, J. M., Peacock, J. M., Borecki, I. B., Arnett, D. K. Erythrocyte fatty acid composition and the metabolic syndrome: a National Heart, Lung, and Blood Institute GOLDN study. Clin Chem. 2008;54(1):154-62.

383. Yary T, Voutilainen, S., Tuomainen, T. P., Ruusunen, A., Nurmi, T., Virtanen, J. K. Omega-6 polyunsaturated fatty acids, serum zinc, delta-5- and delta-6-desaturase activities and incident metabolic syndrome. J Hum Nutr Diet. 2017;30(4):506-14.

384. Barber E, Sinclair AJ, Cameron-Smith D. Comparative actions of omega-3 fatty acids on in-vitro lipid droplet formation. Prostaglandins Leukot Essent Fatty Acids. 2013;89(5):359-66.

385. Rosqvist F, Bjermo, H., Kullberg, J., Johansson, L., Michaelsson, K., Ahlstrom, H., Lind, L., Riserus, U. Fatty acid composition in serum cholesterol esters and phospholipids is linked to visceral and subcutaneous adipose tissue content in elderly individuals: a cross-sectional study. Lipids Health Dis. 2017;16(1):68.

386. Gertow K, Rosell, M., Sjogren, P., Eriksson, P., Vessby, B., de Faire U, Hamsten, A., Hellenius, M. L., Fisher, R. M. Fatty acid handling protein expression in adipose tissue, fatty acid composition of adipose tissue and serum, and markers of insulin resistance. Eur J Clin Nutr. 2006;60(12):1406-13.

387. Iggman D, Arnlov, J., Vessby, B., Cederholm, T., Sjogren, P., Riserus, U. Adipose tissue fatty acids and insulin sensitivity in elderly men. Diabetologia. 2010;53(5):850-7.

388. Bonadonna RC, Del Prato S, Saccomani MP. Transmembrane glucose transport in skeletal muscle of patients with non-insulin-dependent diabetes. Clin Invest. 1993;92:486-94.

389. Chung ST, Courville AB, Onuzuruike AU, Galvan-De La Cruz M, al. e. Gluconeogenesis and risk for fasting hyperglycemia in Black and White women. JCI Insight. 2018;3(18).

390. Ni Y, Zhao L, Yu H, Ma X, Bao Y, Rajani C, et al. Circulating Unsaturated Fatty Acids Delineate the etabolic Status of Obese Individuals. EBioMedicine 2. 2015;2:1513-22.

391. Ntambi JM, Miyazaki M, Stoehr JP, Hong L, Kendziorski CM, Yandell BS, et al. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. Proc Natl Acad Sci USA. 2002:11482-6.

392. Flowers MT, Ntambi JM. Stearoyl-CoA Desaturase and its Relation to High-Carbohydrate Diets and Obesity. Biochim Biophys Acta. 2009;1791(2):85-91.

393. Goff LM, Ladwa M, Hakim O, Bello O. Ethnic distinctions in the pathophysiology of type 2 diabetes: a focus on black African-Caribbean populations. Proc Nutr Soc. 2019:1-10.

394. Tsurutani Y, Inoue K, Sugisawa C, Saito J, Omura M, Nishikawa T. Increased Serum Dihomo-γ-linolenic Acid Levels Are Associated with Obesity, Body Fat Accumulation, and Insulin Resistance in Japanese Patients with Type 2 diabetes. Intern Med. 2018;57:2929-35.

395. Sergeant S, Rahbarb E, Chiltonc FH. Gamma-linolenic acid, Dihommo-gamma linolenic, Eicosanoids and Inflammatory Processes. Eur J Pharmacol. 2016;785:77-86.

396. Virtanen JK, Mursu J, Voutilainen S, Tuomainen TP. The associations of serum n-6 polyunsaturated fatty acids with serum C-reactive protein in men: the Kuopio Ischaemic Heart Disease Risk Factor Study. Eur J Clin Nutr. 2018;72(3):342-8.

397. Purdom T, Kravitz L, Dokladny K, Mermier C. Understanding the factors that effect maximal fat oxidation. J Int Soc Sports Nutr. 2018;15:3.

398. Clifton PM, Nestel PJ. Relationship between plasma insulin and erythrocyte fatty acid composition. Prostaglandins, L eukotrienes and Essential Fatty Acids 1998;59(3):191-4.

399. Jung SH, Park HS, Kim K, Choi WH, Ahn CW, Kim BT, et al. Effect of weight loss on some serum cytokines in human obesity: increase in IL-10 after weight loss. Journal of Nutritional Biochemistry. 2008;19:371-5.

400. Gustafson B, Gogg S, Hedjazifar S, Jenndahl L, Hammarstedt A, Smith U. Inflammation and impaired adipogenesis in hypertrophic obesity in man. Am J Physiol Endocrinol Metab. 2009;297(5):E999-E1003.

401. Devries MC, Hamadeh MJ, Glover AW, Raha S, Samjoo IA, Tarnopolsky MA. Endurance training without weight loss lowers systemic, but not muscle, oxidative stress with no effect on inflammation in lean and obese women. Free Radic Biol Med. 2008;45(4):503-11.

402. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation. 1999;100:2473-6.

403. Bashan N, Kovsan J, Kachko I, Ovadia H, Rudich A. Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species. Physiol Rev. 2009;89(1):27-71.

404. Bloch-Damti A, Bashan N. Proposed Mechanisms for the Induction of Insulin Resistance by Oxidative Stress. Antioxid Redox Signal. 2005;7:1553-67.

405. Bays HE, González-Campoy JM, Bray GA. Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity. Expert Rev Cardiovasc Ther 2008;6:343-68.

406. Goedecke JH, Levitt, N.S., Evans, J., Ellman, N., Hume, D.J., Kotze, L., Tootla, M., Victor, H., Keswell, D. The role of adipose tissue in insulin resistance in women of African ancestry. J Obes. 2012;2013:952916.

407. Després JP. Abdominal obesity as important component of insulin-resistance syndrome. Nutrition. 1993;9:452-9.

408. Manolopoulos KN, Karpe F, Frayn KN. Gluteofemoral body fat as a determinant of metabolic health. Int J Obes (Lond). 2010;34(6):949-59.

409. Keswell D, Adams, K., Dlamini, T., et al. . Ethnic differences in adipocyte cell size and insulin sensitivity in South African women. JEMDSA. 2012;17(32).

410. Blüher M, Michael D, Peroni OD, Ueki K, arter N, Kahn BB, et al. Adipose Tissue Selective Insulin Receptor Knockout Protects against Obesity and Obesity-Related Glucose Intolerance. Developmental Cell. 2002;3:25-38.

411. Lakhdar N, Denguezli M, Zaouali M, Zbidi A, Tabka Z, Bouassida A. Diet and diet combined with chronic aerobic exercise decreases body fat mass and alters plasma and adipose tissue inflammatory markers in obese women. Inflammation. 2013;36(6):1239-47.

412. Libardi CA, De Souza GV, Cavaglieri CR, Madruga VA, Chacon-Mikahil MP. Effect of resistance, endurance, and concurrent training on TNF-alpha, IL-6, and CRP. Med Sci Sports Exerc. 2012;44(1):50-6.

413. Chantler S, Dickie K, Micklesfield LK, Goedecke JH. Determinants of change in body weight and body fat distribution over 5.5 years in a sample of free-living black South African women. Cardiovasc J Afr. 2016;27(6):367-74.

414. Oh S, Tanaka K, Warabi E, Shoda J. Exercise reduces inflammation and oxidative stress in obesity-related liver diseases. Med Sci Sports Exerc. 2013;45(12):2214-22.

415. Kelly AS, Steinberger J, Olson TP, Dengel DR. In the absence of weight loss, exercise training does not improve adipokines or oxidative stress in overweight children. Metabolism. 2007;56(7):1005-9.

416. Bradley AA, Carl FN. Glutathione Metabolism as a Determinant of therapeutic Efficacy: A Review. Cancer Res. 1984;44:4224-32.

417. Rutkowski JM, Stern JH, Scherer PE. The cell biology of fat expansion. J Cell Biol 2015;Vol. 208 (No. 5):501-12.

418. Monteiro R, Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. Mediators Inflamm. 2010;2010.

419. Cristancho AG, Lazar MA. Forming functional fat: a growing understanding of adipocyte differentiation. Nat Rev Mol Cell Biol. 2011;12(11):722-34.

420. Fischer IP, Irmler M, Meyer CW, Sachs SJ, Neff F, Hrabe de Angelis M, et al. A history of obesity leaves an inflammatory fingerprint in liver and adipose tissue. Int J Obes (Lond). 2018;42(3):507-17.

421. Pasarica M, Tchoukalova YD, Heilbronn LK, Fang X, Albu JB, Kelley DE, et al. Differential effect of weight loss on adipocyte size subfractions in patients with type 2 diabetes. Obesity (Silver Spring). 2009;17(10):1976-8.

422. Despres JP, Bouchard C, Savard R, Tremblay A, Marcotte M, Theriault G. Level of physical fitness and adipocyte lipolysis in humans. Journal of Applied Physiology. 1984;56:1157-61.

423. Teixeira AA, Lira FS, Pimentel GD, Oliveira de Souza C, Batatinha H, Biondo LA, et al. Aerobic Exercise Modulates the Free Fatty Acids and Inflammatory Response During Obesity and Cancer Cachexia. Crit Rev Eukaryot Gene Expr. 2016;26(3):187-98.

424. Huang SC, Everts, B., Ivanova, Y., O'Sullivan, D., Nascimento, M., Smith, A.M., Beatty, W., Love-Gregory, L., Lam, W.Y., O'Neill, C.M., Yan, C., Du, H., Abumrad NA, Urban, J.F., Jr., Artyomov, M.N., Pearce, E.L., Pearce, E.J. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat Immunol. 2014;15(9):846-55.

425. Kosteli A, Sugaru E, Haemmerle G, Martin JF, Lei J, Zechner R, et al. Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. J Clin Invest. 2010;120(10):3466-79.

426. Perriotte-Olson C, Adi N, Manickam DS, Westwood RA, Desouza CV, Natarajan G, et al. Nanoformulated copper/zinc superoxide dismutase reduces adipose inflammation in obesity. Obesity (Silver Spring). 2016;24(1):148-56.

427. Khadir A, Kavalakatt S, Cherian P, Warsame S, Abubaker JA, Dehbi M, et al. Physical Exercise Enhanced Heat Shock Protein 60 Expression and Attenuated Inflammation in the Adipose Tissue of Human Diabetic Obese. Front Endocrinol (Lausanne). 2018;9:16.

428. Muniyappa R, Madan R. Assessing Insulin Sensitivity and Resistance in Humans. In: Feingold KR AB, Boyce A, et al.editors, editor. South Dartmouth (MA): MDText.com, Inc.: Endotext [Internet]; 2018.

429. Wang D. Discrepancy between mRNA and protein abundance: insight from information retrieval process in computers. Comput Biol Chem. 2008;32(6):462-8.

430. Yamamoto Y, Gesta S, Lee KY, Tran TT, Saadatirad P, Kahn CR. Adipose Depots Possess Unique Developmental Gene Signatures. Obesity (Silver Spring). 2010;18(5):872-8.

431. Brune JE, Kern M, Kunath A, Flehmig G, Schon MR, Lohmann T, et al. Fat depot-specific expression of HOXC9 and HOXC10 may contribute to adverse fat distribution and related metabolic traits. Obesity (Silver Spring). 2016;24(1):51-9.

432. Passaro A, Miselli MA, Sanz JM, Dalla Nora E, Morieri ML, Colonna R, et al. Gene expression regional differences in human subcutaneous adipose tissue. BMC Genomics. 2017;18(1):202.

433. Rehrer CW, Karimpour-Fard A, Hernandez TL, Law CK, Stob NR, Hunter LE, et al. Regional differences in subcutaneous adipose tissue gene expression. Obesity (Silver Spring). 2012;20(11):2168-73.

434. Billon N, Dani C. Developmental origins of the adipocyte lineage: new insights from genetics and genomics studies. Stem Cell Rev Rep. 2012;8(1):55-66.

435. Gehring WJ, Hiromi Y. Homeotic genes and the homeobox. Ann Rev Genet. 1986;20:147-73.

436. Procino A, Cillo C. The HOX genes network in metabolic diseases. Cell Biol Int. 2013;37:1145-8.

437. Claussnitzer M, Dankel SN, Kim KH, Quon G, Meuleman W, Haugen C, et al. FTO Obesity Variant Circuitry and Adipocyte Browning in Humans. N Engl J Med. 2015;373(10):895-907.

438. Inui M, Tamano M, Kato T, Takada S. CRISPR/Cas9-mediated simultaneous knockout of Dmrt1 and Dmrt3 does not recapitulate the 46,XY gonadal dysgenesis observed in 9p24.3 deletion patients. Biochem Biophys Rep. 2017;9:238-44.

439. Qiu C, Han Z, Li W, Ye K, Xie Y, Wang Z. A high-density genetic linkage map and QTL mapping for growth and sex of yellow drum (Nibea albiflora). Sci Rep. 2018;8(1):17271.

440. du Plessis J, van Pelt J, Korf H, Mathieu C, van der Schueren B, Lannoo M, et al. Association of Adipose Tissue Inflammation With Histologic Severity of Nonalcoholic Fatty Liver Disease. Gastroenterology. 2015;149(3):635-48 e14.

441. Pujar MK, Vastrad B, Vastrad C. Integrative Analyses of Genes Associated with Subcutaneous Insulin Resistance. Biomolecules. 2019;9(2).

442. Ahn J, Wu H, Lee K. Integrative Analysis Revealing Human Adipose-Specific Genes and Consolidating Obesity Loci. Sci Rep. 2019;9(1):3087.

443. Johansson LE, Danielsson AP, Parikh H, Klintenberg M, Norstrom F, Groop L, et al. Differential gene expression in adipose tissue from obese human subjects during weight loss and weight maintenance. Am J Clin Nutr. 2012;96(1):196-207.

444. Vordenbaumen S, Saenger T, Braukmann A, Tahan T, Bleck E, Jose J, et al. Human casein alpha s1 induces proinflammatory cytokine expression in monocytic cells by TLR4 signaling. Mol Nutr Food Res. 2016;60(5):1079-89.

445. Lindgren CM, Heid IM, Randall JC, Lamina C, Steinthorsdottir V, Qi L, et al. Genome-wide association scan meta-analysis identifies three Loci influencing adiposity and fat distribution. PLoS Genet. 2009;5(6):e1000508.

446. Gepner Y, Shelef I, Schwarzfuchs D, Zelicha H, Tene L, Yaskolka Meir A, et al. Effect of Distinct Lifestyle Interventions on Mobilization of Fat Storage Pools: CENTRAL Magnetic Resonance Imaging Randomized Controlled Trial. Circulation. 2018;137(11):1143-57.

447. Fabre O, Ingerslev LR, Garde C, Donkin I, Simar D, Barrès R. Exercise training alters the genomic response to acute exercise in human adipose tissue. Epigenomics. 2018;10(8):1033-50.

448. Hotamisligil GS, Erbay E. Nutrient sensing and inflammation in metabolic diseases. Nat Rev Immunol. 2008;8(12):923-34.

449. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest. 2003;112(12):1821-30.

450. Park Y, Myers M, Potter-Vierra V. Adipose Tissue Inflammation and Metabolic Dysfunction: Role of Exercise. Missouri Medecine. 2014;111(1):65.

451. Grace MS, Formosa MF, Bozaoglu K, Bergouignan A, Brozynska M, Carey AL, et al. Acute effects of active breaks during prolonged sitting on subcutaneous adipose tissue gene expression: an ancillary analysis of a randomised controlled trial. Sci Rep. 2019;9(1):3847.

452. Thompson D, Karpe F, Lafontan M, Frayn K. Physical activity and exercise in the regulation of human adipose tissue physiology. Physiol Rev. 2012;92(1):157-91.

453. Lyngsø D, Simonsen L, Bülow J. Interleukin-6 production in human subcutaneous abdominal adipose tissue: the effect of exercise. The Journal of Physiology. 2002;543(1):373-8.

454. van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P, et al. Interleukin-6 Stimulates Lipolysis and Fat Oxidation in Humans. The Journal of Clinical Endocrinology & Metabolism. 2003;88(7):3005-10.

455. Pasarica M, Tchoukalova YD, Heilbronn LK, Fang X, Albu JB, Kelley DE, et al. Differential effect of weight loss on adipocyte size subfractions in patients with type 2 diabetes. Obesity (Silver Spring). 2009;17(10):1976-8.

456. Joo JI, Yun JW. Gene Expression Profiling of Adipose Tissues in Obesity Susceptible and Resistant Rats under a High Fat Diet. Cell Physiol Biochem. 2011;27:327-40.

457. Pettersson AT, Laurencikiene J, Mejhert N, Naslund E, Bouloumie A, Dahlman I, et al. A possible inflammatory role of twist1 in human white adipocytes. Diabetes. 2010;59(3):564-71.

458. Lu S, Wang H, Ren R, Shi X, Zhang Y, Ma W. Reduced expression of Twist 1 is protective against insulin resistance of adipocytes and involves mitochondrial dysfunction. Sci Rep. 2018;8(1):12590.

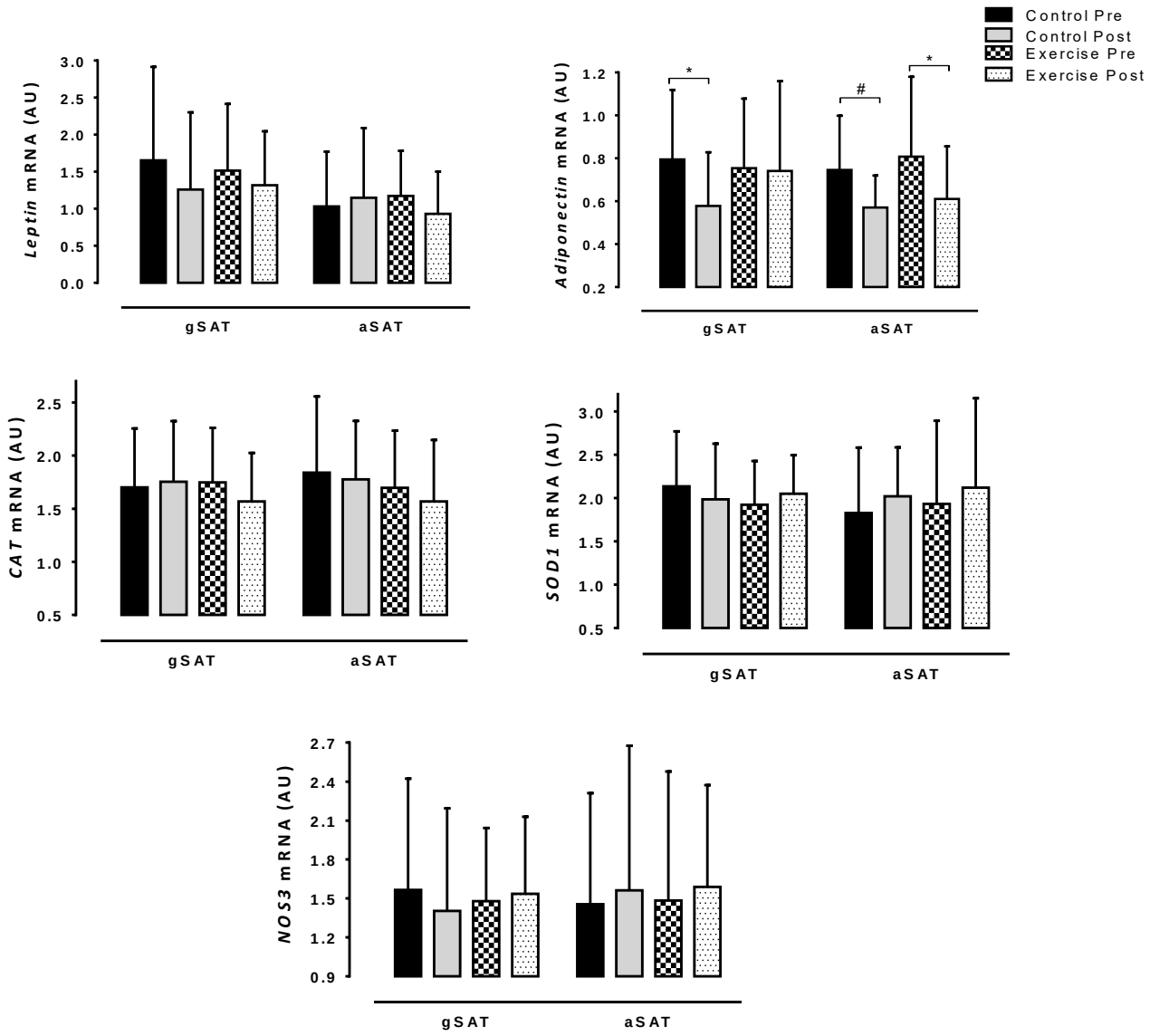
459. Ryden M, Arner P. Tumour necrosis factor-alpha in human adipose tissue -- from signalling mechanisms to clinical implications. J Intern Med. 2007;262(4):431-8.

460. Draper CE, Davidowitz KJ, Goedecke JH. Perceptions relating to body size, weight loss and weight-loss interventions in black South African women: a qualitative study. Public Health Nutr. 2016;19(3):548-56.

