

https://dspace.org/

The effect of exercise on metabolic and cardiovascular parameters changes in sprague-dawley rats receiving a short-term high fat-high sucrose diet

Shogor, Hager Omar

2016-10-14
http://hdl.handle.net/10539/21183

Downloaded from a DSpace repository. https://dspace.org/

THE EFFECT OF EXERCISE ON METABOLIC AND CARDIOVASCULAR PARAMETERS CHANGES IN SPRAGUE-DAWLEY RATS RECEIVING A SHORT-TERM HIGH FAT-HIGH SUCROSE DIET

Hager Omar Shogor

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand,

for the degree of Master of Science in Medicine.

Johannesburg, 2015

Declaration

I, Hager Omar Shogor, declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science of Medicine, in the Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa. The work in this thesis, has not been submitted for any degree or examination in this university, or any other university.

Hager Omar Shogor

Signed on the.....day of……2015

I certify that the studies contained in this dissertation have the approval by the Animal Ethics Screening Committee of the University of the Witwatersrand, Johannesburg. The ethics approval number is 2013/51/ 04

Hager Omar Shogor

.....

Signed on the.....day of…… 2015

....

Frederic Michel (supervisor) Alleta Millen (supervisor)

Date..... Date.....

Publications and presentations

Data presented in this dissertation have been presented at the 42st Annual Conference of the Physiology Society of Southern Africa held at the University of KwaZulu-Natal, September 2014. The title of the presentation was “Exercise does not improve diet-induced hypertriglyceridemia in young non-obese Sprague-Dawley rats”

Although during the course of my MSc I learnt the techniques of echocardiography and vascular reactivity, these are specialized techniques which require a high degree of skill which only comes with experience. Hence in the interests of collecting accurate and hence meaningful data experts collected these data.

Acknowledgements

Dr. Aletta Millen and Dr. Frederic Michel: for their supervision and all the valuable knowledge and skills they taught me. They have been good role models and inspired me a lot.

Prof. Gavin Norton and Prof Angela Woodiwiss: for their assistance in echocardiography.

Miss Monica Gomes: for her assistance in preparing the reagents that I used and for the preparing and processing of tissues for histology, and for assistance in determining plasma insulin content using the ELISA technique.

Miss Lebogang Mokotedi and Mrs Oluwatosin Tade: for their assistance with the feeding trial, experimental work and data collection.

I would like to express my most sincere gratitude to the staff of the Central Animal Services, Faculty of Health Sciences, University of the Witwatersrand, for their assistance with the care and welfare of the animals.

Abstract

Dyslipidaemia is a risk factor for cardiovascular disease. Triglycerides (TG), low density lipoprotein (LDL) and total cholesterol (TChol) concentrations are lower in physically active participants and are improved by exercise training. Whether the effect of exercise on diet-induced dyslipidaemia occurs independent of other metabolic syndrome parameters is nevertheless uncertain.

The aim of the present study was to investigate the effect of exercise training on dyslipidaemia, induced by a high-fat-high-sucrose (HFHS) diet independent of other metabolic syndrome features. 48 three-month-old Sprague-Dawley rats, selected for their ability to run in running wheels, were fed a HFHS diet or a standard chow diet (SC) for 9 weeks. Rats were randomly assigned to 4 groups: sedentary HFHS diet group (n=9); HFHS with exercise group (n=9); sedentary SC diet (n=9); and SC with exercise group (n=9). Both of the exercise groups were allowed to exercise voluntarily during the diet intervention. Fasting blood glucose (BG), insulin, glycated haemoglobin A1c (HbA1c), fasting blood triglyceride (TG) concentrations, body weight, daily food consumption, visceral adipose tissue weight, hepatic glycogen and lipid contents, liver weight, blood pressure, heart function and dimensions and vascular reactivity were measured.

After 9 weeks, body weights were significantly lower in both HFHS diet groups compared to SC diet groups ($p<0.0001$) despite a greater food intake in HFHS diet groups ($p<0.0001$). BG concentrations, insulin concentration, HbA1c, normalized visceral fat tissue, hepatic lipid contents, normalized liver weights, heart function and dimensions, and vascular reactivity were not different between the groups. Fasting TG and total cholesterol concentrations were significantly greater with a HFHS diet compared to SC groups ($p<0.0001$ and $p=0.029$, respectively). HDL concentration was significantly lower with a HFHS diet compared to SC groups ($p<0.0001$) Furthermore, liver glycogen contents were significantly greater with HFHS diet groups compared to SC diet groups ($p<0.0001$). Exercise did not change the blood lipid concentrations and the liver glycogen contents. Systolic blood pressure, but not

diastolic blood pressure, was significantly higher with a HFHS diet groups compared to SC diet groups (p=0.021 and p=0.0573, respectively). Exercise did not change the HFHS-induced increase in SBP.

In conclusion, exercise does not ameliorate HFHS diet-induced dyslipidaemia and elevated blood pressure in adult non-obese Sprague-Dawley rats without other metabolic syndrome parameter changes**.**

Table of contents

Declaration i

Publications and presentations ii

Acknowledgments iii

Abstract iv

Table of contents vi

List of figures x

List of tables xi

List of abbreviations xii

Chapter One: Introduction 1

1.1 Introduction 2

1.2 Problem statement 3

Chapter Two: Literature Review 6

2.1 Western diet 7

2.2 The effects of high fat-high sucrose diet on metabolic parameters 10

2.2.1 Metabolism of balanced and excessive fat intake 10

2.2.2 Metabolism of balanced and excessive carbohydrate intake 11

2.2.3 The effects of high fat-high sucrose diet on metabolic syndrome characteristics 12

2.2.3.1 Hyperglycaemia and insulin resistance 13

2.2.3.2 Obesity 14

2.2.3.3 Dyslipidaemia 15

2.2.3.4 Metabolic effects of a high fat-high sucrose diet in rats 16

2.3 The effects of high fat-high sucrose diet on cardiovascular parameters 23

2.4 Metabolic response to an exercise intervention 27

2.5 The effect of exercise on cardiovascular parameters 33

2.6 Aims and objectives 37

Chapter Three: Materials and Methods 38

3.1 Experimental groups and exercise protocol 39

3.2 Diet compositions and preparation..... 41

3.3 Measurements and procedures 41

3.3.1 Blood pressure measurement..... 41

3.3.2 Blood measurements..... 42

3.3.2.1 Fasting blood glucose and triglyceride 42

3.3.2.2 Lipid profile 42

3.3.2.3 Haemoglobin a1c..... 42

3.3.2.4 Serum insulin..... 42

3.4 Echocardiography 44

3.5 Vascular reactivity 46

3.6 Organs mass 47

3.7 Tibial length 47

3.8 Histology and aortic morphometric study 47

3.9 Measurement of liver lipid and glycogen..... 47

3.9.1 Hepatic lipid content 47

3.9.2 Hepatic glycogen content 48

3.10 Data analysis 50

Chapter Four: results 51

4.1 The effect of short term high fat-high sucrose diet and exercise on food intake, body weights, visceral adipose tissue weights, kidney weights and liver weights..... 52

4.2 The effect of short-term high fat–high sucrose diet and exercise on Cardiovascular parameters 56

4.2.1 The effect of short-term high fat–high sucrose diet and exercise on heart weights, dimensions and function as well as aortic wall thickness 56

4.2.2 The effect of short term high fat-high sucrose diet and exercise on blood pressure. 59

4.2.3 The effect of short term high fat-high sucrose diet and exercise on vascular reactivity 61

4.3 The effect of short-term high fat–high sucrose diet and exercise on metabolic parameters 64

4.3.1The effect of short-term high fat–high sucrose diet and exercise on fasting blood glucose, haemoglobin a1c and insulin level 64

4.3.2 The effect of short term-high fat high-sucrose diet and exercise on fasting blood triglyceride and lipid profile 67

4.3.3 The effect of short term- high fat high-sucrose diet and exercise on hepatic glycogen and lipid contents 70

Chapter Five: Discussion 73

5.1 The effect of High fat-high sucrose diet on body weight and tibial length 74

5.2 The metabolic effects of high fat-high sucrose diet 76

5.2.1 Blood glucose, serum insulin, glycated haemoglobin and hepatic glycogen contents 76

5.2.2 Blood lipids 78

5.3 The effect of high fat-high sucrose diet on cardiovascular parameters 80

5.3.1 Blood pressure 80

5.4 Exercise effect on metabolic and cardiovascular parameters: 81

5.4.1 The effect of exercise on body weight of animals fed a High fat-high sucrose diet 81

5.4.2 Exercise effect on blood glucose, serum insulin, glycated haemoglobin and hepatic glycogen content 83

5.4.3 Exercise and blood lipids 84

5.4.4 Exercise and blood pressure 85

Chapter Six: Conclusion 87

6.1 Conclusions 88

6.2 Limitations and future studies 89

6.3 Uniqueness and strength the current study 90

6.4 Possible clinical implications and recommendations 91

List of figures

Figure 3.1: Protocol with group assignation according to dietary/exercise intervention. 40

Figure 3.2: Measurements and procedures during the diet intervention and at termination. 43

Figure 3.3: M-mode echocardiographic image of the left ventricle of a high fat-high sucrose diet fed rat..... .45

Figure 3.4: photomicrography of thoracic aortic wall section. 49

Figure 4.1: Effect of short term high fat-high sucrose diet and exercise on body weights. .. 53

Figure 4.2: Effect of short term high fat-high sucrose and exercise on food intake. 54

Figure 4.3: Effects of short term high fat-high sucrose diet and exercise on blood pressure. 60

Figure 4.4: Effects of short term high fat-high sucrose diet and exercise on fasting blood glucose. 65

Figure 4.5: Effects of short term high fat-high sucrose diet and exercise on fasting blood triglycerides. 68

Figure 4.6: Effects of short term high fat-high sucrose diet and exercise on lipid profile. 69

List of tables

Table 2.1: Changes in metabolic syndrome parameters in rats consuming a high fat-high sucrose diet and experiencing dyslipidaemia 17

Table 2.2: Effect of high fat-high sucrose diet in rats without obesity..... 20

Table 2.3: Effect of exercise cardio-metabolic parameters in individuals with dyslipidaemia 29

Table 2.4: Effect of high fat-high sucrose diet and exercise in rats..... 32

Table 4.1: Effects of dietary intervention and exercise on tibia lengths, liver weights, right kidney weights and visceral adipose tissue weights. 55

Table 4.2: Effects of the dietary intervention and exercise on heart weights, left ventricular weights and right ventricular weights. 57

Table 4.3: Effect of short term high fat-high sucrose diet and exercise on left ventricular dimensions and functions as assessed in *vivo* 58

Table 4.4: Effect of short-term high fat-high sucrose diet and exercise on vascular reactivity in mesenteric artery 62

Table 4.5: Effect of short-term high fat-high sucrose diet and exercise on vascular reactivity in renal artery 63

Table 4.6: Effects of short-term high fat-high sucrose diet and exercise on Hemoglobin A1c and insulin level. 66

Table 4.7: Effects of short-term high fat-high sucrose diet and exercise on Hepatic glycogen contents and Hepatic lipid contents. 71

Table 4.8: Non-normally distributed data..... 72

List of abbreviations

A.Ch	acetylcholine
APO	apolipoprotein (apo)
Apo-B48	apolipoprotein B48
BG	llood glucose
BP	blood pressure
CHD	coronary heart diseases
ChREBP	carbohydrate response element binding protein
CRP	C - reactive protein
CVD	cardiovascular diseases
DASH	dietary-approach-to stop-hypertension
Emax	maximum contraction response
eNOS	endothelial nitric oxide synthase
EC50	concentration of agonist at 50% maximum response
ET-1	endothelin-1
FFA	free fatty acids
HbA1c	glycated haemoglobin
HDL	high density lipoprotein
HFHS	high-fat-high-sucrose
IDF	International Diabetes Federation
KCl	potassium chloride
LDL	low density lipoprotein

LVEDD	left ventricular end diastolic volume
LVESD	left ventricular end systolic diameter
MDA	malondialdehyde
MG	monoglycerides (MG)
NAFLD	non-alcoholic fatty liver disease
NEFA	non-esterified fatty acid
nfTG	non-fasting triglycerides
NO synthase	nitric oxide synthase
OGTT	oral glucose tolerance test
Phe	phenylephrine
PWED	left ventricular end diastolic posterior wall thickness
PWES	left ventricular end systolic posterior wall thickness
RNS	reactive nitrogen species
ROS	reactive oxygen species
SD	sprague-Dawley
SNP	sodium nitroprusside
SREBBP	sterol regulator element binding protein
TCA	citric acid cycle
TChol	total cholesterol
TG	triglycerides
VLDL	very low density lipoprotein
VPR	volume Pressure Recording

Chapter One: Introduction

1.1 Introduction

A large amount of refined carbohydrates, in addition to a large consumption of saturated fats and a high salt intake, are characteristic of a western diet (Chilton *et al*., 2014). According to current nutritional guidelines a high carbohydrate intake is considered to be > 45% of the total energy consumed per day and high fat to be > 30% of the total energy consumed per day (Feinman *et al*., 2015), while the amount of salt is considered high if it exceeds 2400 mg/day (Rhee *et al*., 2015). The western diet is also characterized by a lack of other nutrients such as vitamins, fiber and omega 3 fatty acids (Myles *et al*., 2014). Consumption of a western diet in different age categories (including children) may impact on growth and may induce nutrition-related diseases (Dearth-Weasley *et al*., 2011; Myles *et al*., 2014). Indeed, over consumption of non-nutritious food with excess caloric intake are associated with various chronic diseases such as immune-mediated diseases, obesity and its related diseases, cardiovascular diseases and disturbances in cognitive ability (Myles *et al*., 2014). A western diet is also one of the leading causes for the metabolic syndrome (Petkevičienė *et al*., 2012; Boers *et al*., 2014; Perez-Cornago *et al*., 2015). The metabolic syndrome is defined by a group of metabolic and cardiovascular risk factors including insulin resistance, dyslipidaemia, hyperglycaemia, obesity and hypertension (Grundy *et al*., 2005). However dyslipidaemia is also an independent risk factor for cardiovascular diseases. The prevalence of both dyslipidaemia and metabolic syndrome have increased significantly in the recent years (Jameson *et al*., 2013; Hong *et al*., 2014; Pinhas-Hamiel *et al*., 2015; Noubiap *et al*., 2015). In this regard current guidelines advocate lifestyle changes, including a healthy diet and physical activity as first line management for the prevention and management of life-style related metabolic and cardiovascular disease risk factors (Tur *et al*., 2013).

"Physical activity is defined as any bodily movement produced by the contraction of skeletal muscles that increases energy expenditure above resting levels and comprises of routine daily tasks such as commuting, occupational tasks, or household activities, as well as purposeful health-enhancing movements/activities" (Diaz *et al*., 2013, Pg.2). Physical

inactivity is a well-recognized behavioural risk factor for the development of non-communicable diseases including metabolic and cardiovascular disease (Milton *et al*., 2015, Horodyska *et al*., 2015) and has consistently been associated with an increased risk of mortality independent of general adiposity (Ekelund *et al*., 2015). Moreover, the evidence supports that physical inactivity, characterizing the western lifestyle, is pro-inflammatory and augments oxidative stress, whereas physical activity is anti-inflammatory (Dean *et al*., 2012). In contrast, intervention studies have demonstrated that regular physical activity reduces morbidity and mortality associated with coronary heart disease, hypertension, diabetes, and stroke (Young *et al*., 2014). In this regard current research focusses on the impact of exercise and dietary interventions in obese individuals and individuals with metabolic syndrome. However, the effects of exercise on metabolic and cardiovascular risk markers while consuming a westernized diet is not certain.

The current study imitated the western diet by utilizing a high fat-high sucrose (HFHS) diet model which is characterized by the concurrent inclusion of two western dietary components (saturated fat and sucrose). This study looked at the HFHS diet with and without exercise to ascertain the impact of the western diet as well as exercise on body weight, cardiovascular and metabolic parameters.

1.2 Problem statement

The worldwide shift in food patterns toward the consumption of a western diet has raised concerns regarding this diet (Amiri *et al*., 2014). The collateral rise in chronic non-communicable diseases has been connected to the western diet (Chambless *et al*. 1997; Hariharan *et al*., 2015; Brigham *et al*., 2015). Although the direct nutritional deficit of a western diet (fiber, minerals and vitamins) plays an important part, the abundance of macronutrients is still thought to be responsible for its chronic consequences.

Recently, several epidemiological studies have documented the rise in consumption of both simple carbohydrates and saturated fat (Baker *et al*., 2014; Bielemann *et al*., 2015; de Souza *et al*., 2015). Previous studies have investigated the effects of only one these compositions upon metabolism and other body functions, which does not reflect the spectrum of essential components regarding the western diet. Therefore, the HFHS (both sucrose and saturated fat) diet model has been defined as the best representative model for the western diet composition and the most suitably designed model to demonstrate the impact of western diet metabolism and its subsequent effect on cardiovascular system performance.

One of the chronic metabolic sequelae induced by the western diet is a high level of blood lipids, defined as dyslipidaemia. Dyslipidaemia is not only affecting older individuals but younger populations as well (Lambert *et al*., 2013; Wilson *et al*., 2015). Some of the well-known consequences of dyslipidaemia include atherosclerosis, coronary heart disease, hypertension, chronic kidney diseases, pancreatitis and fatty liver diseases (Ewang-Emukowhate *et al*., 2014; Li *et al*., 2014). Furthermore, dyslipidaemia has been found to affect vascular reactivity and impair the endothelial function in the coronary and forearm blood vessels of dyslipidemic individuals (Lambert *et al*., 2013). Dyslipidaemia may present distinctively or in association with several other disturbances including hyperglycaemia, insulin resistance, obesity, and hypertension, which are collectively known as metabolic syndrome (Grundy *et al*., 2005). Large epidemiological data revealed that the prevalence of metabolic syndrome in western communities has reached alarming levels and negatively impacts the active working population (Morikawa *et al*. 2013; Lao *et al*., 2014; DiBello *et al*., 2015).

Previous studies indicated that the habitual consumption of a western diet predisposes the human body to the disturbances that constitute metabolic syndrome (Saiki *et al*., 2007; Zhou *et al*., 2014). In this regard, it is crucial to identify and differentiate the contribution of a western diet for a short duration in the pathogenesis of dyslipidaemia and metabolic syndrome characteristics. Dyslipidaemia is under-diagnosed in the clinical practice and this

may be attributed to dyslipidaemia being underrated in non-obese individuals (Liao *et al*., 2004). The present study is therefore designed to provide a better understanding of the effect of a western diet-induced dyslipidaemia independently of other metabolic syndrome features, mainly obesity. Indeed it is important to assess the impact of a western diet in inducing dyslipidaemia and its consequences in non-obese individuals.

The consumption of a western diet is often associated with a sedentary lifestyle and lack of physical fitness. Physical activity in western communities has been decreasing due to long working hours and accessible transport, which makes individuals prone to a higher relative risk of cardiovascular diseases (Thijssen *et al*., 2010). According to current guidelines, implementing lifestyle changes could be effective in improving the metabolic parameters, as well as decreasing cardiovascular disease risk factors (Chobanian *et al*., 2003). In addition to being attainable and economic, exercise interventions may be the simplest in the line of management of cardiovascular disease risk factors. Previous studies have reported that lifestyle interventions including exercise and weight loss can alleviate some adverse cardiovascular and metabolic effects induced by a western diet (Golbid *et al*., 2012; Mohr *et al*., 2014; Prior *et al*., 2014; Beavers *et al*., 2014). However whether exercise can slow down or even prevent the adverse effects of a western diet independently of weight loss is not currently known. In order to institute effective therapeutic strategies, we need to determine to what extent an exercise intervention can slow the progression or prevent any cardiovascular or metabolic disturbances induced by a western diet. In particular, it is crucial to evaluate the effect of an exercise intervention on diet-induced cardiovascular risk factors including dyslipidaemia and metabolic syndrome.

Chapter Two: Literature Review

This chapter focuses on a specific western diet model, a high fat-high sucrose diet (HFHS), which incorporates both saturated fat and sucrose. In this regard the metabolic and cardiovascular disturbances induced by a high fat-high sucrose diet in rats, as well as the effect of a western diet in humans, will be highlighted. Finally, this chapter briefly reviews the effect of exercise on metabolic and cardiovascular changes induced by the consumption of a western diet.

2.1 Western diet

Despite the characteristic abundance of a western diet in both refined carbohydrates and saturated fat, western diet models vary in the nature of these macronutrients and also in the proportions of these macronutrients (Soria *et al*., 2001; Novak *et al*., 2012; Martinez *et al*., 2012; Tremblay *et al*., 2013; Panet *et al*., 2014; Fernando *et al*., 2014). Western diet models have been used for different purposes, such as to investigate the mechanisms by which the diet could predispose to a certain disease (Barnard *et al*., 1993; Bunnag *et al*., 1997; Carmiel-Haggai *et al*., 2005; Christon *et al*., 2005; Bourgoin *et al*., 2008), to compare the effect of different compositions in different models (Buettner *et al*., 2006; Boqué *et al*., 2009; Chun *et al*., 2010) or to investigate the specific effect of an interventional procedure in an already established disease by the western diet (Gonçalves *et al*., 2004; Arias *et al*., 2014; Cheng *et al*., 2014). Importantly, the contribution of the simultaneous inclusion of multiple macronutrients in the pathogenesis of chronic metabolic diseases is the focus of recent investigations (Mariotti *et al*., 2008; Lomba *et al*., 2010; Kabel *et al*., 2014). The HFHS diet has then been established to investigate the effect of a western diet on several physiological functions. The HFHS diet's composition is formulated to reflect the proportions of macronutrients in the modern western diet. In this regard, the principal fat used in the HFHS diet is saturated animal fat (Muraki *et al*., 2011, Ochiai *et al*., 2013) or vegetable fat (Rivera *et al*., 2006; Paulino *et al*., 2010) and the carbohydrate used is sucrose.

Despite recent nutritional recommendations that the consumed amount of saturated fat should not exceed 10% of total daily energy (Weech *et al*., 2014), there has been a considerable increase in the consumption of saturated fat in western societies (Weech *et al*., 2014). The current medical advice is not to decrease the fat intake, but rather change the type of fat to unsaturated fat (Benitez *et al*., 2015). Several studies have found that saturated fat predisposes to several diseases through its systemic inflammatory effect (Clarke *et al*., 2009; Mu *et al*., 2014) and oxidative stress (Lithander *et al*., 2013). Saturated fat increases the risk of metabolic disturbances (Tierney *et al*., 2011) and of high blood pressure (Stamler *et al*., 1996). Indeed, a high intake of saturated fatty acids is associated with greater low density lipoprotein (LDL), total cholesterol (TChol) and triglycerides (TG) (Esser *et al*., 2013; Ortega *et al*., 2013; Virtanen *et al*., 2014; Benitez *et al*., 2015). Saturated fat negatively affects the vascular system by preventing the beneficial anti-inflammatory role of high density lipoprotein (HDL) on the endothelium (Nicholls *et al*., 2008) and by decreasing vascular flow mediated dilatation (Vafeiadou *et al*., 2015). Saturated fat is also involved in the development and the rupture of atherosclerotic plaque (Kralova *et al*., 2013). In contrast, the high levels of unsaturated fat (mono or poly unsaturated fat), present in the Mediterranean diet, has shown to lower blood lipids concentrations (Kralova *et al*., 2013), lower coronary heart disease (CHD) risk (Eschen *et al*., 2004; Benitez *et al*., 2015), to improve vascular reactivity (Newens *et al*., 2011) and to normalise blood pressure (Rasmussen *et al*., 2006).

As described above, saturated fat, as a paramount component of the western diet, has devastating metabolic and cardiovascular effects. Another western diet component that is frequently associated with a negative health outcome is sucrose, a simple carbohydrate predominantly used in western food to enhance its sweet taste (Lowndes *et al*., 2014). Sucrose is a disaccharide carbohydrate composed of glucose and fructose, two monosaccharide molecules (Sørensen *et al*., 2014). Sucrose is hydrolyzed into its constituting monosaccharides by an enzyme, called sucrase, which facilitates the absorption

by the gastro-intestinal tract of both the glucose and fructose. The effects of sucrose start by the absorption of glucose, a rise in blood glucose concentration, and a subsequent increase of pancreatic insulin secretion. Additionally, over consumption of carbohydrates including sucrose, in amounts exceeding the energy requirements and the liver's ability for glycogen deposition, induces *de novo* lipogenesis (Aarsland *et al*., 1997; Strable *et al*., 2010). Although *de novo* lipogenesis can be induced by a diet high in fat, it is mainly triggered by excess carbohydrates (Flatt et *al.*, 19970). *De novo* lipogenesis converts the excessive amounts of carbohydrate into lipids, either by incorporating the excess of FFA as TG for storage or oxidizing it into energy (Strable *et al*., 2010).

The economic affordability and worldwide availability of sucrose has contributed to maximizing its usage (Gangwisch *et al*., 2015). Sucrose has been recognised as being involved in the pathogenesis of several metabolic and cardiovascular diseases (Soria *et al*., 2001; Chicco *et al*., 2003; Harmancy *et al*., 2012; Mather et *al*., 2001; Santure *et al.,* 2002; Bunnag *et al*., 1997). Although the World Health Organisation (WHO) recommendation is to lower sugar intake to less than 10% of the total daily energy (Rugg-Gunn *et al*., 2007), the energy input from consumed sweeteners has risen in the last couple of decades (Lowndes *et al*., 2014).

It is evident that the current upsurge in the consumption of both saturated fat and sucrose, that exceeds the nutritional recommendations, has deleterious side-effects. What is discussed above regarding the composition of the western diet justifies then the integration of saturated fat and sucrose (among other western diet compositions) in a representative western diet called HFHS diet. Further assessment of their excessive intake on cardiovascular and metabolic status is discussed in next section.