SUPPLEMENTARY DATA

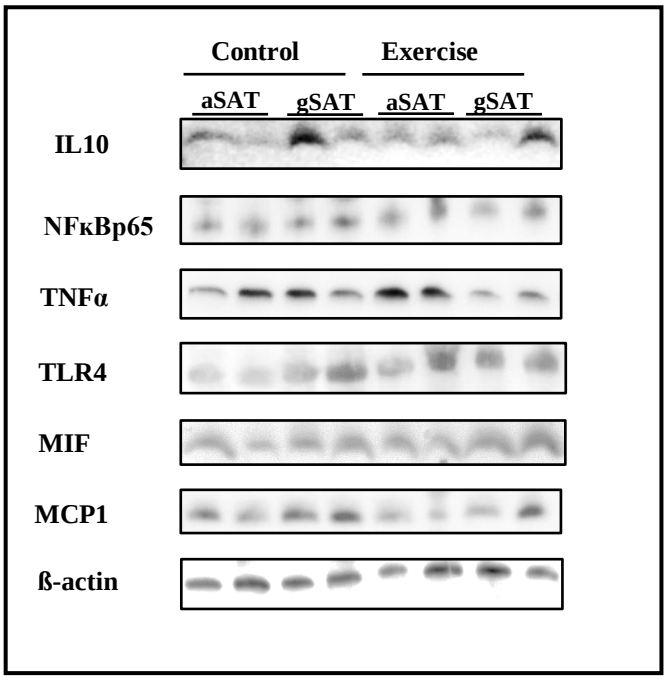
Supplementary Table 2.1. List of genes Taqman IDs

Gene ID	Gene names	Probe ID
RPLPo	Ribosomal protein lateral stalk subunit PO	Hs99999902_m1
LRP10	Low-density lipoprotein receptor-related protein 10	Hs00204094_m1
ADIPOQ	Adiponectin	Hs00605917_m1
MCP1	Monocyte chemoattractant protein 1	Hs00234140_m1
IL10	Interleukin 10	Hs00961622_m1
LEP	Leptin	Hs00174877_m1
MIF	Macrophage migration inhibitory factor	Hs00236988_g1
NFκB1	Nuclear factor-kappa B1	Hs00765730_m1
TLR4	Toll like receptor 4	Hs01060206_m1
TNFα	Tumor necrosis factor-alpha	Hs00174128_m1
PPARγ	Peroxisome proliferator activator receptor gamma	Hs01115513_m1
LPL	Lipoprotein lipase	Hs00173425_m1
DGAT2	Diacylglycerol acyltransferase 2	Hs01045913_m1
PLIN1	Perilipin 1	Hs00160173_m1
ATGL	Adipose triglyceride lipase	Hs00386101_m1
IRS1	Insulin receptor substrate 1	Hs00178563_m1
GLUT4	Glucose transporter 4	Hs00168966_m1
SMG1	Serine/threonine-protein kinase	Hs00979691_m1
CAT	Catalase	Hs00156308_m1
SOD1	Superoxide dismutase	Hs00533490_m1
NOS3	Nitric oxide synthase	Hs01574665_m1



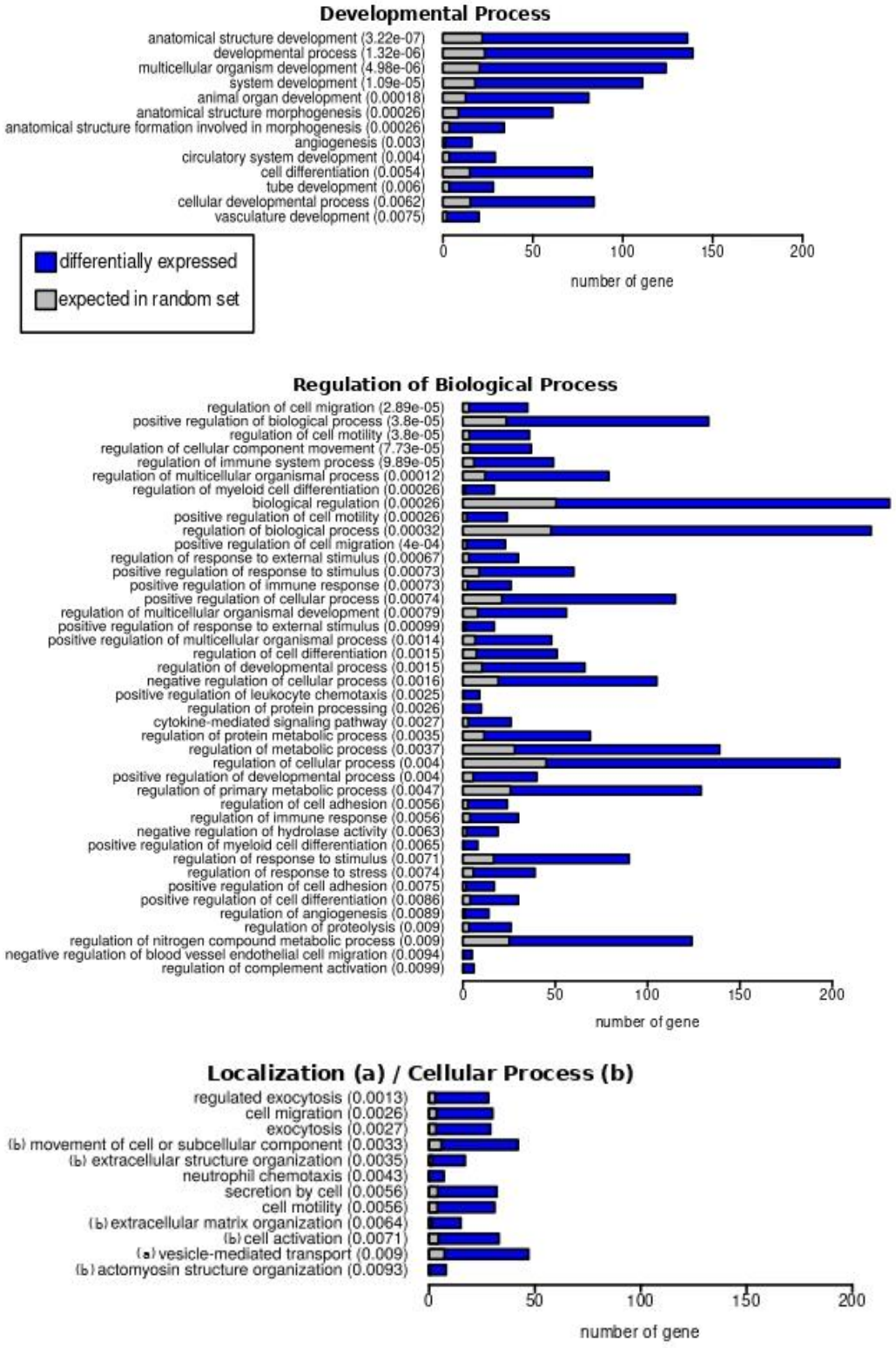
Supplementary Figure 5.1. Effect of exercise training on gluteal (gSAT) and abdominal subcutaneous adipose tissue (aSAT) expression of leptin, adiponectin, catalase (CAT), superoxide dismutase (SOD1) and nitric oxide synthase (NOS3).

$^{\#}P<0.1$ and $*P<0.05$: pre vs post within groups following the intervention (time effect). Control group n=15, both depots and exercise group n=20 in aSAT and n=18 in gSAT at post-training.

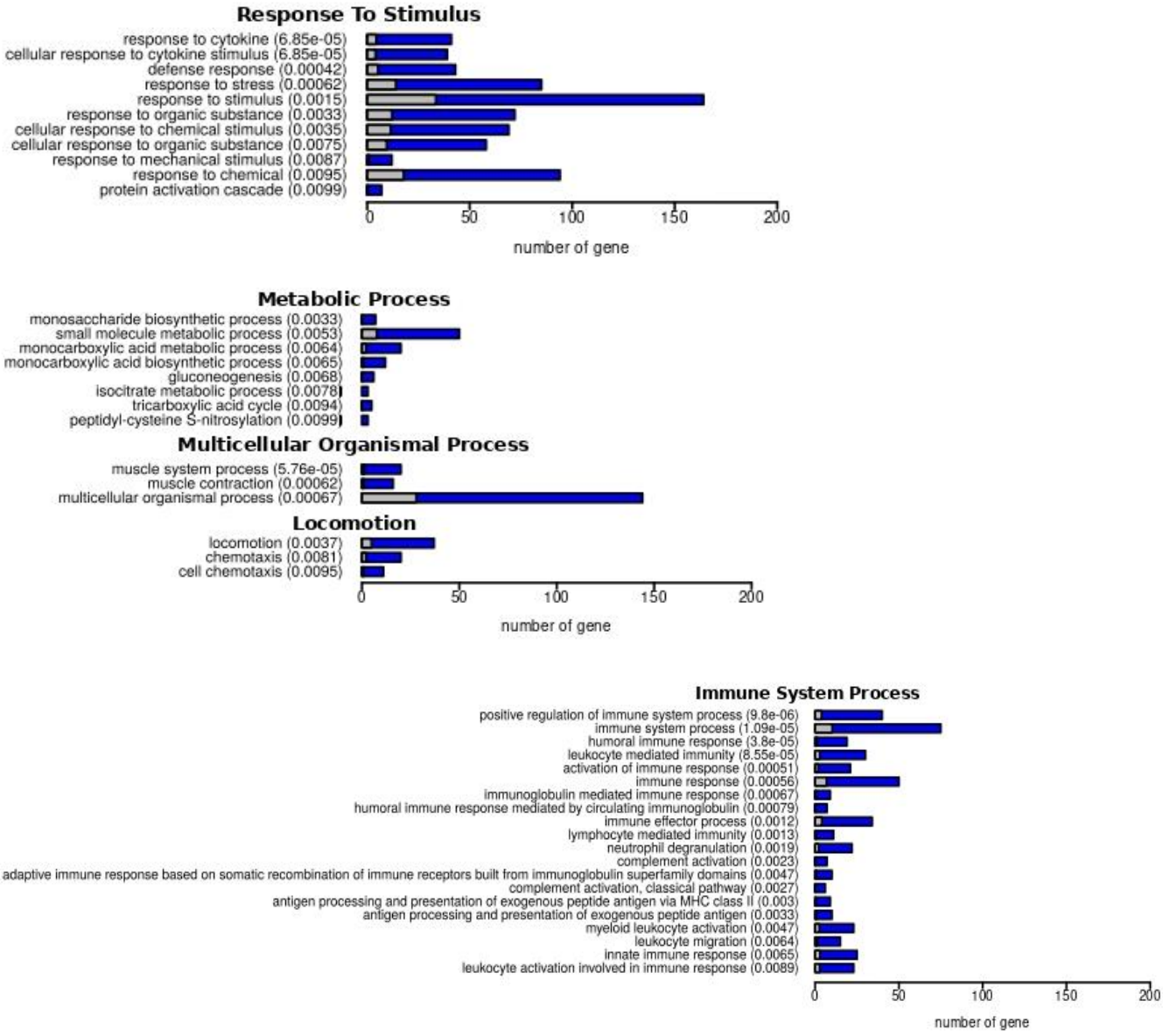


Supplementary Figure 5. 2. Effect of exercise training on gluteal (gSAT) and abdominal subcutaneous adipose tissue (aSAT) inflammatory protein expression: representative.

IL-10: interleukin-10; MCP1: monocyte chemoattractant protein 1; MIF: Macrophage migration inhibitory factor; NFκB: nuclear factor kappa B; TLR4: toll-like receptor 4; TNFα: tumor necrosis factor-alpha.



Supplementary Figure 6.1. GO term enrichment of differentially expressed genes between abdominal and gluteal subcutaneous adipose tissue after exercise training



Supplementary figure 6.1. continued

Supplementary Table 6.1. List of all differentially expressed genes between SAT depots after exercise training based on log2 fold change > 0.58

(up and down-regulated; aSAT: abdominal subcutaneous adipose tissue; gSAT: gluteal subcutaneous adipose tissue)

SYMBOL	SEARCH_KEY	logFC	DEFINITION
Higher in aSAT than gSAT			
HOXA5	NM_019102.2	-1.44	Homo sapiens homeobox A5 (HOXA5), mRNA.
DMRT2	NM_181872.1	-1.41	Homo sapiens doublesex and mab-3 related transcription factor 2 (DMRT2), transcript variant 1, mRNA.
DMRT3	NM_021240.2	-1.35	Homo sapiens doublesex and mab-3 related transcription factor 3 (DMRT3), mRNA.
MYL2	NM_000432.2	-1.15	Homo sapiens myosin, light chain 2, regulatory, cardiac, slow (MYL2), mRNA.
CDKN1B	NM_004064.2	-1.10	Homo sapiens cyclin-dependent kinase inhibitor 1B (p27, Kip1) (CDKN1B), mRNA.
MYH11	NM_002474.1	-1.07	Homo sapiens myosin, heavy chain 11, smooth muscle (MYH11), transcript variant SM1A, mRNA.
ANGPT2	NM_001118888.1	-1.06	Homo sapiens angiopoietin 2 (ANGPT2), transcript variant 3, mRNA.
LOC649841	XM_938906.1	-1.05	PREDICTED: Homo sapiens similar to protein immuno-reactive with anti-PTH polyclonal antibodies (LOC649841), mRNA.
PARM1	NM_015393.2	-1.05	Homo sapiens prostate androgen-regulated mucin-like protein 1 (PARM1), mRNA.
MYH7	NM_000257.1	-1.05	Homo sapiens myosin, heavy chain 7, cardiac muscle, beta (MYH7), mRNA.
FLNC	NM_001458.2	-1.02	Homo sapiens filamin C, gamma (actin-binding protein 280) (FLNC), mRNA.
FNDC1	NM_032532.1	-0.97	Homo sapiens fibronectin type III domain containing 1 (FNDC1), mRNA.
HSPA1B	NM_005346.3	-0.96	Homo sapiens heat shock 70kDa protein 1B (HSPA1B), mRNA.
FAM129A	NM_022083.1	-0.94	Homo sapiens family with sequence similarity 129, member A (FAM129A), transcript variant 2, mRNA.
CLDN11	NM_005602.4	-0.93	Homo sapiens claudin 11 (oligodendrocyte transmembrane protein) (CLDN11), mRNA.
MYADM	NM_138373.3	-0.91	Homo sapiens myeloid-associated differentiation marker (MYADM), transcript variant 2, mRNA.
PER2	NM_022817.1	-0.89	Homo sapiens period homolog 2 (Drosophila) (PER2), mRNA.
LEPR	NM_001003679.1	-0.88	Homo sapiens leptin receptor (LEPR), transcript variant 2, mRNA.

NR5A2	NM_003822.3	-0.88	Homo sapiens nuclear receptor subfamily 5, group A, member 2 (NR5A2), transcript variant 2, mRNA.
NRGN	NM_006176.1	-0.88	Homo sapiens neurogranin (protein kinase C substrate, RC3) (NRGN), mRNA.
RECK	NM_021111.1	-0.87	Homo sapiens reversion-inducing-cysteine-rich protein with kazal motifs (RECK), mRNA.
RSPO3	NM_032784.3	-0.87	Homo sapiens R-spondin 3 homolog (Xenopus laevis) (RSPO3), mRNA.
MSL3	NM_078629.2	-0.85	Homo sapiens male-specific lethal 3 homolog (Drosophila) (MSL3), transcript variant 1, mRNA.
ALDH1A1	NM_000689.3	-0.84	Homo sapiens aldehyde dehydrogenase 1 family, member A1 (ALDH1A1), mRNA.
MUC6	XM_932177.1	-0.84	Homo sapiens mucin 6, oligomeric mucus/gel-forming (MUC6), mRNA.
ATP6V1B1	NM_001692.2	-0.84	Homo sapiens ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B1 (ATP6V1B1), mRNA.
AFAP1L2	NM_001001936.1	-0.84	Homo sapiens actin filament associated protein 1-like 2 (AFAP1L2), transcript variant 1, mRNA.
LOC100134648	XM_001724681.1	-0.83	PREDICTED: Homo sapiens similar to hCG2024106, transcript variant 2 (LOC100134648), mRNA.
TUBB1	NM_030773.2	-0.83	Homo sapiens tubulin, beta 1 (TUBB1), mRNA.
CXCL12	NM_001033886.1	-0.83	Homo sapiens chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1) (CXCL12), transcript variant 2, mRNA.
ARID4B	NM_016374.5	-0.83	Homo sapiens AT rich interactive domain 4B (RBP1-like) (ARID4B), transcript variant 1, mRNA.
MEF2C	NM_002397.2	-0.82	Homo sapiens myocyte enhancer factor 2C (MEF2C), mRNA.
SYT7	NM_004200.2	-0.81	Homo sapiens synaptotagmin VII (SYT7), mRNA.
ATP8B2	NM_020452.2	-0.80	Homo sapiens ATPase, class I, type 8B, member 2 (ATP8B2), transcript variant 1, mRNA.
C1QTNF9	NM_178540.3	-0.80	Homo sapiens C1q and tumor necrosis factor related protein 9 (C1QTNF9), mRNA.
RFX7	NM_022841.5	-0.80	Homo sapiens regulatory factor X, 7 (RFX7), mRNA.
LOC644852	XM_934213.1	-0.79	PREDICTED: Homo sapiens hypothetical protein LOC644852, transcript variant 1 (LOC644852), mRNA.
HOPX	NM_139212.2	-0.79	Homo sapiens HOP homeobox (HOPX), transcript variant 3, mRNA.
MYOM1	NM_003803.2	-0.79	Homo sapiens myomesin 1, 185kDa (MYOM1), transcript variant 1, mRNA.
C7	NM_000587.2	-0.79	Homo sapiens complement component 7 (C7), mRNA.
EFNA1	NM_004428.2	-0.79	Homo sapiens ephrin-A1 (EFNA1), transcript variant 1, mRNA.
TAOK1	NM_020791.1	-0.78	Homo sapiens TAO kinase 1 (TAOK1), mRNA.
RERGL	NM_024730.2	-0.78	Homo sapiens RERG/RAS-like (RERGL), mRNA.
TMEM178	NM_152390.1	-0.77	Homo sapiens transmembrane protein 178 (TMEM178), mRNA.

ZDHHC11	NM_024786.1	-0.77	Homo sapiens zinc finger, DHHC-type containing 11 (ZDHHC11), mRNA.
SLCO3A1	NM_013272.2	-0.77	Homo sapiens solute carrier organic anion transporter family, member 3A1 (SLCO3A1), mRNA.
RUNX1T1	NM_175636.1	-0.77	Homo sapiens runt-related transcription factor 1; translocated to, 1 (cyclin D-related) (RUNX1T1), transcript variant 1, mRNA.
PHACTR2	NM_014721.1	-0.77	Homo sapiens phosphatase and actin regulator 2 (PHACTR2), transcript variant 1, mRNA.
PAK2	NM_002577.3	-0.76	PREDICTED: Homo sapiens p21 (CDKN1A)-activated kinase 2 (PAK2), mRNA.
TMEM154	NM_152680.1	-0.76	Homo sapiens transmembrane protein 154 (TMEM154), mRNA.
HOXA3	NM_153631.1	-0.76	Homo sapiens homeobox A3 (HOXA3), transcript variant 2, mRNA.
HOXB5	NM_002147.2	-0.76	Homo sapiens homeobox B5 (HOXB5), mRNA.
PRMT2	NM_206962.1	-0.76	Homo sapiens protein arginine methyltransferase 2 (PRMT2), transcript variant 1, mRNA.
LOC730525	NM_199327.1	-0.76	PREDICTED: Homo sapiens hypothetical protein LOC730525 (LOC730525), mRNA.
MSL3L1	NM_078630.1	-0.75	Homo sapiens male-specific lethal 3-like 1 (Drosophila) (MSL3L1), transcript variant 1, mRNA.
SEPT5	NM_000407.3	-0.75	Homo sapiens septin 5 (SEPT5), mRNA.
LOC400986	NM_001010914.1	-0.75	PREDICTED: Homo sapiens protein immuno-reactive with anti-PTH polyclonal antibodies (LOC400986), mRNA.
COL4A5	NM_033381.1	-0.75	Homo sapiens collagen, type IV, alpha 5 (COL4A5), transcript variant 1, mRNA.
MAP3K1	XM_042066.10	-0.74	Homo sapiens mitogen-activated protein kinase kinase kinase 1 (MAP3K1), mRNA.
NBPF8	XM_001726946.1	-0.74	PREDICTED: Homo sapiens neuroblastoma breakpoint family, member 8 (NBPF8), mRNA.
C1orf24	NM_052966.1	-0.74	Homo sapiens chromosome 1 open reading frame 24 (C1orf24), transcript variant 2, mRNA.
FEM1C	NM_020177.2	-0.73	Homo sapiens fem-1 homolog c (C. elegans) (FEM1C), mRNA.
	Hs.436134	-0.73	ta96c03.x1 NCI_CGAP_Lu26 Homo sapiens cDNA clone IMAGE:2051908 3, mRNA sequence
CAST	NM_001750.4	-0.73	Homo sapiens calpastatin (CAST), transcript variant 9, mRNA.
LOC440928	XM_942885.1	-0.73	PREDICTED: Homo sapiens hypothetical LOC440928 (LOC440928), mRNA.
ZNF462	NM_021224.3	-0.72	Homo sapiens zinc finger protein 462 (ZNF462), mRNA.
PLCB1	NM_182734.1	-0.72	Homo sapiens phospholipase C, beta 1 (phosphoinositide-specific) (PLCB1), transcript variant 1, mRNA.

LOC644162	XM_933956.1	-0.72	PREDICTED: Homo sapiens similar to septin 7, transcript variant 4 (LOC644162), mRNA.
DKK3	NM_013253.4	-0.71	Homo sapiens dickkopf homolog 3 (Xenopus laevis) (DKK3), transcript variant 2, mRNA.
SAPS2	XM_942540.1	-0.71	PREDICTED: Homo sapiens SAPS domain family, member 2, transcript variant 2 (SAPS2), mRNA.
VPS13C	NM_018080.2	-0.71	Homo sapiens vacuolar protein sorting 13 homolog C (S. cerevisiae) (VPS13C), transcript variant 1B, mRNA.
BRD3	NM_007371.2	-0.71	Homo sapiens bromodomain containing 3 (BRD3), mRNA.
SMARCA1	NM_003069.2	-0.71	Homo sapiens SWI/SNF related matrix associated, actin-dependent regulator of chromatin, subfamily a, member 1 (SMARCA1), transcript variant 1, mRNA.
TM4SF18	NM_138786.1	-0.71	Homo sapiens transmembrane 4 L six family member 18 (TM4SF18), mRNA.
MPZL2	NM_005797.2	-0.71	Homo sapiens myelin protein zero-like 2 (MPZL2), transcript variant 1, mRNA.
C1orf71	NM_152609.1	-0.71	Homo sapiens chromosome 1 open reading frame 71 (C1orf71), mRNA.
SNTB2	NM_006750.2	-0.71	Homo sapiens syntrophin, beta 2 (dystrophin-associated protein A1, 59kDa, basic component 2) (SNTB2), transcript variant 1, mRNA.
IRX2	NM_033267.2	-0.70	Homo sapiens iroquois homeobox 2 (IRX2), mRNA.
MYADM	NM_001020820.1	-0.70	Homo sapiens myeloid-associated differentiation marker (MYADM), transcript variant 4, mRNA.
LBH	NM_030915.1	-0.70	Homo sapiens limb bud and heart development homolog (mouse) (LBH), mRNA.
ETS1	NM_005238.2	-0.70	Homo sapiens v-ets erythroblastosis virus E26 oncogene homolog 1 (avian) (ETS1), mRNA.
FNDC1	NM_032532.1	-0.69	Homo sapiens fibronectin type III domain containing 1 (FNDC1), mRNA.
MTF2	NM_007358.1	-0.69	Homo sapiens metal response element binding transcription factor 2 (MTF2), mRNA.
APCDD1L	NM_153360.1	-0.69	Homo sapiens adenomatosis polyposis coli down-regulated 1-like (APCDD1L), mRNA.
CPNE8	NM_153634.2	-0.69	Homo sapiens copine VIII (CPNE8), mRNA.
STXBP6	NM_014178.6	-0.68	Homo sapiens syntaxin binding protein 6 (amisyn) (STXBP6), mRNA.
ANGPT2	NM_001147.1	-0.68	Homo sapiens angiopoietin 2 (ANGPT2), mRNA.
SMARCC2	NM_003075.2	-0.67	Homo sapiens SWI/SNF related matrix associated, actin-dependent regulator of chromatin, subfamily c, member 2 (SMARCC2), transcript variant 2, mRNA.
C4orf31	NM_024574.2	-0.67	Homo sapiens chromosome 4 open reading frame 31 (C4orf31), mRNA.