2.2 Effect of high fat-high sucrose diet on metabolic parameters

The consumption of food loaded with saturated fat and refined carbohydrates may account for the negative impact of a western diet on metabolism (Riccio *et al*., 2015). Despite the fact that the end products of carbohydrates and fat metabolism may affect the body on the genetic, cellular and systemic levels (Riccio *et al*., 2015), this section focuses only on the systemic effects of these dietary components.

2.2.1 Metabolism of balanced and excessive fat intake

Dietary lipids (mostly triglycerides, TG) are absorbed in the intestine after which it is hydrolysed by the pancreatic enzymes into free fatty acids (FFA) and monoglycerides (MG). Both are combined with bile products (emulsification) to be absorbed by intestinal cells and re-synthesized into TG (Beaslas *et al*., 2009). TG subsequently attach to apolipoprotein in the endoplasmic reticulum of the intestinal cell to produce a chylomicron which is secreted from the intestine into the lymph and then into general circulation (Beaslas *et al*., 2009). Energy demanding situations, including daily activities such as exercise and fasting induce fatty acid oxidation (defined as lipolysis). The production of acetyl CoA and energy is therefore used for the ongoing vital operations in the body such as respiration, maintaining cell structure and skeletal muscles activities (Koppo *et al*., 2010; Frühbeck *et al*., 2014). The equilibrium between fat utilization and storage usually keeps TG in a low and balanced situation, whereas, the consumption of a high amount of either simple carbohydrates or saturated fats may be responsible for metabolic disturbances (Novak *et al*., 2012).

The lipoprotein lipase induces the storage of absorbed fatty acids in adipose tissue and other tissues for later use (Goldberg et al., 1996). The inability of enzymes (involved in fatty acid oxidation and the citric acid cycle (TCA cycle) to handle the high flux of fatty acids results in less utilization of absorbed fatty acids (Bruce *et al*., 2009). Overfeeding of a diet composed of a high amount of fat induces less oxidation of the absorbed fatty acids and

more storage (Horton *et al*., 1995, Gibson *et al*., 2007). In healthy individuals, ingestion of a diet rich in fat results in greater concentrations in LDL and TChol compared to the consumption of a low fat diet (Tremblay *et al*., 2013). Consuming a diet high in fat, even for a short period of time, also leads to an increase in plasma FFA in sedentary individuals (Edwards *et al*., 2010). Hence, a lack of balance between the dietary intake of fatty acid and its processing induces an increased lipid accumulation in different organs which may result in further metabolic disturbances such as impaired insulin secretion (Man *et al*., 1997), lipotoxicity in heart muscles (Turkieh *et al*., 2014) and the expansion of visceral adipose tissue (Lyer *et al*., 2012; Novak *et al*., 2012). Finally, non-alcoholic fatty liver diseases, non-alcoholic steatohepatitis, as well as renal injury and proteinuria through obesity related-glomerulopathy, are ultimate consequences induced by a diet rich in fat and the subsequent elevated plasma FFA concentration (Pan *et al*., 2014).

2.2.2 Metabolism of balanced and excessive carbohydrate intake

Dietary carbohydrates are absorbed in the small intestine and hydrolysed by enzymes into simple carbohydrates (Sørensen *et al*., 2014). Sucrose is hydrolysed by sucrase into glucose and fructose (Sørensen *et al*., 2014). In turn, the resultant simple carbohydrates are oxidized by cellular mitochondria into pyruvate (Flatt *et al*., 19970). Pyruvate is then oxidized into acetyl CoA and by-product energy as adenosine triphosphate (ATP) (Aarsland *et al*., 1997). In this way, the metabolised carbohydrate produces energy for basic use in the cells and maintains the energy balance.

The surplus glucose from the metabolised excess carbohydrate will be stored as glycogen in the liver (in a limited capacity) and in the skeletal muscles under the effect of insulin (Bollen *et al*., 1998; Randle *et al*., 1999). Further exuberant glucose above the body's limited capacity to store glycogen, is directed for *de novo* lipogenesis (Schwarz *et al*., 1995; Kersten *et al*., 2001; Ferré *et a*l., 2010). Furthermore, insulin stimulates the transcriptional factors

sterol regulator element binding proteins (SREBP) and the high blood glucose level induces carbohydrate response element binding protein (ChREBP) which are both responsible transcriptional factors in the upregulation of fat synthesis (Osei-Hyiaman *et al*., 2008). Insulin, released by high blood glucose following high sucrose intake, induces the activation of hepatic fatty acid synthase which is one of the main enzymes in the *de novo* lipogenesis (Flatt *et al*., 19970). The excess glucose oxidation results in the accumulation of acetyl CoA. This accumulated acetyl CoA forms the main substrate for *de novo* lipogeesis and the formation of fatty acids which are released from the liver as triacylglycerol fatty acids (TGFA) and very low density lipoprotein (VLDL) (Schonfeld *et al*., 1971). The high release of secreted VLDL in the blood causes an increase in blood lipids and may be responsible for the development of dyslipidaemia (Schonfeld *et al*., 1971). Additionally, the excessive amount of absorbed fructose causes the dysregulation of hepatic metabolism by directing the resultant carbon into *de novo* lipogenesis and further accumulation of lipids in the blood (Basciano *et al*., 2005; Merino-Aguilar *et al*., 2014).

2.2.3 The effect of a high fat-high sucrose diet on metabolic syndrome characteristics

Metabolic syndrome is a cluster of cardiovascular and metabolic disease risk factors including abdominal obesity, insulin resistance, elevated blood pressure, atherogenic dyslipidaemia, pro-thrombotic state and a pro-inflammatory state (Grundy *et al*., 2005). Different criteria have been proposed for the diagnosis of metabolic syndrome in humans. The WHO requires a marker of insulin resistance and an additional two risk factors of the previously mentioned risk factors. The International Diabetes Federation (IDF) requires obesity as the main cornerstone for the diagnosis of metabolic syndrome in addition to a further two risk factors (Grundy *et al*., 2005). However in rats, consumption of a HFHS diet may induce metabolic syndrome or most of the risk factors constituting metabolic syndrome (Barnard *et al*., 1993; Yamamoto *et al*., 2006; Pranprawit *et al*., 2013). The concurrent consumption of large amounts of both sucrose and saturated fat by rats has been shown to

induce more profound metabolic disturbances than the consumption of a high sucrose or a high fat diet alone (Osabe *et al*., 2008; Ragab *et al*., 2015). Most of the metabolic syndrome characteristics are interrelated and can be induced by consumption of a HFHS diet.

2.2.3.1 Hyperglycaemia and insulin resistance

Blood glucose concentration is marked by the equilibrium between glucose uptake and glucose metabolism (Harber et al., 2005). The abnormally high blood glucose is defined as hyperglycaemia (Matsui et al., 2012). Hyperglycaemia has been reported previously in animal studies with different models of the western diet (Matsui et al., 2012; Martinez et al., 2012; Fernando et al., 2014). Although not all animal studies observed a change in blood glucose concentration with the HFHS diet (Ivanova *et al*., 2011; Medford *et al*., 2012) rats consuming the HFHS diet may develop hyperglycaemia (Sugatani *et al*., 2006; La Favor *et al*., 2013; Zhou *et al*., 2014; Zhang *et al*., 2015). An increased serum insulin concentration and insulin resistance have been also observed in rats fed the HFHS diet (Chun *et al*., 2010; Pierine *et al*., 2014; Cheng *et al*., 2014; Méndez *et al*., 2014; de la Garza *et al*., 2015). In contrast, other studies have reported no change in insulin levels (Ivanova *et al*, 2011; Fisher-Wellman *et al*., 2013; La Favor *et al*., 2013). Hyperglycaemia is a recognized feature of insulin resistance. A majority of the studies have reported a concurrent elevated blood glucose level with an increased insulin concentration (Sugatani *et al*., 2006; Zhou *et al*., 2014; Zhang *et al*., 2015). However, some studies have still reported no change in insulin concentration despite the elevated blood glucose concentration (Fisher-Wellman *et al*., 2013; La Favor *et al*. 2013). It is possible that, in these studies, the rats may not have developed insulin resistance yet. However, this could also be due to the effect of the short duration of the studies on both the glucose and insulin levels. Nevertheless, as discussed above, there is still controversy about whether a HFHS diet will result in insulin resistance and an elevated blood glucose level.

2.2.3.2 Obesity

Despite the role of a genetic predisposition, obesity is mainly attributed to high energy consumption or/and low energy expenditure which might be due to low activity (Roberts *et al*., 2002). An acknowledged dietary factor which influences visceral adiposity is the saturated fat content of the food (Sun *et al*., 2013), while the carbohydrate content of food is the main contributor to weigh t gain (Westman *et al*., 2007; Hession *et al*., 2009; Hite *et al.*, 2010). Thus, obesity is a frequent consequence of the consumption of a western diet (Pratt *et al*., 2010; la Fleur *et al*., 2010; Uriarte *et al*., 2013; Pierine *et al*., 2014) and hence a cornerstone of the development of metabolic syndrome. Obesity is associated with a high incidence of chronic metabolic diseases including diabetes mellitus, dyslipidaemia, non-alcoholic liver disease and chronic kidney diseases (Kamimura *et al*., 2013; Noborisak *et al*., 2013; Belczak *et al*., 2014; Huetter *et al*., 2014). Insulin resistance, associated with obesity, may be the main contributor to the pathogenesis of these chronic diseases (Pierine *et al*., 2014). Increased visceral fat mass (characterizing obesity) induces inflammatory, oxidative and metabolic changes (Pierine *et al*., 2014). Beta-cell dysfunction and the subsequent abnormal insulin secretion, as well as inability to regulate the glucose level, are associated with obesity (Zhang *et al*., 2001). The accumulated adipose tissue also plays an essential role in insulin resistance through increasing the release of FFA which induces muscle to resist insulin action through impairing the insulin signalling pathway (Kelley *et al*., 2001; Jellinger *et al*., 2007). It is also well known that obesity is characterised by inflammation, which may result in insulin resistance (Pradhan *et al*., 2007).

In addition to insulin resistance, obesity has been found to be associated with abnormal glucose metabolism, glucose intolerance and hyperglycaemia (Pozzan *et al*., 1997; Edelstein *et al*., 1997; Stolk *et al*., 1997). Impaired glucose tolerance, as a phase of glucose intolerance, indicates a high risk for development of diabetes (Tuomilehto *et al*., 2001). Insulin resistance in individuals with a high body mass index (BMI) is more likely to progress to diabetes mellitus (Resnick *et al*., 1998; Matsumoto *et al*., 2001). Furthermore, high fasting

blood glucose in obesity indicates a higher than normal incidence of progression to diabetes (Edelstein *et al*., 1997; Tuomilehto *et al*., 2001). Chronic hyperglycaemia is known to be associated with increased protein kinase C and several biochemical pathways like protein glycation, polyol and glucose autoxidation (de Carvalho *et al*., 2012). These pathways subsequently increase the oxidative stress (de Carvalho *et al*., 2012).

2.2.3.3 Dyslipidaemia

Another metabolic syndrome feature resulting from the HFHS diet is dyslipidaemia (Sugatani *et al*., 2008; Bourgoin *et al*., 2008; Pranprawit *et al*., 2013; Zhou *et al*., 2014; Kabel *et al*., 2014). Dyslipidaemia is a metabolic disturbance defined by high concentrations of TChol, TG and LDL, in addition to a low HDL level (Nelson *et al*., 2013). Dyslipidaemia is also marked by the ratio of TChol to HDL, as it integrates information about LDL and TG (Wietlisbach *et al*., 2013). Dyslipidaemia can be classified as primary (genetic defect) or secondary (environmental causes) (Nelson *et al*., 2013; Teramoto *et al*., 2014). In both primary and secondary dyslipidaemia, despite the genetic susceptibility in primary dyslipidaemia, diet is still a strong predisposing environmental factor (Teramoto *et al*., 2014). Nevertheless, the main factor responsible for secondary dyslipidaemia is a diet rich in saturated fat and high in sugar (Bourgoin *et al*., 2008; Kabel *et al*., 2014; Zhou *et al*., 2014). A HFHS diet induces an increase in production and a decrease in clearance of blood lipids (Robert *et al*., 2004). A defect in lipoprotein lipase functioning and a decreased expression of VLDL and LDL receptors are the main contributors to the raised blood lipids concentration (Robert *et al*., 2002). In addition, impaired hepatic catabolism of cholesterol and abnormal hepatic enzyme activities are also responsible for the development of dyslipidaemia (Robert *et al*., 2004; Kabel *et al*., 2015). Moreover, hyperglycaemia and insulin resistance have also been associated with dyslipidaemia (Chicco et al., 2003; Harmancey et al., 2012) and elevated level of TG in the blood, in the liver as well as in the muscle (Ochiai et al., 2013). Indeed, insulin resistance affects apoprotein production and the regulation of lipoprotein lipase in the

liver which may predispose to dyslipidaemia in association to hyperglycaemia (Goldberg et al., 2001).

2.2.3.4 Metabolic effects of a high fat-high sucrose diet in rats

Despite the evidence of altered metabolic status due to a western diet in humans, a number of studies have investigated these effects in animals. A summary of the animal studies assessing the effect of a HFHS diet on metabolic syndrome parameters in rats experiencing dyslipidaemia are provided in Table 2.1. In this table, the various studies sued diets that were composed of sucrose between 20 and 65% and between 20 and 77% fat. Different rat species had been used including Sprague-Dawley, Wistar, Hanover and Fischer rats. The age of the animals varied between 4 weeks and 4 months (Sugatani *et al*., 2008; Ochiai *et al*., 2013; Zhang *et al*. 2015). The studies lasted between 3 weeks and 22 months with the most pronounced metabolic responses noted over the longer duration (Saiki *et al*., 2007; Zhou *et al*., 2014) and with higher carbohydrate content (Bourgoin *et al*., 2008; Sun *et al*., 2013; Pranprawit *et al*., 2013; Zhou *et al*., 2014).

In most of these studies, the HFHS diet-induced dyslipidaemia is associated with a concomitant increase in glucose concentration and secretion of insulin (Bourgoin *et al*., 2008; Sugatani *et al*., 2008; Pranprawit *et al*., 2013; Ochiai *et al*., 2013; Sun *et al*., 2013; Zhou *et al*., 2014; Kabel *et al*., 2014; Zhang *et al*., 2015). This may be attributed to the decompensation of pancreatic beta-cells. However, no changes in blood glucose concentration and plasma insulin concentration were observed in one study (Ivanova *et al*., 2011), which could be attributed to the beta-cell of the pancreases compensating with an increase in mass to maintain normoglycemia. According to table 2.1, dyslipidaemia may also be seen in conjunction with either obesity (Roberts *et al*., 2004; Bourgoin *et al*., 2008; Yamamoto *et al*., 2010; Pranprawit *et al*., 2013; Sun *et al*., 2013; Kabel *et al*., 2014) or without obesity (Roberts *et al*., 2002 ; Saiki *et al*., 2007; Sugatani *et al*., 2008;

Table 2.1: Changes in metabolic syndrome parameters in rats consuming high fat-high sucrose diet and experiencing dyslipidaemia

Article	Strain	Age/B.wt	Diet composition	Duration	Blood lipids	Blood glucose	Insulin	B.wt	Bp
Zhang *et al*., 2015	Male Wistar	6 weeks	20% protein, 34% starch, 15% sucrose, 20% fat	8 weeks	↑	↑	↑	--	##
Kabel *et al*., 2014	Wistar	100–150 g	fat emulsion (77% fat, 14% protein, 9% starch) +18% sucrose solution	8 weeks	↑	↑	↑	↑	##
Zhou *et al*., 2014	Sprague-Dawley	200–250 g	65% sucrose, 25% fat, 10% protein	48 weeks	↑	↑	↑	↑	##
Ochiai *et al.*, 2013	Male Wistar	4 weeks	30% protein, 20% sucrose, 14.8 % starch, 25% fat	8 weeks	↑	↑	↑	--	##
Pranprawit *et al*., 2013	Sprague-Dawley	9 weeks	19.6% protein, 40% fat, 41% sucrose	4 weeks or 8 weeks	↑	↑	↑	↑	##
Sun *et al*., 2013	Sprague-Dawley	180–220 g	16.89% protein, 39.39% fat, 43.7% sucrose	8 weeks	↑	↑	↑	↑	##
Ivanova *et al*. 2011	Male Wistar	12 weeks	34.7% fat, 30% sucrose	48 days	↑	--	--	--	##
Yamamoto *et al*., 2010	Male Sprague-Dawley	7 weeks	15.5% fat, 40 % sucrose, 20.5% protein	4 weeks	↑	##	##	↑	↑
Bourgoin *et al*., 2008	Male Sprague-Dawley	6 weeks	21% protein, 39% fat, 41 % sucrose	4 weeks	↑	↑ unfasted	↑	↑	↑
Sugatani *et al*., 2008	Male Wistar	6 weeks	23.9% fat, 56.8% sucrose, 19.3% protein	3 weeks	↑	↑	↑	--	##
Mariotti *et al*. 2008	Wistar–Hanover	130-140 g	19.25 % protein, 14.5% fat, 48.7%	9 weeks	↑	↑	↑	--	##

	male		sucrose						
Saiki *et al*., 2007	Male Sprague-Dawley, female Wistar Kyoto	4 months	23.4% protein, 11.0% fat, 6.3% starch, 15% sucrose solution	12 months	↑	↑	↑	--	##
Roberts *et al*., 2004	Female Fischer	2 months	40% sucrose, 39% fat	20 months	↑	##	##	↑	↑
Roberts *et al*., 2002	Female Fisher	2 months	40% sucrose, 39% fat	22 months	↑	##	##	--	↑

(↑) increased, (↓) decrease, (--) No change, (≠≠) not measured. B.wt, Body weight; Bp, Blood pressure.

Mariotti *et al*., 2008; Ivanova *et al.*, 2011; Zhang *et al*., 2015). Hence, these studies suggest that a HFHS diet is one of the causes that predispose to dyslipidaemia and may be associated with other metabolic syndrome disturbances. However, the mechanism by which concomitant occurrence of dyslipidaemia and the other metabolic defects occur is still not clear. Furthermore, what is the effect of a HFHS diet on the metabolic status in absence of obesity? The relevant animal studies showing the metabolic impact of a HFHS diet without inducing obesity are summarised in Table 2.2. In these studies, the diets were composed of between 20 and 48% sucrose and 10 and 62% fat and lasted from 3 weeks up to 22 months. The rat species included Sprague-Dawley, Wistar, Hanover and Fischer rats and their ages varied between 4 weeks and 4 months with younger rats more resistant to obesity (Medford *et al*., 2012; La Favor *et al*. 2013; Fisher-Wellman *et al*., 2013). Insulin levels were greater in Wistar rats (Sugatani *et al*., 2008; Ochiai *et al*., 2013; Etxeberria *et al*., 2013; Arias *et al*., 2014; Zhang *et al*., 2015). Longer studies showed that greater glucose concentration (Robert *et al*., 2002; Souza *et al*., 2007; Saiki *et al*., 2007) and dyslipidaemia is noted with high carbohydrate content (Sugatani *et al*., 2008; Mariotti *et al*., 2008; Zhang *et al*., 2015).

From the table, it is shown that the non-obese animals consuming a western diet still showed considerable metabolic side effects. A few of these studies showed that non-obese animals (animals that did not gain a significant amount of weight) consuming the HFHS diet experienced hyperglycaemia (Saiki *et al*., 2007; Souza *et al*., 2007; Mariotti *et al*., 2008; Ochiai *et al*., 2013; Fisher-Wellman *et al*., 2013; Etxeberria *et al*., 2013; Arias *et al*, 2014). Most of the studies with an increased glucose concentration, report a simultaneous elevated insulin concentration (Saiki *et al*., 2007; Mariotti *et al*., 2008; Etxeberria *et al*., 2013; Ochiai *et al*., 2013; Arias *et al*., 2014). This suggests that hyperglycaemia and insulin resistance induced by a western diet may be present without obesity. It is also interesting to note that no change in glucose levels (Ivanova *et al*., 2011; Medford *et al*., 2012) or a glucose level lower than the control group (which may be the result of liver disease) (Hirotani *et al*., 2012) has also been reported in a rats fed a HFHS diet without obesity. Similarly, several studies in

Table 2.2: Effect of high fat-high sucrose diet in rats without obesity

Article	Strain	Age/B.wt.	Diet composition	Duration	Blood glucose	Insulin	Blood lipids	Others
Zhang *et al*., 2015	Male Wistar	6 weeks	20% protein, 34% starch, 15% sucrose, 20% fat	8 weeks	↑	↑	↑	
Arias *et a.l*, 2014	male Wistar	6 weeks	22.5% fat, 20% sucrose	6 weeks.	↑	↑	##	No difference in free fatty acids
Ochiai *et al.*, 2013	male Wistar	4 weeks	30% protein, 20% sucrose, 14.8 % starch, 25% fat	8 weeks	↑	↑after OGTT	↑TChol --TG	↓Adiponectin,↑ liver TG,↓ Lpl activity
Fisher-Wellman *et al.*, 2013	Male Sprague-Dawley	5 weeks	14.8% protein, 44.6% fat, 34 % sucrose, 6.2% starch	12 weeks	↑	--	--	↓mitochondrial H_2O_2 in heart, ↑in RG
Etxeberria *et al.*, 2013	Male Wistar	12 weeks	20% proteins, 35% sucrose, 45% fat	63 days	↑	↑	##	Hyperleptinemia, no difference in serum total protein, ↓serum urea
Hirotani *et al*., 2012	Male Wistar	180-190 g	23% protein, 25.3% sucrose, 35% fat	12 weeks	Lower than control	##	TG TChol lower than control	↑liver weight, ↑AST, non-alcoholic fatty liver disease
La Favor *et al*., 2013	male Sprague-Dawley	5, 9 &13 weeks	34% sucrose, 6% starch, 23% fat, 17.3% protein.	12weeks	↑	--	--	Vascular reactivity changes
Medford *et al*., 2012	Male Sprague-Dawley	4 weeks	40% fat, 15% protein, 31% sucrose, 14% starch	52 weeks	--	##	-- TG-TChol	↑HbA1c, lower indices of renal function, no different in (tibia length, liver& kidney weight)
Ivanova *et al.*, 2011	Male Wistar	12 weeks	34% fat, 30% sucrose	48 days	--	--	↑(TG,LDL, VLDL)	No difference in heart weight and systolic BP,↓ collagen I and III in LV
Sugatani *et al*., 2008	Male Wistar	6 weeks	23.9% fat, 56.8% sucrose, 19.3% protein	3 weeks	↑	↑	↑	

Roberts *et al*., 2002	Female Fisher	2 months	40% sucrose, 39% fat	22 months	↑	##	##	Increased BP
Mariotti *et al.*, 2008	Wistar–Male Hanover	130-140 g	19.25 % protein, 14.5% fat, 48.7% sucrose	9 weeks	↑	↑	↑TAG↑HDL, ↓ T.Chol	↓blood glutathione. No change in blood pressure
Saiki *et al.*, 2007	Male Sprague-Dawley, female Wistar Kyoto	4 months	23.4% protein, 11.0% fat, 6.3% starch, 15% sucrose solution	12 months	↑	↑	↑TChol	↑liver (cholesterol, TG, relative weight), ↑plasma ALT, oxidative stress and ↑lipid peroxide
Souza *et al.*, 2007	Wistar	60-day	25% sucrose, 23% starch, 25% protein, 10% fat	4 months	↑ by GTT	##	##	(Altered insulin sensitivity), ↑ Protein oxidation in frontal cortex
Rivera *et al.*, 2006	Male Wistar	300–325 g	32% fat, 48% sucrose, 20% protein	6 weeks	##	--	##	No difference in leukocyte number in blood, Occasional cytoplasmic lipid accumulation in liver
Grimditch *et al.*, 1987	Female Sprague-Dawley	125-150g	21% protein, 40% fat, 39 % sucrose	10-12 weeks	--by IVGTT	↑	##	No different in sarcolemmal vesicles orientation

(↑) increased, (↓) decrease, (--) No change, (≠≠) not measured. B.Wt, Body weight; BP, Blood pressure, HbA1c, LPL, lipoprotein lipas

rats have not reported a change in insulin concentration (Riviera *et al*., 2006; Ivanova *et al*., 2011; Medford *et al*., 2012). To note, one study showed an increased in insulin concentration without a change in blood glucose concentration (Grimditch *et al.*, 1987). From these studies we can conclude that the simultaneous occurrence of a high insulin level and hyperglycaemia (in the absence of obesity) can be attributed to the strain and the age of the rats or the duration and the composition of the diet. However, it is not clear what the main metabolic defect is that contributes to these changes.

A HFHS diet may induce dyslipidaemia with an absence of obesity (Saiki *et al*., 2007; Mariotti *et al*., 2008; Ivanova *et al*., 2011; Ochiai *et al*., 2013). A HFHS diet induces metabolic abnormalities such as lipid accumulation in the liver and abnormal liver enzymes (Ochiai *et al*., 2013; Saiki *et al*., 2007). A HFHS diet in non-obese animals has been shown to induce decreased activity of lipoprotein lipase and adiponectin, an enzyme and hormone responsible for blood lipid processing (Ochiai *et al*., 2013). Another noticeable consequence of consuming a HFHS diet in the absence of obesity is the increase of adipose tissue (Ivanova *et al*., 2011; Fisher-Wellman *et al*., 2013). Adipose tissue may secrete substances affecting lipid metabolism (Ivanova *et al*., 2011; Fisher-Wellman *et al*., 2013). In this respect, adipocytes are well known to produce adipo(cyto)kines and other inflammatory substances (Zhou *et al*., 2014), as well as an elevation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which, subsequently, can affect lipid metabolism (Boqué *et al*., 2009). On the other hand, some studies did not report dyslipidaemia with a HFHS diet in the absence of obesity (Medford *et al*., 2012; Etxeberria *et al*., 2013; Fisher-Wellman *et al*., 2013; La Favor *et al*. 2013). An increase in the activity of leptin, an adipo(cyto)kine secreted by adipose tissue and responsible for energy equilibrium, may explain the absence of dyslipidaemia and obesity with a HFHS diet (Etxeberria *et al*., 2013).