ADO	NM_032804.5	-0.67	Homo sapiens 2-aminoethanethiol (cysteamine) dioxygenase (ADO), mRNA.
DACH1	NM_080759.3	-0.67	Homo sapiens dachshund homolog 1 (Drosophila) (DACH1), transcript variant 2, mRNA.
ALDH1A1	NM_000689.3	-0.67	Homo sapiens aldehyde dehydrogenase 1 family, member A1 (ALDH1A1), mRNA.
FRMD3	NM_174938.3	-0.67	Homo sapiens FERM domain containing 3 (FRMD3), mRNA.
NAP1L1	NM_139207.1	-0.66	Homo sapiens nucleosome assembly protein 1-like 1 (NAP1L1), transcript variant 1, mRNA.
TMEM47	NM_031442.2	-0.66	Homo sapiens transmembrane protein 47 (TMEM47), mRNA.
PSMC1	XM_928629.1	-0.66	Homo sapiens proteasome (prosome, macropain) 26S subunit, ATPase, 1 (PSMC1), mRNA.
GALNTL1	NM_020692.1	-0.66	Homo sapiens UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 1 (GALNTL1), mRNA.
CHD1	NM_001270.2	-0.66	Homo sapiens chromodomain helicase DNA binding protein 1 (CHD1), mRNA.
EIF2C2	NM_012154.2	-0.66	Homo sapiens eukaryotic translation initiation factor 2C, 2 (EIF2C2), mRNA.
BAT2D1	NM_015172.2	-0.66	Homo sapiens BAT2 domain containing 1 (BAT2D1), mRNA.
SAFB2	NM_014649.2	-0.65	Homo sapiens scaffold attachment factor B2 (SAFB2), mRNA.
CNN3	NM_001839.2	-0.65	Homo sapiens calponin 3, acidic (CNN3), mRNA.
ARID4B	NM_016374.5	-0.65	Homo sapiens AT rich interactive domain 4B (RBP1-like) (ARID4B), transcript variant 1, mRNA.
TSHZ3	NM_020856.1	-0.65	Homo sapiens tea shirt zinc finger homeobox 3 (TSHZ3), mRNA.
PTRF	NM_012232.2	-0.65	Homo sapiens polymerase I and transcript release factor (PTRF), mRNA.
ULK1	XM_942125.1	-0.65	Homo sapiens unc-51-like kinase 1 (C. elegans) (ULK1), mRNA.
SULF1	NM_015170.1	-0.65	Homo sapiens sulfatase 1 (SULF1), mRNA.
TNRC6B	NM_001024843.1	-0.65	Homo sapiens trinucleotide repeat containing 6B (TNRC6B), transcript variant 2, mRNA.
HELZ	NM_014877.2	-0.64	Homo sapiens helicase with zinc finger (HELZ), mRNA.
CXCR7	NM_020311.1	-0.64	Homo sapiens chemokine (C-X-C motif) receptor 7 (CXCR7), mRNA.
SERPINI1	NM_005025.2	-0.64	Homo sapiens serpin peptidase inhibitor, clade I (neuroserpin), member 1 (SERPINI1), mRNA.
	Hs.388347	-0.64	Homo sapiens mRNA; cDNA DKFZp686J0156 (from clone DKFZp686J0156)
ULK1	NM_003565.1	-0.64	Homo sapiens unc-51-like kinase 1 (C. elegans) (ULK1), mRNA.
ZBTB20	NM_015642.2	-0.64	Homo sapiens zinc finger and BTB domain containing 20 (ZBTB20), mRNA.
PTGDS	NM_000954.5	-0.64	Homo sapiens prostaglandin D2 synthase 21kDa (brain) (PTGDS), mRNA.

LOC651309	XM_942586.1	-0.64	PREDICTED: Homo sapiens hypothetical protein LOC651309 (LOC651309), mRNA.
PCDH17	NM_014459.2	-0.64	Homo sapiens protocadherin 17 (PCDH17), mRNA.
	Hs.555252	-0.64	DA371742 BRTHA2 Homo sapiens cDNA clone BRTHA2001741 5, mRNA sequence
PLS3	NM_005032.3	-0.64	Homo sapiens plastin 3 (T isoform) (PLS3), mRNA.
RAD21	NM_006265.1	-0.64	Homo sapiens RAD21 homolog (S. pombe) (RAD21), mRNA.
	Hs.379253	-0.64	Homo sapiens mRNA; cDNA DKFZp686J23256 (from clone DKFZp686J23256)
LOC441155	XM_930970.1	-0.64	PREDICTED: Homo sapiens similar to Zinc finger CCCH-type domain-containing protein 11A, transcript variant 2 (LOC441155), mRNA.
C10orf140	NM_207371.3	-0.63	Homo sapiens chromosome 10 open reading frame 140 (C10orf140), mRNA.
FAM179B	NM_015091.1	-0.63	Homo sapiens family with sequence similarity 179, member B (FAM179B), mRNA.
CFI	NM_000204.1	-0.63	Homo sapiens complement factor I (CFI), mRNA.
NFAT5	NM_173215.1	-0.63	Homo sapiens nuclear factor of activated T-cells 5, tonicity-responsive (NFAT5), transcript variant 5, mRNA.
SENP7	NM_001077203.1	-0.63	Homo sapiens SUMO1/sentrin specific peptidase 7 (SENP7), transcript variant 2, mRNA.
RIPK5	NM_199462.1	-0.62	Homo sapiens receptor interacting protein kinase 5 (RIPK5), transcript variant 2, mRNA.
DMWD	NM_004943.1	-0.62	Homo sapiens dystrophia myotonica, WD repeat-containing (DMWD), mRNA.
NFKBIA	NM_020529.1	-0.62	Homo sapiens nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (NFKBIA), mRNA.
PDE5A	NM_033437.2	-0.62	Homo sapiens phosphodiesterase 5A, cGMP-specific (PDE5A), transcript variant 1, mRNA.
C4orf32	NM_152400.1	-0.62	Homo sapiens chromosome 4 open reading frame 32 (C4orf32), mRNA.
LRRC17	NM_001031692.1	-0.62	Homo sapiens leucine-rich repeat-containing 17 (LRRC17), transcript variant 2, mRNA.
FAM13B	NM_001101800.1	-0.62	Homo sapiens family with sequence similarity 13, member B (FAM13B), transcript variant 2, mRNA.
CPE	NM_001873.1	-0.62	Homo sapiens carboxypeptidase E (CPE), mRNA.
C21orf7	NM_020152.2	-0.62	Homo sapiens chromosome 21 open reading frame 7 (C21orf7), mRNA.
COL8A1	NM_020351.2	-0.61	Homo sapiens collagen, type VIII, alpha 1 (COL8A1), transcript variant 2, mRNA.
ABTB1	NM_172027.1	-0.61	Homo sapiens ankyrin repeat and BTB (POZ) domain containing 1 (ABTB1), transcript variant 1, mRNA.

SERPINE2	NM_006216.2	-0.61	Homo sapiens serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2 (SERPINE2), mRNA.
PDCD10	NM_145860.1	-0.61	Homo sapiens programmed cell death 10 (PDCD10), transcript variant 2, mRNA.
EDN1	NM_001955.2	-0.61	Homo sapiens endothelin 1 (EDN1), mRNA.
	Hs.184721	-0.61	EST366269 MAGE resequences, MAGC Homo sapiens cDNA, mRNA sequence
KLHL28	NM_017658.2	-0.61	Homo sapiens kelch-like 28 (Drosophila) (KLHL28), mRNA.
BST2	NM_004335.2	-0.61	Homo sapiens bone marrow stromal cell antigen 2 (BST2), mRNA.
VCAN	NM_004385.2	-0.61	Homo sapiens versican (VCAN), mRNA.
	Hs.571887	-0.61	Homo sapiens cDNA: FLJ21429 fis, clone COL04205
RNF19A	NM_183419.1	-0.61	Homo sapiens ring finger protein 19A (RNF19A), transcript variant 1, mRNA.
LRRC1	NM_018214.3	-0.60	Homo sapiens leucine-rich repeat-containing 1 (LRRC1), mRNA.
DOCK10	NM_014689.1	-0.60	Homo sapiens dedicator of cytokinesis 10 (DOCK10), mRNA.
MBNL3	NM_133486.1	-0.60	Homo sapiens muscleblind-like 3 (Drosophila) (MBNL3), transcript variant R, mRNA.
ISM1	XM_939699.1	-0.60	Homo sapiens isthmin 1 homolog (zebrafish) (ISM1), mRNA.
KIAA1600	NM_020940.2	-0.60	Homo sapiens KIAA1600 (KIAA1600), mRNA.
CDCA7	NM_145810.1	-0.60	Homo sapiens cell division cycle associated 7 (CDCA7), transcript variant 1, mRNA.
C6	NM_000065.1	-0.59	Homo sapiens complement component 6 (C6), mRNA.
DPYSL5	NM_020134.2	-0.59	Homo sapiens dihydropyrimidinase-like 5 (DPYSL5), mRNA.
MLLT10	NM_004641.2	-0.59	Homo sapiens myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 10 (MLLT10), transcript variant 1, mRNA.
HOXA9	NM_152739.2	-0.59	Homo sapiens homeobox A9 (HOXA9), mRNA.
PGM5	XM_936702.1	-0.59	Homo sapiens phosphoglucomutase 5 (PGM5), mRNA.
KIAA1267	NM_015443.2	-0.59	Homo sapiens KIAA1267 (KIAA1267), mRNA.
TMEM200A	NM_052913.2	-0.59	Homo sapiens transmembrane protein 200A (TMEM200A), mRNA.
C17orf58	NM_181656.1	-0.59	Homo sapiens chromosome 17 open reading frame 58 (C17orf58), transcript variant 2, mRNA.
PDPN	NM_001006625.1	-0.59	Homo sapiens podoplanin (PDPN), transcript variant 4, mRNA.
SORBS2	NM_003603.4	-0.59	Homo sapiens sorbin and SH3 domain containing 2 (SORBS2), transcript variant 1, mRNA.
SORL1	NM_003105.3	-0.59	Homo sapiens sortilin-related receptor, L(DLR class) A repeats-containing (SORL1), mRNA.
ZNF385D	NM_024697.1	-0.59	Homo sapiens zinc finger protein 385D (ZNF385D), mRNA.
CBX6	NM_014292.3	-0.59	Homo sapiens chromobox homolog 6 (CBX6), mRNA.

Lower in aSAT than gSAT			
CSN1S1	NM_001025104.1	1.37	Homo sapiens casein alpha s1 (CSN1S1), transcript variant 1, mRNA.
SPP1	NM_001040058.1	1.25	Homo sapiens secreted phosphoprotein 1 (SPP1), transcript variant 1, mRNA.
LOC644936	NR_004845.1	1.22	Homo sapiens cytoplasmic beta-actin pseudogene (LOC644936), non-coding RNA.
IFI30	NM_006332.3	1.17	Homo sapiens interferon, gamma-inducible protein 30 (IFI30), mRNA.
LAPTM5	NM_006762.1	1.17	Homo sapiens lysosomal multispanning membrane protein 5 (LAPTM5), mRNA.
SPP1	NM_000582.2	1.16	Homo sapiens secreted phosphoprotein 1 (SPP1), transcript variant 2, mRNA.
FCGBP	NM_003890.1	1.15	Homo sapiens Fc fragment of IgG binding protein (FCGBP), mRNA.
TM4SF19	NM_138461.1	1.13	PREDICTED: Homo sapiens transmembrane 4 L six family member 19, transcript variant 2 (TM4SF19), mRNA.
CSN1S1	NM_001890.1	1.12	Homo sapiens casein alpha s1 (CSN1S1), transcript variant 1, mRNA.
FOSB	NM_006732.1	1.10	Homo sapiens FBJ murine osteosarcoma viral oncogene homolog B (FOSB), mRNA.
LOC55908	NM_018687.3	1.10	Homo sapiens hepatocellular carcinoma-associated gene TD26 (LOC55908), mRNA.
LOC645313	XR_017585.2	1.09	PREDICTED: Homo sapiens misc_RNA (LOC645313), miscRNA.
CD163	NM_203416.1	1.06	Homo sapiens CD163 molecule (CD163), transcript variant 2, mRNA.
APOC1	NM_001645.3	1.02	Homo sapiens apolipoprotein C-I (APOC1), mRNA.
G0S2	NM_015714.2	1.01	Homo sapiens G0/G1switch 2 (G0S2), mRNA.
LOC649679	XM_945045.1	1.00	PREDICTED: Homo sapiens similar to Tubulin beta-4q chain, transcript variant 2 (LOC649679), mRNA.
TUBB4Q	NM_020040.3	0.98	Homo sapiens tubulin, beta polypeptide 4, member Q (TUBB4Q), mRNA.
CD163	NM_203416.1	0.98	Homo sapiens CD163 molecule (CD163), transcript variant 2, mRNA.
FCER1G	NM_004106.1	0.96	Homo sapiens Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide (FCER1G), mRNA.
FCGBP	XM_940656.1	0.95	PREDICTED: Homo sapiens Fc fragment of IgG binding protein (FCGBP), mRNA.
MYOC	NM_000261.1	0.95	Homo sapiens myocilin, trabecular meshwork inducible glucocorticoid response (MYOC), mRNA.
LAMB3	NM_000228.2	0.94	Homo sapiens laminin, beta 3 (LAMB3), transcript variant 1, mRNA.
CYR61	NM_001554.3	0.93	Homo sapiens cysteine-rich, angiogenic inducer, 61 (CYR61), mRNA.
MYOC	NM_000261.1	0.92	Homo sapiens myocilin, trabecular meshwork inducible glucocorticoid response (MYOC), mRNA.
PLIN2	NM_001122.2	0.92	Homo sapiens perilipin 2 (PLIN2), mRNA.

TNMD	NM_022144.1	0.91	Homo sapiens tenomodulin (TNMD), mRNA.
ITGAX	NM_000887.3	0.91	Homo sapiens integrin, alpha X (complement component 3 receptor 4 subunit) (ITGAX), mRNA.
FPR3	NM_002030.3	0.90	Homo sapiens formyl peptide receptor 3 (FPR3), mRNA.
PEMT	NM_148173.1	0.87	Homo sapiens phosphatidylethanolamine N-methyltransferase (PEMT), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA.
HLA-DQA1	XM_936120.1	0.86	PREDICTED: Homo sapiens major histocompatibility complex, class II, DQ alpha 1, transcript variant 10 (HLA-DQA1), mRNA.
CHI3L1	NM_001276.2	0.86	Homo sapiens chitinase 3-like 1 (cartilage glycoprotein-39) (CHI3L1), mRNA.
FBP1	NM_000507.2	0.86	Homo sapiens fructose-1,6-bisphosphatase 1 (FBP1), mRNA.
CAPG	NM_001747.2	0.86	Homo sapiens capping protein (actin filament), gelsolin-like (CAPG), mRNA.
VSIG4	NM_007268.1	0.84	Homo sapiens V-set and immunoglobulin domain containing 4 (VSIG4), transcript variant 1, mRNA.
VEGFB	NM_003377.3	0.84	Homo sapiens vascular endothelial growth factor B (VEGFB), mRNA.
LOC644237	XR_039184.1	0.84	PREDICTED: Homo sapiens misc_RNA (LOC644237), miscRNA.
PLA2G7	NM_005084.2	0.82	Homo sapiens phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma) (PLA2G7), mRNA.
TUBB	NM_178014.2	0.82	Homo sapiens tubulin, beta (TUBB), mRNA.
C1QC	NM_172369.2	0.81	Homo sapiens complement component 1, q subcomponent, C chain (C1QC), mRNA.
ACP5	NM_001611.2	0.80	Homo sapiens acid phosphatase 5, tartrate resistant (ACP5), mRNA.
C1QB	NM_000491.2	0.80	Homo sapiens complement component 1, q subcomponent, B chain (C1QB), mRNA.
ANKRD33	NM_182608.2	0.80	Homo sapiens ankyrin repeat domain 33 (ANKRD33), mRNA.
TREM2	NM_018965.1	0.77	Homo sapiens triggering receptor expressed on myeloid cells 2 (TREM2), mRNA.
IDH2	NM_002168.2	0.77	Homo sapiens isocitrate dehydrogenase 2 (NADP+), mitochondrial (IDH2), nuclear gene encoding mitochondrial protein, mRNA.
CISH	NM_145071.1	0.77	Homo sapiens cytokine-inducible SH2-containing protein (CISH), mRNA.
RBP4	NM_006744.3	0.75	Homo sapiens retinol binding protein 4, plasma (RBP4), mRNA.
CPVL	NM_031311.3	0.75	Homo sapiens carboxypeptidase, vitellogenic-like (CPVL), transcript variant 1, mRNA.
SRPR	NM_003139.2	0.74	Homo sapiens signal recognition particle receptor (docking protein) (SRPR), mRNA.
TM7SF4	NM_030788.2	0.74	Homo sapiens transmembrane 7 superfamily member 4 (TM7SF4), mRNA.

MRAS	NM_012219.2	0.73	Homo sapiens muscle RAS oncogene homolog (MRAS), transcript variant 1, mRNA.
CYP4B1	NM_000779.2	0.73	Homo sapiens cytochrome P450, family 4, subfamily B, polypeptide 1 (CYP4B1), transcript variant 2, mRNA.
LOC100133678	XM_001719804.1	0.72	PREDICTED: Homo sapiens similar to hCG2042724 (LOC100133678), partial mRNA.
TUBB2C	NM_006088.5	0.72	Homo sapiens tubulin, beta 2C (TUBB2C), mRNA.
CARS	NM_001751.4	0.71	Homo sapiens cysteinyl-tRNA synthetase (CARS), transcript variant 4, mRNA.
ALCAM	NM_001627.2	0.71	Homo sapiens activated leukocyte cell adhesion molecule (ALCAM), mRNA.
FTHL3	NR_002201.1	0.71	Homo sapiens ferritin, heavy polypeptide-like 3 (FTHL3), non-coding RNA.
DPP7	NM_013379.2	0.71	Homo sapiens dipeptidyl-peptidase 7 (DPP7), mRNA.
KIAA1598	NM_018330.3	0.70	Homo sapiens KIAA1598 (KIAA1598), mRNA.
ABCC6	XM_936351.1	0.70	Homo sapiens ATP-binding cassette, sub-family C (CFTR/MRP), member 6 (ABCC6), transcript variant 1, mRNA.
CXCL9	NM_002416.1	0.70	Homo sapiens chemokine (C-X-C motif) ligand 9 (CXCL9), mRNA.
SLC6A8	NM_005629.1	0.70	Homo sapiens solute carrier family 6 (neurotransmitter transporter, creatine), member 8 (SLC6A8), mRNA.
AHNAK	NM_024060.2	0.69	Homo sapiens AHNAK nucleoprotein (AHNAK), transcript variant 2, mRNA.
DECR1	NM_001359.1	0.69	Homo sapiens 2,4-dienoyl CoA reductase 1, mitochondrial (DECR1), nuclear gene encoding mitochondrial protein, mRNA.
MS4A6E	NM_139249.2	0.69	Homo sapiens membrane-spanning 4-domains, subfamily A, member 6E (MS4A6E), mRNA.
RASSF4	NM_178145.1	0.68	Homo sapiens Ras association (RalGDS/AF-6) domain family member 4 (RASSF4), mRNA.
CCL22	NM_002990.3	0.68	Homo sapiens chemokine (C-C motif) ligand 22 (CCL22), mRNA.
MAL2	NM_052886.1	0.68	Homo sapiens mal, T-cell differentiation protein 2 (MAL2), mRNA.
NNMT	NM_006169.2	0.68	Homo sapiens nicotinamide N-methyltransferase (NNMT), mRNA.
FASN	NM_004104.4	0.68	Homo sapiens fatty acid synthase (FASN), mRNA.
ZNF385A	NM_015481.1	0.68	Homo sapiens zinc finger protein 385A (ZNF385A), transcript variant 3, mRNA.
GAPDH		0.68	Homo sapiens glyceraldehyde-3-phosphate dehydrogenase (GAPDH), mRNA.
IRF8	NM_002163.2	0.67	Homo sapiens interferon regulatory factor 8 (IRF8), mRNA.
C1QA	NM_015991.1	0.67	Homo sapiens complement component 1, q subcomponent, alpha polypeptide (C1QA), mRNA.

LGALS12	NM_033101.2	0.67	Homo sapiens lectin, galactoside-binding, soluble, 12 (LGALS12), mRNA.
NCKAP1L	NM_005337.2	0.67	Homo sapiens NCK-associated protein 1-like (NCKAP1L), mRNA.
INDO	NM_002164.3	0.67	Homo sapiens indoleamine-pyrrole 2,3 dioxygenase (INDO), mRNA.
ATP6V0C	NM_001694.2	0.67	PREDICTED: Homo sapiens ATPase, H+ transporting, lysosomal 16kDa, V0 subunit c (ATP6V0C), mRNA.
REEP6	NM_138393.1	0.67	Homo sapiens receptor accessory protein 6 (REEP6), mRNA.
FOLR2	NM_000803.2	0.67	Homo sapiens folate receptor 2 (fetal) (FOLR2), mRNA.
CRYBB2	NM_000496.1	0.67	Homo sapiens crystallin, beta B2 (CRYBB2), mRNA.
CD74	NM_004355.2	0.67	Homo sapiens CD74 molecule, major histocompatibility complex, class II invariant chain (CD74), transcript variant 2, mRNA.
CLMN	NM_024734.2	0.66	Homo sapiens calmin (calponin-like, transmembrane) (CLMN), mRNA.
SAA1	NM_199161.1	0.66	Homo sapiens serum amyloid A1 (SAA1), transcript variant 2, mRNA.
ME3	NM_006680.2	0.66	Homo sapiens malic enzyme 3, NADP(+)-dependent, mitochondrial (ME3), nuclear gene encoding mitochondrial protein, transcript variant 2, mRNA.
PTPLA	NM_014241.2	0.66	Homo sapiens protein tyrosine phosphatase-like (proline instead of catalytic arginine), member A (PTPLA), mRNA.
CTSA	NM_000308.1	0.66	Homo sapiens cathepsin A (CTSA), transcript variant 1, mRNA.
LY86	NM_004271.3	0.66	Homo sapiens lymphocyte antigen 86 (LY86), mRNA.
AP1B1	NM_145730.1	0.66	Homo sapiens adaptor-related protein complex 1, beta 1 subunit (AP1B1), transcript variant 2, mRNA.
GPC1	NM_002081.1	0.66	Homo sapiens glypican 1 (GPC1), mRNA.
DMPK	NM_004409.2	0.66	Homo sapiens dystrophia myotonica-protein kinase (DMPK), transcript variant 4, mRNA.
DAP	NM_004394.1	0.65	Homo sapiens death-associated protein (DAP), mRNA.
GAPDH	NM_002046.2	0.65	Homo sapiens glyceraldehyde-3-phosphate dehydrogenase (GAPDH), mRNA.
ITPK1	NM_014216.3	0.65	Homo sapiens inositol 1,3,4-triphosphate 5/6 kinase (ITPK1), mRNA.
IDH1	NM_005896.2	0.65	Homo sapiens isocitrate dehydrogenase 1 (NADP+), soluble (IDH1), mRNA.
PPP1R1A	NM_006741.2	0.65	Homo sapiens protein phosphatase 1, regulatory (inhibitor) subunit 1A (PPP1R1A), mRNA.
ITGB5	NM_002213.3	0.64	Homo sapiens integrin, beta 5 (ITGB5), mRNA. XM_944688 XM_944693
LIPA	NM_000235.2	0.64	Homo sapiens lipase A, lysosomal acid, cholesterol esterase (LIPA), transcript variant 2, mRNA.
C6orf145	NM_183373.2	0.64	PREDICTED: Homo sapiens chromosome 6 open reading frame 145 (C6orf145), mRNA.

HSD11B1	NM_181755.1	0.64	Homo sapiens hydroxysteroid (11-beta) dehydrogenase 1 (HSD11B1), transcript variant 2, mRNA.
NPLOC4	NM_017921.1	0.64	Homo sapiens nuclear protein localization 4 homolog (S. cerevisiae) (NPLOC4), mRNA.
LOC92755	XR_018705.2	0.64	PREDICTED: Homo sapiens misc_RNA (LOC92755), miscRNA.
LGALS8	NM_201545.1	0.64	Homo sapiens lectin, galactoside-binding, soluble, 8 (LGALS8), transcript variant 4, mRNA.
GDE1	NM_016641.3	0.63	Homo sapiens glycerophosphodiester phosphodiesterase 1 (GDE1), mRNA.
KLC1	NM_182923.2	0.63	Homo sapiens kinesin light chain 1 (KLC1), transcript variant 1, mRNA.
PGD	NM_002631.2	0.63	Homo sapiens phosphogluconate dehydrogenase (PGD), mRNA.
RARRES1	NM_206963.1	0.63	Homo sapiens retinoic acid receptor responder (tazarotene induced) 1 (RARRES1), transcript variant 1, mRNA.
CD74	NM_001025158.1	0.63	Homo sapiens CD74 molecule, major histocompatibility complex, class II invariant chain (CD74), transcript variant 1, mRNA.
PCK1	NM_002591.2	0.63	Homo sapiens phosphoenolpyruvate carboxykinase 1 (soluble) (PCK1), mRNA.
AADACL1	NM_020792.3	0.62	Homo sapiens arylacetamide deacetylase-like 1 (AADACL1), mRNA.
VAC14	NM_018052.3	0.62	Homo sapiens Vac14 homolog (S. cerevisiae) (VAC14), mRNA.
ELAC2	NM_018127.4	0.62	Homo sapiens elaC homolog 2 (E. coli) (ELAC2), mRNA.
CD52	NM_001803.2	0.62	Homo sapiens CD52 molecule (CD52), mRNA.
PLCD4	NM_032726.2	0.62	Homo sapiens phospholipase C, delta 4 (PLCD4), mRNA.
FMO3	NM_006894.4	0.62	Homo sapiens flavin-containing monooxygenase 3 (FMO3), transcript variant 1, mRNA.
MED8	NM_001001653.1	0.62	Homo sapiens mediator of RNA polymerase II transcription, subunit 8 homolog (S. cerevisiae) (MED8), transcript variant 5, mRNA.
P8	NM_012385.1	0.62	Homo sapiens p8 protein (candidate of metastasis 1) (P8), mRNA.
MATK	NM_139354.2	0.62	Homo sapiens megakaryocyte-associated tyrosine kinase (MATK), transcript variant 3, mRNA.
PAK1	NM_002576.3	0.62	Homo sapiens p21/Cdc42/Rac1-activated kinase 1 (STE20 homolog, yeast) (PAK1), mRNA.
ERGIC3	NM_198398.1	0.62	Homo sapiens ERGIC and golgi 3 (ERGIC3), transcript variant 1, mRNA.
ALDOC	NM_005165.2	0.62	Homo sapiens aldolase C, fructose-bisphosphate (ALDOC), mRNA.
FADS3	NM_021727.3	0.62	Homo sapiens fatty acid desaturase 3 (FADS3), mRNA.
SH3GLB1	NM_016009.2	0.61	Homo sapiens SH3-domain GRB2-like endophilin B1 (SH3GLB1), mRNA.
CBR1	NM_001757.2	0.61	Homo sapiens carbonyl reductase 1 (CBR1), mRNA.

SLC6A10P	NM_198857.1	0.61	Homo sapiens solute carrier family 6 (neurotransmitter transporter, creatine), member 10 (pseudogene) (SLC6A10P) on chromosome 16.
RRAS2	NM_012250.3	0.61	Homo sapiens related RAS viral (r-ras) oncogene homolog 2 (RRAS2), mRNA.
OLFM2	NM_058164.1	0.61	Homo sapiens olfactomedin 2 (OLFM2), mRNA.
TFRC	NM_003234.1	0.61	Homo sapiens transferrin receptor (p90, CD71) (TFRC), mRNA.
CIDEC	NM_022094.2	0.61	Homo sapiens cell death-inducing DFFA-like effector c (CIDEC), mRNA.
ACO2	NM_001098.2	0.61	Homo sapiens aconitase 2, mitochondrial (ACO2), nuclear gene encoding mitochondrial protein, mRNA.
CALB2	NM_001740.2	0.61	Homo sapiens calbindin 2 (CALB2), transcript variant CALB2c, mRNA.
TENC1	NM_198316.1	0.61	Homo sapiens tensin like C1 domain containing phosphatase (tensin 2) (TENC1), transcript variant 3, mRNA.
DYNLL2	NM_080677.1	0.60	Homo sapiens dynein, light chain, LC8-type 2 (DYNLL2), mRNA.
NMB	NM_021077.3	0.60	Homo sapiens neuromedin B (NMB), transcript variant 1, mRNA.
CS	NM_004077.2	0.60	Homo sapiens citrate synthase (CS), nuclear gene encoding mitochondrial protein, mRNA.
CPVL	NM_031311.2	0.60	Homo sapiens carboxypeptidase, vitellogenic-like (CPVL), transcript variant 2, mRNA.
SPOCD1	NM_144569.3	0.60	Homo sapiens SPOC domain containing 1 (SPOCD1), mRNA.
MOCOS	NM_017947.1	0.60	Homo sapiens molybdenum cofactor sulfurase (MOCOS), mRNA.
MAGED2	NM_201222.1	0.60	Homo sapiens melanoma antigen family D, 2 (MAGED2), transcript variant 3, mRNA.
MS4A7	NM_206938.1	0.60	Homo sapiens membrane-spanning 4-domains, subfamily A, member 7 (MS4A7), transcript variant 2, mRNA.
ALDH3B1	NM_001030010.1	0.60	Homo sapiens aldehyde dehydrogenase 3 family, member B1 (ALDH3B1), transcript variant 1, mRNA.
CD151	NM_139030.2	0.60	Homo sapiens CD151 molecule (Raph blood group) (CD151), transcript variant 5, mRNA.
RBPMS	NM_001008712.1	0.60	Homo sapiens RNA binding protein with multiple splicing (RBPMS), transcript variant 3, mRNA.
RGS20	NM_170587.1	0.60	Homo sapiens regulator of G-protein signaling 20 (RGS20), transcript variant 1, mRNA.
LOC730908	XM_001717941.1	0.60	PREDICTED: Homo sapiens hypothetical LOC730908, transcript variant 2 (LOC730908), mRNA.
HSPB7	NM_014424.3	0.59	Homo sapiens heat shock 27kDa protein family, member 7 (cardiovascular) (HSPB7), mRNA.
LOC731486	NM_198277.1	0.59	PREDICTED: Homo sapiens hypothetical protein LOC731486 (LOC731486), mRNA.

CRYAB	NM_001885.1	0.59	Homo sapiens crystallin, alpha B (CRYAB), mRNA.
RETSAT	NM_017750.2	0.59	Homo sapiens retinol saturase (all-trans-retinol 13,14-reductase) (RETSAT), mRNA.
MEGF9	NM_001080497.1	0.59	Homo sapiens multiple EGF-like-domains 9 (MEGF9), mRNA.
NEU1	NM_000434.2	0.59	Homo sapiens sialidase 1 (lysosomal sialidase) (NEU1), mRNA.
DNASE2B	NM_021233.2	0.59	Homo sapiens deoxyribonuclease II beta (DNASE2B), transcript variant 1, mRNA.
GRN	NM_002087.2	0.59	Homo sapiens granulin (GRN), mRNA.

Supplementary Table 6.2. List of all differentially expressed genes in gluteal subcutaneous adipose tissue (gSAT) in response to exercise training based on log2 fold change > 0.58 ***(up and down-regulated)***

SYMBOL	SEARCH_KEY	logFC	DEFINITION
Up-regulated genes in gSAT after exercise training			
MMP9	NM_004994.2	-1.44	Homo sapiens matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase) (MMP9), mRNA.
SPP1	NM_001040058.1	-1.24	Homo sapiens secreted phosphoprotein 1 (SPP1), transcript variant 1, mRNA.
SPP1	NM_000582.2	-1.20	Homo sapiens secreted phosphoprotein 1 (SPP1), transcript variant 2, mRNA.
APOC1	NM_001645.3	-1.14	Homo sapiens apolipoprotein C-I (APOC1), mRNA.
ITGAX	NM_000887.3	-1.04	Homo sapiens integrin, alpha X (complement component 3 receptor 4 subunit) (ITGAX), mRNA.
TM4SF19	NM_138461.2	-1.04	Homo sapiens transmembrane 4 L six family member 19 (TM4SF19), mRNA.
IFI30	NM_006332.3	-1.02	Homo sapiens interferon, gamma-inducible protein 30 (IFI30), mRNA.
PLA2G7	NM_005084.2	-0.94	Homo sapiens phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma) (PLA2G7), mRNA.
LAPTM5	NM_006762.1	-0.93	Homo sapiens lysosomal multispanning membrane protein 5 (LAPTM5), mRNA.
CISH	NM_145071.1	-0.89	Homo sapiens cytokine-inducible SH2-containing protein (CISH), mRNA.
TM4SF19	NM_138461.1	-0.85	PREDICTED: Homo sapiens transmembrane 4 L six family member 19, transcript variant 2 (TM4SF19), mRNA.
DHRS9	NM_005771.3	-0.84	Homo sapiens dehydrogenase/reductase (SDR family) member 9 (DHRS9), transcript variant 1, mRNA.
COL1A1	NM_000088.2	-0.83	Homo sapiens collagen, type I, alpha 1 (COL1A1), mRNA.
FCER1G	NM_004106.1	-0.82	Homo sapiens Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide (FCER1G), mRNA.
CCL22	NM_002990.3	-0.80	Homo sapiens chemokine (C-C motif) ligand 22 (CCL22), mRNA.
FCGBP	NM_003890.1	-0.79	Homo sapiens Fc fragment of IgG binding protein (FCGBP), mRNA.
APOE	NM_000041.2	-0.79	Homo sapiens apolipoprotein E (APOE), mRNA.
HP	NM_005143.2	-0.78	Homo sapiens haptoglobin (HP), mRNA.
CHI3L1	NM_001276.2	-0.77	Homo sapiens chitinase 3-like 1 (cartilage glycoprotein-39) (CHI3L1), mRNA.

LOC653879	XM_936226.1	-0.77	PREDICTED: Homo sapiens similar to Complement C3 precursor (LOC653879), mRNA.
SREBF1	NM_004176.3	-0.76	Homo sapiens sterol regulatory element binding transcription factor 1 (SREBF1), transcript variant 2, mRNA.
MATK	NM_139354.2	-0.74	Homo sapiens megakaryocyte-associated tyrosine kinase (MATK), transcript variant 3, mRNA.
NNMT	NM_006169.2	-0.74	Homo sapiens nicotinamide N-methyltransferase (NNMT), mRNA.
CAPG	NM_001747.2	-0.73	Homo sapiens capping protein (actin filament), gelsolin-like (CAPG), mRNA.
ERGIC3	NM_198398.1	-0.73	Homo sapiens ERGIC and golgi 3 (ERGIC3), transcript variant 1, mRNA.
F13A1	NM_000129.2	-0.72	Homo sapiens coagulation factor XIII, A1 polypeptide (F13A1), mRNA.
ACP5	NM_001611.2	-0.72	Homo sapiens acid phosphatase 5, tartrate resistant (ACP5), mRNA.
FAIM3	NM_005449.3	-0.71	Homo sapiens Fas apoptotic inhibitory molecule 3 (FAIM3), mRNA.
PLTP	NM_006227.2	-0.69	Homo sapiens phospholipid transfer protein (PLTP), transcript variant 2, mRNA.
SLC43A3	NM_017611.2	-0.69	Homo sapiens solute carrier family 43, member 3 (SLC43A3), mRNA.
LIPA	NM_000235.2	-0.69	Homo sapiens lipase A, lysosomal acid, cholesterol esterase (LIPA), transcript variant 2, mRNA.
GRN	NM_002087.2	-0.68	Homo sapiens granulin (GRN), mRNA.
FCGBP	XM_940656.1	-0.68	PREDICTED: Homo sapiens Fc fragment of IgG binding protein (FCGBP), mRNA.
TM7SF4	NM_030788.2	-0.66	Homo sapiens transmembrane 7 superfamily member 4 (TM7SF4), mRNA.
TYROBP	NM_003332.2	-0.66	Homo sapiens TYRO protein tyrosine kinase binding protein (TYROBP), transcript variant 1, mRNA.
FBP1	NM_000507.2	-0.66	Homo sapiens fructose-1,6-bisphosphatase 1 (FBP1), mRNA.
COL6A2	NM_058174.1	-0.65	Homo sapiens collagen, type VI, alpha 2 (COL6A2), transcript variant 2C2, mRNA.
NPL	NM_030769.1	-0.64	Homo sapiens N-acetylneuraminate pyruvate lyase (dihydrodipicolinate synthase) (NPL), mRNA.
RABGGTA	NM_182836.1	-0.64	Homo sapiens Rab geranylgeranyltransferase, alpha subunit (RABGGTA), transcript variant 1, mRNA.
NPL	NM_030769.1	-0.64	Homo sapiens N-acetylneuraminate pyruvate lyase (dihydrodipicolinate synthase) (NPL), mRNA.
RNH1	NM_203385.1	-0.61	Homo sapiens ribonuclease/angiogenin inhibitor 1 (RNH1), transcript variant 4, mRNA.
C3	NM_000064.1	-0.61	Homo sapiens complement component 3 (C3), mRNA.
LGMN	NM_001008530.1	-0.61	Homo sapiens legumain (LGMN), transcript variant 2, mRNA.

MVP	NM_005115.3	-0.61	Homo sapiens major vault protein (MVP), transcript variant 2, mRNA.
CILP	NM_003613.2	-0.60	Homo sapiens cartilage intermediate layer protein, nucleotide pyrophosphohydrolase (CILP), mRNA.
CYP27A1	NM_000784.2	-0.60	Homo sapiens cytochrome P450, family 27, subfamily A, polypeptide 1 (CYP27A1), nuclear gene encoding mitochondrial protein, mRNA.
GFPT2	NM_005110.1	-0.60	Homo sapiens glutamine-fructose-6-phosphate transaminase 2 (GFPT2), mRNA.
LOC92755	XR_018705.2	-0.59	PREDICTED: Homo sapiens misc_RNA (LOC92755), miscRNA.
CD163	NM_203416.1	-0.59	Homo sapiens CD163 molecule (CD163), transcript variant 2, mRNA.
LOC646294	XR_019565.2	-0.59	PREDICTED: Homo sapiens misc_RNA (LOC646294), miscRNA.
EMILIN2	NM_032048.2	-0.59	Homo sapiens elastin microfibril interfacer 2 (EMILIN2), mRNA.
ALCAM	NM_001627.2	-0.59	Homo sapiens activated leukocyte cell adhesion molecule (ALCAM), mRNA.
LOC649679	XM_945045.1	-0.59	PREDICTED: Homo sapiens similar to Tubulin beta-4q chain, transcript variant 2 (LOC649679), mRNA.
CLDN7	NM_001307.3	-0.59	Homo sapiens claudin 7 (CLDN7), mRNA.
Down-regulated genes in gSAT after exercise training			
LOC651309	XM_942586.1	0.64	PREDICTED: Homo sapiens hypothetical protein LOC651309 (LOC651309), mRNA.
PCDH9	NM_020403.3	0.64	Homo sapiens protocadherin 9 (PCDH9), transcript variant 1, mRNA.
NTM	NM_016522.2	0.63	Homo sapiens neurotrimin (NTM), transcript variant 2, mRNA.
SLIT2	NM_004787.1	0.62	Homo sapiens slit homolog 2 (Drosophila) (SLIT2), mRNA.
NUTF2	NM_005796.1	0.61	Homo sapiens nuclear transport factor 2 (NUTF2), mRNA.
	Hs.99472	0.60	Homo sapiens mRNA; cDNA DKFZp564O0862 (from clone DKFZp564O0862)
FAM13A	NM_014883.2	0.59	Homo sapiens family with sequence similarity 13, member A (FAM13A), transcript variant 1, mRNA.

Supplementary Table 6.3. List of all differentially expressed genes in abdominal subcutaneous adipose tissue (aSAT) in response to exercise training based on log2 fold change > 0.58 ***(up and down-regulated)***

SYMBOL	SEARCH_KEY	logFC	DEFINITION
Up-regulated genes in aSAT after exercise training			
ACTA1	NM_001100.3	-1.59	Homo sapiens actin, alpha 1, skeletal muscle (ACTA1), mRNA.
FOLR3	NM_000804.2	-1.23	Homo sapiens folate receptor 3 (gamma) (FOLR3), mRNA.
MYL2	NM_000432.2	-1.15	Homo sapiens myosin, light chain 2, regulatory, cardiac, slow (MYL2), mRNA.
CHI3L2	NM_004000.2	-1.13	Homo sapiens chitinase 3-like 2 (CHI3L2), transcript variant 1, mRNA.
MYH7	NM_000257.1	-1.09	Homo sapiens myosin, heavy chain 7, cardiac muscle, beta (MYH7), mRNA.
COL1A1	NM_000088.2	-1.02	Homo sapiens collagen, type I, alpha 1 (COL1A1), mRNA.
FLNC	NM_001458.2	-0.98	Homo sapiens filamin C, gamma (actin-binding protein 280) (FLNC), mRNA.
CKM	NM_001824.2	-0.96	Homo sapiens creatine kinase, muscle (CKM), mRNA.
LTB	NM_002341.1	-0.93	Homo sapiens lymphotoxin beta (TNF superfamily, member 3) (LTB), transcript variant 1, mRNA.
FNDC1	NM_032532.1	-0.90	Homo sapiens fibronectin type III domain containing 1 (FNDC1), mRNA.
CFB	NM_001710.4	-0.89	Homo sapiens complement factor B (CFB), mRNA.
HOPX	NM_139212.2	-0.88	Homo sapiens HOP homeobox (HOPX), transcript variant 3, mRNA.
SLN	NM_003063.1	-0.84	Homo sapiens sarcolipin (SLN), mRNA.
CXCL12	NM_000609.4	-0.82	Homo sapiens chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1) (CXCL12), transcript variant 2, mRNA.
ALPL	NM_000478.2	-0.82	Homo sapiens alkaline phosphatase, liver/bone/kidney (ALPL), transcript variant 1, mRNA.
MB	NM_005368.2	-0.81	Homo sapiens myoglobin (MB), transcript variant 1, mRNA.
MYBPC1	NM_002465.2	-0.80	Homo sapiens myosin binding protein C, slow type (MYBPC1), transcript variant 2, mRNA.
PTGDS	NM_000954.5	-0.80	Homo sapiens prostaglandin D2 synthase 21kDa (brain) (PTGDS), mRNA.
C1QTNF9	NM_178540.3	-0.79	Homo sapiens C1q and tumor necrosis factor related protein 9 (C1QTNF9), mRNA.
CXCL12	NM_001033886.1	-0.79	Homo sapiens chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1) (CXCL12), transcript variant 2, mRNA.

CPXM1	NM_019609.3	-0.75	Homo sapiens carboxypeptidase X (M14 family), member 1 (CPXM1), mRNA.
IGDCC4	NM_020962.1	-0.74	Homo sapiens immunoglobulin superfamily, DCC subclass, member 4 (IGDCC4), mRNA.
BASP1	NM_006317.3	-0.71	Homo sapiens brain abundant, membrane attached signal protein 1 (BASP1), mRNA.
FNDC1	NM_032532.1	-0.69	Homo sapiens fibronectin type III domain containing 1 (FNDC1), mRNA.
SMOC2	NM_022138.1	-0.69	Homo sapiens SPARC related modular calcium binding 2 (SMOC2), mRNA.
ISLR	NM_005545.3	-0.69	Homo sapiens immunoglobulin superfamily containing leucine-rich repeat (ISLR), transcript variant 1, mRNA.
IL7R	NM_002185.2	-0.68	Homo sapiens interleukin 7 receptor (IL7R), mRNA.
IL7R	XM_937367.1	-0.68	PREDICTED: Homo sapiens interleukin 7 receptor (IL7R), mRNA.
PDPN	NM_001006625.1	-0.67	Homo sapiens podoplanin (PDPN), transcript variant 4, mRNA.
HP	NM_005143.2	-0.67	Homo sapiens haptoglobin (HP), mRNA.
GNLY	NM_012483.1	-0.66	Homo sapiens granulysin (GNLY), transcript variant 519, mRNA.
ATP8B2	NM_020452.2	-0.66	Homo sapiens ATPase, class I, type 8B, member 2 (ATP8B2), transcript variant 1, mRNA.
CTSG	NM_001911.2	-0.66	Homo sapiens cathepsin G (CTSG), mRNA.
HRC	NM_002152.2	-0.64	Homo sapiens histidine-rich calcium-binding protein (HRC), mRNA.
FAM129A	NM_022083.1	-0.64	Homo sapiens family with sequence similarity 129, member A (FAM129A), transcript variant 2, mRNA.
F10	NM_000504.2	-0.64	Homo sapiens coagulation factor X (F10), mRNA.
APCDD1L	NM_153360.1	-0.64	Homo sapiens adenomatosis polyposis coli down-regulated 1-like (APCDD1L), mRNA.
GZMA	NM_006144.2	-0.63	Homo sapiens granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3) (GZMA), mRNA.
C7	NM_000587.2	-0.63	Homo sapiens complement component 7 (C7), mRNA.
CD3D	NM_001040651.1	-0.63	Homo sapiens CD3d molecule, delta (CD3-TCR complex) (CD3D), transcript variant 2, mRNA.
TCAP	NM_003673.2	-0.63	Homo sapiens titin-cap (telethonin) (TCAP), mRNA.
TMEM154	NM_152680.1	-0.62	Homo sapiens transmembrane protein 154 (TMEM154), mRNA.
KBTBD10	NM_006063.2	-0.62	Homo sapiens kelch repeat and BTB (POZ) domain containing 10 (KBTBD10), mRNA.
CHST15	NM_015892.2	-0.61	Homo sapiens carbohydrate (N-acetylgalactosamine 4-sulfate 6-O) sulfotransferase 15 (CHST15), mRNA.
CD48	NM_001778.2	-0.61	Homo sapiens CD48 molecule (CD48), mRNA.