The above discussion highlights that a HFHS diet, as the model for a western diet, can induce considerable metabolic disturbances. Furthermore, the evidence from this discussion shows that controversy still exists over the occurrence of simultaneous metabolic changes

associated with dyslipidaemia in the absence of obesity. Mainly, it is not clear whether dyslipidaemia induced by a HFHS diet is associated with other metabolic syndrome defects without obesity. Therefore, it is crucial to illuminate the role of a HFHS diet in the development of dyslipidaemia with or without metabolic syndrome characteristics in animals which present no obesity.

2.3 The effect of a high fat-high sucrose diet on cardiovascular parameters

An important system affected by the consumption of a western diet is the cardiovascular system. The different components of the western diet, namely high saturated fat and high sucrose, affect the cardiovascular system in different ways. Among several causes, one factor thought to increase the prevalence and to further exacerbate cardiovascular diseases is the unhealthy composition of the western diet (Robert *et al*., 2004; Baumann *et al*., 2015). Although lifestyle intervention and dietary intervention are considered first line therapy in the management of cardiovascular disease (Folsom *et al*., 2015; Masset *et al*., 2015), it remains important to ascertain to what extent the western diet predisposes or initiates the cardio-metabolic risk factors and to determine whether HFHS diet in absence of obesity may induce dyslipidaemia and other metabolic syndrome features.

Clinical trials have shown that cardiac and metabolic risk factors are linked. For instance, a HFHS diet predispose to dyslipidaemia (Sugatani *et al*., 2008; Bourgoin *et al*., 2008; Pranprawit *et al*., 2013; Zhou *et al*., 2014; Kabel *et al*., 2014), which is associated with numerous cardiovascular risk factors including high blood pressure and atherosclerosis (Barnard *et al*., 1998; Yamamoto *et al*., 2006). Furthermore hypertension, one of the leading risk factors for cardiovascular disease, is strongly associated with obesity, insulin resistance and uncontrolled diabetes (Roca *et al*., 2005). Despite the fact that cardiovascular disease risk factors in relation to metabolic syndrome has been widely investigated, and despite the considerable effect of a western diet in inducing cardiovascular diseases, the mechanism by

which a western diet predisposes to simultaneous multiple cardiovascular risk factors is still not clear and needs to be elaborated. In this regard, a HFHS diet has been shown to induce considerable cardiovascular disturbances, including an increase in cardiac output (Seymour *et al*., 2015), raised atherogenic index (Kabel *et al*., 2014), predisposing to oxidative stress (Lomba *et al*., 2010; Fisher-Wellman *et al*., 2014), inducing vascular dysfunction (Bourgoin *et al*., 2008; La Favor *et al*., 2013), and increased blood pressure (Barnard *et al*., 1993; Robert *et al*., 2001; Bourgoin *et al*., 2008; Yamamoto *et al*., 2006; Xie *et al*., 2013; Kong *et al*., 2014).

One of the major risk factors of cardiovascular disease is hypertension. Substituting a HFHS diet with a diet low in fat and high in complex carbohydrates reversed the elevated blood pressure, which emphasises the impact of a HSFS diet on blood pressure elevation (Roberts *et al*., 2002). Moreover, in a dietary-approach-to-stop-hypertension (DASH) clinical trial, following a diet high in fat with a healthy diet intervention led to a lowering of blood pressure (Appel *et al*., 1997). In this regard, DASH study enrolled 459 individuals whose blood pressure less than160/95 mmHg. The participants received a diet high in fruit, vegetable and low-fat dairy product and low in saturated fat (Appel *et al*., 1997). This diet intervention last for 8 weeks. Furthermore, several animal studies have shown the impact of a HFHS diet in the development of hypertension. The elevation of blood pressure has been reported in a longer duration (20 to 24 months) studies (Barnard *et al*., 1993; Robert *et al*., 2001; Kong *et al*., 2014) but also in a shorter duration (3 to 4 weeks) diet intervention with a HFHS diet (Yamamoto *et al*., 2006; Bourgoin *et al*., 2008). Dyslipidaemia and endothelial dysfunction have been found to be responsible for elevated blood pressure following consumption of a HFHS diet (Bourgoin *et al*., 2008). Dyslipidaemia and endothelial dysfunction are, however, not the only recognized factors in inducing the elevation of blood pressure. A HFHS diet has been found to predispose to renal injury and glomerulopathy (Kong *et al*., 2011) as well as oxidative stress through enhancing dietary lipid peroxidation (Roberts *et al*., 2002) in addition to increasing inflammatory markers which all predispose to elevate blood pressure (Robert

et al., 2005; Pierine *et al*., 2014). Furthermore, a HFHS diet causes atherosclerosis (Munshi *et al*., 2014). Inflammation is known to be involved in the pathogenesis of atherosclerosis (Koizumi *et al*., 2013).

Although metabolic syndrome is associated with the risk of developing cardiovascular disease, obesity is associated with the development of cardiovascular disease (Klein *et al*., 2004). First, there is evidence to support that obesity may be a major contributor to the development of hypertension (Harris *et al*., 2000). In this regard, obesity induces vascular dysfunction which results from abnormal vasomotor tone, enhanced vasoconstriction and decreased vasodilatation (Steinberg *et al*., 1996). Vascular dysfunction associated with obesity may be mediated by a decreased Nitric Oxide synthase (NOS) activity (Walther *et al*., 2015). Vascular abnormalities related to obesity are associated with inflammation and oxidative stress, which leads to endothelial dysfunction and decreased nitric oxide bioavailability (Steinberg *et al*., 1996). In addition, obesity causes an increased activation of the renin-angiotensin-aldosterone system, ultimately resulting in an elevated blood pressure (Hajer *et al*., 2008). Furthermore, obesity induces micro and macro vascular dysfunction. Obesity leads to anatomical vascular changes, such as an increased wall to lumen ratio and the microvascular rarefaction (a decrease in the number of arterioles or capillaries) of various tissues, which contributes to an increased blood pressure (Serné *et al*., 2007). Obesity is associated with increased plasma FFA concentration (Brotman *et al*., 2002). This vascular impairment is believed to be mediated through FFA and inflammatory mediators released from the adipose tissue (de Jongh *et al*., 2004). Table 2.2 summarises studies of the HFHS model without obesity. HFHS without obesity is still able to induce the impairment of vascular reactivity (La Favor *et al*., 2013) and cause oxidative stress (Saiki *et al*., 2007). Other changes in the hearts of rats consuming the HFHS diet (lower collagen I and collagen III in the ventricular tissue) were noticed (Ivanova *et al*., 2011). Finally, a HFHS diet intervention did not elevate the blood pressure in rats without obesity (Mariotti *et al*., 2008; Ivanova *et al*., 2011). However one study reported an increase in blood pressure in the

absence of obesity (Roberts *et al*., 2002). The discrepancy may be attributed to the different responses from different rat species. While the studies that reported an increase of blood pressure used Sprague-Dawley and Fischer rats, the ones that did not report an increase of blood pressure used Wistar rats.

Dyslipidaemia is a well-recognized risk factor for coronary heart disease (CHD) (Fattore *et al*., 2014). Although a low level of HDL is an independent risk factor for anticipating cardiovascular events (Ono *et al*., 2012), LDL plays the most important part in the atherogenicity of blood lipids (Chan *et al*., 2006; Jia *et al*., 2008). Table 2.1 highlights the studies for rats affected with dyslipidaemia following consumption of a HFHS diet. Even though not all of the studies in tables 2.1 measured blood pressure or assess cardiovascular function, dyslipidaemia has been shown to be associated with changes in heart and vascular functions. Three studies reported an increase in blood pressure accompanied with dyslipidaemia in obese rats (Roberts *et al*., 2004; Bourgoin *et al*., 2008; Yamamoto *et al*., 2010). Dyslipidaemia in obese rats has been reported simultaneously with a high atherogenic index, as well as an increased insulin level or a raised C-reactive protein (CRP) concentration (Robert *et al*., 2004). These are all cardio-metabolic risk factors. Animals consuming a HFHS diet, but without inducing obesity, exhibit also some adverse effects on the heart and the vasculature as well (Saiki *et al*., 2007; Ivanova *et al*., 2011; Ochiai *et al*., 2013).

From the aforementioned investigations, it is clear that consuming a HFHS diet and obesity both have profound negative impacts on cardiovascular performance. Nevertheless, it has been shown that a HFHS diet is still able to induce cardiovascular disturbances without obesity. Despite the abundant information on the western diet effect, the mechanism by which a HFHS diet could predispose to cardiovascular disturbances in the absence of obesity is still not clear. Furthermore, we need to illuminate more on the mechanism by which a western diet induces development of concurrent cardiovascular risk factors in the absence of the obesity.

2.4 Metabolic response to an exercise intervention

The metabolic effect of various exercise training regimens has been widely investigated in different population groups, including healthy persons (Schneider *et al*., 2009; Oberbach *et al*., 2010; Stepto *et al*., 2012; Heinonen *et al*., 2012) and persons with obesity (Coker *et al*., 2006; Bocalini *et al*., 2012; Khadir *et al*., 2015), diabetes mellitus (Yavari *et al*., 2010; Kadoglou *et al*., 2013; Motahari-Tabari *et al*., 2014; Lowenstein *et al*., 2014; Kluding *et al*., 2015) or hypertension (Erikson *et al*., 1997; Whelton *et al*., 2002; Mohr *et al*., 2014). Although the effect of exercise training has been studied in persons with metabolic abnormalities, these studies are often confounded by weight loss, as it is well known that weight reduction is associated with an improved metabolic profile (Lang *et al*., 2011., Mason *et al*., 2011; Fisher *et al*., 2012; Prior *et al*., 2014; Beavers *et al*., 2014). Therefore, the effect of exercise training in persons with metabolic abnormalities in the absence of weight loss is not clear. In this section I will highlight some studies showing whether exercise can ameliorate the characteristic metabolic abnormalities in metabolic syndrome with or without weight change.

Exercise is one lifestyle intervention that may attenuate the different risk factors constituting the metabolic syndrome. The effect of exercise on the risk factors of metabolic syndrome will be discussed in relation to a change in body weight. One of those risk factors is insulin resistance. Firstly, aerobic exercise enhances insulin sensitivity independent of changes in body weight or body composition (Larsen *et al*., 2014). Secondly, improvement in insulin sensitivity is accompanied by an improvement in mitochondrial function in diabetics (Meex *et al*., 2010). Thirdly, high intensity exercise has been shown to improve insulin–stimulated glucose disposal in obese subjects without decreasing body weight (Coker *et al*., 2006), whereas medium intensity exercise does not (Coker *et al*., 2006). However, exercise accompanied with weight reduction results in a down-regulation of myostatin mRNA and improves insulin sensitivity in obese older individuals (Ryan *et al*., 2013). Conversely, Trachta *et al* (2014) reported no change in insulin levels and blood pressure following

aerobic exercise despite decreased body weight. Furthermore, exercise did not improve the insulin resistance in individuals with metabolic syndrome without loss of body weight (Stuart *et al*., 2013). Although there is evidence that exercise improves insulin sensitivity, there is clearly controversy on this matter. What is the evidence that exercise mitigates hyperglycaemia?

Evidence from clinical trials showed an improvement in hyperglycaemia with aerobic exercise. In this regard, aerobic exercise with concomitant weight loss in diabetics and prediabetics improved impaired glucose tolerance (Vancea *et al*., 2009; Malin *et al*., 2012; Solomon *et al*., 2013). This improvement is mediated through the enhancement of insulin sensitivity (Malin *et al*., 2013). Although some studies showed no improvement in glycated haemoglobin (HbA1c) following aerobic exercise (Vancea *et al*., 2009; McNeilly *et al*., 2012, Tunar 2012), other clinical trials showed that aerobic exercise can improve HbA1c (Sung *et al*., 2012; O'Neil *et al*., 2012; del Pozo-Cruz *et al*., 2014). Despite the controversial results obtained with regard to glycated haemoglobin, exercise may be effective in reducing the fasting blood glucose (Arciero *et al*., 1999; Vancea *et al*., 2009; Malin *et al*., 2013). Therefore, there is still a debate regarding the impact of exercise on glucose metabolism. Moreover, the effect of exercise on other metabolic disturbances, such as dyslipidaemia and obesity, need to be discussed further.

Although the effect of exercise on glucose metabolism is controversial, the effects of exercise on other metabolic syndrome characteristics, specifically obesity and dyslipidaemia, have also been investigated. There is a large amount of evidence provided by previous studies regarding the effect of aerobic exercise on dyslipidemic individuals. A summary of the studies on the effect of aerobic exercise on metabolic and cardiovascular diseases risk markers in humans is provided in Table 2.3. In this table, aerobic exercise was the only lifestyle intervention method introduced. In addition, whether exercise in dyslipidemic participants resulted in weight loss or not and whether the weight loss was associated with

Table 2.3: Effect of exercise on cardio-metabolic parameters in individuals with dyslipidaemia

Article	Starting BMI/B.Wt	Number of participants	Intensity	Duration	Blood pressure	Glucose	Insulin	Body weight	Blood lipid
Ficker *et al*., 2010	27±5	40	Moderate	4 months	##	##	##	--	↓ LDL ↑HDL –TG
Camhi *et al*., 2010	25±2	328	60-80% max HR	1 year	--	--	--	--	--
Sixt *et al*., 2010	90-98 kg	23	80% of max HR	6 months	##	↓	##	--	↓TC↓LDL
Watkins *et al*. 2003	>25	53	70-80% max HR	3 months	--	--	↓	--	--
Green *et al*., 2003	26.8-28.8	75	70-85% max HR	8 weeks	--	--	↓	--	--
Lewis *et al*., 1999	29	9	65% max HR	4 weeks	##	##	##	--	---
Watt *et al*., 1976	179-183Ib	60	Low intensity	12 weeks	##	##	##	--	--
Coghill *et al*., 2008	28	67	Moderate	12 weeks	↓SBP	--	--	↓	↓TG↓LDL↑ HDL
Yoshida *et al*., 2010	24.6	25	Moderate	16 weeks	≠≠	≠≠	≠≠	↓	↓TC↓LDL
Yoo *et al*., 2003	26	54	Aquatic	12 weeks	--	--	≠≠	↓	↓TG↑ HDL
Roussel *et al*., 2009	29-35	153	60 % max HR	12 months	↓	≠≠	≠≠	↓	↓TG↓TC↓LDL↑ HDL
Drevenhorn *et al*., 2007	28-30	177	Intermediate to high	15 months	↓SBP	≠≠	≠≠	↓	↓TG
Slentz *et al.,* 2005	29.6	225	Vigorous/ moderate	8 months	≠≠	≠≠	≠≠	↓	↓ LDL
Stewart *et al*., 2005	29.4	104	60-90% max HR	6 months	↓	--	--	↓	↓TC↓LDL↓TG↑ HDL
Kraus *et al.,* 2002	29.3	111	High /moderate	6-8 months	≠≠	≠≠		↓	↓LDL↓TG↑ HDL
Couillard *et al*., 2001	24-28	200	55-75% vo_2 max	20 weeks	≠≠	≠≠	↓	↓	↓TG↑ HDL

(↑) increased, (↓) decrease, (--) No change, (≠≠) not measured, (SBP) systolic blood pressure

changes in cardiovascular or metabolic risk factors, was highlighted. Some studies showed an improvement in TChol levels as well as LDL, TG and HDL levels following a period of aerobic exercise and significant loss of body weight (Kraus *et al*., 2002; Stewart *et al*., 2005; Drevenhorn *et al*., 2007; Roussel *et al*., 2009; Yoshida *et al*., 2010). In a few other studies, there was an improvement in dyslipidaemia following exercise despite an unchanged body weight (Ficker *et al*., 2010; Sixt *et al*., 2010). Yet, in some other studies, there was no improvement of dyslipidaemia and no loss of body weight following aerobic exercise (Watt *et al*., 1976; Lewis *et al*., 1999; Watkins *et al*., 2003; Green *et al*., 2003; Camhi *et al*., 2010). In particular, the duration and intensity of the exercise was the same in two studies (Rousell *et al*., 2009; and Camhi *et al*., 2010). However, there was no change observed in blood lipid and body weight in Camhi study whereas both lipid concentrations and body weight were improved in Rousell study. The observed difference may be attributed to the fact that the population in Camhi study was selected as metabolic syndrome while the Rousell study population were dyslipidemic individuals. The presence of metabolic syndrome characteristics could delay/prevent the improvement of blood lipids.

It is evident from these studies that the simultaneous effect of exercise and weight loss on dyslipidaemia was beneficial. This can be reflected positively in patients with metabolic syndrome in that a decrease in body weight accompanied by improved dyslipidaemia will reduce the cardiovascular risk factors in the metabolic syndrome figure. The reviewed studies showed that exercise intensity was inversely proportional to the impact on blood lipid and body weight (Stewart *et al*., 2005; Slentz *et al*., 2005; Drevenhorn *et al*., 2007). In the aforementioned studies the number of participants was clearly larger with committed weight reduction. Furthermore, from the studies in table 2.3, we can summarize that a greater loss of body weight is seen in individuals with a higher starting body mass index. Importantly, these studies present with a number of limitations such as the short duration of some studies that did not give enough time for exercise to induce significant changes. Also, not all the studies measured all the risk factors of metabolic syndrome and this may restrict a

comprehensive comparison with the measured parameters in the current study. Moreover, when interpreting these studies, one must take into consideration the differences between the studies with regards to in the intensity and duration of the exercise interventions. Thus, we can extract from Table 2.3 that once exercise ameliorates obesity it will improve the accompanied dyslipidaemia. On the other hand, exercise was less effective in the improvement of dyslipidaemia without decreased body weight.

The acute effect of exercise following consumption of a specific meal and postprandial hyperlipidemia was widely investigated. Postprandial hypelipidemia is attenuated following high intensity exercise (Ferreira *et al*., 2011; Gabriel *et al*., 2012). It is preferable to investigate exercise and postprandial hyperlipidemia in humans rather than intervention with a diet known to be harmful, which cannot be followed for a long time because of ethical considerations. To my knowledge there have not been many exercise studies with a western diet over a long period of time. Therefore, animal models have been developed for longer interventions in order to investigate the effect of diet and exercise on the metabolic status of rats and the results are subsequently translated for human benefit. In this regard the extent of aerobic exercise on metabolic abnormalities induced by a western diet studied in animal model of the HFHS diet is shown in Table 2.4. In these studies, the effect of a HFHS diet without exercise was compared to the effect of exercise in conjunction with a HFHS diet. The HFHS diet in this table composed of a sucrose or a sucrose and starch from the rat's chow. A HFHS diet without exercise induced obesity (Grimditch *et al*., 1988; Gauthier *et al*., 2006; Paulino *et al*., 2010; Ennequin *et al*., 2015), high blood glucose level (De Moraes *et al*., 2008; La Favor *et al*., 2013), blood TG level (De Moraes *et al*., 2008; Gerbaix *et al*., 2013) and insulin resistance (Grimditch *et al*., 1988; De Moraes *et al*., 2008). The addition of aerobic exercise (running on a treadmill or running wheels) to the HFHS diet prevented most of these changes, including the elevated TG level (De Moraes *et al*., 2008; Gerbaix *et al*., 2013), increased blood glucose (Grimditch *et al*., 1988; Fisher-Wellman *et al*., 2013) and insulin

Table 2.4 Effect of high fat-high sucrose diet and exercise in rats

Article	Strain	Age	Diet	Duration	Effect of diet	Effect of exercise
Grimditch *et a*l., 1988	Male Sprague-Dawley	10 weeks	40 % fat 39 % sucrose, 21 % protein	10 weeks	↑ body weight glucose intolerance Insulin resistance	↓ body weight Prevent glucose intolerance Prevent insulin resistance
Gauthier *et al*., 2003	Female Sprague-Dawley	6 weeks	42 % fat, 36 % sucrose, 22 % protein	8 weeks	↑ body weight ↑hepatic lipid	↓body weight ↓hepatic lipid
De Moraes *et al*., 2008	Male Wistar	10 weeks	26 % fat, 56 % sucrose, 18 % protein	12 weeks	↑ triglycerides↑glucose ↑insulin impaired (V.R)	↓ triglycerides No effect on glucose↓ insulin ↓body weight improves (V.R)
Paulino *et al*., 2010	Male Wistar	4 weeks	54% starch,20% fat, 20% protein, 30% sucrose solution	Diet for 25 weeks Exercise for 10 weeks	↑ body weight, LV systolic dysfunction, No effect on BP	↓ body weight Prevent ↓ LV fractional shortening, No effect on BP
La Favor *et al*., 2013	Male Sprague-Dawley	5 weeks	34% sucrose, 6% starch, 23% fat, 17.3% protein.	12 weeks	↑ body weight ↑ glucose – insulin oxidative stress-impaired (V.R)	↓body weight, ↓total cholesterol ↓oxidative stress-improve (V.R)
Fisher-Wellman *et al*., 2013	Male Sprague-Dawley	5 weeks	14.8% protein, 44.6% fat, 34 % sucrose, 6.2% starch	12 weeks	↑ fat mass ↑fasting blood glucose – insulin Oxidative stress	Prevent ↑ fat mass, Prevent ↑ blood glucose, Improve oxidative stress
Gerbaix *et al*., 2013	Male Wistar	11 months	24.4 % fat, 22.2 % sucrose, 22.2% starch 20.4% protein	8 weeks exercise4 months diet	Obesity, ↑triglycerides ↓leptin ↓adiponectin	↓body weight, ↓ triglycerides ↓ leptin ↓adiponectin ↑NEFA
Ennequin *et al*., 2015	Male Wistar	7 months	24.4 % fat, 22.2 % sucrose, 22.2% starch, 20.4% protein	16 weeks + 8 weeks	↑ body weight, did not alter signalling pathway of neuregulin 1-no effect on glucose and insulin	↓body weight, improve signalling pathway of neuregulin 1-no effect on glucose and insulin

(↑) increased, (↓) decrease, (--) No change, (≠≠) not measured, ↑NEFA (non-esterified fatty acid), a (artery), LV left ventricular

resistance (Grimditch *et al*., 1988; De Moraes *et al*., 2008). Importantly however, the exercise intervention in a majority of these studies modified the metabolic status and obesity. Hence this concurrent change does not allow the assessment of the impact of exercise alone, independent of a change in body weight.

To conclude, both human and animal evidence provided by the literature argued that exercise can ameliorate metabolic disturbances. Although exercise may improve some metabolic changes, there is still debate about whether exercise can do so without a loss of body weight, in particular, if exercise can ameliorate dyslipidaemia without a loss of body weight. Therefore, it is very important to elucidate the potential role of exercise in improving dyslipidaemia in animals that does not exhibit weight loss.

2.5 Effect of exercise on cardiovascular parameters

Although it is well known that exercise has a beneficial effect on cardiovascular disease risk factors (Williams *et al*., 2013; Mohr *et al*., 2014; Rodrigues., 2014) in persons with established metabolic syndrome risk factors, it is not clear whether exercise alone can prevent the progression to cardiovascular diseases, independent of intervention with a healthy diet (Steven *et al*., 2002; Christou *et al.,* 2005; Millen *et al.,* 2014).

Nevertheless physical activity is widely recommended as an important lifestyle change that may help in the prevention of hypertension (Diaz *et al*., 2013; Huang *et al*., 2013; Brown *et al*., 2013). As hypertension is strongly associated with cardiovascular diseases risk, early intervention, either at the general population level or by targeting high risk populations such as overweight, physically inactive persons or those with a family history of hypertension, could lower the incidence of hypertension and its subsequent adverse effects (Erikson *et al*., 1997; Whelton *et al*., 2002; Golbid *et al*., 2012; Mohr *et al*., 2014). In this regard, several mechanisms have been suggested for blood pressure reduction as a result of exercise training including, changes in blood vessel function and structure, modulation of the renin-

angiotensin system and decreased sympathetic nervous system stimulation (Golbid *et al*., 2012). On the other hand, aerobic exercise interventions have shown controversial results in hypertensive individuals with other metabolic syndrome risk factors. While some studies showed no improvement in the blood pressure of hypertensives with metabolic syndrome characteristics (Arsenault *et al*., 2009; Lima *et al*., 2012), others showed a decrease in blood pressure. However those studies that have shown an improvement in blood pressure after an exercise intervention reported an accompanied weight reduction (Malin *et al*., 2012; Choo *et al*., 2014). What is the evidence that exercise, independent of weight loss, can improve cardiovascular risk in people who already have dyslipidaemia? A summary of the most pertinent studies assessing the effect of aerobic exercise on cardiovascular disease risk factors in dyslipidemic individuals with other cardio-metabolic risk factors is provided in Table 2.3. Four studies reported that aerobic exercise in dyslipidemic individuals resulted in improved blood pressure in conjunction with weight loss (Stewart *et al*., 2005; Drevenhorn *et al*., 2007; Coghill *et al*., 2008; Roussel *et al*., 2009). However, it is not known whether it is the exercise per se or the combination of exercise and weight loss that resulted in the improved blood pressure. Moreover, there was no change in blood pressure in those dyslipidemic individuals after the aerobic exercise intervention without a change in body weight (Watkins *et al*., 2003; Green *et al*., 2003; Camhi *et al*., 2010). Yoo *et al* (2003) showed no change in blood pressure despite the decreased body weight. Furthermore, what is the evidence that exercise can improve other cardiovascular risk markers in individuals with dyslipidaemia?

In this regard Table 2.3 shows several studies with improved blood pressure in conjunction with the improvement of dyslipidaemia (Stewart *et al*., 2005; Drevenhorn *et al*., 2007; Coghill *et al*., 2008; Roussel *et al*., 2009;). The simultaneous improvement of dyslipidaemia and blood pressure following exercise may emphasise the role of high blood lipids in the pathogenesis of blood pressure. Nevertheless, Yoo *et al* (2003) reported that blood pressure did not decrease despite the improved dyslipidaemia in another study. Additionally, in these

studies the improvement of dyslipidaemia and blood pressure was accompanied by weight loss. Hence, the effect of aerobic exercise on blood pressure was obviously in positive association with the change in body weight, where the decrease of body weight was accompanied by an improvement of blood pressure as well as dyslipidaemia. On the other hand, the effect of exercise on dyslipidaemia and blood pressure without loss of body weight is still not clear and needs to be investigated further.

Further to cardiovascular disease risk factors, aerobic exercise is also reportedly effective in improving cardiac function in several diseases. Aerobic exercise in diabetic patients improves maximum oxygen consumption and insulin sensitivity (Schrauwen-Hinderling *et al*., 2011). In heart failure patients, exercise leads to a simultaneous increase in left ventricular end diastolic volume (LVEDD) and arterial volume (Sahlén *et al*., 2011), and increases left ventricular filling pressure (Borlaug*et al*., 2011) in patients with heart failure with a preserved ejection fraction. Furthermore, the effect of aerobic exercise has been investigated on several vasculatures as the renal and cerebral vasculatures (Murrel *et al*., 2013). Without loss in body weight, exercise has been shown to improve the endothelial function in pre-hypertensive and hypertensive individuals (Kouamé *et al*., 1995; Beck *et al*., 2014). Furthermore, exercise improves cerebrovascular reactivity and decreases stroke risk and age related cerebral atrophy (Linkis *et al*., 1995; Murrel *et al*., 2013).

Similarly, animal studies provide evidence regarding the effect of exercise on cardiovascular disease risk factors. Animal studies where an aerobic exercise intervention was implemented with a HFHS diet model, are summarized in Table 2.4. All of these studies were performed exclusively on rats. From the results, it is seen that in one study, despite the increased body weight, the blood pressure did not change with consumption of a HFHS diet and did not change with exercise, despite the loss of body weight (Paulino *et al*., 2010). Further to the cardiovascular disease risk factors, one study reported that the exercise intervention resulted in improved coronary artery endothelial function (La Favor *et al*., 2013) and two others reported a decrease in oxidative stress, a well-known contributing factor to

endothelial dysfunction (Fisher-Wellman *et al*., 2013; La Favor *et al*., 2013). Furthermore, Paulino *et al* (2010) studied the impact of exercise on the heart. They reported that exercise prevented the worsening of cardiac function in a long duration intervention with a HFHS diet (Paulino *et al*., 2010). Notably, these studies induced obesity with a HFHS diet in their animals and the exercise intervention decreased the body weight of these rats (Paulino *et al*., 2010; Fisher-Wellman *et al*., 2013; La Favor *et al*., 2013). Hence, the effect of exercise could be mediated through the weight reduction. Therefore, it is not clear whether the impact of exercise on the heart and vascular reactivity dysfunction induced by a HFHS diet is due to the loss of weight or due to the impact of the exercise itself. Therefore, it is essential to investigate the cardiovascular effect of exercise on animals that do not display weight loss to clarify the effect of exercise rather than the impact of weight loss.

In summary, although exercise is a well-recognized, important lifestyle modification tool which is widely recommended in the management of cardiovascular risk factors, whether or not the effect of exercise is independent from the body characteristic, metabolic and cardiovascular parameters is still not clear. Therefore, further evaluation of the metabolic and cardiovascular role of exercise in non-obese animals without the confounding impact of weight loss needs to be investigated. In this regard a number of questions that arose from the above discussion will be addressed in the following chapters. Firstly, what is the impact of exercise on the metabolic status and blood pressure, particularly with regards to dyslipidaemia, in non-obese animals? Secondly, is the impact of exercise independent of weight change? Thirdly, does exercise ameliorate the other concomitant metabolic disturbances associated with dyslipidaemia in non-obese animals, particularly the lipid status? Additionally, what is the effect of a HFHS diet on the other metabolic parameters associated with dyslipidaemia, and what is the effect of a HFHS diet on these metabolic changes in the absence of an obesity. Finally what is the effect of a HFHS diet on the cardiovascular system without a change in body weight?

2.6 Aims and objectives

The main aim of this study is to determine the impact of a westernized diet model intervention in inducing cardiovascular and metabolic changes and to investigate the effect of exercise on the cardiovascular and metabolic status in Sprague-Dawley (SD) rats.

The objectives of this project are:

1- To determine the effects of a short-term HFHS diet intervention in inducing the characteristics dyslipidaemia with or without the metabolic syndrome characteristics.
2- To determine the effects of a short-term HFHS diet intervention on body weight, lipid profile, blood glucose, insulin concentration and glycated haemoglobin in 3-month-old SD rats.
3- To determine the effects of a short-term HFHS diet intervention on blood pressure, heart functions and dimensions in 3-month-old SD rats.
4- To determine the effect of exercise on metabolic and cardiovascular changes induced by a HFHS diet.

Chapter Three: Materials and Methods

3.1 Experimental groups and exercise protocol

Rats were provided by the Central Animal Service (CAS) of The University of the Witwatersrand. The study protocol was approved by the animal ethics screening committee (AESC) of The University of the Witwatersrand (AESC number: 2013/51/04) and was conducted as shown in Figure 2.1. All rats were individually housed in rooms that were lighted between 07:00 and 19:00 with the room temperature between 23 and 26°C. Rats received the water and food *ad libitum*. Rats were weighed weekly using a digital scale (Snowrex Electronic Scale, Clover Scales, Johannesburg South Africa).

The selection of rats was performed at the age of 4 weeks. The rats were housed in individual cages and had free access to a running wheel through an opening between the cage and the wheel. The running wheels were designed to allow rotation in only one direction to prevent unmeasured running. Cat Eye Micro Cyclocomputers (CC-6000, Cat Eye) were used for monitoring of distance (Kilometres) and duration of exercise (hours/day) (Woodiwiss et al., 2000). The rats were chosen over a 10-day period of voluntary training selection according to their performance and ability to run 2 or more kilometres per day (Woodiwiss et al., 2000). The “non-runners” were returned to stock. Of the 92 male Sprague-Dawley (SD) rats, 48 “runners” SD male rats were selected. The rats continued to exercise until the age of 3 months for all groups.

The diet and/or exercise intervention started at the age of 3 months and continued for a period of 9 weeks. The selected “runners” were randomly assigned into 4 groups. Rats in 2 groups (group 2 and 4) continued to have free access to a running wheel and exercise whereas exercise was withheld in the other 2 groups (group1 and 3). Activities of rats from group 1 and 3 were not assessed but their activities cannot be referred as exercise. Groups 2 and 4 were running 2.87 and 3.89 km/day, respectively ($p=0.24$). The rats in 2 groups (group 3 and 4) were fed a diet rich in fat and sucrose (high fat-high sucrose diet) whereas rats in the other 2 groups (group 1 and 2) continue to receive a standard rat diet.

Rats were assigned as follow:

group1: Standard diet (ST) without exercise (n=12)

group2: Standard diet (ST+E) with exercise (n=12)

group3: High fat-high sucrose diet (HFHS) without exercise (n=12)

group 4: High fat-high sucrose diet (HFHS+E) with exercise (n=12).

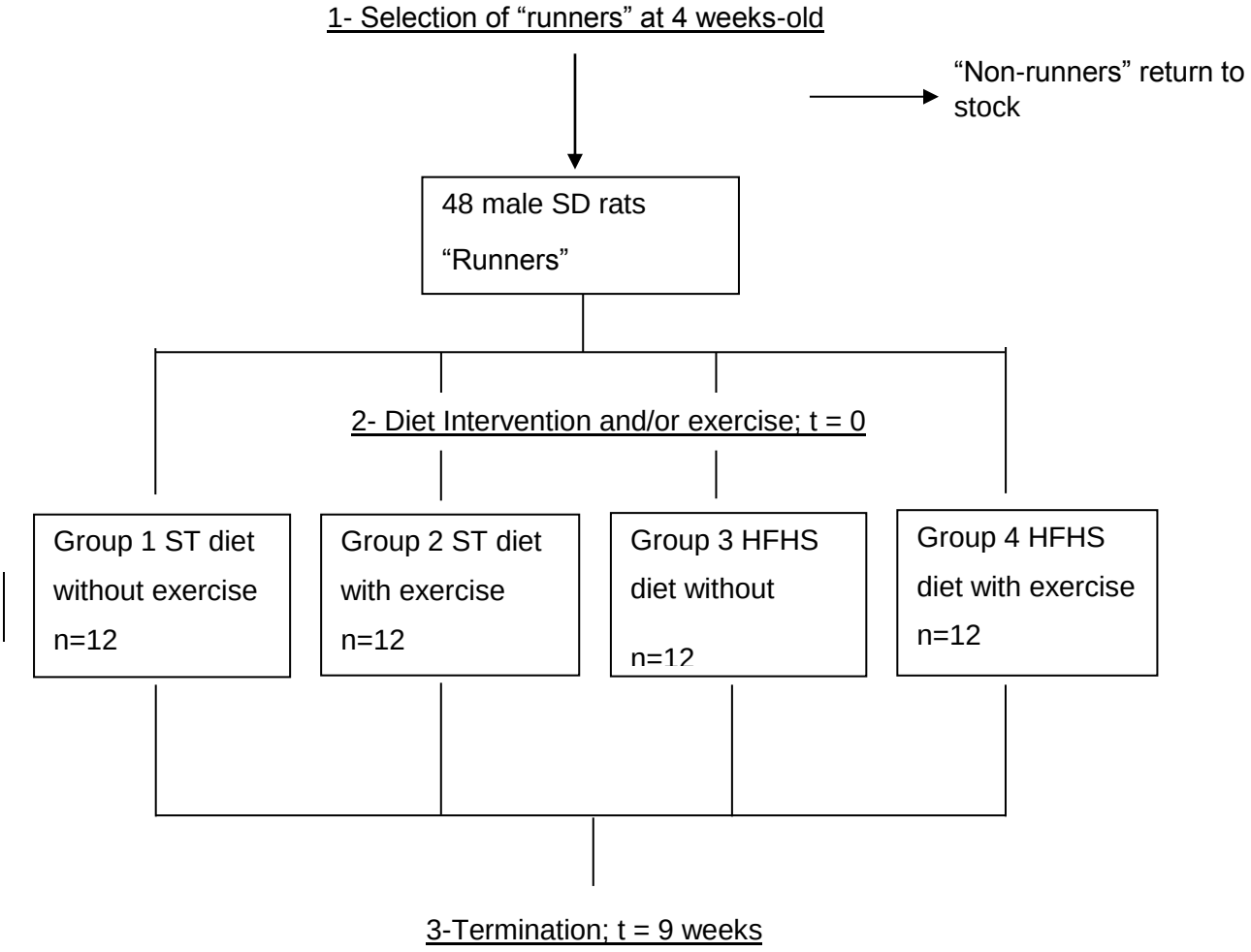


Figure 3.1 Protocol with group assignation according to dietary/exercise intervention. ST, standard diet; HFHS, high fat-high sucrose diet; t, time; SD, Sprague-Dawley.

3.2 Diet compositions and preparation

The standard animal chow diet (Epol, Centurion, South Africa) was composed of 2.78% fat, 17.99% protein as well as 6.54% sugar and 28.76% starch. The HFHS diet consisted of 39% fat, 40% sucrose and 21% rat chow and starch (Barnard et al., 1998). The HFHS diet was composed of lecithinated fat powder (Berg+Schmidt, Hamburg, Germany), sugar (Selati, Malalane, South Africa) and the standard animal chow. Five percent water was added to the mentioned compositions. Rats ate new pellets every day; the pellets were prepared the day before and were allowed to dry and be hard enough for the animal to eat. Throughout the time of the dietary intervention, the daily amount of food consumed by each animal was determined on a daily basis using a digital scale. The daily food intake was calculated by subtracting the lifted amount of food of everyday from the total amount of the given food that day.

3.3 Measurements and procedures

3.3.1 Blood Pressure (BP) Measurement

Blood pressure (BP) was measured at mid-day using a tail-cuff technique which employs Volume Pressure Recording (VPR) technology (CODA, Kent Scientific, Torrington USA) (Feng et al., 2009) every 3 weeks (Figure 2.2). The VPR sensor utilizes a specially designed differential pressure transducer to non-invasively measure the blood volume in the tail. In a warm and calm room, conscious rats were placed in restrainers and rat tails were placed on a heating platform. Two cuffs were positioned on the tail. An occlusion cuff was positioned in the middle of the tail and a VPR-cuff was positioned near the base of the tail. Both cuffs were connected to the CODA system. Rats were habituated before the first measurement for three consecutive days, in order to enable them to adapt to the procedure. Measurements were taken at midday to avoid diurnal variation. BP determined by the mean of 10 readings.

3.3.2 Blood measurements

3.3.2.1 Fasting blood glucose and triglycerides (TG):

The rats were fasted for 12 hours (overnight) before blood glucose and triglycerides measurements were determined. Measurements were done before starting the dietary intervention and then at the 3rd, 5th and 7th week and at termination (Figure 2.2). A passively fallen drop of blood (approximately 20µl) was obtained by a pin prick (using 25 gauge needle) into the tail vain (Parasuraman *et al*., 2010) and a glucometer (Ascensia Elite, Bayer USA) was used to assess fasting blood glucose by using a reagent strip. Another drop of blood was obtained by a pinprick into the tail vain to assess the fasting TG using a TG meter (Accutrend Triglycerides, Roche diagnostics, Germany). The glucometer and TG meter were calibrated by the code stripes with each new package of test strips.

3.3.2.2 Lipid profile

Blood was obtained at termination by cardiac puncture. 4 ml of whole blood was centrifuged and 2 ml of serum was collected and stored at -20 °C. High density lipoprotein (HDL), non-fasting triglycerides (nfTG) and total cholesterol (TC) were measured by enzymatic colorimetric method (Cobas 6000, Roche diagnostic, Germany).

3.3.2.3 Haemoglobin A1C

Glycated haemoglobin (HbA1c) was assessed by radioimmunoassay RIA (ADIVA HbA1c, Siemens Germany) using 100-µl of whole blood (+EDTA) as described above in section 3.3.2.2.

3.3.2.4 Serum insulin

Serum insulin was measured using a solid phase two-site enzyme immunoassay (DRG Ultra-Sensitive Rat Insulin ELISA, DRG USA) Using 25-µl of serum. The Ultrasensitive Rat Insulin ELISA is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule.

- Once a week for every animal:

Body weight

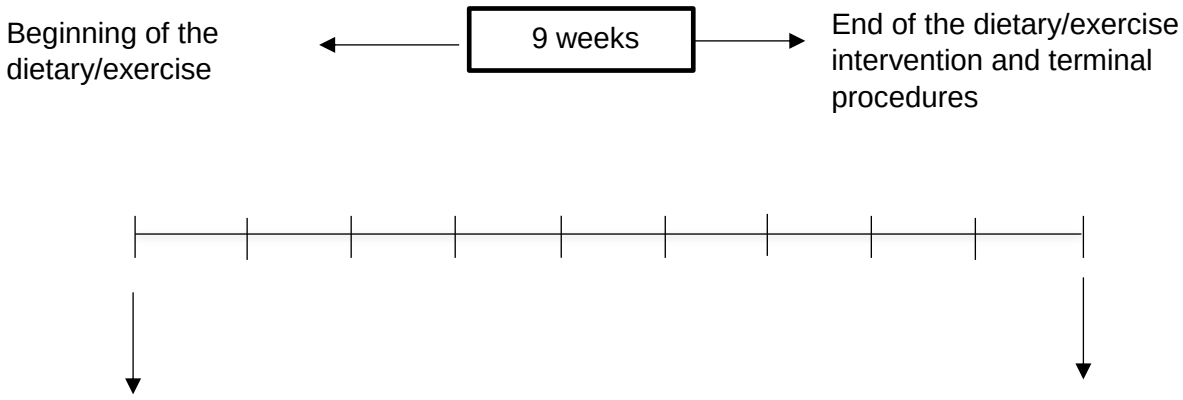
Running distance measurements

- Once every three weeks:

Blood pressure measurements

- Blood sampling (measurement of

Blood glucose and blood triglycerides); at 0, 3, 6, and 9 weeks



3 month-old male ‘’runner’’ SD rats (n=48)

Time=0

Blood pressure measurement

Blood sampling

Time=9 weeks

Non-invasive blood pressure measurements

Echocardiography

Blood and tissue collection

Blood vessel reactivity

Figure 3.2 Measurements and procedures during the diet/exercise intervention and at termination.

3.4 Echocardiography

Echocardiography was obtained at termination (Figure 3.2) by an experienced member of the research team from the Cardiovascular Pathophysiology and Genomic Research Unit (CPGRU). Echocardiography is a non-invasive technique, conferring minimal or no pain or discomfort to the animals (Woodiwiss et al., 2001). Rats were anaesthetised 15 minutes before the procedure by intramuscular injections of ketamine ($100mg.kg^{-1}$) and xylazine ($5mg.kg^{-1}$). An ultrasonic probe was placed on the chest of the animal. Left ventricular function, intrinsic myocardial systolic function and left ventricular dimensions were determined *in vivo* using two-dimensional directed M-mode imaging. During three consecutive beats (to ensure that there is no variability in vagal autonomic activity and heart rate), the internal dimensions and wall thickness of the left ventricle were measured in systole and diastole. From these dimensions, left ventricular endocardial fractional shortening (FSend), midwall fractional shortening (FSmid) and relative wall thickness (RWT) were determined using the following calculation:

FSend = (LVEDD-LVESD)/LVEDD x 100

FSmid = [(LVEDD+LVED PWT) -(LV ESD+PWES)]/ (LV EDD +PWED) x 100

RWT = PWED/ (LVEDD/2)

Where PWED = left ventricular end diastolic posterior wall thickness, PWES = left ventricular end systolic posterior wall thickness, LVEDD = left ventricular end diastolic diameter and LVESD = left ventricular end systolic diameter (Anarmourlis et al., 2006; Norton et al., 2008).

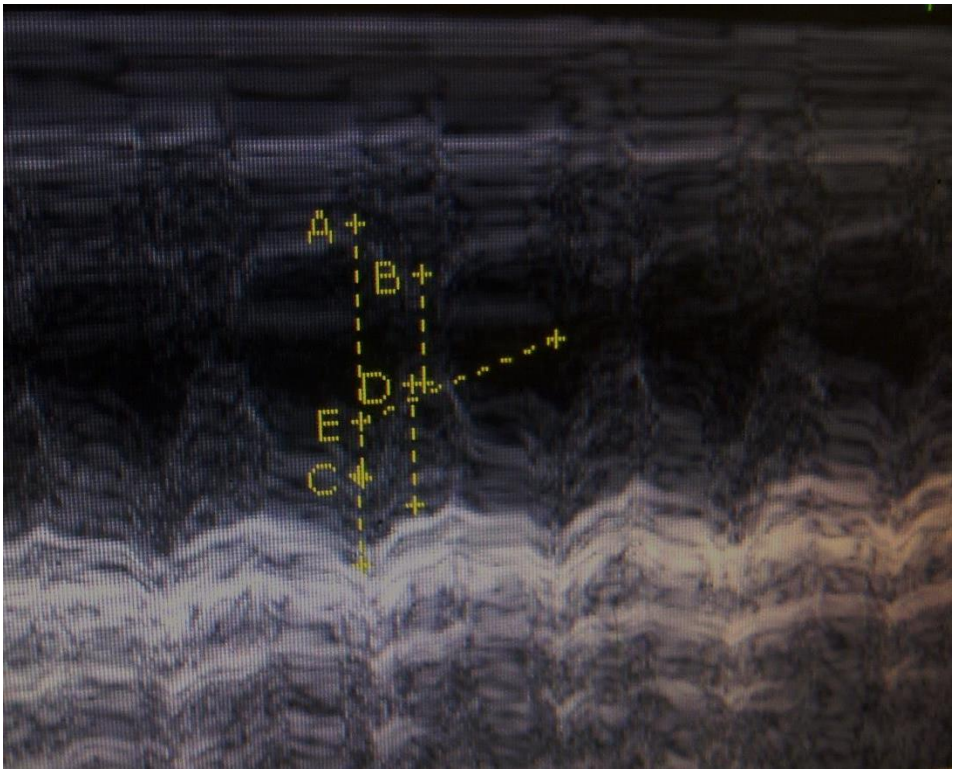


Figure 3.3 Echocardiographic image of the left ventricle of a HFHS diet fed rat (M-mode). A, left ventricular end diastolic diameter (LVEDD); B, left ventricular end systolic diameter (LVESD); C, left ventricular end systolic posterior wall thickness (PWES); D, left ventricular end diastolic posterior wall thickness in systole (PWED).

3.5 Vascular reactivity

After the echocardiography, the anaesthetised rats underwent a thoracotomy and the heart was removed from the thoracic cavity. Thoracic aorta, kidneys and mesentery were also removed and placed in a cold modified Krebs-Ringer bicarbonate (control) solution (g/l: 6.89 NaCl, 0.35 KCl, 0.27 $CaCl_2$, 0.14 MgSO4, 0.16 KH2PO4, 2.1 NaHCO3, and 1.99 glucose).

The modified Krebs solution was used in previous studies in our laboratory to preserve the tissues in a physiological environment identical to the body environment and prepared daily by the same person. Arteries were then isolated. Rings of renal and mesenteric arteries (2 mm long) were cleaned of fat and connective tissue and were suspended in a wire myograph (model 610M, Danish Myo Technology, Aahrus, Denmark). Arteries were mounted as a ring and threaded over two parallel stainless steel wires. The wires were secured to two supports or "jaws" with one support attached to a precision micrometer (allowing manual control of vessel circumference and stretch). The other support was attached to a force transducer for measurements of force/tension development. The preparation was kept in a heated chamber filed with an oxygenated physiological salt solution at 37°C. The baseline tension of mesenteric artery was 1.56±0.07 mN and of renal artery was 2.01±0.01 mN.

Vasoactive drugs were added into the chamber and the concentration-response (contractions or relaxations) curves of the preparation for the different vasoactives drugs were determined under isometric conditions (Christon et al., 2005). The following vasoactives drugs were added into the reactivity chamber in an increasing concentration-dependent manner. Contraction responses to Phenylephrine (Phe, 1 nM - 0.1mM) and Potassium chloride (KCl, 1 mM to 120mM) were recorded. Blood vessels were dilated with Acetylcholine (ACh, 0.1 nM–100 µM) and Sodium nitroprusside (SNP, 1nM-100µM) under phenylephrine-induced contraction (Phe concentration=0.3-1µM). ACh is an endothelium-dependant vasodilator whereas SNP (1µM-100µM) causes an endothelium independent relaxation.

3.6 Organs Mass:

Immediately after termination of the animals, right kidneys, liver, adipose tissue were removed and weighed. Measurements were done by using a digital scale. These organs were stored at -80 °C. The heart was removed and weighted, and then dissected. The free wall of the right ventricle was trimmed off the septum of the heart and weighed; subsequently, the left ventricle with the septum was then weighed.

3.7 Tibial length:

The Tibia was disarticulated from the femur then defleshed. The bone was dried (50 °C) for 5 days in the lab oven (Salvis lab, Switzerland) then weighed. Tibial length was measured by using a pair of Vernier calipers (Hi-impact, Djuca, South Africa). Bone density then calculated by using the tibial length and weight.

Bone density: tibial weight (mg)/ tibial length (mm).

3.8 Histology and aortic morphometric study

Thoracic aortic artery was fixed in 10% buffered formaldehyde solution then embedded in paraffin wax and sectioned. Cross-sections (5μm) were stained with haematoxylin and eosin (H&E) and stained with orcein (figure 2.4). The stained sections were viewed with the light microscope (Nikon Optiphot, JAPAN). Aortic wall thickness (AWT) was determined by mean of 3 measures of the thickness at 3 different angles, including 0, 90, and 180 °C. The commercial software AUTOCAD 2014 was used to measure the thickness.

3.9 Measurement of liver lipid and glycogen

3.9.1 Hepatic lipid content

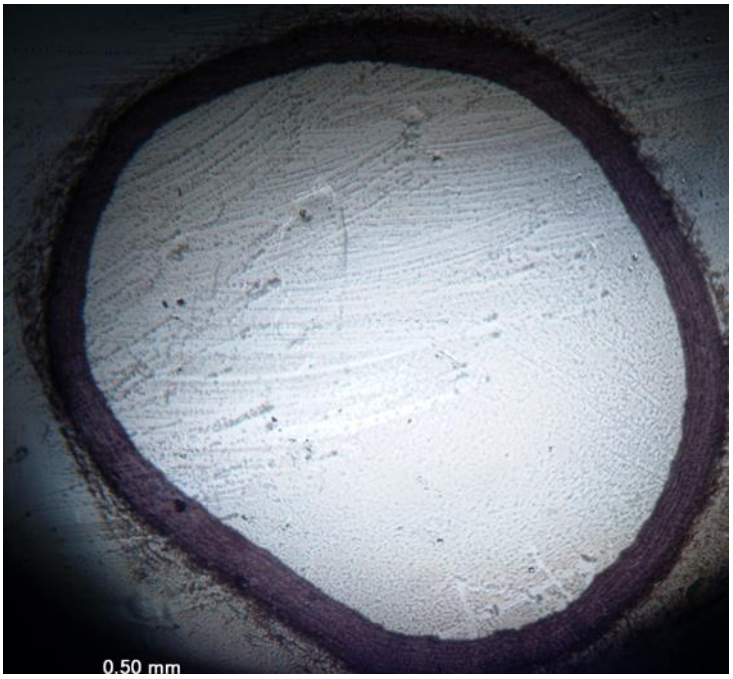
Hepatic lipid content was determined using the procedure by Bligh and Dyer (1959). Firstly the frozen liver samples were thawed, thereafter 5-6 grams of each liver sample were immersed in 150 ml of a chloroform: methanol solution (2:1 ratio) (MERCK chemicals, South Africa). The samples were then sealed with Accu-seal and incubated at 4 °C overnight.