KLRB1	NM_002258.2	-0.61	Homo sapiens killer cell lectin-like receptor subfamily B, member 1 (KLRB1), mRNA.
MYOZ1	NM_021245.2	-0.61	Homo sapiens myozenin 1 (MYOZ1), mRNA.
GALNTL1	NM_020692.1	-0.61	Homo sapiens UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 1 (GALNTL1), mRNA.
LTB	NM_002341.1	-0.60	Homo sapiens lymphotoxin beta (TNF superfamily, member 3) (LTB), transcript variant 1, mRNA.
C1orf54	NM_024579.1	-0.60	Homo sapiens chromosome 1 open reading frame 54 (C1orf54), mRNA.
CILP	NM_003613.2	-0.59	Homo sapiens cartilage intermediate layer protein, nucleotide pyrophosphohydrolase (CILP), mRNA.
LILRB3	NM_006864.1	-0.59	Homo sapiens leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3 (LILRB3), transcript variant 2, mRNA.
SEMA4D	NM_006378.2	-0.59	Homo sapiens sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4D (SEMA4D), mRNA.
TPSAB1	NM_003294.3	-0.59	Homo sapiens tryptase alpha/beta 1 (TPSAB1), mRNA.
CD79A	NM_001783.2	-0.59	Homo sapiens CD79a molecule, immunoglobulin-associated alpha (CD79A), transcript variant 2, mRNA.
Down-regulated genes in aSAT after exercise training			
FOS	NM_005252.2	1.05	Homo sapiens v-fos FBJ murine osteosarcoma viral oncogene homolog (FOS), mRNA.
FOSB	NM_006732.1	0.89	Homo sapiens FBJ murine osteosarcoma viral oncogene homolog B (FOSB), mRNA.
CSN1S1	NM_001025104.1	0.87	Homo sapiens casein alpha s1 (CSN1S1), transcript variant 1, mRNA.
MYOC	NM_000261.1	0.82	Homo sapiens myocilin, trabecular meshwork inducible glucocorticoid response (MYOC), mRNA.
TWIST1	NM_000474.3	0.77	Homo sapiens twist homolog 1 (Drosophila) (TWIST1), mRNA.
LOC643911	XR_042101.1	0.76	PREDICTED: Homo sapiens hCG1815491 (LOC643911), miscRNA.
CSN1S1	NM_001890.1	0.76	Homo sapiens casein alpha s1 (CSN1S1), transcript variant 1, mRNA.
MYOC	NM_000261.1	0.75	Homo sapiens myocilin, trabecular meshwork inducible glucocorticoid response (MYOC), mRNA.
THBS4	NM_003248.3	0.75	Homo sapiens thrombospondin 4 (THBS4), mRNA.
LOC643911	XM_931911.1	0.72	PREDICTED: Homo sapiens hypothetical LOC643911 (LOC643911), mRNA.

TSTD1	NM_001113206.1	0.71	Homo sapiens thiosulfate sulfurtransferase (rhodanese)-like domain containing 1 (TSTD1), transcript variant 2, mRNA.
GLDN	NM_181789.1	0.65	Homo sapiens gliomedin (GLDN), mRNA.
TMEM100	NM_018286.1	0.65	Homo sapiens transmembrane protein 100 (TMEM100), transcript variant 2, mRNA.
IRX5	NM_005853.4	0.64	Homo sapiens iroquois homeobox protein 5 (IRX5), mRNA.
TNMD	NM_022144.1	0.64	Homo sapiens tenomodulin (TNMD), mRNA.
SYN2	NM_133625.2	0.63	Homo sapiens synapsin II (SYN2), transcript variant IIa, mRNA.
IRX5	NM_005853.4	0.63	Homo sapiens iroquois homeobox 5 (IRX5), mRNA.
AHNAK	NM_024060.2	0.61	Homo sapiens AHNAK nucleoprotein (AHNAK), transcript variant 2, mRNA.
AHNAK	NM_024060.2	0.60	Homo sapiens AHNAK nucleoprotein (AHNAK), transcript variant 1, mRNA.
NPY5R	NM_006174.2	0.60	Homo sapiens neuropeptide Y receptor Y5 (NPY5R), mRNA.
CALB2	NM_001740.2	0.59	Homo sapiens calbindin 2 (CALB2), transcript variant CALB2c, mRNA.
GRB14	NM_004490.2	0.59	Homo sapiens growth factor receptor-bound protein 14 (GRB14), mRNA.

APPENDIX

RNA Extraction from adipose tissue samples

- Weigh ~70 mg of the frozen samples. Do not allow the thawing of the tissue, work on dry ice.
- Place the tissue in a suitably sized vessel containing 1 ml QIAzol lysis reagent and place the tip of the probe into the vessel. Operate the TissueRuptor at full speed until the lysate is uniformly homogeneous (20-40 s).
- Place the tube containing the homogenate on the benchtop at room temperature (15-25ºC) for 5 min.
- Centrifuge a 2 ml tube at 12,000-16,000 x g for 20-30 s and transfer the homogenate to the tube.
- Add 200 µL of chloroform to the tube, securely cap the tube, and shake it vigorously for 15 s. Do not vortex.
- Place the tube on the benchtop at room temperature for 2-3 min
- Centrifuge at 12,000 x g for 15 min at 4ºC
- Transfer the upper, aqueous phase to another 2mL tube. Add 1 volume of 70% ethanol and mix thoroughly by vortexing. Do not centrifuge.
- Transfer up to 700 µl of the sample to an RNeasy Mini spin column placed in a 2 ml collection tube. Close the lid gently, and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm) at room temperature (15-25ºC). Discard the flow-through.
- Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm) to wash the membrane. Discard the flow-through.
- Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the membrane. Discard the flow-through.
- Place the RNeasy spin column in a new 2 ml collection tube and discard the old collection tube with the flow-through. Close the lid gently, and centrifuge at full speed for 1 min.
- Place the RNeasy spin column in a new 1.5 ml collection tube. Add 30-50 µl RNase-free water directly to the spin column membrane. Close the lid gently and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the RNA.

RNA dilution and cDNA synthesis - workflow

- Calculate the amount of RNA that contains 1200 ng and the amount of Rnase free water to add for the final volume of 30 uL per sample.
- Thaw RNA on ice, gently vortex and quick microfuge each tube/sample.
- Pipette the volume of RNA per sample in the plate, for all samples (work on ice)
- Pipette the corresponding volume of water to make up to 30 uL of solution for each sample.
- Add 30 uL of RT master mix to each well and insert the plate in the machine to perform reverse transcription in a thermal cycler.

Note: Work on the ice during the whole process, put samples in a randomized manner on the plate.

RT master mix preparation (for 30 µL reaction)

	RT master mix sol	RT buffer
10xRT-buffer	960 µl	6 µl
25x dNTP	384 µl	2.4 µl
10 x RT Random primers	960 µl	6 µl
Multiscribe Reverse Transcriptase	480 µl	0 µl
RNAse inhibitor	480 µl	3 µl
Nuclease free water	1536 µl	12.6 µl
Total	**4800 µl**	**30 µl**

- Pipette 30 µL of RT master mix solution and add to each well (containing 30uL of RNA diluted with Rnase free water).
- Reverse transcript.

Gene Expression Analysis workflow

- Storage: Store TaqMan Gene Expression Assay products at -15 to -25 ℃ and keep them protected from light. To minimize freeze-thaw cycles, consider diluting 60x assays to 20x working stocks and dividing the solutions into smaller aliquots.
- Prepare the PCR reaction mix before transferring it to the reaction plate for thermal cycling and fluorescence analysis.
- Add 2 µl from each sample to one tube and mix well. Dilute the cDNA mixture to 5 different concentrations between 1-40 ng (eg: 2,5 ng, 5ng, 10 ng, 20 ng, and 40 ng).
- Add in triplicates the five different concentrations of cDNA standards (1-40 ng) for each gene (including the endogenous controls). The concentration that signals between (Ct) 16-30 is suitable to use.
- Thaw the reagents on ice, completely re-suspend by gentle vortex, and briefly centrifuge to bring the liquid to the bottom of the tube:

• TaqMan Gene Expression Assays (20x)

• cDNA samples

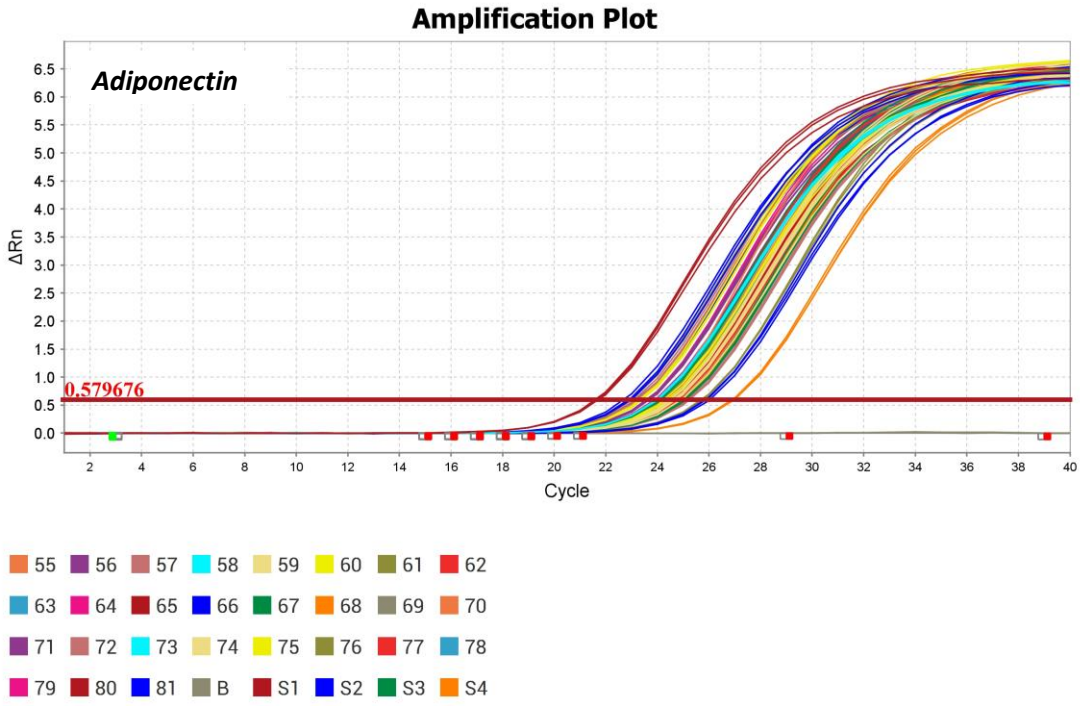
- Mix the master mix reagent by gently swirling the bottle
- Calculate the number of reactions (for 20uL volume)

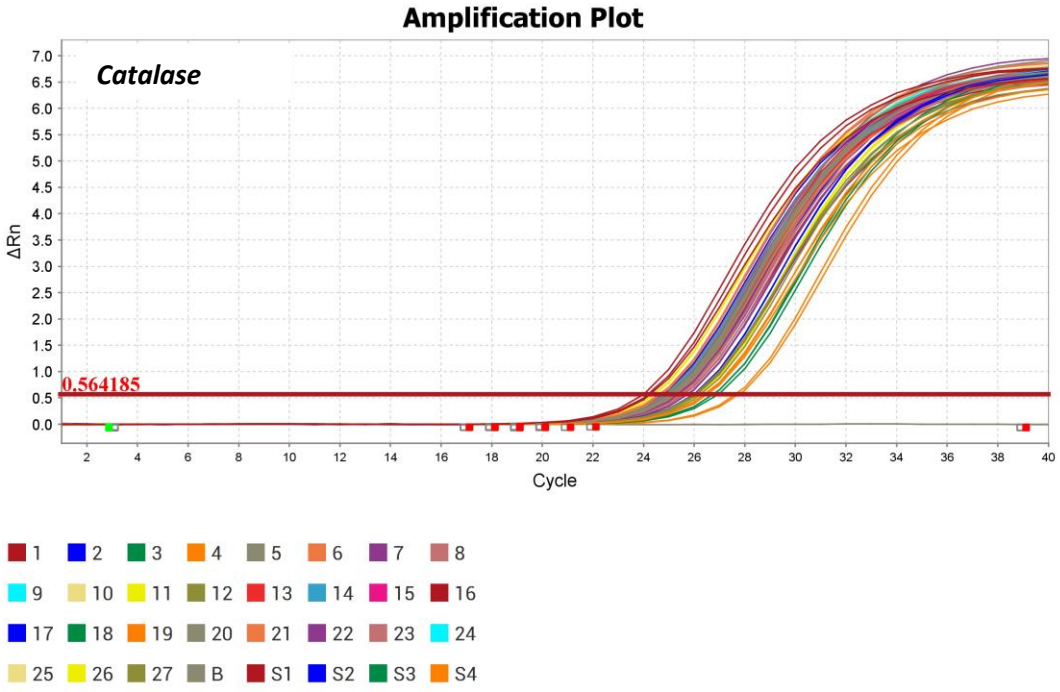
PCR reaction mix component	**single reaction**	**Triplicate**
20x Taqman gene expression Assay	1.0 µl	3.0 µl
2X Taqman gene expression master mix	10.0 µl	30 µl
RNase-free water	5.0 µl	15 µl
cDNA template (1 to 100 ng)	4.0 µl	12 µl

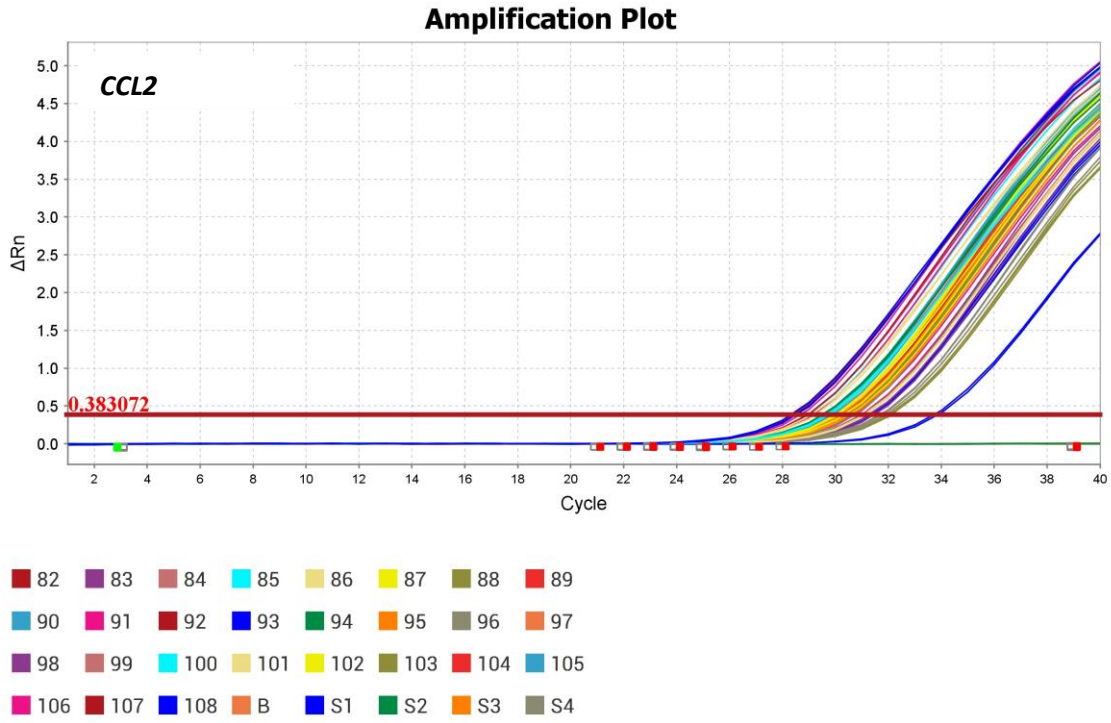
- Cap the tube and invert it several times to mix the reaction components.
- Centrifuge the tube briefly.
- Transfer 16 µL of PCR reaction mix into each well of the reaction plate
- Transfer 4 uL of each cDNA standard, control, and sample in triplicate
- Seal the plate and centrifuge briefly
- Load the plate into the instrument and run the plate

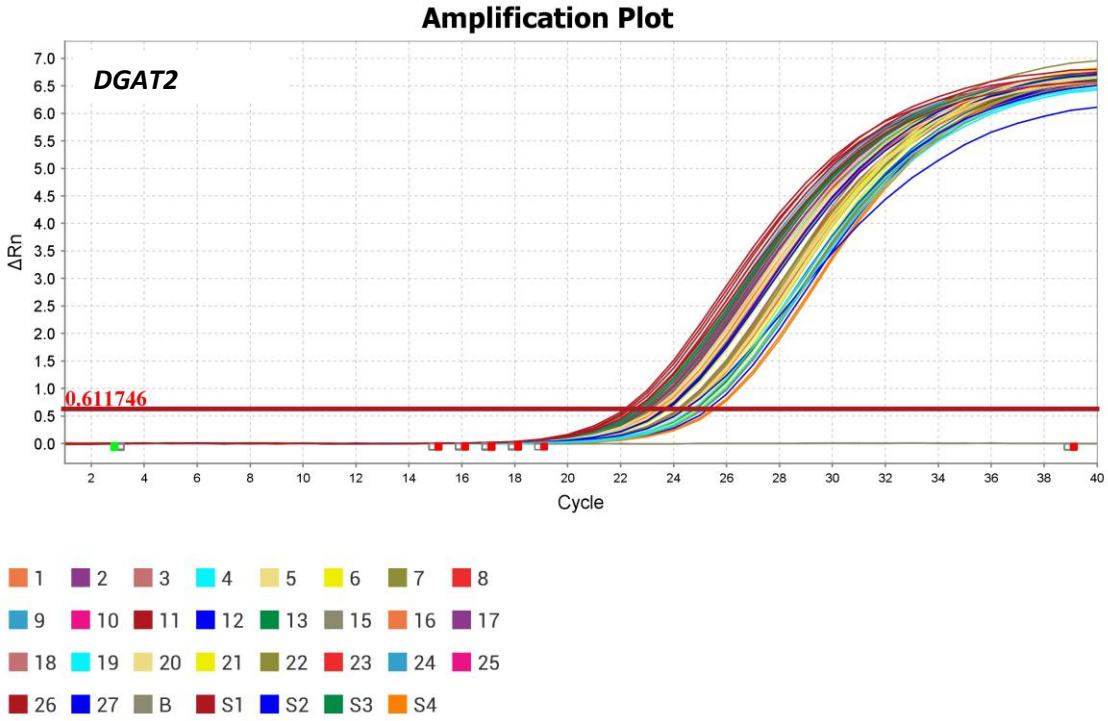
REAL-TIME PCR Amplification Plots

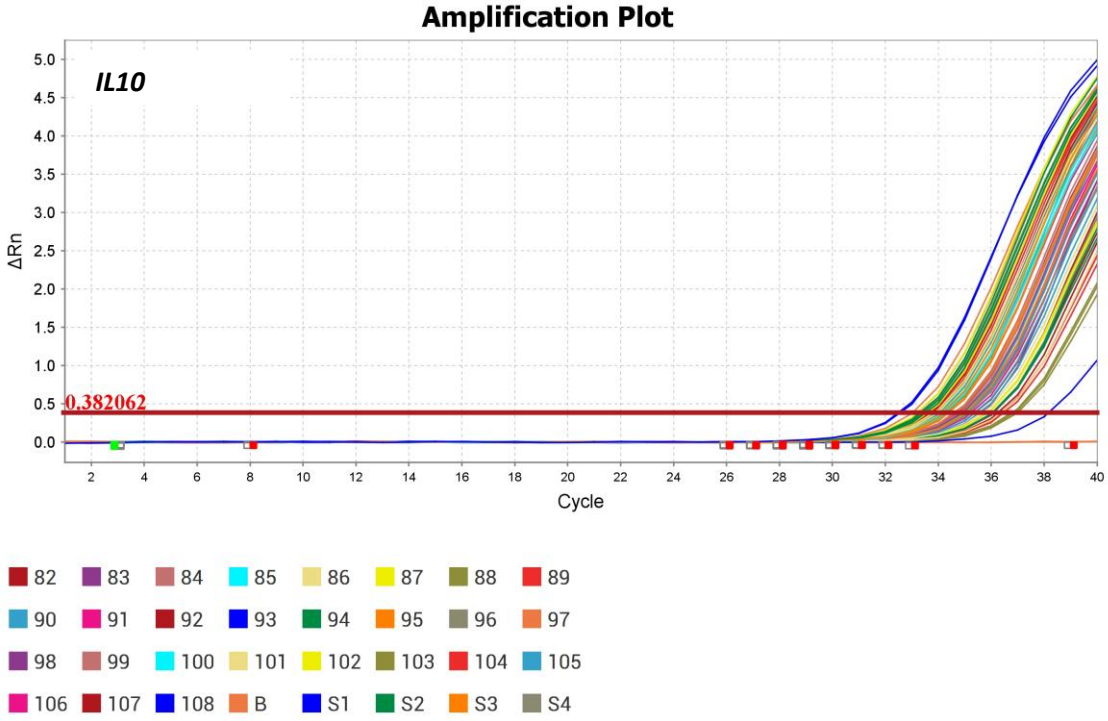
Real-Time PCR (RT-PCR) was performed in triplicate for each sample using Applied Biosystems QuantStudioTM^3 Real-Time PCR system with predesigned Taqman assays from Applied Biosystems (Warrington, UK). The evaluated genes were selected based on the physiological pathways involved in adipogenesis (*PPARγ*), lipid metabolism (*LPL*, *DGAT2*, *PLIN1*, *ATGL*), insulin signalling (*IRS1*, *GLUT4*, serine/threonine-protein kinase; *SMG1*), inflammation (*adiponectin*, *leptin*, *MIF*, *CCL2 (orMCP1)*, *IL-10*, *TLR4*, *NFκB1* and *TNFα*) and oxidative stress (nitric oxide synthase 3 (*NOS3*), *CAT*, *SOD1*). The amplification plots show the variation of log (ΔRn) with PCR cycle number. This indicates the level of expression of each gene for a given set of samples and is presented below in alphabetic order.