Following the incubation period, the samples where then filtered through filter paper (Whatmann, No. 1 size 50mm, England) into a separating funnel. 30ml of 0.9% saline solution were then added and mixed fully. The solution was then incubated at 4 °C overnight in order to separate into two distinct layers. Following the overnight incubation, the bottom layer in the separating funnel containing chloroform was titrated into a round bottom flask (SCOTT DURAN, Germany). The lipids were then extracted by evaporating the solvent obtained at 37 °C using a rotor evaporator for approximately 30 minutes (Labocon (Pty) Ltd, Krugersdorp, Transvaal, South Africa). The residual oil obtained was then made up to 20ml by adding chloroform. The 20ml solution was then extracted from the round bottom flask using a pipette and stored in a vial. Small vials were then re-dried at 50 °C for 15 minutes. To determine the percentage of lipids in each liver sample an aliquot of 2 ml was extracted from the 20ml vial solution and placed in a dry- pre-weighed vial. The vial was then re-dried at 50 degrees Celsius using an oven (Salvis R, Oakton Instruments, and USA). The vial was then weighed on a digital lab scale and the mass of the lipid obtained was multiplied by a factor of 10, and recorded as a percentage of the original liver sample weight.

3.9.2 Hepatic Glycogen Content

Hepatic glycogen was determined using the process of indirect hydrolysis that was proposed by Passonneau and Lauderdale (1974). Firstly 0.1g of the liver sample was homogenized in 1ml 0.03M hydrochloric acid using an ultra turrex homogenizer (Janke & Kunkel GmbH & Co IKA-Werk D7813, Staufen). 1 milliliter of 1M hydrochloric acid was then added in order to hydrolyse the liver glycogen. The test tubes were then sealed and placed in boiling water for 2 hours. 1ml 1M sodium hydroxide was then added to each test tube in order to neutralize the acid before determining the glucose concentration. The glucose concentration was determined using a glucose-oxidase based reaction on the Accu-Check Active Glucose meter (Roche, Germany). Glycogen concentration was recorded as the glucose equivalents in the homogenate.

A



B

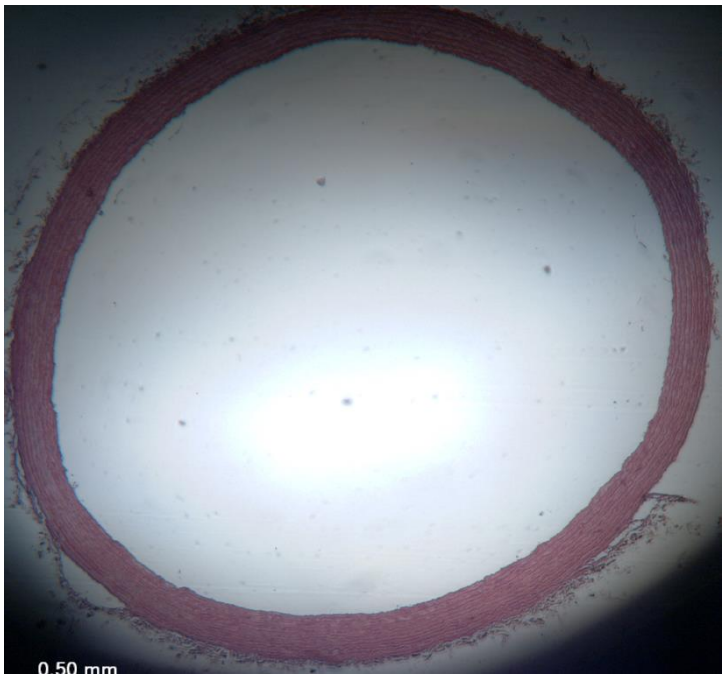


Figure 3.4 photomicrography of thoracic aortic wall section**.** (A) Aortic wall section stained with orcein stain. (B) Aortic wall section stained with H&E (Hematoxylin and eosin).

3.10 Data analysis

Data is expressed as mean ± SD. Data analysis was performed using SAS software v. 9.3 (SAS institute Inc, Carry, NC) and GraphPad Prism 5 software (Graph-pad Software Inc., San Diego, USA). The mixed model was used to analyse parameters measured each week namely blood pressure, fasting blood glucose, fasting blood triglycerides and body weight. A two way ANOVA followed by a Tukey-Kramer *post hoc* analysis was used to determine the difference between groups of the parameters measured at termination; namely non-fasting blood TG, HDL cholesterol, total cholesterol, insulin level, HbA1C, echocardiography, vascular reactivity, the heart weights, hearts function, heart dimensions, liver weights, hepatic glycogen contents, hepatic lipid contents, visceral adipose tissue weights, right kidney weights and thoracic aortic wall thickness. Median and range was reported for the non-normally distributed data. Differences were considered statistically significant at $P \leq 0.05$.

The mixed model used for repeated measurements including blood glucose, blood triglycerides, blood pressures and body weight are:

$Y_{abcd} = \sum + E_a + R_b + T_C + D_d + \partial$

Y_{abcd} =blood glucose at time T fasting blood glucose

$\sum$= mean for all observations

E_a = effect of exercise on blood glucose (n=0, 2….9)

R_b = fixed effect of each rat (n=1, 2…48)

T_C = fixed effect of time on blood glucose (n=1, 2….5)

D_d = fixed effect of diet on blood glucose (n=0, 2….9)

∂ = random error

The model was the same for blood triglycerides, blood pressures and body weight. The factors used in the analysis were diet, exercise and diet-exercise.

Chapter Four: Results

4.1 The effect of short term high fat-high sucrose diet and exercise on food intake, body weights, visceral adipose tissue weights, kidney weights and liver weights

Figure 4.1 shows the effect of short term HFHS diet and exercise on weekly body weight in the different groups. Body weights of rats fed with standard diet increased throughout the 9 weeks. Body weights of rats fed HFHS diet decreased in the first 2 weeks then increased until the end of the dietary intervention. Rats fed with HFHS diet were significantly lighter compared to the rats fed with standard diet by the end of first week ($p<0.01$) and continued to be lighter up to the ninth week ($p<0.0001$). Figure 4.2 shows the food intake of rats fed the HFHS diet or the standard diet with or without exercise. Food intake was significantly higher in rats fed the HFHS diet compared to the standard diet ($p<0.0001$). No differences in body weight and food intake were observed between the exercising and non-exercising groups ($p =0.5517$ and $p=0.1806$, respectively).

Table 4.1 shows the effect of short term HFHS diet and exercise on weights of the liver, right kidney and visceral adipose tissue as well as tibiae lengths. Liver and right kidney weights of rats fed the HFHS diet were significantly lighter than those of rats fed the standard diet ($p<0.0006$ and $p=0.0032$, respectively). Liver, right kidney and visceral adipose tissue weights relative to body weight of rats fed the HFHS diet were similar to those of rats fed the standard diet ($p=0.9798$, $p=0.8106$ and $p=0.3137$, respectively). No difference was observed in liver and right kidney weights of rats that were exercising compared to those that were not exercising ($p=0.9165$ and $p=.01944$, respectively). Visceral adipose tissue weight in rats fed the HFHS were similar to those of rats fed the standard diet ($p=0.3392$). No difference was observed in visceral tissue weights of rats that were exercising compared to those that were not exercising ($p=0.0553$). Tibial lengths were similar in the 4 experimental groups ($p=0.7381$ for diet and $p=0.9927$ for exercise).

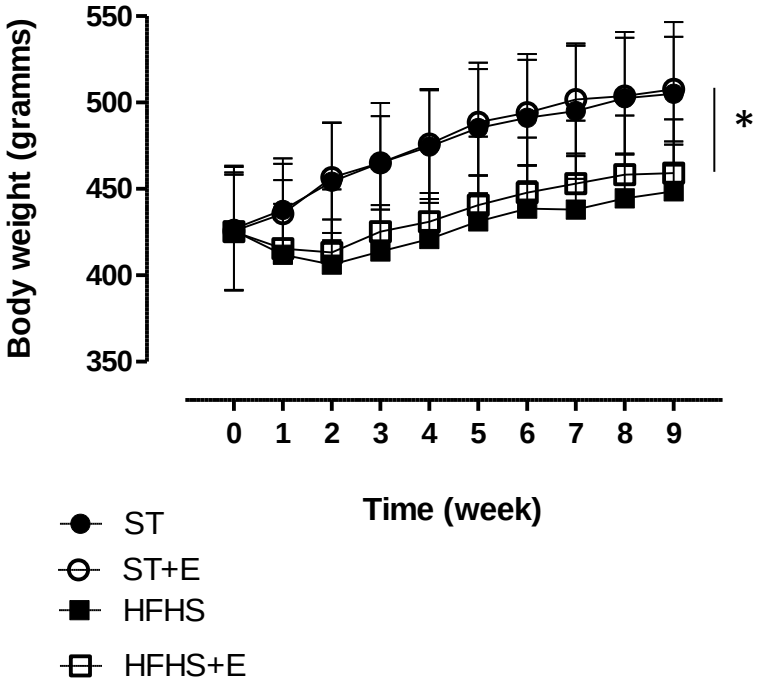


Figure 4.1: Effect of short term HFHS diet and exercise on body weights. Data are expressed as means ± SD. n=12 per group. ST, standard diet; ST+E, standard diet with exercise; HFHS, high fat high sucrose diet; HFHS+E, high fat high sucrose diet with exercise. * $P < 0.05$ for standard diet compared to high fat-high sucrose diet.

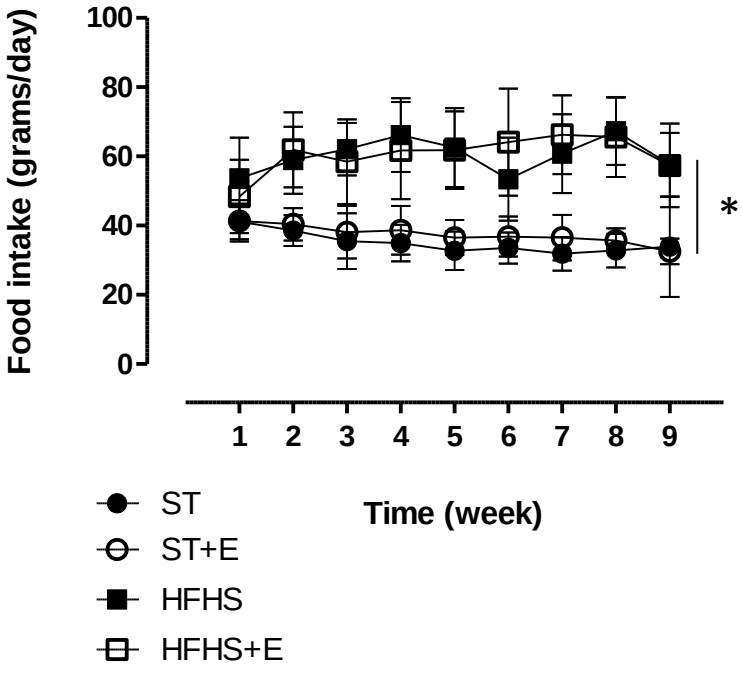


Figure 4.2: Effect of short term high fat-high sucrose and exercise on food intake. Data are expressed as means ± SD, n=12 per group. ST, standard diet; ST+E, standard diet with exercise; HFHS, high fat high sucrose; HFHS+E, high fat high sucrose with exercise. * $P < 0.05$ for standard diet compared to high fat-high sucrose diet.

Table 4.1 Effects of dietary intervention and exercise on tibia lengths, liver weights, right kidney weights and visceral adipose tissue weights.

	ST	ST+E	HFHS	HFHS+E	Significance level	
					Diet	exercise
TL (cm)	4.50±0.18	4.51±0.14	4.53±0.17	4.52±0.1	ns	ns
LW (g)	16.48±1.7	16.03±1.3	14.36±1.12*	14.80±1.85*	P<0.0006	ns
RKW (g)	1.70±0.18	1.82±0.21	1.56±0.19*	1.59±0.21*	P=0.0032	ns
VATW (g)	6.43±1.10	5.41±1.87	7.01±2.13	5.84±2.73	ns	ns
LW/BWx100 (%)	3.27±0.28	3.16±0.27	3.21±0.28	3.23+0.40	ns	ns
RKW/BWx100 (%)	0.34±0.11	0.35±0.11	0.34±0.11	0.35±0.05	ns	ns
VATW/BWx100	1.26±0.21	1.06±0.36	1.56±0.43	1.26±0.56	ns	ns

Data are expressed as means ± SD, n=12 per group. TL, tibia length; LW, liver weight; RKW, right kidney weight; VATW, visceral adipose tissue weight; ST, standard diet; ST+E, standard diet with exercise; HFHS, high fat-high sucrose; HFHS+E, high fat-high sucrose with exercise. * $P < 0.05$ for standard diet compared to high fat-high sucrose diet. ns, non-significant.

4.2 The effect of short-term high fat–high sucrose diet and exercise on cardiovascular parameters

4.2.1The effect of high fat–high sucrose diet and exercise on heart weights, dimensions and function as well as aortic wall thickness

Table 4.2 shows the effect of short term HFHS and exercise on heart weight (HW), right and left ventricular weights (RVW and LVW, respectively) and thoracic aortic wall thickness (AWT). At 9 weeks, HW, RVW, LVW and thoracic aortic wall thickness in rats fed with HFHS diet were similar to those of rats fed the standard diet (P=0.2727, p=0.6625, p=0.6086 and p=0.7898, respectively). HW, LVW and thoracic aorta wall thickness of rats that were exercising were similar to those rats that were not exercising, despite the dietary intervention (p=0.1520 p=0.5906 and p=0.3794 respectively). RVW of rats that underwent exercise were heavier compared to those that did not perform exercise (p=0.0462).

Table 4.3 shows the effect of short term HFHS and exercise on the left ventricular end diastolic (LVEDD) and end systolic diameters (LVESD), posterior wall thickness in diastole (PWED) and systole (PWES) and relative wall thickness (RWT). No differences were observed in LVEDD, LVESD, PWED, PWES and RWT of rats fed with HFHS diet compared to those of rats fed with standard diet (p=0.4046, p=0.4824, p=0.8115, p=0.7171 and p=0.6316, respectively) or in rats that underwent exercise compared to those rats that did not exercise (p=0.7318, p=0.6167, p=0.1742, p=4529, and p=0.3404respectively).

Table 4.3 shows the effect of short term HFHS and exercise on the left ventricular systolic chamber function as indexed by endocardial fractional shortening (FSend) and intrinsic myocardial systolic function as indexed by intrinsic midwall fractional shortening (FSmid). FSend and FSmid were similar in the 4 experimental groups (p=0.7051 and 0.4224, respectively, for diet and p=0.4776 and p=0.3906, respectively, for exercise).

TABLE 4.2 Effects of the dietary intervention and exercise on heart weights, left ventricular weights and right ventricular weights.

					Significance level	
	ST	**ST+E**	**HFHS**	**HFHS+E**	Diet	exercise
HW (g)	1.21±	1.16±0.12	1.18±0.10	1.22±0.11	ns	ns
RVW (g)	0.25±0.03	0.29±0.04*	0.27±0.05	0.28±0.05*	ns	p=0.0462
LVW(g)	0.96±0.12	0.97±0.10	0.90±0.11	0.94±0.11	ns	ns
HW/BWx100	0.24±0.01	0.25±0.02	0.26±0.02	0.27±0.03	ns	ns
RVW/BWx100	0.05±.01	0.06±0.01	0.06±0.01	0.06±0.01	ns	ns
LVW/BWx100	0.19±0.01	0.19±0.02	0.20±.03	0.20±0.02	ns	ns
AWT (μm)	195±0.15	132±0.09	173±0.13	150±0.09	ns	ns

Data are expressed as means ± SD, n=12 per group. HW, heart weight; RVW, right ventricular weight; LVW, left ventricular weight; AWT, aortic wall thickness; ST, standard diet; ST+E, standard diet with exercise; HFHS, high fat-high sucrose diet; HFHS+E, high fat-high sucrose diet with exercise. * $P < 0.05$ groups with exercise compared to groups without exercise. ns, non-significant.

Table 4.3 Effect of short term high fat-high sucrose diet and exercise on left ventricular dimensions and functions as assessed in *vivo*

					Significance level	
	ST	**ST+E**	**HFHS**	**HFHS+E**	Diet	exercise
LVEDD (mm)	6.78±0.66	6.83±0.48	6.76±0.26	6.64±0.36	ns	ns
LVESD (mm)	3.11±0.72	3.16±0.75	3.21±0.32	2.95±0.35	ns	ns
PWED (mm)	1.98±0.32	2.12±0.48	1.98±0.20	2.13±0.27	ns	ns
PWES (mm)	2.94±0.40	2.89±0.41	2.8±0.29	2.94±0.39	ns	ns
FS end (%)	54.18±6.6	53.94±8.52	52.57±6.3	55.72±4.09	ns	ns
FS mid (%)	30.82±4.7	32.21±6.85	31.33±5.5	32.96±4.33	ns	ns
RWT (mm)	0.59±0.12	0.63±0.15	0.59±0.07	0.64±0.08	ns	ns

Data are expressed as means ± SD, n=12 per group.LV, left ventricle; EDD end diastolic diameter; ESD, end systolic diameter; PWED, posterior wall thickness at end diastole; PWES, posterior wall thickness at end systole; FSend, endocardial fractional shortening; FSmid, midwall fractional shortening; RWT, relative wall thickness; ST, standard diet; ST+E, standard diet with exercise; HFHS, high fat-high sucrose diet; HFHS+E, high fat-high sucrose diet with exercise. ns, non-significant.

4.2.2 The effects of short term high fat-high sucrose diet and exercise on blood pressure.

Figure 3.3 shows the effect of short-term HFHS diet and exercise on systolic and diastolic blood pressure (SBP and DBP respectively). Figure 3.3 (A) and (B) show the SBP and DBP, respectively, in rats fed the HFHS and rats fed the standard diet at baseline (0), 3, 6 and 9 weeks. SBP of rats fed the HFHS diet was significantly higher compared to that of rats fed the standard diet at the 6^{th} ($p< 0.05$) and 9^{th} week ($p<0.05$). There was a trend towards significance in the DBP of rats fed the HFHS diet compared to that of rats fed the standard diet ($p=0.0573$).

No differences were observed in SBP or DBP between the exercising and non-exercising groups. ($p=0.3954$ and $p=0.3954$, respectively).

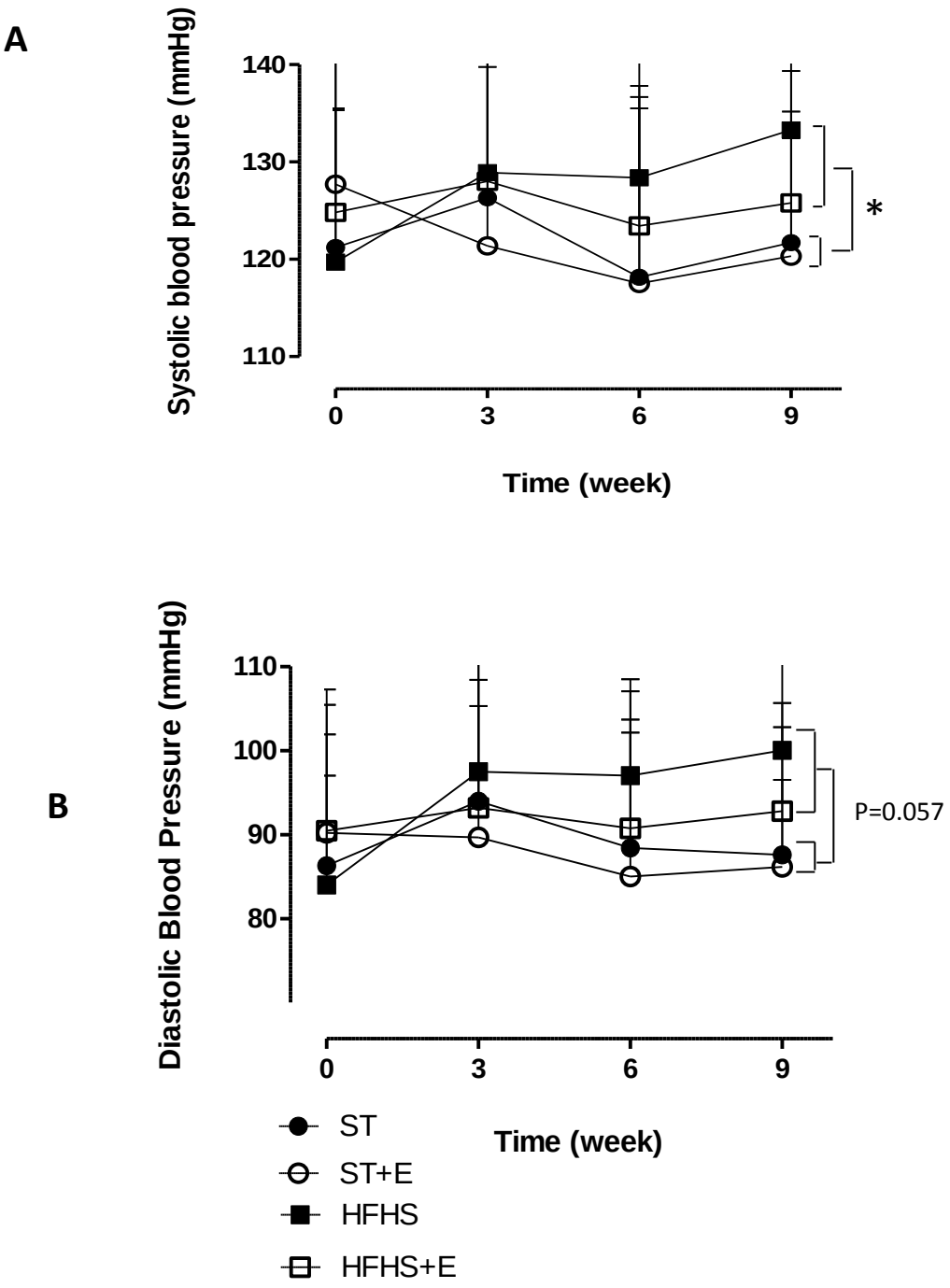


Figure 4.3 Effects of short term high fat-high sucrose diet and exercise on blood pressure. Panel A shows systolic blood pressure assessed in several points of time. Panel B shows diastolic blood pressure assessed in several points of time. Data expressed as means ± SD, n=12 per group. ST, standard diet; ST+E, standard diet with exercise; HFHS, high fat-high sucrose diet; HFHS+E, high fat-high sucrose diet with exercise. * $P < 0.05$ for the standard diet compared to high fat-high sucrose diet.

4.2.3The effects of short term high fat-high sucrose diet and exercise on vascular reactivity

Table 4.4 and 4.5 show the effect of short-term HFHFS diet and exercise on vascular reactivity in renal and mesenteric artery rings. The results are expressed in Emax and EC50; Emax is the maximum contraction response and EC50 is the half maximum contraction response. No differences were observed in phenylephrine-induced contractions in mesenteric and renal arteries of rats fed the HFHS diet compared to those of rats fed the standard diet ($p=0.0884$ and $p=0.9811$ for Emax, respectively, and $p=0.3728$ and $p=0.5232$ for EC50, respectively,). No differences were observed in KCl-induced contractions in mesenteric and renal arteries of rats fed the HFHS diet compared to those of rats fed the standard diet ($p=0.5629$ and $p=0.1716$ for Emax, respectively, and $p=0.2222$ and $p=0.4591$ for EC50, respectively). Phenylephrine and KCl-induced contractions in rats that underwent exercise were similar to those in rats that did not exercising ($p>0.05$).

Acetylcholine-induced relaxations in mesenteric and renal arteries of rats fed the HFHS were similar to those of rats fed the standard diet in ($p=0.2246$ and $p=0.5589$ for Emax, respectively, and $p=0.4420$ and $p=0.6991$ for EC50, respectively). Sodium nitroprusside-induced relaxations in mesenteric and renal arteries of rats fed the HFHS were similar to those of rats fed the standard diet ($p=0.4563$ and $p=0.4538$ for Emax, respectively, and $p=0.9679$ and $p=0.7289$ for EC50, respectively). Acetylcholine and SNP-induced relaxations in rats that underwent exercise were similar to those in rats that did not exercise ($p>0.05$).

Table 4.4 Effect of short-term high fat-high sucrose diet and exercise on vascular reactivity in mesenteric artery

	ST	ST+E	HFHS	HFHS+E	Significance level	
Phe					Diet	exercise
Emax (mN)	21.02±10.15	16.75±7.4	21.41±8.00	25.40±5.91	ns	ns
EC50 (µm)	1.7±6.12	1.03±7.1	1.77±12.9	9.83±3.00	ns	ns
KCL						
Emax (mN)	11.42±4.68	7.85±3.09	11.59±1.49	12.14±3.91	ns	ns
EC50 (µm)	0.06±0.03	0.05±0.04	0.03±0.02	0.05±0.03	ns	ns
ACh						
Emax (%)	31.02±18.0	16.2±26.08	17.7±21.57	12.45±16.1	ns	ns
EC50 (µm)	9.36±12.89	1.26±14.73	3.06±12.81	1.69±11.13	ns	ns
SNP						
Emax (%)	17.79±12.47	28.51±30.24	13.68±8.72	23.96±13.6	ns	ns
EC50 (µm)	1.66±21.46	5.35±4.77	1.41±12.03	7.41±7.47	ns	ns

Data expressed as means ± SD, n=12 per group. Phe, Phenylephrine; KCl, Potassium chloride; A.Ch, Acetylcholine; SNP, Sodium nitroprusside; Emax, maximum contraction response, EC50; half maximum contraction; Emax for Phe and KCl expressed in mN; Emax for Ach and SNP expressed as % of Phe-induced contraction, EC50 expressed in µmole (µm). ST, standard diet; ST+E, standard diet with exercise; HFHS, high fat-high sucrose diet; HFHS+E, high fat-high sucrose diet with exercise. ns, non-significant.

Table 4.5 Effect of short-term high fat-high sucrose diet and exercise on vascular reactivity in renal artery

	ST	ST+E	HFHS	HFHS+E	Significance level	
Phe					Diet	exercise
Emax (mN)	16.21±5.35	17.28±5.98	14.21±5.20	19.20±4.28	ns	ns
EC50 (µm)	5.42±3.81	6.77±5.79	9.83±8.55	4.92±3.15	ns	ns
KCL						
Emax (mN)	9.88±6.76	21.81±12.93	7.88±4.92	14.40±5.94	ns	ns
EC50 (µm)	0.04±0.02	0.11±0.08	0.07±0.10	0.04±0.02	ns	ns
ACh						
Emax (%)	32.32±21.48	19.62±11.68	15.46±20.9	28.98±21.01	ns	ns
EC50 (µm)	1.1±14.07	6.17±6.36	2.99±3.63	1.08±24.21	ns	ns
SNP						
Emax (%)	25.34±18.52	24.48±13.32	26.45±11.9	30.62±15.35	ns	ns
EC50 (µm)	2.76±5.85	4.59±5.52	1.96±4.95	2.29±5.43	ns	ns

Data expressed as means ± SD, n=12 per group. Phe, Phenylephrine; KCl, Potassium chloride; A.Ch, Acetylcholine; SNP, Sodium nitroprusside; Emax, maximum contraction response, EC50; half maximum contraction; Emax for Phe and KCl expressed in mN; Emax for Ach and SNP expressed as % of Phe-induced contraction, EC50 expressed in µmole (µm). ST, standard diet; ST+E, standard diet with exercise; HFHS, high fat-high sucrose diet; HFHS+E, high fat-high sucrose diet with exercise. ns, non-significant.

4.3 The effect of high fat–high sucrose diet and exercise on metabolic parameters

4.3.1 The effects of high fat–high sucrose diet and exercise on fasting blood glucose, Hemoglobin A1c and insulin level

Figure 4.4 shows the effect of short term HFHS diet and exercise on fasting blood glucose level in rats fed the HFHS diet and rats fed the standard diet at baseline (0), 3, 5, 7 and 9 weeks. Fasting blood glucose of rats fed the HFHS was similar to those of rats fed the standard diet (p=0.17). No difference was observed in fasting blood glucose of the rats that were exercising compared to those that were not exercising (p=0.84). Table 4.6 shows the effect of short term HFHS diet and exercise on hemoglobin A1C and insulin concentration in the different groups. No difference was observed in HbA1c concentration of rats fed the HFHS diet compared to those of rats fed the standard diet (p=0.61). No difference was observed in HbA1C of the rats that were exercising compared to those that were not exercising (p=0.20). Insulin level of rats fed the HFHS was similar to those of rats fed the standard diet (p=0.52) and also between the rats that were exercising compared to those that were not exercising (p=0.6).

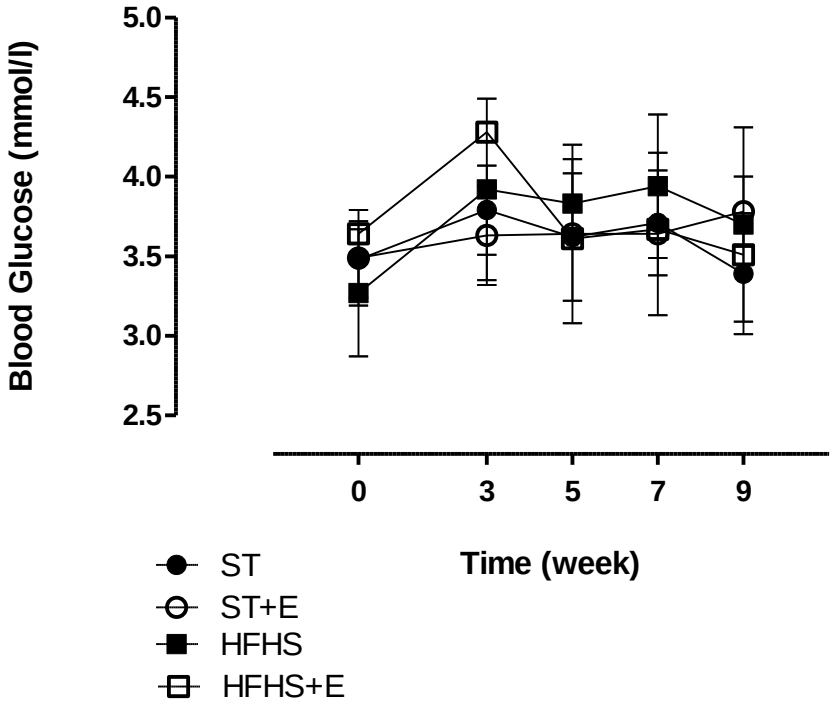


Figure 4.4 Effects of short term high fat-high sucrose diet and exercise on fasting blood glucose. Data are expressed as means ± SD, n=12. ST, standard diet; ST+E, standard diet with exercise; HFHS, high fat high sucrose diet; HFHS+E, high fat high sucrose diet with exercise.

Table 4.6 Effects of short-term high fat-high sucrose diet and exercise on Hemoglobin A1c and insulin level.

	ST	ST+E	HFHS	HFHS+E	Significance level
HbA1c (%)	3.50±0.20	3.40±0.24	3.50±0.39	3.39±0.22	ns
Insulin (µg/l)	0.044±0.00	0.044±0.00	0.043±0.00	0.044±0.00	ns

Data are expressed as means ± SD, n=12 per group. HbA1c, hemoglobin A1C; ST, standard diet; ST+E, standard diet with exercise; HFHS, high fat-high sucrose diet; HFHS+E, high fat-high sucrose diet with exercise. ns, non-significant.

4.3.2 Effect of short term- high fat–high sucrose diet and exercise on fasting blood triglyceride and lipid profile

Figure 4.5 shows the effect of short-term HFHS diet and exercise on fasting blood triglycerides at baseline (0), 3, 5, 7 and 9 weeks. Fasting triglycerides of rats fed the HFHS diet were significantly higher compared to those of rats fed the standard diet on the 3rd ($p<0.001$), 5th ($p<0.001$), 7th ($p<0.001$) and 9th week ($p<0.01$). No differences were observed in fasting blood triglycerides of the rats that were exercising compared to those that were not exercising ($p=0.2131$).

Figure 4.6 shows the effect of short-term HFHS diet and exercise on the lipid profile in the different groups, including non-fasting blood triglycerides, TC and HDL cholesterol. Non-fasting triglycerides and cholesterol of rats fed the HFHS diet were significantly higher compared to those of rats fed the standard diet ($p<0.0001$ and $p=0.029$, respectively). HDL cholesterol of rats fed the HFHS diet was significantly lower compared to those of rats fed the standard diet ($p<0.0001$). No differences were observed in non-fasting triglycerides, TC and HDL of the rats that were exercising compared to those that were not exercising ($p=0.41$, $p=0.66$ and $p=0.22$, respectively).

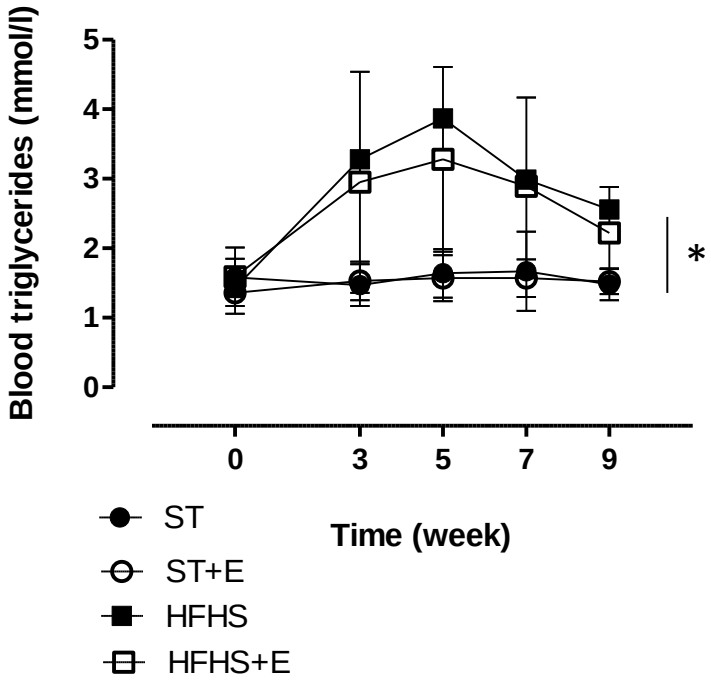
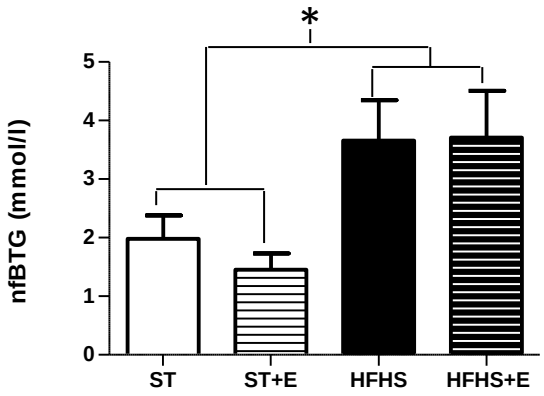
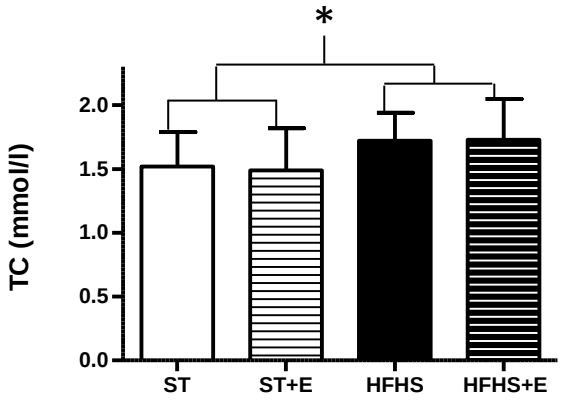


Figure 4.5 Effects of short term high fat-high sucrose diet and exercise on fasting blood triglycerides. Data are expressed as means ± SD, n=12 per group. ST,standard diet; ST+E, standard diet with exercise; HFHS,high fat-high sucrose diet; HFHS+E, high fat high sucrose diet with exercise. * $P < 0.05$ for standard diet compared to HFHS diet.

A



B



A

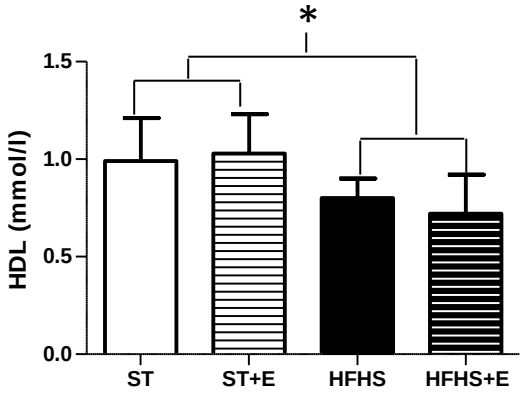


Figure 4.6 Effects of short term high fat-high sucrose diet and exercise on lipid profile. Data are expressed in means ±SD, n=12 per group. Panel A shows non-fasting blood triglycerides (nfBTG), panel B shows total blood cholesterol (TC) and panel C shows high density lipoprotien cholesterol (HDL). ST,standard diet; ST+E, standard diet with exercise; HFHS,high fat high sucrose diet; HFHS+E, high fat high sucrose diet with exercise. * P < 0.05 standard diet compared to HFHS diet.

4.3.3 Effect of short term- high fat–high sucrose diet and exercise on hepatic glycogen and lipid contents

Table 4.7 shows the effect of short term HFHS and exercise on hepatic glycogen and hepatic lipid contents. Hepatic glycogen contents of rats fed the HFHS diet were significantly higher compared to those of rats fed the standard diet ($p<0.02$). Hepatic lipid contents of rats fed the HFHS were similar to those of rats fed with standard diet ($p=0.73$). No differences were observed in hepatic glycogen and lipid contents of the rats that were exercising compared to those that were not exercising ($p=0.86$, $p=0.85$, respectively).

Table 4.7 Effects of short-term high fat-high sucrose diet and exercise on Hepatic glycogen contents and Hepatic lipid contents.

	ST	ST+E	HFHS	HFHS+E	Significance level	
					Diet	exercise
Glycogen (GE)	5.56±1.59	5.61±1.70	6.65±1.40*	6.76±2.34*	P<0.02	ns
Lipid (%)	5.28±2.19	6.01±1.35	6.12±2.54	5.68±2.59	ns	ns

Data are expressed in means ±SD. Glycogen, hepatic glycogen contents (expressed as glucose equivalents (GE) in the liver homogenate); Lipid, hepatic lipid contents (expressed as percentage of the liver sample weight); ST, standard diet; ST+E, standard diet with exercise; HFHS, high fat high sucrose diet; HFHS+E, high fat high sucrose diet with exercise. * $P < 0.05$ standard diet compared to HFHS diet. ns, non-significant.

Table 4.8 Non-normally distributed data.

	ST	ST+E	HFHS	HFHS+E
Food intake (g/day)				
Range	56.17	29.11	61.55	55.14
Median	32.33	35.67	59.71	57.87
TG (mmol/l)				
Range	2.58	1.63	5.2	5.40
Median	1.54	1.50	2.94	2.31
Glycogen (GE)				
Range	4.6	3.9	4.6	5.9
Median	5.2	5.2	6.3	6.6
nfTG (mmol/l)				
Range	1.6	1	2.5	2.8
Median	1.8	1.4	3.75	3.7
TCol (mmol/l)				
Range	0.8	1.2	0.6	1.1
Median	**1.5**	**1.4**	**1.75**	**1.6**

TG, fasting triglycerides; Glycogen, hepatic glycogen; nfTG, non-fasting triglycerides; TCol, total cholesterol

Chapter Five: Discussion

The discussion highlights the effects induced by feeding high fat-high sucrose (HFHS) diet for 9 consecutive weeks. The main finding of the current study is the development of dyslipidaemia and elevated systolic blood pressure accompanied by lower body weight gain in rats fed a HFHS diet for 9 weeks compared to the rats fed standard diet. Furthermore, the rats fed a HFHS diet also had a higher glycogen deposition in the liver. In addition this study showed that short-term exercise training for nine consecutive weeks did not attenuate the effects induced by the HFHS diet on blood lipids, systolic blood pressure as well as liver glycogen.

5.1 The effect of a high fat–high sucrose diet on body weight and tibial length

Studies have shown that a HFHS diet rat model has been used previously to model western diet-induced metabolic abnormalities such as obesity and metabolic syndrome (Barnard *et al*., 1998; Yamamoto *et al*., 2006; Bourgoin *et al*., 2008; Kong *et al*., 2011). Although a number of studies have reported an increase in body weight after a HFHS diet intervention (Barnard *et al*., 1998; Yamamoto *et al*., 2006; Bourgoin *et al*., 2008; Kong *et al*., 2011), not all studies that employed a HFHS diet observed a difference in the body weight gain of the rats (Arias *et al*., 2014, Pierine *et al*., 2014). In the current study, rats fed a HFHS diet weighed significantly less than the control groups. In agreement with our results, a previous study has reported that the rats that administered a HFHS diet for 12 weeks, weighed less than rats receiving a control diet (Hirotani *et al*., 2012). However, in this study, rats experienced liver disease. Furthermore, the results from Hirotani *et al*. (2012) should be interpreted carefully as the food intake of rats fed the HFHS diet was lower than the control group and hence the body weight gain of the rats was lower. Additionally, in the intervention group, lower blood glucose and triglycerides were justified by a decrease in body weight and abnormal lipid metabolism (Hirotani *et al*., 2012).

In the current study, the food intake of rats fed the HFHS diet was greater than that of rats fed the control diet. However, in the first two weeks of diet intervention, the body weights of rats fed HFHS diet were decreased. This could possibly be explained by that rats fed with the HFHS may have eaten less than they were supposed to eat in the first two weeks of the intervention as a result of introducing a new diet; hence rats fed the HFHS diet were lighter throughout the experiment. However, following the first 2 weeks, the growth rate was similar between the groups. Secondly, a habituation time which is suggested to adapt to a new diet (Ferraris *et al*., 1995; Levin *et al*., 2002) was not done in this study, but food intake may have corrected for the decreased body weight, where food intake in rats fed HFHS diet increased and was higher than the standard diet group. Thirdly, the HFHS diet was prepared in our laboratory and was mixed with water, in contrast to the standard diet which constituted of dry compositions only. This could have affected the percentage of vitamins and minerals in the diet, food efficiency and could have affected the caloric compositions of the diet. The essential vitamins and minerals were not added to the HFHS diet, which could lead to malnutrition of rats fed HFHS diet and subsequent lower body weight. Finally, the fat used in our diet was vegetarian fat, in contrast to the fat used in the other studies which was mainly lard (Yamamoto *et al*., 2002; Kong *et al*., 2011). It has been reported in previous studies that the diet which contains lard induces an increase of body weight more than other types of fats (Carmiel-Haggai *et al*., 2005; Buettner *et al*., 2006; Lionetti *et al*., 2014). All these reasons may explain the weight loss in the rats fed the HFHS diet during the first 2 weeks. Subsequent to the first two weeks, the food intake of rats fed the HFHS increased and the increase in body weight was similar between the experimental and control groups. However this increased food intake was not enough for rats fed the HFHS diet to catch up or exceed the weights of the control groups.

Tibial length, another growth parameter, was not affected by consumption of the HFHS diet. This observation suggested that the growth of animals were similar between the experimental groups. We therefore cannot assume that malnutrition occured with the HFHS

diet. We can therefore conclude that, in our model and despite some experimental bias, the rats were not obese following consumption a HFHS diet for 9 weeks. In agreement with this conclusion, the visceral adipose tissue weights were not different between the experimental groups.

5.2. The metabolic effects of a high fat–high sucrose diet

5.2.1 Blood glucose, serum insulin, glycated haemoglobin and hepatic glycogen contents

In the current study fasting blood glucose concentrations at termination were not different compared to the baseline readings in any of the groups. There are contradictory reports with regards to the effects of a HFHS diet on blood glucose with some studies reporting that such diets elevate blood glucose concentration (Barnard *et al*., 1998; Sugatani *et al*., 2006; Zhang *et al*. 2015) and others reporting no effect of HFHS diet on blood glucose concentration (Yamamoto *et al*., 2006; Chun *et al*., 2010; Stephenson *et al*., 2012). This discrepancy may be explained by the difference in the duration of the dietary intervention. Indeed, blood glucose concentration increases with the longer diet interventions (Barnard *et al*., 1998). In this study, the blood glucose concentrations did not change and this may be as a result of short duration of the intervention. A study by Sugatani and colleagues (2006) has shown an increase in blood glucose following short-term dietary intervention. However the percentage of carbohydrates in the former study was greater than in my study. The age of the animals at the beginning of the dietary intervention may possibly determine the incidence of increased blood glucose concentration. In the present study, the young age of the animals may allow them to handle the load of sucrose in the HFHS diet. In this study, glucose may have been transformed into lipids by *de novo lipogenesis* (Strable *et al*., 2010), thus blood glucose levels did not change.

In the current study, similar to glucose, no difference was observed in serum insulin concentration between the groups. Some studies observed similar results where no change was observed in serum insulin concentration (Sugatani *et al*., 2006; Yamamoto *et al*., 2006; Muraki *et al*., 2011; Cheng *et al*., 2014). Other studies reported contrasting results with an elevated insulin concentration following HFHS consumption (Barnard *et al*., 1998 and Zhang *et al*., 2015). This discrepancy may be explained in some case by a concurrent increase in blood glucose. Hyperglycaemia has been previously reported to compensate for the insulin resistance induced by a western diet (Commerford *et al*., 2001). Insulin is well known as the principle hormone responsible for glucose regulation in the blood. In the present study, the absence of change in insulin concentration may be explained in conjunction with an unchanged blood glucose concentration. As both blood glucose and serum insulin did not change with the 9 weeks consumption of HFHS diet, these findings suggest that these rats have no tendency to diabetes mellitus yet.

Haemoglobin A1C (HbA1c) explains the glycosylation of haemoglobin and it reflects the status of blood glucose for at least 3 consecutive months. In the present study no difference was observed in HbA1c concentration between the groups. HbA1c concentration was in agreement with the unchanged blood glucose and insulin concentration. Hence, the absence of change in HbA1c could be explained by the absence of hyperglycaemia. Previous investigations observed similar results where no change was observed in HbA1c (Rosety-Rodriguez *et al*., 2012; Cheng *et al.*, 2014). Some studies found contrasting results with elevated HbA1c concentration when a HFHS diet was consumed (Medford *et al*., 2012). However in the former study the dietary intervention lasted 52 weeks which could explain the lack of change in the current study (Medford *et al*., 2012). Additionally in the present study, the absence of hyperglycaemia and HbA1c change may be explained by a low ambient temperature in the central animal unit. Rats fed HFHS diet may have used the glucose as an energy source for thermoregulation in order to maintain their body temperature (Balmagiva *et al*., 1983).

Finally, in the current study the rats fed the HFHS diet had an increased hepatic glycogen deposition. Liver is the main storage site for glucose in the form of glycogen. Previous studies have reported an increased glycogen deposition in the liver following consumption of a western diet (Mattar *et al*., 2010; Mousavi *et al*., 2012; Kjaergaard *et al*., 2014), whereas others found contrasting results without any change observed in hepatic glycogen deposition with HFHS diet consumption (, Commerford *et al*., 2001; Medford *et al*., 2012). A diet high in simple carbohydrates has been shown to promote glycogen deposition in the liver (Garrido *et al*., 1996; de Castro *et al*., 2013; Nasri *et al*., 2015; Maslak *et al*., 2015). In my study, because insulin concentration was within normal range, it is possible that glucose handling is normal and that glucose is either stored as glycogen in the liver or converted to fat to be stored instead of resulting in altered glucose metabolism and insulin resistance.

5.2.2 Blood lipids

High concentrations of triglycerides (TG), low density lipoprotein (LDL), total cholesterol (T,Chol) and low concentrations of HDL are considered markers of dyslipidaemia (Wietlisbach *et al*., 2013). In the current study high fasting and non-fasting TG, high TChol and a low HDL concentrations was observed following consumption of a HFHS diet compared to the standard diet group. It is well known that a diet high in fat induces elevation of blood lipids (Tzeng *et al*., 2012; Kumar *et al*., 2014; Zhukova *et al*., 2014), and therefore the high fat component in the present study may be responsible for the elevated blood lipids. Dyslipidaemia can be explained by the fact that the HFHS diet is composed of 39% fat. Fat content in the diet increases apolipoprotein concentration and subsequently increases the TG, LDL and TChol concentrations and lowers HDL concentration (Barnard *et al*., 1998). Furthermore, a high sugar load is well known to induce *de novo* lipogenesis (Strable *et al*., 2010). Given the presence of an excessive amount of sugar that would metabolise into fat, this may be further increasing the blood lipids. On the other hand, glucose derived from metabolised carbohydrate either would be used for energy or directed to be stored in the

liver as glycogen (Foufelle *et al*., 2002; Ferrer *et al*., 2003). The large amount of glucose derived from HFHS diet exposes the liver to a large amount of glucose and would be stored it as glycogen (Bollen *et al*., 1998). Once the liver reaches the maximum storage capacity for glycogen the liver would use glucose for the *de novo* liponeogenesis and the formed lipids would be excreted as blood lipids (Foufelle *et al*., 2002).

However, the visceral adipose tissue weights were not different between the experimental groups. It is well-known that lipids from the diet could be stored in the form of visceral adipose tissues. In our model, ingestion of high concentrations of fat and carbohydrate resulted in an elevated blood lipid concentration but no change in the storage of fat.

Similarly to visceral adipose tissue, in the current study no change was observed in the hepatic lipid contents despite the elevated blood lipids concentration. This finding was in disagreement with findings from previous studies which reported an increased hepatic lipid contents (Sugatani *et al*., 2006; Muraki *et al*., 2011). This may be explained by the room temperature at the central animal unit which was less than the desired temperature for the rats (Maloney *et al*., 2014). Fat from the diet may have been used as an energy source to maintain normal body temperature (thermoregulation) of the rats (Balmagiva *et al*., 1983). Moreover my results could be explained by lack of obesity in the animals of the current study. In previous studies, obesity has been reported to induce an increase of lipid deposition in the liver (Uriarte *et al*., 2013; Kabel *et al*., 2014; Chu *et al.,* 2015). Although liver weights of animals fed a HFHS diet were lower than the control group, the liver weights relative to body weights (normalized) showed no differences between the groups. These findings were similar to the findings from previous studies which found no difference in liver weights and liver weights relative to body weight following consumption of HFHS diet (Bourgoin *et al*., 2008 and Muraki *et al*., 2011, respectively). These observations (no change of the liver lipid contents and no change in liver weights) indicate that despite elevated blood lipids, the liver is well functioning. The aforementioned results suggest that the consumption

of a HFHS diet induces elevation of blood lipids without increased body weight and visceral adipose tissue and without affecting the liver function.