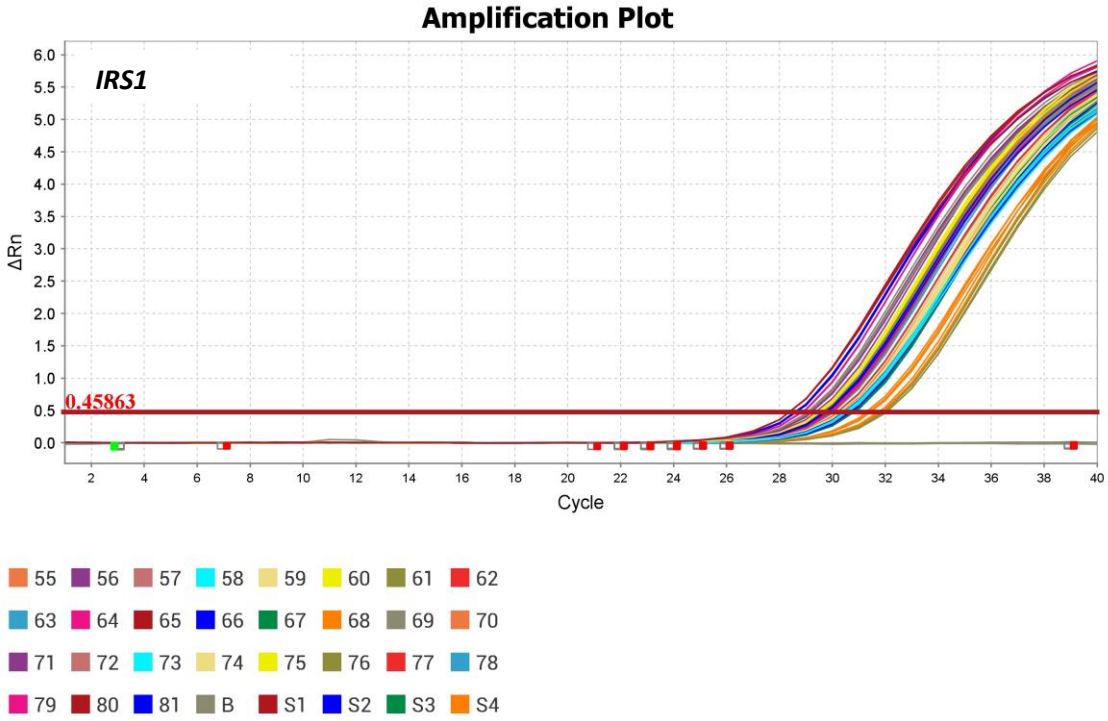


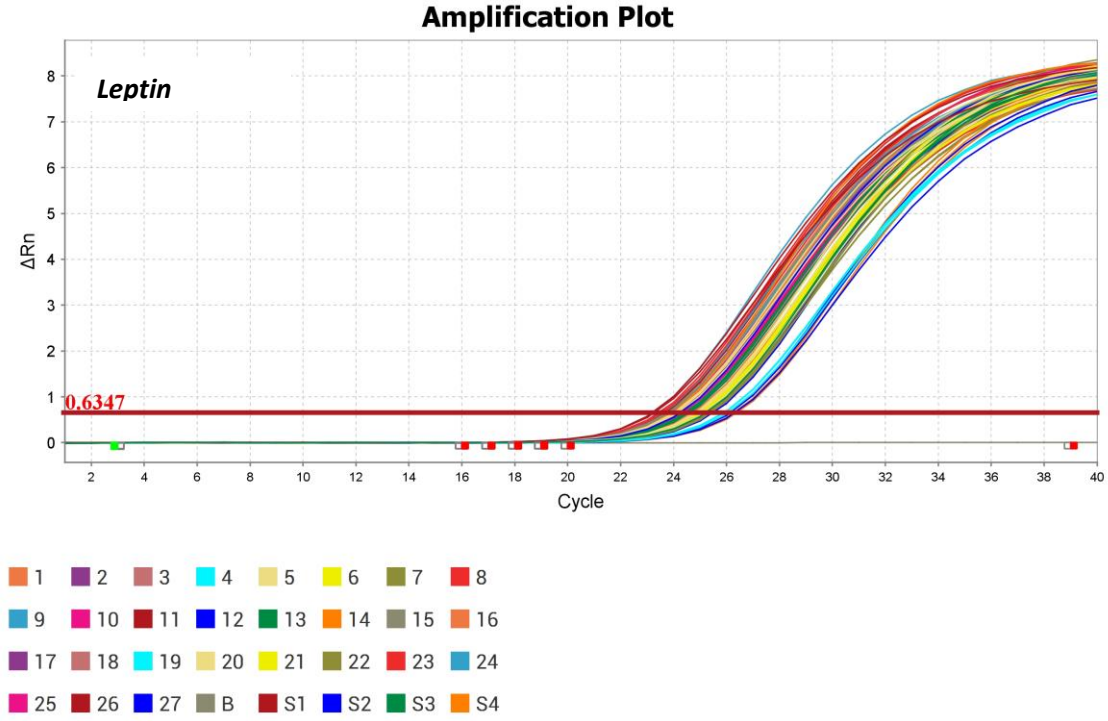

Amplification Plot
Catalase
ΔRn
Cycle
0.564185
1 2 3 4 5 6 7 8
9 10 11 12 13 14 15 16
17 18 19 20 21 22 23 24
25 26 27 B S1 S2 S3 S4

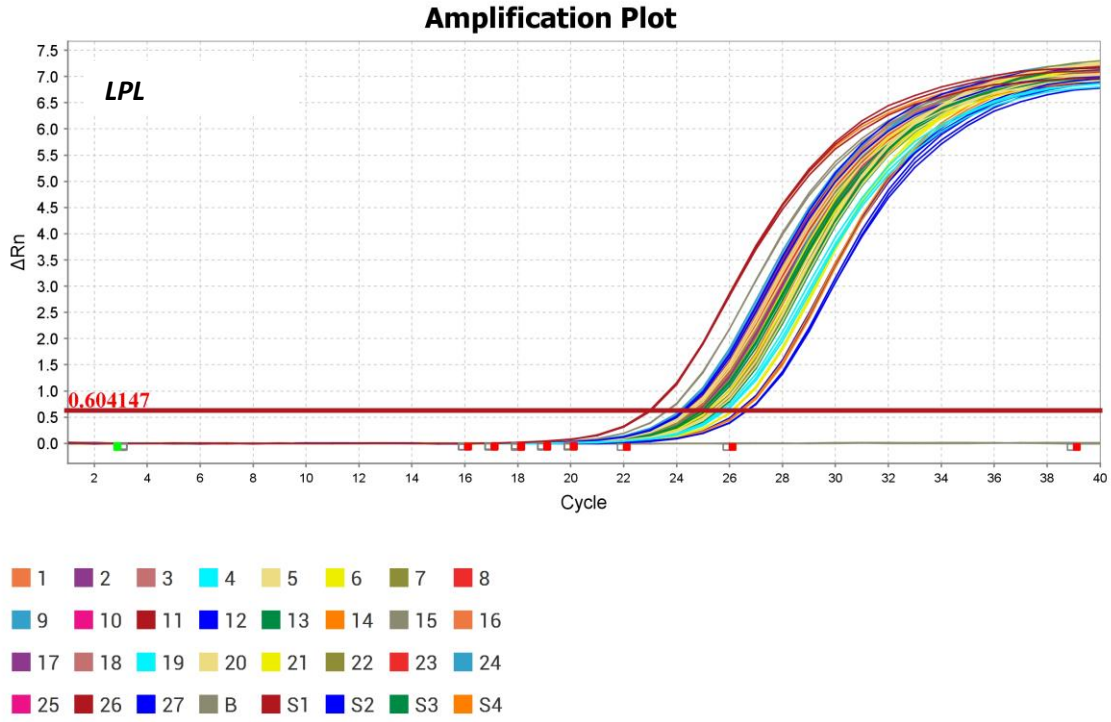

Amplification Plot
CCL2
ΔRn
Cycle
0.383072
82 83 84 85 86 87 88 89
90 91 92 93 94 95 96 97
98 99 100 101 102 103 104 105
106 107 108 B S1 S2 S3 S4

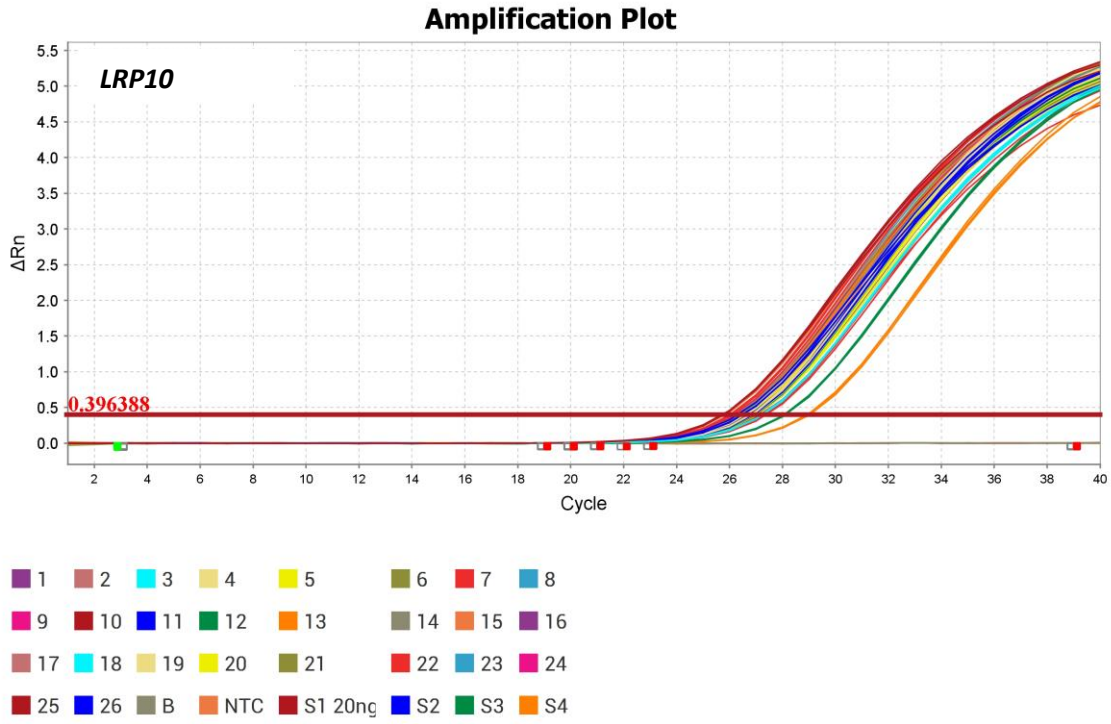

Amplification Plot
DGAT2
ΔRn
Cycle
0.611746
1 2 3 4 5 6 7 8
9 10 11 12 13 15 16 17
18 19 20 21 22 23 24 25
26 27 B S1 S2 S3 S4

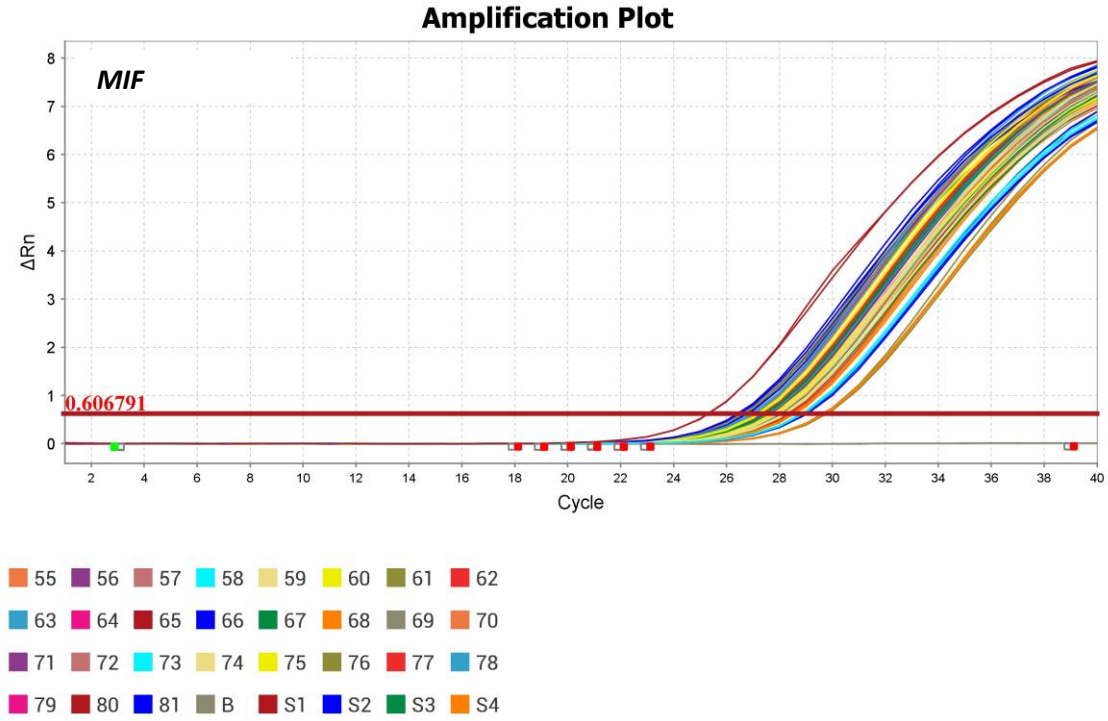

Amplification Plot
IL10
ΔRn
Cycle
0.382062
82 83 84 85 86 87 88 89
90 91 92 93 94 95 96 97
98 99 100 101 102 103 104 105
106 107 108 B S1 S2 S3 S4

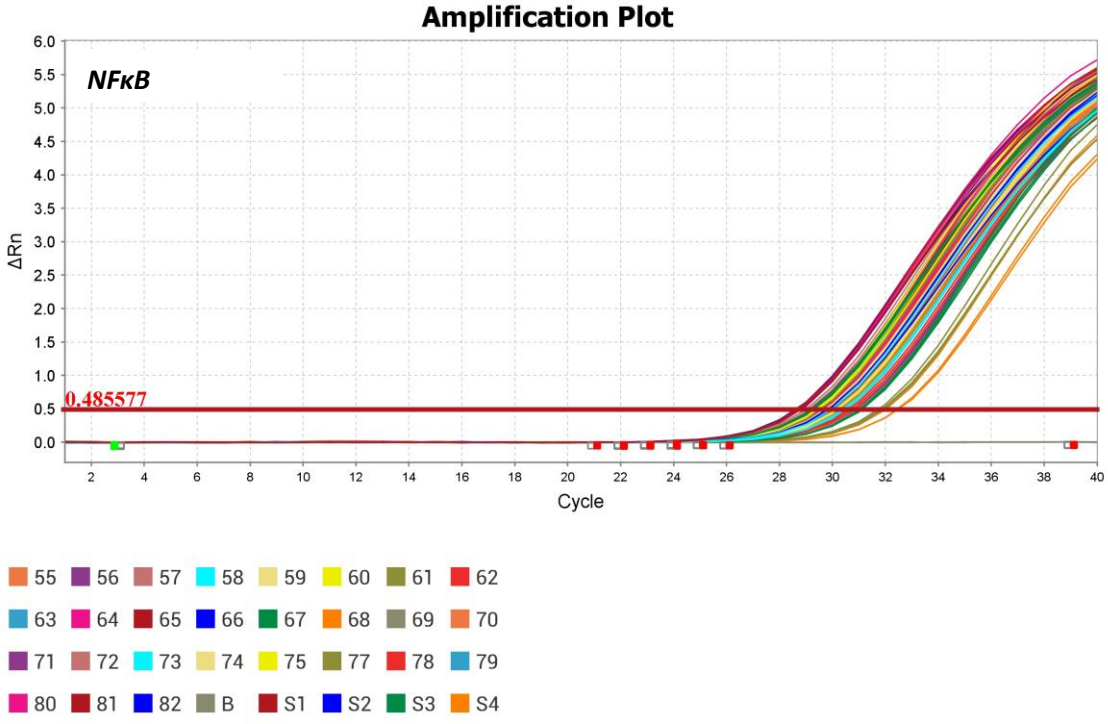

Amplification Plot
IRS1
ΔRn
0.45863
Cycle
55 56 57 58 59 60 61 62
63 64 65 66 67 68 69 70
71 72 73 74 75 76 77 78
79 80 81 B S1 S2 S3 S4

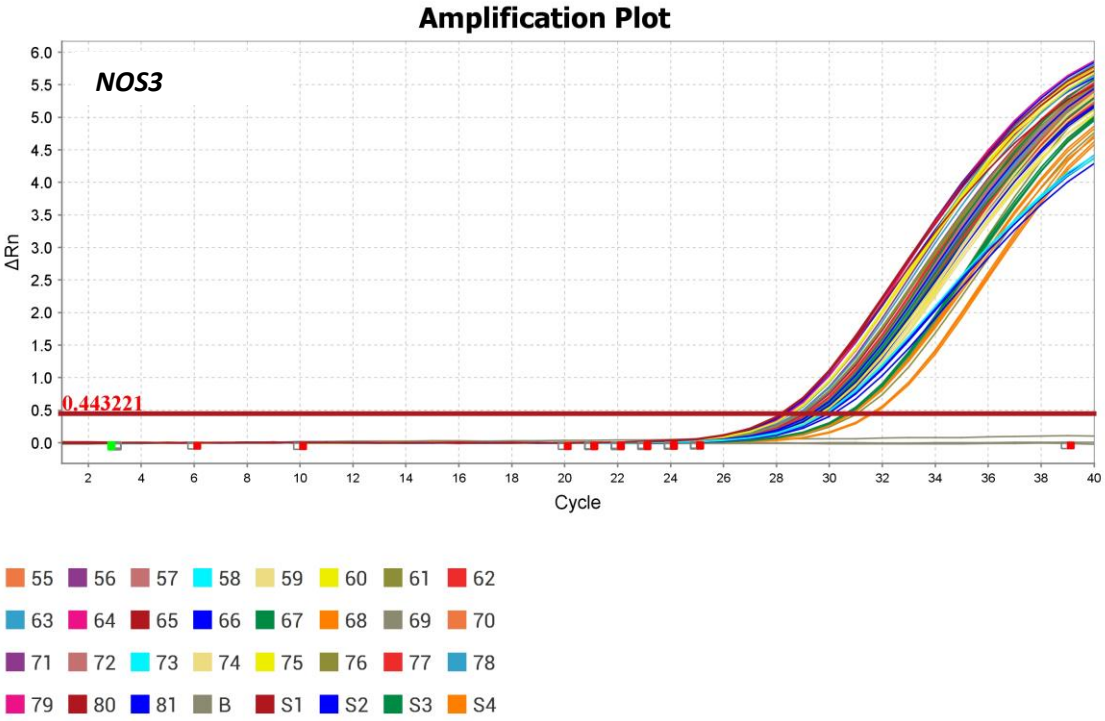

Amplification Plot
Leptin
ΔRn
0.6347
Cycle
1 2 3 4 5 6 7 8
9 10 11 12 13 14 15 16
17 18 19 20 21 22 23 24
25 26 27 B S1 S2 S3 S4

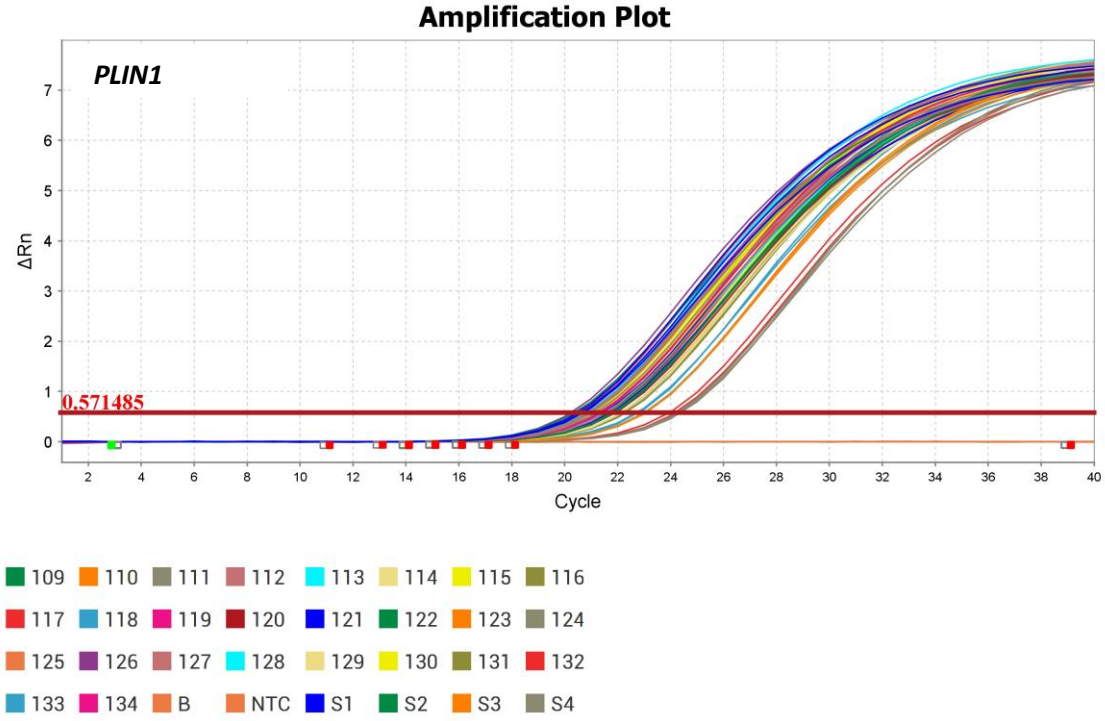

Amplification Plot
LPL
ΔRn
0.604147
Cycle
1 2 3 4 5 6 7 8
9 10 11 12 13 14 15 16
17 18 19 20 21 22 23 24
25 26 27 B S1 S2 S3 S4

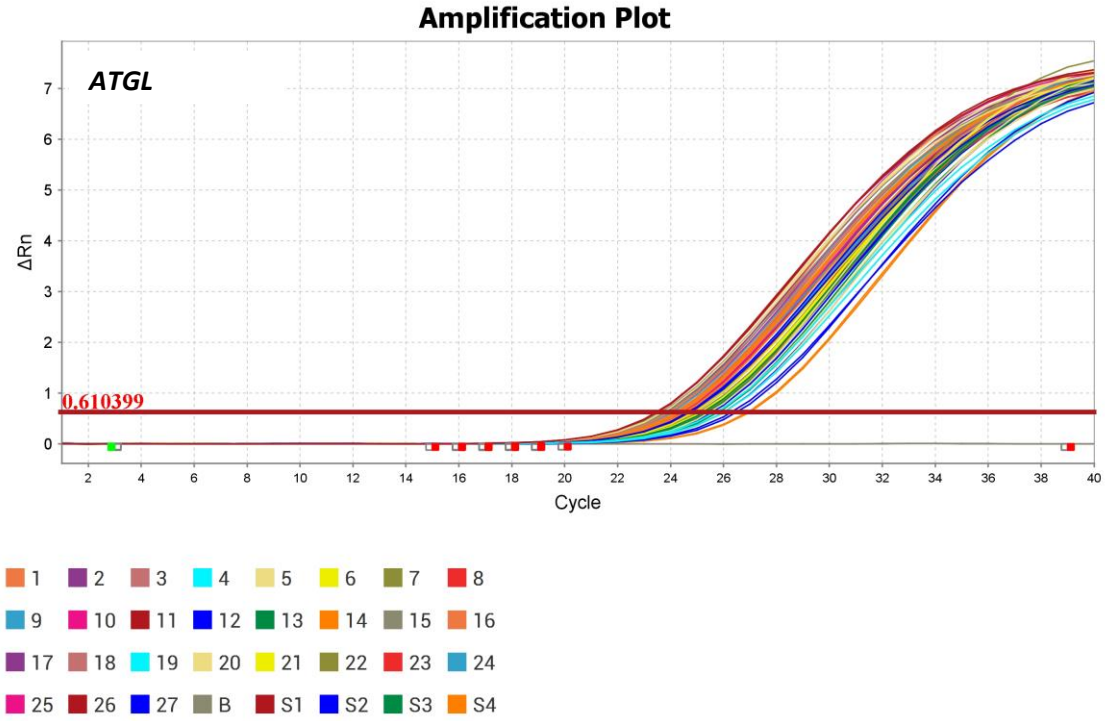

Amplification Plot
LRP10
ΔRn
0.396388
Cycle
1 2 3 4 5 6 7 8
9 10 11 12 13 14 15 16
17 18 19 20 21 22 23 24
25 26 B NTC S1 20ng S2 S3 S4