5.3 The effect of a high fat–high sucrose diet on cardiovascular parameters

5.3 .1 blood pressure

In the current study the consumption of a HFHS diet for 9 weeks resulted in significant increase of systolic blood pressure (SBP) and there was a trend towards an increase in diastolic blood pressure (DBP) compared to the rats fed the control diet. Despite a lack of obesity, SBP still increased following the consumption of HFHS diet. In the present study, blood lipid concentration increased in conjunction with the raised SBP. The increased blood lipids could be responsible for the increased SBP as previous studies have associated the rise in blood pressure with dyslipidaemia (Barnard *et al*., 1998; Yamamoto *et al*. 2006; Bourgoin *et al*., 2008). An important factor that could be responsible for the raised SBP in these studies is the nature of the fat used, namely lard (saturated animal fat). Saturated fat is well known to predispose rats to hypertension (Oliveira *et a*l., 2013). The fat used in the current study was lecithinated fat (saturated vegetable fat). Lecithinated fat is composed of phosphatidyl choline, which is metabolised by the gastrointestinal tract into TMAO (Trimethylamine-N-oxide) (Mendelsohn *et al*., 2013). TMAO has been recently recognised to have adverse effects on the vasculature. In this regard TMAO has shown to induce atherosclerotic changes and increase oxidative stress, both which affects blood vessels remodeling and subsequently may predispose to an increased blood pressure (Mendelsohn *et al*., 2013).

Inflammation is another suggested mechanism which has been linked to development of elevated blood pressure (Utimura *et al*., 2003; Gonçalves *et al*., 2004). In this regard Bourgoin *et al* (2013) linked the elevated blood pressure to the presence of endothelin-1 in the adipose tissue surrounding the blood vessel of animals consuming a HFHS diet. In the

current study, to determine the possible mechanism responsible for the raised blood pressure, the aortic wall thickness was measured as an indicator of vascular anatomical changes including atherosclerosis, increased wall to lumen ratio and microvascular rarefaction (Serné *et al*., 2007). Moreover, the reactivity of the medium-sized blood vessels was assessed to determine whether the increased blood pressure is associated with changes in vasodilation or vasoconstriction responses. No differences were observed in aortic wall thickness and vascular reactivity of the renal and mesenteric arteries between the rats fed a HFHS diet and rats fed the control diet. Hence the increased BP cannot be explained by changes in aortic wall thickness and vascular reactivity. Further investigations are therefore required to illuminate the mechanism responsible for the elevation in blood pressure.

In order to determine the effect of raised blood pressure on the heart; heart weight as well as heart dimension and function were measured. Hypertension is one of the most common causes responsible for development of heart failure (Natsume *et al*., 1993; Carll *et al*., 2011). In the current study, although the animals fed the HFHS diet had dyslipidaemia and their blood pressure were elevated, no differences were observed between the groups with regards to heart weight, heart function or dimension. These findings suggest that the hearts of these animals have no tendency to hypertrophy or progress to heart failure. The absence of cardiac remodeling is most likely as a result of the short duration of raised blood pressure induced by the HFHS diet.

5.4 Exercise effects on cardiovascular and metabolic parameters

5.4.1 The effect of exercise on body weight of animals fed high fat–high sucrose diet

Several studies have reported a decrease in body weight following exercise in animals consuming a HFHS diet (Paulino *et al*., 2010; La Favor *et al*., 2013; Ennequin *et al*., 2015). The present work shows that the exercise and sedentary groups had the same pattern of

change in body weight in both the control and HFHS dietary groups, suggesting that exercise for a short duration in dyslipidemic non-obese animals did not change the body weight of these animals. The discrepancy may be attributed to an increased body weight induced by the diet in the previous studies. Notably, some of the previous studies have induced obesity before starting the exercise intervention (Paulino *et al.*, 2010; Gerbaix *et al*., 2013; Ennequin *et al*., 2015) to ensure that those animals are obese. In the current experiment, the HFHS diet did not induce obesity, despite the higher food intake in rats fed HFHS diet compared to rats fed ST diet. The higher food intake and lesser gain in body weight could be attributed to the initial weight loss and the water content of HFHS diet. Although the HFHS diet composed of a higher proportion of two macronutrients (fat and carbohydrates), it composed of a lower protein compared to the standard diet. The low protein content of the HFHS diet could affect the metabolism and caused a lower body weight as a result of protein energy malnutrition despite the higher food intake (de Melo Montenegro *et al*., 2012; Malta *et al*., 2014). Secondly a (slightly) shorter duration in my study compared to prior studies may have impacted the change in body weight. Furthermore, all selected rats have the ability to run voluntarily (runner). This particular feature may protect the rats from diet induced obesity (Ruegsegger *et al*., 2015).

Furthermore, in the current study, no differences were observed in the visceral adipose tissue weights between the exercise and sedentary groups. Additionally, exercise did not change the food intake pattern for the exercise groups in comparison to the sedentary groups. These observations suggest that exercise did not modify the animals' appetite as well as body weight regulation mechanism which subsequently did not change the body weight.

Consumption of HFHS diet, obesity (Zernicke *et al*., 1995) and loss of body weight (Bodnar *et al*., 2012) are recognized factors that may predispose to negative impacts on bone characteristics. In the current study, exercise for 9 weeks did not change the bone length or bone mineral density in both dietary groups compared to the sedentary groups (data not

shown). This finding could be justified by the fact that the animals in the current study were not obese and also did not lose weight by exercise intervention. Data from these observations suggest that 9 weeks of exercise in non-obese animals did not change the growth pattern of the exercise groups. Human studies showed controversial results regarding the effect of exercise on body weight. Some studies found similar findings to our finding that exercise did not lower the body weight (Watt *et al*., 1976; Lewis *et al*., 1999; Green *et al*., 2003). In these studies, the absence of loss in body weight could be attributed to the effect of exercise on the muscular compartment. Green *et al* (2003) recognised an increase of lean body mass (muscle mass) which indicated by decreased the percentage of body fat and no change in total body weight. In contrast, other human studies reported that exercise resulted in decreased body weight (Slentz *et al*., 2005; Drevenhorn *et al*., 2007; Roussel *et al*., 2009). The discrepancy in the resulted body weight could be attributed to the differences in the exercise protocol used in these studies with different intensity, duration and the concurrent diet consumed by the participants. Therefore, the effect of exercise in conjunction with HFHS diet needs further investigation.

5.4.2 Exercise effect on blood glucose, serum insulin, glycated haemoglobin and hepatic glycogen contents

Exercise is known to affect the control of blood glucose concentration. Exercise has been shown to ameliorate the raised blood glucose in animals fed HFHS diet (Fisher-Wellman *et al*., 2013), improved hyperinsulinemia (Grimditch *et al*.,1988) and to lower the high HbA1c concentrations in rats fed high fat diet (Heo *et al*., 2013). In the current study, fasting blood glucose, insulin and HbA1c concentrations were the same in both exercising groups and the sedentary groups, regardless of the diet consumed. Our findings are in agreement with those of previous studies showing that exercise did not change these parameters. Indeed, in animals fed HFHS diet, exercise did not change blood glucose (De Moraes *et al*., 2008) and insulin (La Favor *et al*., 2013) concentrations. However in these studies the blood glucose

(De Moraes *et al*., 2008) and insulin (La Favor *et al*., 2013) concentrations were increased with a HFHS diet. However, the length of the intervention was 10 weeks in Grimditch (1988) study and 12 weeks in both De Moraes (2008) and Fisher-wellman (2013). While the length of intervention in a study by Heo *et al* (2013) was 4 weeks. Nevertheless, we can assume that, similar to these studies, exercise did not drop blood glucose, insulin as well as HbA1c concentrations. Additionally, in my study the rats did not develop insulin resistance or increased blood glucose, hence with a normal glucose metabolism, exercise is unlikely to alter the metabolic pathways.

In addition to the unchanged glucose, insulin and HbA1c exercise for 9 weeks in non-obese animals did not ameliorate the elevated liver glycogen content in the animal fed HFHS diet. Gluconeogenesis induced by exercise is known to utilize different substrates to synthetize the glucose including lipid, glycogen or lactate (Turcotte *et al*., 1990). In particular, the use of the dietary glucose as substrate for gluconeogenesis could be the cause of sparing the blood glucose and the subsequent prevented glycogenolysis of the elevated hepatic glycogen that induced by HFHS consumption. These observations suggest that exercise for 9 weeks did not change the glucose metabolism of the non-obese animals and did not ameliorate the hepatic glycogen content.

5.4.3 Exercise and blood lipids

Previous animal studies have shown that exercise can ameliorate the raised blood lipids induced by HFHS diet consumption (De Moraes *et al*., 2008; Gerbaix *et al*., 2013; La Favor *et al.,* 2013). In contrast to the previously mentioned studies, the results of the current study suggest that an exercise intervention for 9 weeks did not modify the blood lipid profile of the dyslipidaemic rats. This discrepancy can be explained by the fact that both De Moraes *et al* (2008) and La Favor *et al* (2013) reported decreased blood lipids in conjunction with decreases of body weight. Indeed, previous human studies have reported that a decrease in

blood lipid concentration is more likely to occur in conjunction with weight loss (Coghill *et al*., 2008; Roussel *et al*., 2009; Yoshida *et al.,* 2010). In my study, the animals did not lose body weight in response to exercise which may explain the absence of improvement of blood lipids. However the rats in the current study did not have an increased body weight regardless of diet. Hence it is unlikely that they will lose even more weight. The findings from several exercise studies in rats consuming a high fat diet were in agreement with our findings that exercise training did not change the elevated blood lipids (Cambri *et al*., 2011; Teixeira de Lemos *et al*., 2011; Delghingaro-Augusto et al., 2012). Similarly, previous studies in humans did not record improvement of blood lipids following exercise (Arsenault *et al*., 2009; Lima *et al*., 2012). Thus, the findings from the current study suggest that exercise for 9 weeks; despite a low body weight did not ameliorate dyslipidaemia induced by HFHS diet consumption.

5.4.4 Exercise and blood pressure

Exercise training is known to decrease BP in humans, especially in individuals with a high baseline BP (Coghill *et al*., 2008; Roussel *et al*., 2009). Moreover, in individuals with dyslipidaemia exercise training results in a decreased BP when accompanied by a decrease in blood lipids and body weight (Stewart *et al*., 2005; Drevenhorn *et al*., 2007; Coghill *et al*., 2008; Roussel *et al*., 2009). In contrast to these findings in the current study, exercise did not alter the elevated blood pressure induced by consumption of the HFHS diet. The discrepancy may be explained by the fact that exercise did not induce weight loss and did not decrease blood lipids; therefore exercise did not improve the elevated blood pressure induced by HFHS diet consumption. Similarly, a previous study in rats fed a HFHS diet showed no change in BP in the absence of a change in lipids and body weight after exercise training (Paulino *et al*., 2010). Moreover, several human studies reported similar results whereby blood pressure was unchanged (Watkins *et al*., 2003; Green *et al*., 2003; Camhi *et al*., 2010). These observations suggest that exercise for 9 consecutive weeks without loss of

body weight and without decrease of blood lipid did not improve the elevated blood pressure induced by HFHS diet consumption.

Chapter Six: Conclusion

6.1 Conclusion

The current study investigated the effect of 9 weeks of HFHS diet consumption and exercise on 3-month-old rats. The metabolic and cardiovascular parameters investigated were blood glucose, insulin concentration, glycated haemoglobin, fasting and non- fasting blood lipids, hepatic glycogen and lipid content as well as blood pressure, vascular reactivity and heart dimensions and functions. Findings of the current study showed that 9 weeks of HFHS diet consumption had induced dyslipidaemia and increased liver glycogen content and that was without inducing obesity in these animals. While HFHS diet did not induce obesity, it also did not induce an increase in adipose tissue or organ weight. Furthermore, dyslipidaemia was not associated with other metabolic disturbances such as hyperglycaemia, insulin resistance or a change in the hepatic lipid content. In addition to the metabolic changes, short-term HFHS diet consumption resulted in elevated blood pressure in non-obese animals. There were no changes in heart dimension and function as well as vascular reactivity associated with the high blood pressure during the time of the study. These observations suggest that HFHS diet consumption for 9 weeks induced elevated BP without inducing the complications associated with hypertension. We can conclude from the current study that short-term consumption of HFHS diet induced dyslipidaemia and elevated hepatic glycogen content in non-obese animals without further metabolic disturbances. Additionally, HFHS diet induced elevated blood pressure in non-obese animals without further impact on vascular reactivity and wall thickness. The elevation in BP may be linked directly to the increased in blood lipids concentration.

Additionally, the current study evaluated the impact of 9 weeks of exercise on the metabolic and cardiovascular changes induced by the short term HFHS diet. Indeed, exercise for 9 weeks without weight loss did not ameliorate dyslipidaemia or the high hepatic glycogen content in the animals consuming the HFHS diet. In addition exercise did not change the adipose tissue weight, blood glucose, glycated HbA1c or insulin concentrations in both dietary interventions. Moreover, exercise did not change the bone length or bone density or

organs weight. Furthermore, exercise did not drop the elevated blood pressure and did not induce changes in the heart function and dimensions or vascular reactivity. Hence exercise alone is not effective in combatting the adverse effects of a HFHS diet.

6.2 Limitations and future studies

The possible limitations of the current study are as follow: firstly, the most important limitation is the lack of recommended vitamins and minerals in the diet compositions. The vitamins and minerals are essential micro-compositions required for the animal's wellbeing, general health and growth. In this regard, in future studies an additional group is suggested to be added for better comparison between the animals' growth in rats consuming the HFHS diet with or without essential vitamins and minerals. Secondly, the aim of this study was to investigate the effect of a HFHS diet for a short duration. However, a HFHS diet administration for longer duration may have resulted in more adverse outcomes. Therefore, the effect of a long duration dietary intervention need to be further investigated to compare the findings from the current study to the impact of chronic consumption of HFHS diet. Thirdly, the present study investigated the effects of a HFHS diet and exercise in males only. Future studies needs to include female rats to compare diet and exercise effect in both genders. Fourthly, the mechanism responsible for the concurrent development of dyslipidaemia and high blood pressure needs further investigation, as neither inflammatory markers, quantitative measurements of oxidative stress substances or molecular technology were measured in this study. Additionally, low room temperature in the animal unit is another limitation to be considered, because at higher temperature an increased body weight and hence an altered glucose metabolism may have been observed. Another limitation in the current study is that we did not measure the liver enzymes. Therefore, we could not investigate the effect of HFHS diet on the liver functions. On the other hand, further studies need to include a high fat diet group and a high sucrose diet group to be investigate the effects of each component independently and to compare it to the HFHS diet effects.

Furthermore, the current study did not investigate the effect of exercise on the musculature which has an important role in insulin sensitivity and glycogen storage capacity. Therefore, the effect of a HFHS diet and exercise in the muscle need to be investigated. In addition to the previously mentioned limitations, all the selected rats were runners and exercised voluntarily. Therefore, the effect of exercise needs to be investigated in the runners compared to non-runners animals to eliminate the effect of the period of exercise in the non-exercising group. Finally, the exercise intervention in dyslipidemic animals did not ameliorate the dyslipidaemia and blood pressure. Further studies are needed with different interventions to elucidate this finding.

6.3 Uniqueness and strength of current study

The uniqueness of current project is in studying the effect of HFHS diet without the confounding effect of obesity and its associated disturbances. In non-obese animals, a HFHS diet elevated liver glycogen content, induced dyslipidaemia and elevated blood pressure. Additionally, this study investigated the impact of exercise on the metabolic and cardiovascular disturbances induced by HFHS diet consumption without the confounding effect of weight loss. In this regard to my knowledge there are limited studies that investigated the effect of exercise in a HFHS diet model and that have evaluated the effect of exercise in non-obese animals without the confounding effect of weight loss.

The study design of this research where there was a control group for each interventional variable, made it possible to evaluate the effect of each variable independently. While the standard diet groups acted as a control for the HFHS diet groups, the sedentary groups served as a control for the exercising groups.

6.4 Possible clinical implications and recommendations

The findings from this study could be translated to humans. In this regard the HFHS diet model imitated the consumed western diet. The short-term HFHS diet intervention induced elevated liver glycogen content, dyslipidaemia and elevated blood pressure in non-obese animals. These findings suggest that a western diet for even a short period of time can predispose lean individuals to metabolic and cardiovascular disturbances. Therefore, public education and awareness about the side effects of a western diet and the encouragement of healthy eating habits should take priority.

In the current study an exercise intervention did not ameliorate the metabolic and cardiovascular changes induced by the HFHS diet in non-obese animals. Therefore it can be suggested that exercise training alone may not be sufficient in counteracting the negative effects of a western diet, even in lean individuals. More aggressive management such as an intense lifestyle intervention or pharmacological therapy may be suggested in lean individuals with the elevated blood pressure associated with dyslipidaemia.

REFERENCES

Aarsland A, Chinkes D, Wolfe RR (1997). Hepatic and whole-body fat synthesis in humans during carbohydrate overfeeding. *The American journal of clinical nutrition.* 65(6):1774-82.

Amiri M, Majid HA, Hairi F, Thangiah N, Bulgiba A, Su TT (2014). Prevalence and determinants of cardiovascular disease risk factors among the residents of urban community housing projects in Malaysia. *BMC public health*. 14 Suppl 3:S3.

Anamourlis C, Badenhorst D, Gibbs M, Correia R, Veliotes D, Osadchii O, Norton GR, Woodiwiss AJ. Phosphodiesterase inhibition promotes the transition from compensated hypertrophy to cardiac dilatation in rats. Pflugers Arch. 2006 Jan; 451(4):526-33.

Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, Bray GA, Vogt TM, Cutler JA, Windhauser MM, Lin PH and Karanja N (1997). A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *The New England journal of medicine.* 336(16):1117-24.

Arciero PJ, Vukovich MD, Holloszy JO, Racette SB and Kohrt WM (1999). Comparison of short-term diet and exercise on insulin action in individuals with abnormal glucose tolerance. *Journal of Applied Physiology.* 86(6):1930-5.

Arias N, Macarulla MT, Aguirre L, Martínez-Castaño MG and Portillo MP (2014). Quercetin can reduce insulin resistance without decreasing adipose tissue and skeletal muscle fat accumulation. *Genes & Nutrition.* 9(1):361.

Arsenault BJ, Côté M, Cartier A, Lemieux I, Després JP, Ross R, Earnest CP, Blair SN and Church TS (2009). Effect of exercise training on cardiometabolic risk markers among sedentary, but metabolically healthy overweight or obese post-menopausal women with elevated blood pressure. *Atherosclerosis.* 207(2):530-3.

Baker P, Friel S (2014). Processed foods and the nutrition transition: evidence from Asia. *Obesity reviews*. 15(7):564-77.

Balmagiya T and Rozovski SJ (1983). Thermoregulation in young adult rats during short- and long-term protein malnutrition. *The Journal of Nutrition.* 113(2):228-38.

Barnard RJ, Faria DJ, Menges JE and Martin DA (1993). Effects of a high-fat, sucrose diet on serum insulin and related atherosclerotic risk factors in rats. *Atherosclerosis*. 100(2):229-36.

Barnard RJ, Roberts CK, Varon SM and Berger JJ (1998). Diet-induced insulin resistance precedes other aspects of the metabolic syndrome. *Journal of Applied Physiology*. 84(4):1311-1315.

Basciano H, Federico L, Adeli K (2005). Fructose, insulin resistance, and metabolic dyslipidaemia. *Nutrition & metabolism*. 2(1):5.

Baumann S, Toft U, Aadahl M, Jørgensen T and Pisinger C (2015). The long-term effect of screening and lifestyle counseling on changes in physical activity and diet: the Inter99 Study - a randomized controlled trial. The *International Journal of Behavioural Nutrition and Physical Activity.* 12:33.

Béaslas O, Cueille C, Delers F, Chateau D, Chambaz J, Rousset M and Carrière V (2009). Sensing of dietary lipids by enterocytes: a new role for SR-BI/CLA-1. *PLoS One*. 4(1):e4278.

Beavers DP, Beavers KM, Loeser RF, Walton NR, Lyles MF, Nicklas BJ, Shapses SA, Newman JJ and Messier SP (2014). The independent and combined effects of intensive weight loss and exercise training on bone mineral density in overweight and obese older adults with osteoarthritis. *Osteoarthritis Cartilage/ OARS, osteoarthritis research society.* 22(6):726-33.

Beck DT, Martin JS, Casey DP and Braith RW (2014). Exercise training improves endothelial function in resistance arteries of young prehypertensives. *Journal of Human Hypertension*. 28(5):303-9.

Behall KM, Scholfield DJ and Hallfrisch J (2006). Whole-grain diets reduce blood pressure in mildly hypercholesterolemic men and women. *Journal of the American Dietetic Association.* 106(9):1445-9.

Belczak CE, de Godoy JM, Belzack SQ, Ramos RN and Caffaro RA (2014). Obesity and worsening of chronic venous disease and joint mobility. *Phlebology/ Venous Forum of the royal Society of Medicine.* 29(8):500-4.

Benítez R, Núñez Y, Fernández A, Isabel B, Fernández AI, Rodríguez C, Barragán C, Martín-Palomino P, López-Bote C, Silió L, Óvilo C (21015). Effects of dietary fat saturation on fatty acid composition and gene transcription in different tissues of Iberian pigs. *Meat science*. 102:59-68.

Bielemann RM, Santos Motta JV, Minten GC, Horta BL, Gigante DP (2015). Consumption of ultra-processed foods and their impact on the diet of young adults. *Revista de saude publica*. 49:28.

Bligh EG and Dyer WJ. A rapid method of total lipid extraction and purification (1959). *Journal of Biochemistry and Physiology.* 37(8): 911-917.

Bocalini DS, Lima LS, de Andrade S, Madureira A, Rica RL, Dos Santos RN, Serra AJ, Silva JA Jr, Rodriguez D, Figueira A Jr and Pontes FL Jr (2012). Effects of circuit-based exercise programs on the body composition of elderly obese women. *Clinical Intervention in Aging.* 7:551-6.

Bodinham CL, Hitchen KL, Youngman PJ, Frost GS and Robertson MD (2011). Short-term effects of whole-grain wheat on appetite and food intake in healthy adults: a pilot study. *The British Journal of Nutrition. 1*06(3):327-30.

Bodnar M, Skalicky M, Viidik A and Erben RG (2012). Interaction between exercise, dietary restriction and age-related bone loss in a rodent model of male senile osteoporosis. *Gerontology.* 58(2):139-49.

Boers I, Muskiet FA, Berkelaar E, Schut E, Penders R, Hoenderdos K, Wichers HJ, Jong MC (2014). Favourable effects of consuming a Palaeolithic-type diet on characteristics of the metabolic syndrome: a randomized controlled pilot-study. *Lipids in health and disease*. 11;13:160.

Bollen M, Keppens S, Stalmans W (1998). Specific features of glycogen metabolism in the liver. *The Biochemical journal.* 336 (Pt 1):19-31.

Boqué N, Campión J, Paternain L, García-Díaz DF, Galarraga M, Portillo MP, Milagro FI, Ortiz de Solórzano C and Martínez JA (2009). Influence of dietary macronutrient composition on adiposity and cellularity of different fat depots in Wistar rats. *Journal of Physiology Biochemistry.* 65(4):387-95.

Borlaug BA, Jaber WA, Ommen SR, Lam CS, Redfield MM and Nishimura RA (2011). Diastolic relaxation and compliance reserve during dynamic exercise in heart failure with preserved ejection fraction. *Heart (British Cardiac Society).* 97(12):964-9.

Bourgoin F, Bachelard H, Badeau M, Mélançon S, Pitre M, Larivière R and Nadeau A (2008). Endothelial and vascular dysfunctions and insulin resistance in rats fed a high-fat, high-sucrose diet. *The American Journal of Physiology, Heart and circulatory Physiology.* 295(3):1044-55.

Brigham EP, Kolahdooz F, Hansel N, Breysse PN, Davis M, Sharma S, Matsui EC, Diette G, McCormack MC (2015). Association between Western diet pattern and adult asthma: a focused review. *Annals of allergy, asthma & immunology*. (4):273-80.

Brotman DJ and Girod JP (2002). The metabolic syndrome: a tug-of-war with no winner. *Cleveland Clinic Journal of Medecine.* 69(12):990-4.

Brown AT, Smith TP, Cruz CP, Poirier LA, Simmons D, Williams DK, Wang Y, Eidt JF and Moursi MM (2003). Intimal hyperplasia following carotid endarterectomy in an insulin-resistant rat model. *Metabolism: clinical and experimental.* 52(7):834-9.

Bruce CR, Hoy AJ, Turner N, Watt MJ, Allen TL, Carpenter K, Cooney GJ, Febbraio MA, Kraegen EW (2009). Overexpression of carnitine palmitoyltransferase-1 in skeletal muscle is sufficient to enhance fatty acid oxidation and improve high-fat diet-induced insulin resistance. *Diabetes*. 58(3):550-8.

Buettner R, Parhofer KG, Woenckhaus M, Wrede CE, Kunz-Schughart LA, Schölmerich J and Bollheimer LC (2006). Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. *Journal of Molecular Endocrinology.* 36(3):485-501.

Bunnag P, Hori MT, Ormsby B, Berger ME, Golub MS and Tuck ML (1997). Impaired in vivo adrenergic responses in diet-induced hypertensive rats. *Hypertension Research.* 20(1):17-21.

Cambri LT, de Araujo GG, Ghezzi AC, Botezelli JD and Mello MA (2011). Metabolic responses to acute physical exercise in young rats recovered from fetal protein malnutrition with a fructose-rich diet. *Lipids in Health and Disease.* 10:164.

Camhi SM, Stefanick ML, Katzmarzyk PT and Young DR (2010). Metabolic syndrome and changes in body fat from a low-fat diet and/or exercise randomized controlled trial. *Obesity.*18(3):548-54.

Carll AP, Willis MS, Lust RM, Costa DL and Farraj AK (2011). Merits of non-invasive rat models of left ventricular heart failure. *Cardiovascular Toxicology.* 11:91-112.

Carmiel-Haggai M, Cederbaum AI and Nieto N (2005). A high-fat diet leads to the progression of non-alcoholic fatty liver disease in obese rats. *FASEB Journal.* 19:136-8.

Castro MC, Francini F, Gagliardino JJ, Massa ML (2014). Lipoic acid prevents fructose-induced changes in liver carbohydrate metabolism: role of oxidative stress. *Biochimica et biophysica acta.* 1840(3):1145-51.

Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, Clegg LX (1997). Association of coronary heart disease incidence with carotid arterial wall thickness an d major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987-1993. *American journal of epidemiology*. 146(6):483-94.