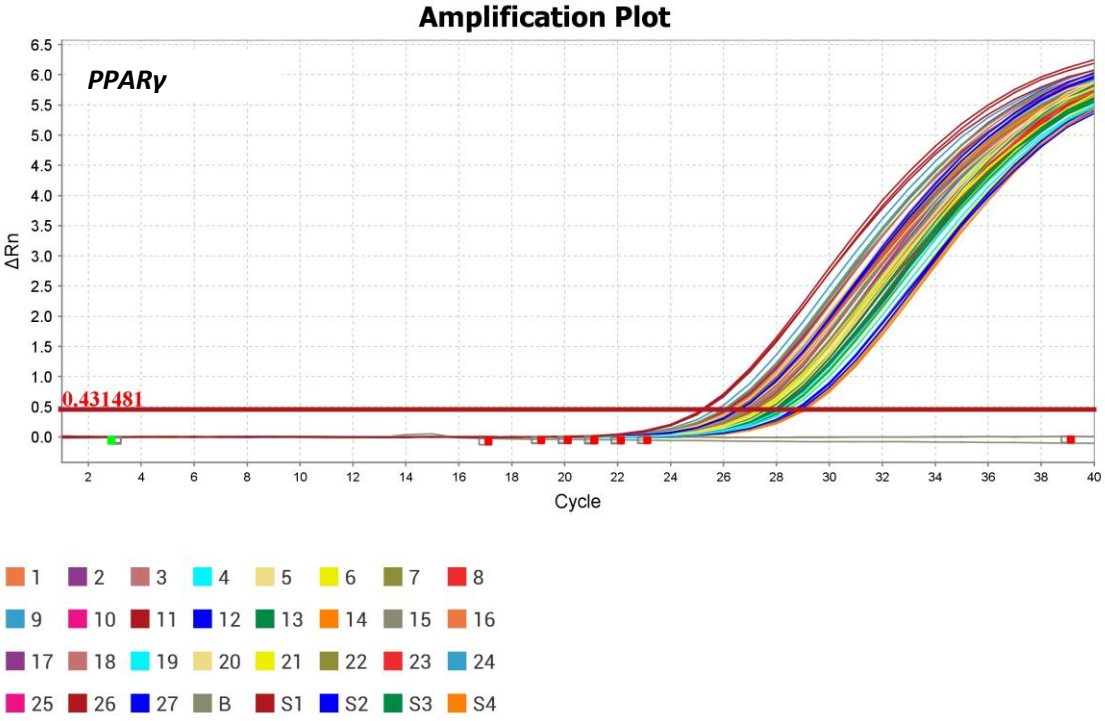

Amplification Plot
MIF
ΔRn
0.606791
Cycle
55 56 57 58 59 60 61 62
63 64 65 66 67 68 69 70
71 72 73 74 75 76 77 78
79 80 81 B S1 S2 S3 S4

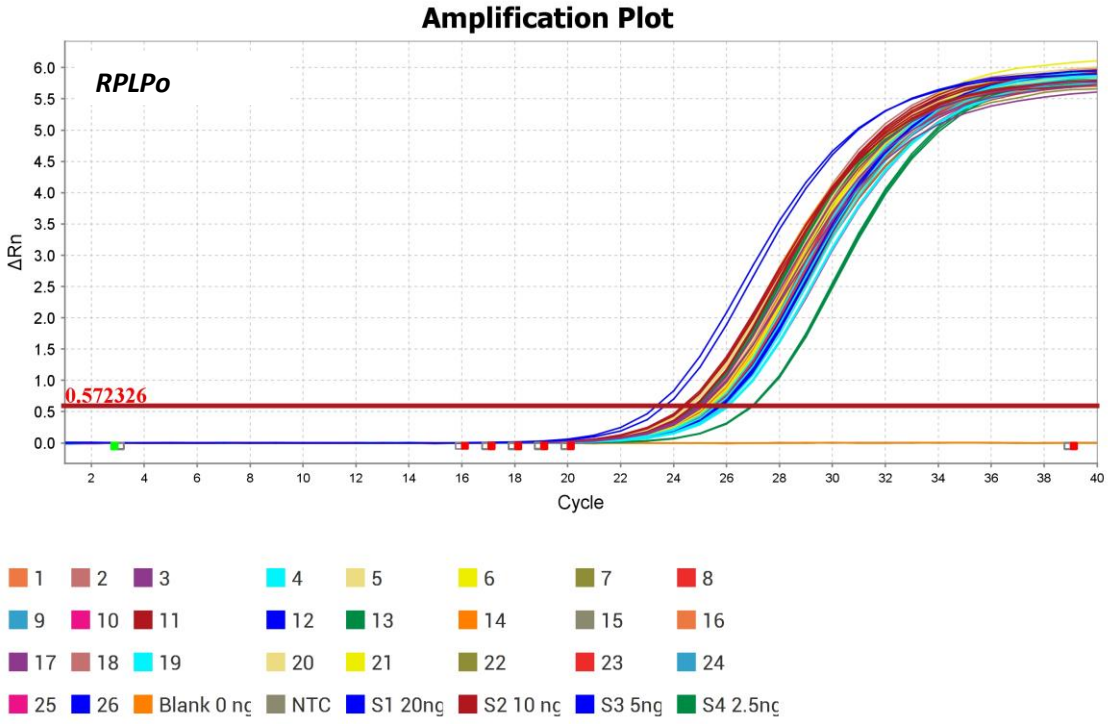

Amplification Plot
NFκB
ΔRn
0.485577
Cycle
55 56 57 58 59 60 61 62
63 64 65 66 67 68 69 70
71 72 73 74 75 77 78 79
80 81 82 B S1 S2 S3 S4

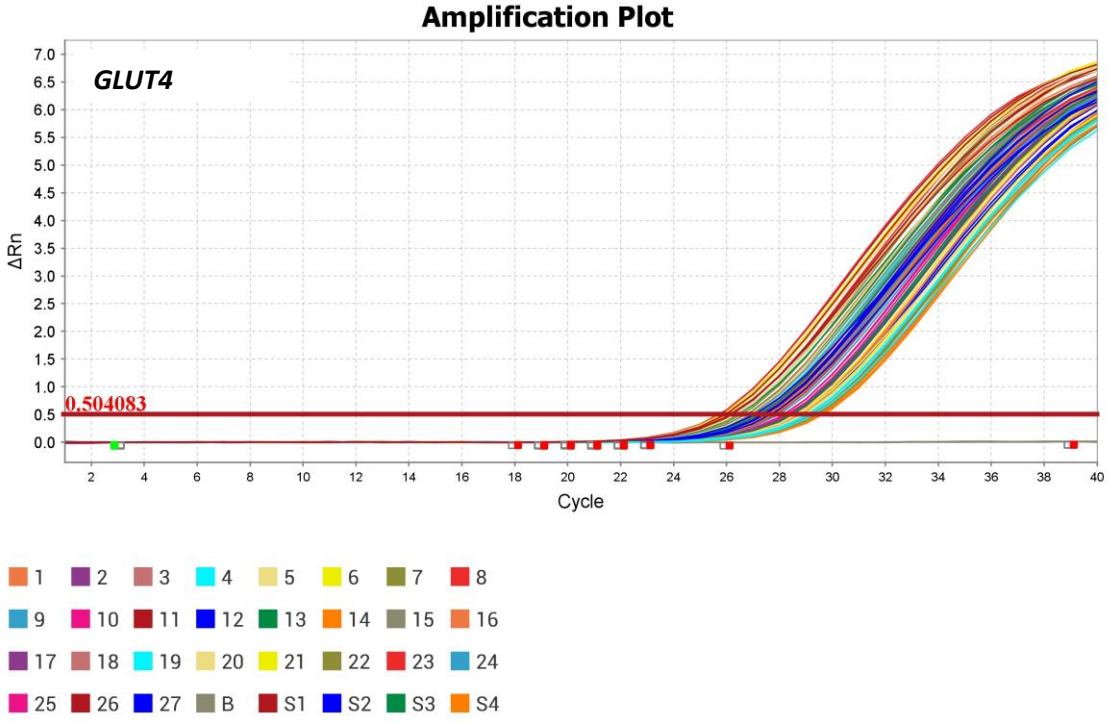

Amplification Plot
NOS3
ΔRn
Cycle
0.443221
55 56 57 58 59 60 61 62
63 64 65 66 67 68 69 70
71 72 73 74 75 76 77 78
79 80 81 B S1 S2 S3 S4

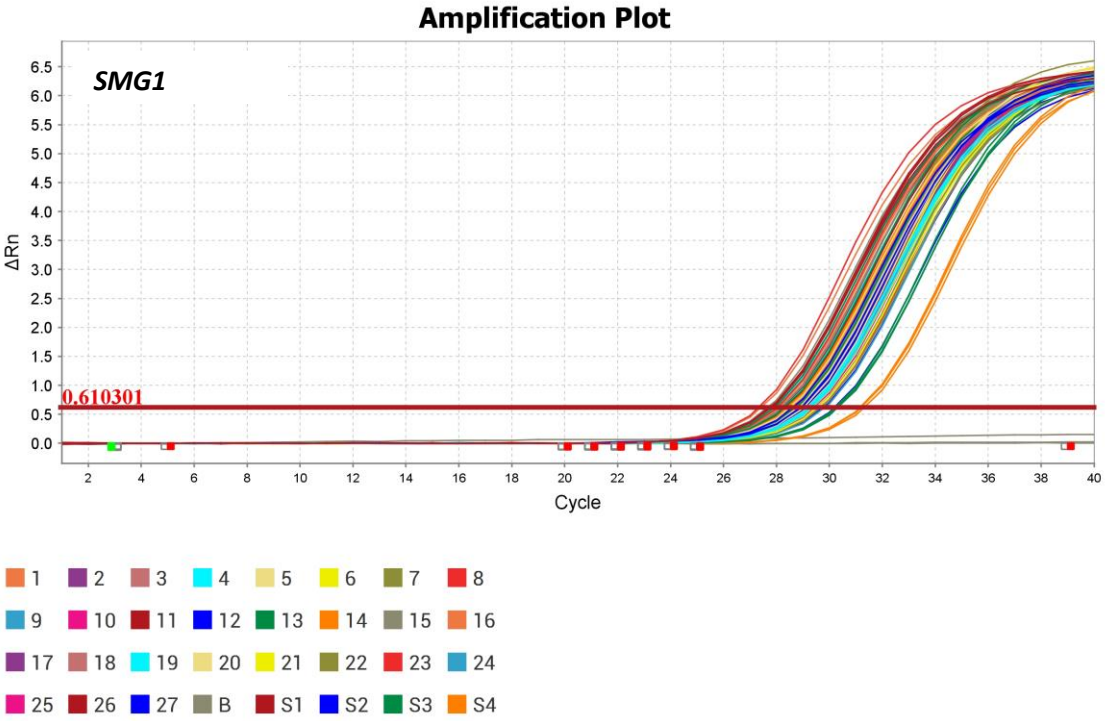

Amplification Plot
PLIN1
ΔRn
Cycle
0.571485
109 110 111 112 113 114 115 116
117 118 119 120 121 122 123 124
125 126 127 128 129 130 131 132
133 134 B NTC S1 S2 S3 S4

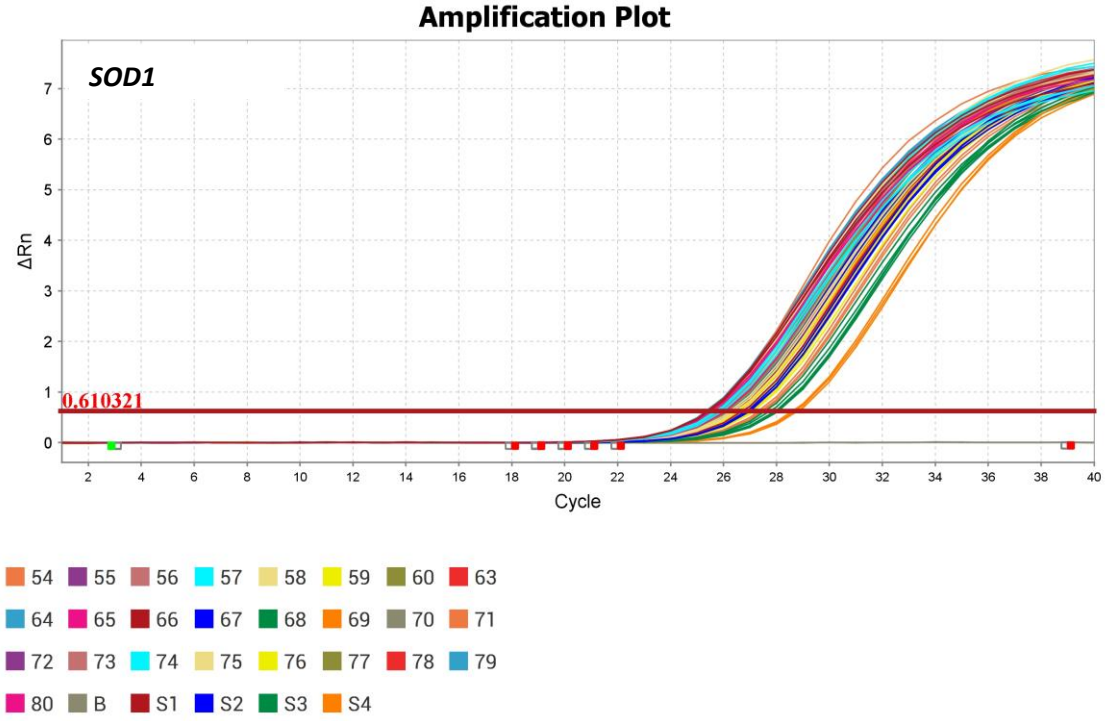

Amplification Plot
ATGL
ΔRn
0.610399
Cycle
1 2 3 4 5 6 7 8
9 10 11 12 13 14 15 16
17 18 19 20 21 22 23 24
25 26 27 B S1 S2 S3 S4

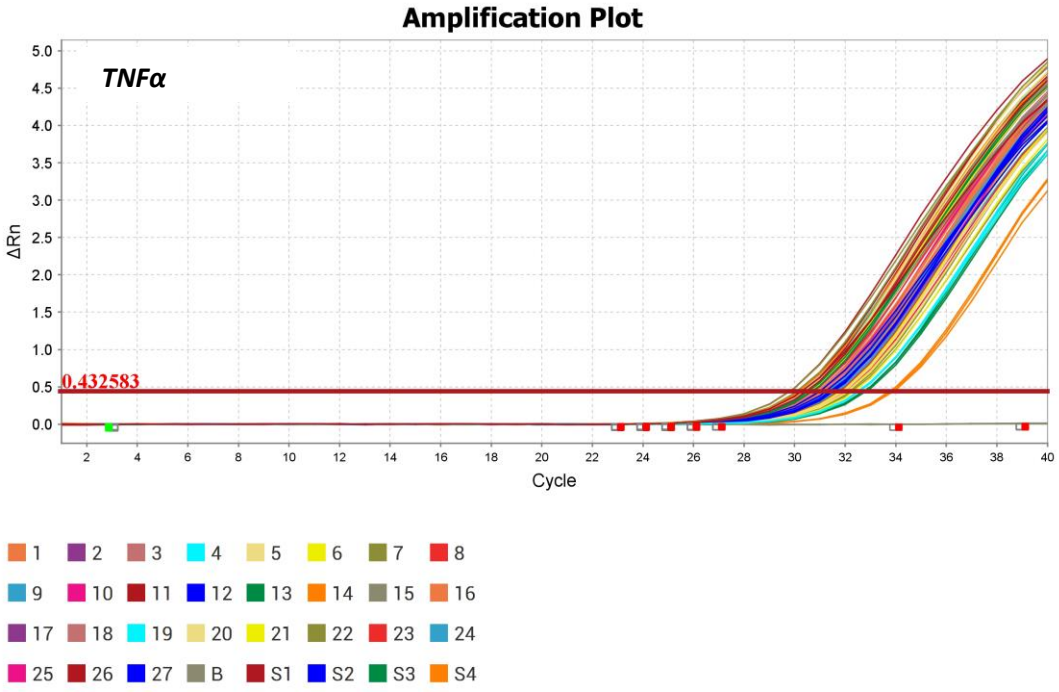

Amplification Plot
PPARγ
ΔRn
0.431481
Cycle
1 2 3 4 5 6 7 8
9 10 11 12 13 14 15 16
17 18 19 20 21 22 23 24
25 26 27 B S1 S2 S3 S4

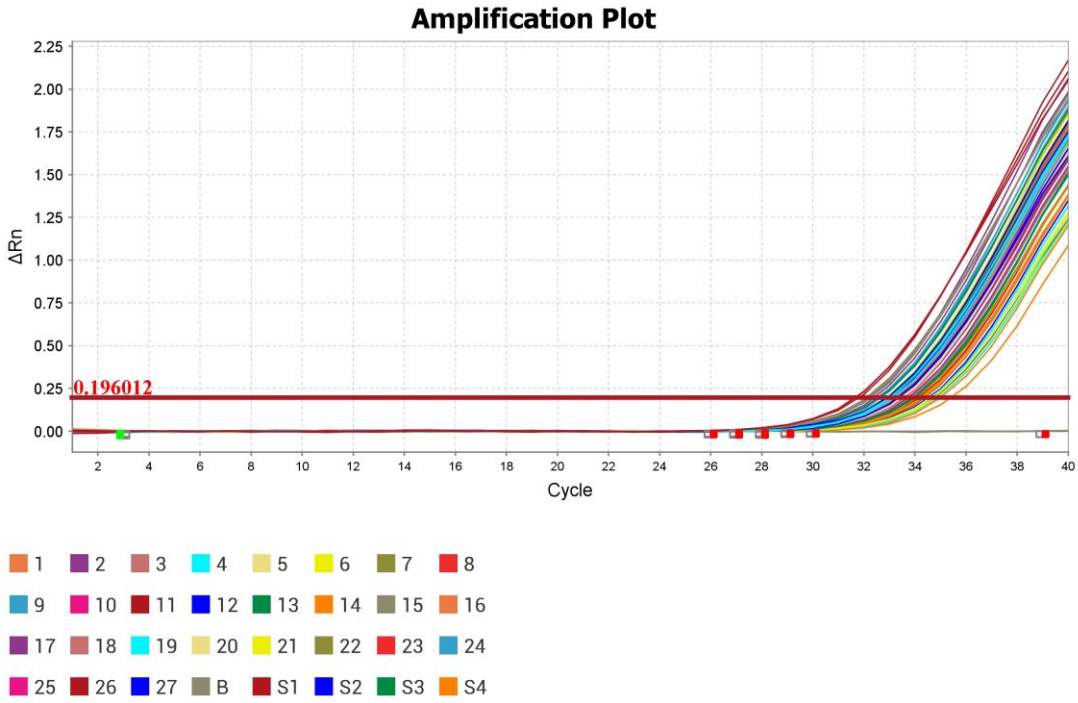

Amplification Plot
RPLPo
ΔRn
0.572326
Cycle
1 2 3 4 5 6 7 8
9 10 11 12 13 14 15 16
17 18 19 20 21 22 23 24
25 26 Blank 0 ng NTC S1 20ng S2 10 ng S3 5ng S4 2.5ng


Amplification Plot
GLUT4
ΔRn
0.504083
Cycle
1 2 3 4 5 6 7 8
9 10 11 12 13 14 15 16
17 18 19 20 21 22 23 24
25 26 27 B S1 S2 S3 S4


Amplification Plot
SMG1
ΔRn
Cycle
0.610301
1 2 3 4 5 6 7 8
9 10 11 12 13 14 15 16
17 18 19 20 21 22 23 24
25 26 27 B S1 S2 S3 S4


Amplification Plot
SOD1
ΔRn
Cycle
0.610321
54 55 56 57 58 59 60 63
64 65 66 67 68 69 70 71
72 73 74 75 76 77 78 79
80 B S1 S2 S3 S4





The numbers below each plot represent the different samples loaded on each plate; B: Blank; S1-S4: standard samples with different concentrations. Ct: cycle threshold; IL-10: interleukin-10; MCP1: monocyte chemoattractant protein 1; MIF: Macrophage migration inhibitory factor; NFκB: nuclear factor kappa B; TLR4: toll-like receptor 4; TNFα: tumor necrosis factor-alpha; PPARγ: Peroxisome proliferator activator receptor gamma; LPL: Lipoprotein lipase; DGAT2: Diacylglycerol acyltransferase 2; PLIN1: Perilipin 1; ATGL: Adipose triglyceride lipase; IRS1: Insulin receptor substrate 1; GLUT4: Glucose transporter 4; SMG1: Serine/threonine-protein kinase; SOD1: Superoxide dismutase; NOS3: Nitric oxide synthase; RPLPo: Ribosomal protein lateral stalk subunit PO; LRP10: Low-density lipoprotein receptor-related protein 1.

Western blot Bench Protocol

- ***Preparation of Radio Immuno Precipitation Assay buffer (RIPA II)***

  150 mM (0.15M) NaCl, 5M (3 mL)

  1.0% NP40 (Triton X-100) (10 mL of 10% NP40)

  0.5 % Sodium deoxycholate (10 mL of 5% SOD stored at -20°C)

  0.1% SDS – sodium dodecyl sulfate (1mL of 10% SDS)

  50 mM Tris pH 8.0 (5 mL of 1M Tris)

  5 mM EDTA (1ml 0.5 M EDTA)

  70 mL distillate water

  Total volume 100 mL

- Aliquot the prepared RIPA buffer in 10mL tubes.
- Add 1 tablet of proteinase inhibitor in one of the aliquots (working solution).
- Cut the frozen tissue, place it into the rupture-tube, add 200 µL of RIPA buffer (working solution) and disrupt the tissue.
- Pipette the lysate in a new labelled tube and centrifuge at 10 000rpm at 4°C for 15 min
- Pipette the supernatant in a new tube, add 200 uL of RIPA buffer and centrifuge at 10 000rpm at 4°C for 15 min
- Keep the protein lysate at -20°C for later use or prepare for loading ton to gel.

- ***Laemmli buffer 2X preparation:***

  4%SDS

  10% 2-mercaptoethanol

  20% Glycerol

  0.004% bromophenol blue

  0.125 M tris HCL

  pH ≈ 6.

- ***Sample preparation***

  Protein lysate + Laemmli buffer 6:1 (6X Laemmli)

  Heat 20 min at 70°C (do not heat the ladder and do not add Laemmli buffer to the ladder).

Gel Run

- Assemble the chamber and check if it is tight (fill the tank with running buffer to approx. 1/3).
- Load 3 µL ladder, and 20 µg of total protein per lane for each sample on the precast gel.
- Run the gel at 100 V and 10 mA for 15 min, and 200 V and 20 mA for approx. 1-1.5 h.
- Place/rinse the PVDF-membrane first in methanol and equilibrate in transfer buffer (for about 1h).

Blotting and staining

- After running, wash the gel in transfer buffer
- Build the blot: Filter paper- membrane- gel (all layers should be well moisturized with transfer buffer and free of air bubbles).
- Blot for 60 min (6x8 cm membrane 100 mA).
- Prepare the blocking buffer (5% BSA in TBST buffer).
- Place the membrane in ponceau S and incubate for 1 min.
- Wash the membrane 2 times in distillate water.

Antibody coloring/catching

- Shortly rinse the membrane with TBST buffer.
- Incubate 60 min in blocking buffer.
- Incubate with the primary antibody (dilute antibody with fresh blocking buffer) overnight at approx. 4°C.
- The next day, rinse the membrane with distillate water, 3 x for 10 min with TBST buffer.
- Incubate with the secondary antibody (dilute the antibody with blocking buffer) for approx. 1 h.
- Rinse with distillate water and wash the membrane 3x for 10 min each with TBST.
- Prepare the membrane for visualization.

Western Blot Buffers

10% SDS
10 g SDS
100 ml H_2O

Upper Gel Buffer (Collection)
30.3 g Tris -> 0,5 M
20 ml 10% SDS -> 0,4%
pH = 6.8
add 500 ml H_2O

Lower Gel Buffer (Separation)
90.85 g Tris -> 1.5 M
20 ml 10% SDS -> 0.4%
pH = 8.8
add 500 ml H_2O

10% APS
0.5 g APS
5 ml H_2O
14 d at 4°C

10 x Glycine Buffer
30 g Tris
144 g Glycine
ad 1000 ml H_2O

1 x Running Buffer
20 ml 10% SDS
200 ml 10 x Glycine Buffer
ad 2000 ml H_2O

Transfer Buffer
100 ml Glycine Buffer
200 ml Methanol
ad 1000 ml H_2O

10 x TBS-Wash-Buffer
24 g Tris (HCL)
80 g NaCl
pH = 7.6
ad 1000 ml H_2O

1 x TBST-Wash-Buffer
100 ml 10 x TBS-Wash-Buffer
10 ml 10% Tween
ad 1000 ml H_2O

Tween
10 ml Tween
90 ml H_2O

Blocking Buffer
2.5 g BSA -> 5%
50 ml TBST-Wash-Buffer

Oxidative stress markers analyses

Thiobarbituric reactive substance (TBARS) assay

Assay principle:

Malonic dialdehyde (MDA) is a reactive compound formed during lipid peroxidation caused by ROS and can be measured by the thiobarbituric acid reactive substances (TBARS) assay. During this assay, blood samples (serum) are mixed with thiobarbituric acid and malonic dialdehyde resulting from the lipid peroxidation within the sample form a pink colour, indicating the levels of TBARS. These last were measured spectrophotometrically and absorbance was recorded at 532 nm.

Assay procedure:

- Add 50 µL serum + 6.25 µL butylated hydroxytoluene (BHT; 4nM in absolute ethanol) + 50µL ortho-phosphoric acid (0.2M) in 2 mL tubes
- Vortex 10 s
- Add 6.25 uL thiobarbituric acid reagent (TBA; 0.793g in 50 mL 0.1M NaOH)
- Vortex 10s
- Microfuge for 2 min at 3000 rpm, 4°C (to collect volumes at the bottom)
- Place tubes in heating bock for 45 min, 90°C (color should change to pink)
- Place tube on ice for 5 min
- Add 500 uL of n-butanol + 50uL saturated NaCl
- Vortex for 10 s
- Microfuge for 2 min at 12000rpm, 4°C (two phases will be formed, top butanol phase and bottom protein phase)
- Pipette 300 uL of top butanol phase into the wells of the UV readable plate
- Read at 532 nm at room temperature
- Calculate the concentrations of malonic dialdehyde using the Beer-Lamberts Law with an extinction coefficient of $1.54 \times 10^5\ M^{-1}/cm^{-1}$.

- **Jentzsch AM, Heinin B, H., Furst P, Biesalski HK.** Improved analysis of malondialdehyde in human body fluids. Free Radical Biology & Medicine. 1996;20(2):251-6

Oxygen radical absorbance capacity assay

Assay Principle

Oxygen radical capacity absorbance (ORAC) measures the total antioxidant capacity a sample by determining the ability of this last to scavenge free radicals.

This assay is based on the principle that free radicals are generated from the thermal decomposition of 2, 2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH), on the signal intensity from the fluorescent probe in the presence of an oxygen radical absorbing substance. Strong absorbance capacity indicates more free radicals scavenged, which maintain the intensity of the fluorescent signal observed. The area under the curve of the fluorescence intensity versus time is subtracted from that of the blank, to determine the antioxidant capacity of the sample, in Trolox equivalents. Trolox is a vitamin-E analog used as the standard measure of antioxidant capacity.

Assay Protocol

Phosphate buffer: mix K2HPO4·3H2O (0.75M) and NaH2PO4·H2O (0.75M) to a final concentration of 0.75M and pH of 7.4.

Standard curve:

- Dilute 0.0050g of trolox (6-OH-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) in 200 µL ethanol (100M) ("Solution 1")
- Add 100 µL of "Solution 1" to 9.9mL of phosphate buffer (1000 µM) ("Solution 2").
- Take 1mL of "Solution 2" to 9 mL of phosphate buffer (5nmol/L) ("Solution 3").
- Add 300 µL of "Solution 3" to 300 µL of phosphate buffer (2.5nmo/L).
- Add 300 µL of the previous solution to 300 µL of phosphate buffer (1.25nmol/L) and prepare further dilution (0.625nmol/L, 0.313nmol/L, 0.156nmol/L and 0.078nmol/L).

Fluorescein solutions:

- Add 0.0225g fluorescein to 50mL of phosphate buffer (0.0012 mol/L) for Stock 1 solution.
- Add 50 µL of stock 1 to 10mL of phosphate buffer (5.98 µmol/L) for the Stock 2 solution.
- Add 320 µL of the Stock 2 solution to 20mL of phosphate buffer (95.7 nmol/L) to prepare the working solution.

AAPH solution: Add 0.087g of AAPH to 980 µL of pre-warm (37°C) phosphate buffer, to a final concentration of 0.33M.

Sample dilution:

- Pipette 100 µL of serum in a 2 mL tubes, add 500 µL of phosphate buffer (0.075M, pH 7.4) and vortex (dilution A).
- Pipette 160 µL of dilution A in a tube, add 840 µL of phosphate buffer and vortex for 10 seconds (dilution B).

- Add 100 µL of dilution B to 700 µL of phosphate buffer (dilution C)
- Pipette 200µL of the previous dilution C and add 200 µL of phosphate buffer. This formed the final sample to use for the experiment (726x dilution).

Assay protocol plate setting

- Add 300 µL of phosphate buffer to the wells A1 and A2 (blank), 200 µL of phosphate buffer in A3 and A4 plus 100 µL of fluorescein.
- To well A5 and A6, add 100 µL of phosphate buffer and 100 µL of fluorescein, into wells A7 and A8, add 50 µL of phosphate buffer and 100µL of fluorescein.
- Add 50 µL of phosphate buffer in in wells A7 to B8, and 100µL of fluorescein and 50µL of trolox (0.078nmol/L, 0.156nmol/L, 0.313nmol/L, 0.625nmol/L, 1.25nmol/L, 2.5nmol/L and 0.078nmol/L respectively).
- Into the rest of the wells (C1 to H12), add 50µL of phosphate buffer, 100µL of fluorescein and 50µL of samples (duplicated).
- After the plate is set, quickly add 100 µL of APPH all the wells (except blank: A1-A4) and insert the 96-well plate into the plate reader.
- Read the plate at an excitation wavelength of 485nm and an emission wavelength of 520nm, with a microplate data acquisition program.

- **Prior R.L. HH, Gu L., Wu X., Bacchiocca M., Howard L., Hampsch-Woodill M., Huang D., Ou B., Jacob R**. Assays for Hydrophilic and Lipophilic Antioxidant Capacity (oxygen radical absorbance capacity (ORACFL)) of Plasma and Other Biological and Food Samples. J Agric Food Chem 2003;51:3273-9.
- **Huang D, Ou B, Hampsch-Woodill M, Flanagan JA, Prior RL.** High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. 2002;50:4437-44. J Agric Food Chem. 2002;50: 4437-44.
- **Maarman GJ.** Melatonin as a novel cardioprotective therapy in pulmonary hypertension University of Cape Town; 2014

Catalase activity assay

- Catalase is an antioxidant enzyme that catalyzes the reduction of hydrogen peroxide into water.
- Dilute serum samples with phosphate buffer (K2HPO4.3H2O/NaH2-PO4.H2O, 0.075 mol/L, pH 7.4) (1:10 dilution).
- Dilute 30% hydrogen peroxide to the concentration of 12nM (150uL of hydrogen peroxide into 100mL phosphate buffer).
- On a UV-readable 96-well plate, pipette 10µL of phosphate buffer into the first two wells (A1 and A2).
- Add 10µL of phosphate buffer + 290µL of hydrogen peroxide solution into the wells A3 and A4.
- Insert the plate into the plate reader and read at 240nm to ascertain that the absorbance of the diluted hydrogen peroxide is approximately 0.523 ± 0.025.
- Pipette 10µL of diluted samples into the empty wells and add 290µL of diluted hydrogen peroxide to each of the samples well.
- Read at time point zero and one minute after point zero.
- Calculate catalase activity by the difference between the values recorded at this two time-point.

Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-6.

Superoxide dismutase activity (SOD) assay

Assay principle

The superoxide radical is produced enzymatically by the reaction catalyzed by Xanthine Oxidase (XOD): Oxidation of xanthine to uric acid with production of superoxide:

$$\text{Xanthine} + O_2 + H_2O \xrightarrow{XOD} \text{Uric acid} + O_2^{-}\cdot + H^+$$

Oxidized cytochrome C is reduced by superoxide radical and the rate of reduction is measurable spectrophotometrically at 550nm.

$$\text{Cytochrome}^{3+}\ c + O_2^{-}\cdot \longrightarrow \text{Cytochrome}^{2+}\ c + O_2$$

SOD inhibits/diminishes the reduction of cytochrome c by competing for the superoxide radicals.

$$2\ O_2^{-}\cdot + 2\ H^+ \xrightarrow{SOD} O_2 + H_2O_2$$

Reagents and Solution preparation

- Potassium phosphate buffer (216 mM; pH 7.8; 25°C): Add 49.3 g of Potassium phosphate dibasic trihydrate (HK_2PO_4) to 500 ml of purified water (H_2O) and adjust the pH to 7.8 before completion to the final volume of 1L.
- Ethylenediaminetetraacetic Acid Solution (EDTA, 10.7mM): Add 0.02 g of EDTA to 5 mL of H_2O.
- Cytochrome C solution (1.1mM): Dilute 14.6mg in 1mL of H_2O.
- Xanthine solution (0.108 mM): Dissolve 1.64 mg of xanthine in 90 mL of purified H_2O and adjust the pH to 7.7 with 1 N KOH. Transfer the solution to a 100ml volumetric flask and complete it to a final volume of 100 mL with purified H_2O.
- Cocktail reaction solution: Add 25 mL of phosphate buffer, 1 mL of EDTA, 1 mL of Cytochrome C, 50 mL of Xanthine solution and 28 mL of purified H_2O for a final volume of 100 mL in a container. Mix the solution and adjust the pH to 7.8 at 25°C.
- Immediately before use, prepare the SOD stock solution (5000U/mL) to make the standards as follows:

  Standard 1 (10U/mL): 2uL SOD stock in 998 µL of ice H_2O
  Standard 2 (20U/mL): 4uL SOD stock in 996 µL of ice H_2O
  Standard 3 (40U/mL): 8uL SOD stock in 992 µL of ice H_2O
  Standard 4 (80U/mL): 16uL SOD stock in 984 µL of ice H_2O

- Xanthine oxidase stock solution (5U/ml): Add 490 µL of Xanthine Oxidase to 510 µL of cold purified water and place on ice. Immediately before use, prepare 0.05U/mL XOD working solution from the stock solution (10 µL of stock XOD to 990 µL cold water).
- Check the XOD working solution by pipetting 280uL of cocktail solution in 4 wells of the 96 well-plate followed by 20 µL of H_2O in the 2 first wells (Blank) and 10

µL of H_2O + 10 µL XOD in the remaining wells (uninhibited reaction) and read the absorbance read at 550nm (A_{550}) every minutes during 5 min.

- Subtract the A_{550} of XOD by that of the blank. Values must be in the range 0.025 ± 0.005, otherwise, adjust the XOD concentration.
- Pipette the prepared reagents solution in suitable wells in duplicate:

  A1 – A2: Blank (280 µL cocktail solution + 20 µL H_2O)
  B1 – B2: XOD (280 µL cocktail solution + 16 µL H_2O + 4 µL XOD)
  C1 – C2 to F1 - F2: Standards (280 µL cocktail solution + 16 µL H_2O + 4 µL XOD + 10 µL SOD standards 1-4)
  H1 – H2 …: Samples (280 µL cocktail solution + 16 µL H_2O + 4 µL XOD + 10 µL serum)
- Read the plate using a microplate reader and record values for approx. 5 min.
- Calculate the fastest linear rate over the one-minute interval for the inhibited reaction and use this time interval to obtain the rates for each test (standards or samples) and blank.
- Calculate the final serum SOD activity using the SOD standard curve.

- **McCord, J. M., and Fridovich, I.** Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 1969; 244, 6049-55.

Recruitment questionnaire

Name: ……

Age: …… Occupation: ……..

Contact numbers: ……..

Address: ……

Email: ……

Country of Birth ……. Xhosa ……...

Weight: …… Height: ……

BMI: …… Weight stability last 6 months …….

Do you know your BP ……. Glucose …… Cholesterol …….

Do you donate blood or can blood be drawn from your arm easily? .....

Chronic medication……
……...
……...

Alcohol consumption drinks per week? ……..

Smoking……Contraception ……..

Are you pregnant or breastfeeding? .....

Menstrual cycle Regular …… Normal ….. Last start date …….

Disease info: HIV Status ……..
Hepatitis …….
Anemic……..

Availability 1 full day and when? ……
Morning and when? …….
Afternoon and when …….
Saturday

Exercise Intervention - Participant recruitment

Name	age	Weight	Height	BMI	Smoke?	Meds?	Contracep	Preg/BF?	Time?	HIV test?	Ex Train?	Phone nos.

IDENTIFICATION AND CONTACT DETAILS	
Name _____ ID number: _____ Date of Birth _____ Age: _____ Physical Address: _____ _____ Postal Address: _____ _____ E-mail_____ Tel No's: _____ (h) _____ (w) _____ (Cel) Alternative contact Person: _____ Tel No: _____ **TO BE KEPT SEPARATE FROM QUESTIONNAIRE DATA**	**SUBJECT CODE:**

CHECKLIST:

Date screened ☐ _____

Informed consent: ☐ _____ Subject info sheet ☐ _____

Pregnancy test: ☐ _____

BP ☐ _____

Fasting bloods: ☐ _____

OGTT: ☐ _____

Basic Anthropometry: ☐ _____

PAR Questionnaire: ☐ _____

FITNESS TEST ☐ _____

HIV Test: ☐ _____

DXA: ☐ _____

CT: ☐ _____

FEEDBACK ☐ _____ Sign sheet ☐ _____

INFORMED CONSENT

MOLECULAR CHANGES OF ADIPOSE TISSUE IN RELATION TO INSULIN SENSITIVITY IN BLACK SA WOMEN IN RESPONSE TO AN EXERCISE INTERVENTION.

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: 30-35 kg/m^2)
III. No known diseases or not taking medication for any diseases;
IV. Not smoking or taking recreational drugs;
V. Not currently pregnant or breast feeding;
VI. Self-reported race or ethnicity as black South African
VII. No muscle or joint pains or medical problems that prevent you from exercising;
VIII. Able to attend four exercise sessions per week for the 12 week study period;
IX. Weight stable (weight not changed more than 5 kg or no change in your clothes size over the past 6 months);
X. Are using injectable contraceptives to prevent pregnancy, for a minimum of 1 month prior to testing;
XI. Not currently taking part in organized physical 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 completesome tests on one day at David Flude Memorial Church hall 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 clinicif you are found to be HIV positive. If you test negative, you will be able to participate in the trial.
- **A urine pregnancy test** will be performed. If you are not pregnant 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 asked 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 at David Flude Memorial Church hall 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 3 testing sessions before and another 3 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, at the Academy of Plastic Surgery, Claremont, or CUBIC at 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:	**Screening session**	**Testing session 1**	**Testing session 2**	**Testing session 3**
Location:	David Flude Memorial Church hall, Khayelitsha	SSISA	CUBIC	Academy of Plastic Surgery
Time of day:	Any	8 am	8 am	8 am
Preparation:	None	Overnight fast and no exercise training for 72 hrs prior	Overnight fast and no exercise training for 72 hrs prior	Overnight fast and no exercise training for 72 hrs prior
Duration:	2 hrs	4 hrs	2 hrs	1 hr
Procedure:	-Trial information -Informed consent -Weight and height -Blood pressure -HIV screening - HbA1C -Urine test -Questionnaires -Accelerometer and Actipal fitted	-FSIGT -DXA scan	-MRS scan	-Fat biopsy

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 72hours (3 days) 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 on various measures of social and economic status (including your education, where you live and how many people share your home, the work that you do, how you spend your spare time), as well as questions on food security (access to food), 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.

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 mass. 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 motion sensors to wear (accelerometer, which measures how fast you move and an Actipal that measures how little you move) for 7 days. Both devices are the size of a small match box and will be attached to your waist with a lightweight belt. You should wear the monitors at all times, except when swimming, bathing, showering and sleeping.

Testing Session 2: Early morning before breakfast - CUBIC at Groote Schuur Hospital - 2 hours

Magnetic resonance spectroscopy (MRS) scans

The MRS scans will be performed at CUBIC at Groote Schuur Hospital, and will take approximately 1 hour. It 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.

Session 3: Early morning before breakfast – at the Academy of Plastic Surgery - 1 hour

You will be requested to go to the Academy of Plastic Surgery in Claremont in the morning after an overnight fast. In other words, you must not eat or drink anything, except water from 10 pm the night before (at least 10 hours). You may not take part in any exercise training for 72 hours (3 days) before this test:

Fat biopsy:

You will be requested to undergo a fat biopsy from your buttocks (bum) and your abdominal area (tummy). The samples will be used to analyse the proteins and hormones produced by the fat cells, which may influence your disease risk. A medical doctor (plastic surgeon) will perform the biopsy. A local anaesthetic will be administered prior to the procedure. Please inform us if you have had any previous reactions to any other anaesthetics, for example at the dentist. After the anaesthetic has taken effect, a small incision (0.5-1 cm) will be made in the skin, and fat samples (1.5 cm^3) will be removed using a needle connected to a syringe. After this procedure, a waterproof sterile dressing will be applied.

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 normal 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:

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, 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 monitors:

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

Magnetic resonance spectroscopy (MRS) scans

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.

Fat biopsy:

For the fat biopsies, you may feel some local stinging for a few seconds after the local anaesthetic is given.You will experience some discomfort during the biopsies, and after the biopsies you may experience some bruising, which will generally feel better within 2-3 days. We have performed many fat biopsies and have had very few adverse events. These included the temporary loss of feeling in the area around the biopsy site, which resolved on its own after a short while; and local infection, which healed without any problem after treatment. Rarely there may be an allergic reaction to the local anaesthetic or to the preservative in it, methylparaben, which could cause itching and if severe, wheezing or low blood pressure that are symptoms of anaphylactic shock. Severe reactions will be treated with adrenaline, which will be available during the procedure.

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. The researchers are trained in first aid and basic life support, and a phone will be carried at all times should medical assistance be required.

Are there any benefits to you for being in the study?

You will receive your own results, including body composition (weight, height, waist circumference and fat mass), 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. Detailed analysis of the tissues samples will take more time. However, once these 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 and fat samples be stored and used for research in the future?

The researchers will ask your permission to store your blood and fat 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 or tissue 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 or tissue for future research. If you decide not to donate blood or tissue 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, either transport will be provided or you will receive R60/day for transport to the testing facilities at UCT (R50 x 8 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: 021-6504570(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) 0729 255 347 (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:

MOLECULAR CHANGES OF ADIPOSE TISSUE IN RELATION TO INSULIN SENSITIVITY IN OBESE BLACK SOUTH AFRICAN WOMEN IN RESPONSE TO AN EXERCISE INTERVENTION

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 40-60 min of aerobic exercise training 4 days/week by a trained facilitator in David Flude Church 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 3 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 to have my weight, dietary intake and physical activity measured every month. I understand that the testing sessions will be performed at the Sports Science Institute, CUBIC at Groote Schuur Hospital and the Academy of Plastic Surgery in Claremont, 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, liver and pancreas, as well as a test to measure insulin secretion and sensitivity. I also understand that fat samples (~1.5 cm^3) will be taken from the fat stores in my abdominal (belly) and gluteal (bottom) area.
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. My 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 _____

I have fully and carefully explained the study to the person named above and confirm that to the best of my knowledge, they clearly understand the nature, risks and benefits of taking part in the study. I confirm that I have given them an opportunity to ask questions and answered their questions to the best of my ability.

Print Name of Researcher_____

Signature of Researcher _____

Date _____

Copy provided to participant _____ (initialled by researcher)

For illiterate participants (If possible the witness should be selected by the participant and not be part of the research team)

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ AND Thumb print of participant

Signature of witness _____

Date _____

Subject code:

CONSENT FOR HIV TESTING:

<u>Additional consent to:</u> Molecular changes of adipose tissue in relation to insulin sensitivity in obese black South African women in response to an exercise intervention.

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 "Molecular changes of adipose tissue in relation to insulin sensitivity in obese black South African women in response to an exercise intervention", conducted by the Division of Exercise Science and Sports Medicine, within the Department of Human Biology at the University of Cape Town.I am 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 _____

I have fully and carefully explained the study to the person named above and confirm that to the best of my knowledge, they clearly understand the nature, risks and benefits of taking part in the study. I confirm that I have given them an opportunity to ask questions and answered their questions to the best of my ability.

Print Name of Researcher_____

Signature of Researcher _____

Date _____

Subject code:

CONSENT FOR STORAGE AND FUTURE USE OF UNUSED SAMPLES:

Additional consent to: Molecular changes of adipose tissue in relation to insulin sensitivity in obese black South African women in response to an exercise intervention.

Information sheet:

We are seeking permission to store your unused blood and fat samples for possible future in either our own research or collaborators' research studies. After we have analysed your blood and fat samples for the current study we might want to do additional analyses on these samples. However, we require your permission before we can do any additional analyses on your blood and fat samples. The reasons why we might need to do additional analyses not specified at the start of the study could be due to new research that we are at present not aware of, but may be important in our understanding of the risk for type 2 diabetes. You can inform us if you do not want us to use your stored samples for any further analyses or only for certain types of testing such as genetic testing. 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: 021-6504570 (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 Committeemay 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:"Molecular changes of adipose tissue in relation to insulin sensitivity in obese black South African women in response to an exercise intervention".
   ☐ 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.

2) If any of the **fat** 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 **fat** sample to be destroyed immediately.
   ☐ I want my **fat** sample to be destroyed after ____ years.
   ☐ I give permission for my **fat** sample to be stored indefinitely

   AND if my **fat** sample is to be stored:
   ☐ I give permission for my **fat** sample to be stored and used in future research but only on the same subject as the current research project: "Molecular changes of adipose tissue in relation to insulin sensitivity in obese black South African women in response to an exercise intervention".

□ I give my permission for my **fat** sample to be stored and used in future research of any type, which has been properly approved

□ I give permission for my **fat** sample to be stored and used in future research except for research about _____

AND

□ I want my identity to be removed from my **fat** sample.

□ I want my identity to be kept with my **fat** 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 _____

For illiterate participants (If possible the witness should be selected by the participant and not be part of the research team)

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ **AND Thumb print of participant**

Signature of witness _____

Date _____
Day/month/year

I have accurately read or witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print Name of Researcher_____

Signature of Researcher _____

Date _____
Day/month/year

Copy provided to participant _____(initialled by researcher)

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

Room E53-46 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

28 November 2016

HREC REF: 827/2016

A/Prof J Goedecke
Division of ESSM
3rd Floor
Sports Science Institute
Newlands-7700

Dear A/Prof Goedecke

PROJECT TITLE: MOLECULAR CHANGES OF ADIPOSE TISSUE IN RELATION TO INSULIN SENSITIVITY IN OBESE BLACK SOUTH AFRICAN WOMEN IN RESPONSE TO AN EXERCISE INTERVENTION (PhD-candidate P Nankam) sub-study linked to 054/2015

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

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 November 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 student, P Nankam 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 to avoid exposure online

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.