Chan L, Terashima T, Fujimiya M and Kojima H (2006). Chronic diabetic complications: the body's adaptive response to hyperglycaemia gone awry? *Transactions of the American Clinical and Climatological Association.* 117: 341-51.

Cheng HS, Kong JM, Ng AX, Chan WK, Ton SH and Abdul Kadir K (2014). Novel Inhibitory Effects of Glycyrrhizic Acid on the Accumulation of Advanced Glycation End Product and Its Receptor Expression. *Natural Product and Bioprospecting.* [Epub ahead of print]

Chicco A, D'Alessandro ME, Karabatas L, Pastorale C, Basabe JC and Lombardo YB (2003). Muscle lipid metabolism and insulin secretion are altered in insulin-resistant rats fed a high sucrose diet. *The Journal of Nutrition.* 133(1):127-33.

Chicco AJ, Sparagna GC, McCune SA, Johnson CA, Murphy RC, Bolden DA, Rees ML, Gardner RT and Moore RL (2008). Linoleate-rich high-fat diet decreases mortality in hypertensive heart failure rats compared with lard and low-fat diets. *Hypertension.* 52(3):549-55.

Chilton FH, Murphy RC, Wilson BA, Sergeant S, Ainsworth H, Seeds MC and Mathias RA (2014). Diet-gene interactions and PUFA metabolism: a potential contributor to health disparities and human diseases. *Nutrients*. 6(5):1993-2022.

Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ; Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure and National Heart, Lung, and Blood Institute; National High Blood Pressure Education Program Coordinating Committee (2003). Seventh report of the Joint National Committee on

Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension.* 42(6):1206-52.

Choo J, Lee J, Cho JH, Burke LE, Sekikawa A and Jae SY (2014). 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 Cardiovascular Disorders.* 14:82.

Christon R, Marette A, Badeau M, Bourgoin F, Mélançon S and Bachelard H (2005). Fatty acid-induced changes in vascular reactivity in healthy adult rats. *Metabolism*. 54(12):1600-9.

Chu MJ, Hickey AJ, Jiang Y, Petzer A, Bartlett AS and Phillips AR (2015). Mitochondrial dysfunction in steatotic rat livers occurs because a defect in complex i make the liver susceptible to prolonged cold ischemia. *Liver Transplantation.* 21(3):396-407.

Chun MR, Lee YJ, Kim KH, Kim YW, Park SY, Lee KM, Kim JY and Park YK (2010). Differential effects of high-carbohydrate and high-fat diet composition on muscle insulin resistance in rats. *Journal of Korean Medical Science.* 25(7):1053-9.

Clarke MC, Talib S, Figg NL, Bennett MR (2010). Vascular smooth muscle cell apoptosis induces interleukin-1-directed inflammation: effects of hyperlipidemia-mediated inhibition of phagocytosis. *Circulation research.* 106(2):363-72.

Clarke G, Fitzgerald P, Hennessy AA, Cassidy EM, Quigley EM, Ross P, Stanton C, Cryan JF, Dinan TG (2010). Marked elevations in pro-inflammatory polyunsaturated fatty acid metabolites in females with irritable bowel syndrome. *Journal of lipid research*. 51(5):1186-92.

Coghill N and Cooper AR (2008). The effect of a home-based walking program on risk factors for coronary heart disease in hypercholesterolaemic men. A randomized controlled trial. *Preventive Medicine*. 46(6):545-51.

Coker RH, Hays NP, Williams RH, Brown AD, Freeling SA, Kortebein PM, Sullivan DH, Starling RD and Evans WJ (2006). Exercise-induced changes in insulin action and glycogen metabolism in elderly adults. *Medicine and Science in Sports and Exercise*. 38(3):433-8.

Commerford SR, Bizeau ME, McRae H, Jampolis A, Thresher JS and Pagliassotti MJ (2001). Hyperglycaemia compensates for diet-induced insulin resistance in liver and skeletal muscle of rats. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology.* 281(5):1380-9.

de Andrade EN, Gonçalves GK, de Oliveira TH, Santos CS, Souza CL, Firmes LB, de Magalhães AC, Soares Tde J, Reis AM and Belo Nde O (2011). Natriuretic peptide system: a link between fat mass and cardiac hypertrophy and hypertension in fat-fed female rats. *Regulatory Peptides.* 167(1):149-55.

Dean E and Gormsen Hansen R (2012). Prescribing optimal nutrition and physical activity as "first-line" interventions for best practice management of chronic low-grade inflammation associated with osteoarthritis: evidence synthesis. *Arthritis.* 2012:560634.

Dearth-Wesley T, Gordon-Larsen P, Adair LS, Siega-Riz AM, Zhang B and Popkin BM (2011). Less traditional diets in Chinese mothers and children are similarly linked to socioeconomic and cohort factors but vary with increasing child age. *The Journal of Nutrition.* 141(9):1705-11.

de Carvalho Vidigal F, Guedes Cocate P, Gonçalves Pereira L and de Cássia Gonçalves Alfenas R (2012). The role of hyperglycaemia in the induction of oxidative stress and inflammatory process. *Nutricion Hospitalaria.* 27(5):1391-8.

de Castro UG, dos Santos RA, Silva ME, de Lima WG, Campagnole-Santos MJ, Alzamora AC (2013). Age-dependent effect of high-fructose and high-fat diets on lipid metabolism and lipid accumulation in liver and kidney of rats. *Lipids in health and disease.* 18; 12:136.

de Jongh RT, Serné EH, IJzerman RG, de Vries G and Stehouwer CD (2004). Impaired microvascular function in obesity: implications for obesity-associated microangiopathy, hypertension, and insulin resistance. *Circulation.* 109(21):2529-35.

de la Garza AL, Etxeberria U, Haslberger A, Aumueller E, Martínez JA and Milagro FI (2013). Helichrysum and Grapefruit Extracts Boost Weight Loss in Overweight Rats Reducing Inflammation. *Journal of Medicinal Food.* [Epub ahead of print].

Del Boca J, Flatt JP (1969). Fatty acid synthesis from glucose and acetate and the control of lipogenesis in adipose tissue. *European journal of biochemistry/FEBS*. 11(1):127-34.

Delghingaro-Augusto V, Décary S, Peyot ML, Latour MG, Lamontagne J, Paradis-Isler N, Lacharité-Lemieux M, Akakpo H, Birot O, Nolan CJ, Prentki M and Bergeron R (2012). Voluntary running exercise prevents β-cell failure in susceptible islets of the Zucker diabetic fatty rat. American Journal of Physiology. *Endocrinology and Metabolism.* 302(2):254-64.

del Pozo-Cruz B, Alfonso-Rosa RM, del Pozo-Cruz J, Sañudo B and Rogers ME (2014). Effects of a 12-wk whole-body vibration based intervention to improve type 2 diabetes. Maturitas. 77(1):52-8.

de Mélo Montenegro IH, Moita L, Dos Reis FK, de Oliveira E, Lisboa PC, de Moura EG, Manhães-de-Castro R, Leandro CG (2012). Effects of a moderate physical training on the leptin synthesis by adipose tissue of adult rats submitted to a perinatal low-protein diet. *Hormone and metabolic research*. 44(11):814-8.

de Moraes C, Davel AP, Rossoni LV, Antunes E and Zanesco A (2008). Exercise training improves relaxation response and SOD-1 expression in aortic and mesenteric rings from high caloric diet-fed rats. *BMC Physiol.* 8:12.

de Souza RJ, Mente A, Maroleanu A, Cozma AI, Ha V, Kishibe T, Uleryk E, Budylowski P, Schünemann H, Beyene J, Anand SS (2015).

Intake of saturated and trans unsaturated fatty acids and risk of
all cause mortality, cardiovascular disease,
andtype 2 diabetes: systematic review and meta-analysis of observational studies. *BMJ (clinical research ed)*. 11;351:h3978.

Diaz KM and Shimbo D (2013). Physical activity and the prevention of hypertension. *Current Hypertension Reports.* 15(6):659-68.

DiBello JR, Ioannou C, Rees J, Challacombe B, Maskell J, Choudhury N, Kastner C, Kirby M (2015). Prevalence of metabolic syndrome and its components among men with and without clinical benign prostatic hyperplasia: a large, cross-sectional, UK epidemiological study. *BJU International*.

Drevenhorn E, Kjellgren KI and Bengtson A (2007). Outcomes following a programme for lifestyle changes with people with hypertension. *Journal of Clinical Nursing*.16(7B):144-51.

Dutta K, Podolin DA, Davidson MB and Davidoff AJ (2001). Cardiomyocyte dysfunction in sucrose-fed rats is associated with insulin resistance. *Diabetes.* 50(5):1186-92.

Edelstein SL, Knowler WC, Bain RP, Andres R, Barrett-Connor EL, Dowse GK, Haffner SM, Pettitt DJ, Sorkin JD, Muller DC, Collins VR, and Hamman RF (1997). Predictors of progression from impaired glucose tolerance to NIDDM: an analysis of six prospective studies. *Diabetes.* 46(4):701-10.

Edwards LM, Murray AJ, Holloway CJ, Carter EE, Kemp GJ, Codreanu I, Brooker H, Tyler DJ, Robbins PA and Clarke K (2011). Short-term consumption of a high-fat diet impairs whole-body efficiency and cognitive function in sedentary men*. FASEB Journal.* 25(3):1088-96.

Ekelund U, Ward HA, Norat T, Luan J, May AM, Weiderpass E, Sharp SJ, Overvad K, Østergaard JN, Tjønneland A, Johnsen NF, Mesrine S,Fournier A, Fagherazzi G, Trichopoulou A, Lagiou P, Trichopoulos D, Li K, Kaaks R, Ferrari P, Licaj I, Jenab

M, Bergmann M, Boeing H, Palli D,Sieri S, Panico S, Tumino R, Vineis P, Peeters PH, Monnikhof E, Bueno-de-Mesquita HB, Quirós JR, Agudo A, Sánchez MJ, Huerta JM, Ardanaz E,Arriola L, Hedblad B, Wirfält E, Sund M, Johansson M, Key TJ, Travis RC, Khaw KT, Brage S, Wareham NJ and Riboli E (2015). 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). *The American Journal of Clinical Nutrition.* 101(3):613-21.

Ennequin G, Boisseau N, Caillaud K, Chavanelle V, Gerbaix M, Metz L, Etienne M, Walrand S, Masgrau A, Guillet C, Courteix D, Niu A, Li YP, Capel F and Sirvent P (2015). Exercise training and return to a well-balanced diet activate the neuregulin 1 / ErbB pathway in skeletal muscle of obese rats. *The Journal of Physiology.* 593(12):2665-77.

Erdos B, Kirichenko N, Whidden M, Basgut B, Woods M, Cudykier I, Tawil R, Scarpace PJ and Tumer N (2011). Effect of age on high-fat diet-induced hypertension. *American Journal of Physiology- Heart and Circulatory Physiology.* 301(1):H164-72.

Eriksson J, Taimela S and Koivisto VA (1997). Exercise and the metabolic syndrome. *Diabetologia.* 40(2):125-35.

Eschen O, Christensen JH, De Caterina R, Schmidt EB (2004). Soluble adhesion molecules in healthy subjects: a dose-response study using n-3 fatty acids. *Nutrition, metabolism, and cardiovascular diseases: NMCD*. 14(4):180-5.

Esser D, van Dijk SJ, Oosterink E, Müller M, Afman LA (2013). A high-fat SFA, MUFA, or n3 PUFA challenge affects the vascular response and initiates an activated state of cellular adherence in lean and obese middle-aged men. *The Journal of nutrition.* 143(6):843-51.

Etxeberria U, de la Garza AL, Martínez JA and Milagro FI (2013). Diet-induced hyperinsulinemia differentially affects glucose and protein metabolism: a high-

throughput metabolomic approach in rats. *Journal of Physiology and Biochemistry.* 69(3):613-23.

Ewang-Emukowhate M, Perera D, Wierzbicki AS (2014). Dyslipidaemia related to insulin resistance and cardiovascular disease in South Asian and West African populations. *Current pharmaceutical design*. 20(40):6270-5.

Flatt JP (1970). Conversion of carbohydrate to fat in adipose tissue: an energy-yielding and, therefore, self-limiting process. *Journal of lipid research*. 11(2):131-43.

Feinman RD, Pogozelski WK, Astrup A, Bernstein RK, Fine EJ, Westman EC, Accurso A, Frassetto L, Gower BA, McFarlane SI, Nielsen JV, Krarup T, Saslow L, Roth KS, Vernon MC, Volek JS, Wilshire GB, Dahlqvist A, Sundberg R, Childers A, Morrison K[21], Manninen AH, Dashti HM, Wood RJ, Wortman J, Worm N (2015). Dietary carbohydrate restriction as the first approach in diabetes management: critical review and evidence base. *Nutrition*. 31(1):1-13.

Feng M and DiPetrillo K (2009). Non-invasive bloosd pressure measurement in mice. *Methods in Molecular Biology*.573:45-55.

Fernando HA, Chandramouli C, Rosli D, Lam YL, Yong ST, Yaw HP, Ton SH, Kadir KA and Sainsbury A (2014). Glycyrrhizic acid can attenuate metabolic deviations caused by a high-sucrose diet without causing water retention in male Sprague-Dawley rats. *Nutrients.* 6(11):4856-71.

Ferraris RP, Vinnakota RR (1995). The time course of adaptation of intestinal nutrient uptake in mice is independent of age. *The Journal of nutrition*. 125(8):2172-82.

Ferrer JC, Favre C, Gomis RR, Fernández-Novell JM, García-Rocha M, de la Iglesia N, Cid E, Guinovart JJ (2003). Control of glycogen deposition. *FEBS letters.* 546(1):127-32.

Ferré P, Foufelle F (2010). Hepatic steatosis: a role for de novo lipogenesis and the transcription factor SREBP-1c. *Diabetes, obesity & metabolism*. 12 Suppl 2:83-92.

Ferreira AP, Ferreira CB, Souza VC, Córdova CO, Silva GC, Nóbrega Ode T and França NM (2011). The influence of intense intermittent versus moderate continuous exercise on postprandial lipemia. *Clinics (Sao Paulo. Brazil).* 66(4):535-41.

Ficker ES, Maranhão RC, Chacra AP, Neves VC, Negrao CE, Martins VC and Vinagre CG (2010). Exercise training accelerates the removal from plasma of LDL-like nanoemulsion in moderately hypercholesterolemic subjects. *Atherosclerosis.* 212(1):230-6.

Fisher G, Hunter GR, Gower BA (2012). Aerobic exercise training conserves insulin sensitivity for 1 yr following weight loss in overweight women. *Journal of applied physiology*. 112(4):688-93.

Fisher-Wellman KH, Mattox TA, Thayne K, Katunga LA, La Favor JD, Neufer PD, Hickner RC, Wingard CJ and Anderson EJ (2013). Novel role for thioredoxin reductase-2 in mitochondrial redox adaptations to obesogenic diet and exercise in heart and skeletal muscle. *The Journal of Physiology.* 591(Pt 14):3471-86.

Folsom AR, Shah AM, Lutsey PL, Roetker NS, Alonso A, Avery CL, Miedema MD, Konety S, Chang PP and Solomon SD (2015). American Heart Association's Life's Simple 7: Avoiding Heart Failure and Preserving Cardiac Structure and Function. *The American Journal of Medicine.* pii: S0002-9343(15)00318-6.

Foufelle F, Ferré P (2002). New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a role for the transcription factor sterol regulatory element binding protein-1c. *The Biochemical journal*. 366(Pt 2):377-91.

Frühbeck G, Méndez-Giménez L, Fernández-Formoso JA, Fernández S, Rodríguez A (2014). Regulation of adipocyte lipolysis. *Nutrition research reviews*. 27(1):63-93.

Gabir MM, Hanson RL, Dabelea D, Imperatore G, Roumain J, Bennett PH, Knowler WC (2000). The 1997 American Diabetes Association and 1999 World Health Organization criteria for hyperglycaemia in the diagnosis and prediction of diabetes. *Diabetes care*. 23(8):1108-12.

Gabriel B, Ratkevicius A, Gray P, Frenneaux MP and Gray SR (2012). High-intensity exercise attenuates postprandial lipaemia and markers of oxidative stress. *Clinical Science.* 123(5):313-21.

Gangwisch JE, Hale L, Garcia L, Malaspina D, Opler MG, Payne ME, Rossom RC, Lane D (2015). High glycemic index diet as a risk factor for depression: analyses from the Women's Health Initiative. *The American journal of clinical nutrition.* 102(2):454-63.

Garrido G, Guzmán M and Odriozola JM (1996). Effect of different types of high carbohydrate diets on glycogen metabolism in liver and skeletal muscle of endurance-trained rats. *European Journal of Applied Physiology Occupational Physiology.* 74(1-2):91-9.

Gauthier MS, Couturier K, Latour JG and Lavoie JM (2003). Concurrent exercise prevents high-fat-diet-induced macrovesicular hepatic steatosis. *The Journal of Applied Physiology.* 94(6):2127-34.

Gerbaix M, Metz L, Mac-Way F, Lavet C, Guillet C, Walrand S, Masgrau A, Vico L and Courteix D (2013). A well-balanced diet combined or not with exercise induces fat mass loss without any decrease of bone mass despite bone micro-architecture alterations in obese rat. *Bone.* 53(2):382-90.

Gibson SA (2007). Are diets high in non-milk extrinsic sugars conducive to obesity? An analysis from the Dietary and Nutritional Survey of British Adults. *Journal of human nutrition and dietetics*. 20(3):229-38.

Golbidi S, Badran M and Laher I (2012). Antioxidant and anti-inflammatory effects of exercise in diabetic patients. *Experimental Diabetes Research.* 2012:941868.

Goldberg IJ (1996). Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *Journal of lipid research*. 37(4):693-707.

Goldberg IJ (2001). Clinical review 124: Diabetic dyslipidaemia: causes and consequences. *The Journal of clinical endocrinology and metabolism.* 86(3):965-71.

Gonçalves AR, Fujihara CK, Mattar AL, Malheiros DM, Noronha Ide L, de Nucci G and Zatz R (2004). Renal expression of COX-2, ANG II, and AT1 receptor in remnant kidney: strong renoprotection by therapy with losartan and a nonsteroidal anti-inflammatory. *American Journal of Physiology- Renal Physiology.* 286(5):F945-54.

Green DJ, Walsh JH, Maiorana A, Best MJ, Taylor RR and O'Driscoll JG (2003). Exercise-induced improvement in endothelial dysfunction is not mediated by changes in CV risk factors: pooled analysis of diverse patient populations. *American Journal of Physiology- Heart and Circulatory Physiology.* 285(6):H2679-87.

Grimditch GK, Barnard RJ, Sternlicht E, Whitson RH and Kaplan SA (1987). Effect of diet on insulin binding and glucose transport in rat sarcolemmal vesicles. *The American Journal of Physiology.* 252(3 Pt 1):420-5.

Grimditch GK, Barnard RJ, Hendricks L and Weitzman D (1988). Peripheral insulin sensitivity as modified by diet and exercise training. *The American Journal of Clinical Nutrition.* 148(1):38-43.

Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F and American Heart Association; National Heart, Lung, and Blood Institute (2005). Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation.* 112(1):2735-52.

Hajer GR, vanHaefen TW and Visseren FL (2008). Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. *European Heart Journal.* 29(24):2959-71.

Hanley AJ, Karter AJ, Williams K, Festa A, D'Agostino RB Jr, Wagenknecht LE and Haffner SM (2005). Prediction of type 2 diabetes mellitus with alternative definitions of the metabolic syndrome: the Insulin Resistance Atherosclerosis Study. *Circulation.* 112(24):3713-21.

Harber MP, Schenk S, Barkan AL and Horowitz JF (2005). Alterations in carbohydrate metabolism in response to short-term dietary carbohydrate restriction. *American Journal of Physiology. Endocrinology and Metabolism.* 289(2):306-12.

Hariharan D, Vellanki K, Kramer H (2015). The Western Diet and Chronic Kidney Disease. *Current hypertension reports*. 17(3):16.

Harmancey R, Lam TN, Lubrano GM, Guthrie PH, Vela D and Taegtmeyer H (2012). Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. *FASEB Journal.* 26(8):3118-26.

Harris MM, Stevens J, Thomas N, Schreiner P and Folsom AR (2000). Associations of fat distribution and obesity with hypertension in a bi-ethnic population: the ARIC study. Atherosclerosis Risk in Communities Study. *Obesity Research.* 8(7):516-24.

Heinonen I, Nesterov SV, Kemppainen J, Fujimoto T, Knuuti J and Kalliokoski KK (2012). Increasing exercise intensity reduces heterogeneity of glucose uptake in human skeletal muscles. *PLoS One.* 7(12):e52191.

Heo M and Kim E (2013) Effects of Endurance Training on Lipid Metabolism and Glycosylated Hemoglobin Levels in Streptozotocin-induced Type 2 Diabetic Rats on a High-fat Diet. *Journal of physical therapy Science.* 25(8):989-92.

Hession M, Rolland C, Kulkarni U, Wise A, Broom J (2009). Systematic review of randomized controlled trials of low-carbohydrate vs. low-fat/low-calorie diets in the management of obesity and its comorbidities. Obesity reviews. 10(1):36-50.

Hirotani Y, Doi A, Takahashi T, Umezawa H, Urashima Y and Myotoku M (2012). Protective effects of the herbal medicine goshajinkigan in a rat model of non-alcoholic fatty liver disease. *Biomedical Research.* 33(6):373-6.

Hite AH, Feinman RD, Guzman GE, Satin M, Schoenfeld PA, Wood RJ (2010). In the face of contradictory evidence: report of the Dietary Guidelines for Americans Committee. *Nutrition*. 26(10):915-24.

Hong S, Lee J, Park J, Lee M, Kim JY, Kim KC, Kim SH, Im JA, Chu SH, Suh SH, Kim SH, Jeon JY (2014). Association between cardiorespiratory fitness and the prevalence of metabolic syndrome among Korean adults: a cross sectional study. *BMC public health*. 21; 14:481.

Horodyska K, Luszczynska A, van den Berg M, Hendriksen M, Roos G, De Bourdeaudhuij I and Brug J (2015). Good practice characteristics of diet and physical activity interventions and policies: an umbrella review. *BMC Public Health.* 15:19.

Horton TJ, Drougas H, Brachey A, Reed GW, Peters JC, Hill JO (1995). Fat and carbohydrate overfeeding in humans: different effects on energy storage. *The American journal of clinical nutrition*. 62(1):19-29.

Huang G, Shi X, Gibson CA, Huang SC, Coudret NA and Ehlman MC (2013). Controlled aerobic exercise training reduces resting blood pressure in sedentary older adults. *Blood Pressure.* 22(6):386-94.

Huetter ML, Fuchs M, Hänle MM, Mason RA, Akinli AS, Imhof A, Kratzer W, Lorenz R and EMIL study group (2014). Prevalence of risk factors for liver disease in a random population sample in southern Germany. *Zeitschrift Gastroenterologie.* 52(6):558-63.

Ivanova M, Janega P, Matejikova J, Simoncikova P, Pancza D, Ravingerova T and Barancik M (2011). Activation of Akt kinase accompanies increased cardiac resistance to ischemia/reperfusion in rats after short-term feeding with lard-based high-fat diet and increased sucrose intake. *Nutrition Research.* 31(8):631-43.

Jellinger PS (2007). Metabolic consequences of hyperglycaemia and insulin resistance. *Clinical Cornerstone*. 7:S30-42.

Jameson K, Amber V, D'Oca K, Mills D, Giles A, Ambegaonkar B (2013). Impact of lipid-lowering therapy on the prevalence of dyslipidaemia in patients at high-risk of cardiovascular events in UK primary care - a retrospective database study. *International journal of clinical practice*. 67(12):1228-37.

Jia X, Chen Y, Zidichouski J, Zhang J, Sun C and Wang Y (2008). Co-administration of berberine and plant stanols synergistically reduces plasma cholesterol in rats. *Atherosclerosis.* 201(1):101-7.

Kabel AM, Abd Elmaaboud MA and Albarraq AA (2014). Ameliorative potential of omega 3 fatty acids and HMG-CoA reductase inhibitors on experimentally-induced non-alcoholic steatohepatitis. Prostaglandins Leukotrienes, and Essential Fatty Acids. *Prostaglandins, Leukotrienes and Essential Fatty Acids.* 96: 1-9

Kadoglou NP, Fotiadis G, Kapelouzou A, Kostakis A, Liapis CD and Vrabas IS (2013). The differential anti-inflammatory effects of exercise modalities and their association with early carotid atherosclerosis progression in patients with type 2 diabetes. *Diabetic Medicine: a Journal of the British Diabetic association.* 30(2):41-50.

Kamimura MA, Carrero JJ, Canziani ME, Watanabe R, Lemos MM and Cuppari L (2013). Visceral obesity assessed by computed tomography predicts cardiovascular events in chronic kidney disease patients. *Nutrition, Metabolism, and Cardiovascular Diseases.* 23(9):891-7.

Kazemian P, Kazemi-Bajestani SM, Alherbish A, Steed J and Oudit GY (2012). The use of ω-3 poly-unsaturated fatty acids in heart failure: a preferential role in patients with diabetes. *Cardiovascular Drugs and Therapy.* 26(4):311-20.

Kelly AS, Wetzsteon RJ, Kaiser DR, Steinberger J, Bank AJ and Dengel DR (2004). Inflammation, insulin, and endothelial function in overweight children and adolescents: the role of exercise. *The Journal of Paediatrics.* 145(6):731-6.

Kelley DE, Williams KV, Price JC, McKolanis TM, Goodpaster BH, Thaete FL (2001). Plasma fatty acids, adiposity, and variance of skeletal muscle insulin resistance in type 2 diabetes mellitus. *The Journal of clinical endocrinology and metabolism.* 86(11):5412-9.

Kersten S (2001). Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO reports.* 2(4):282-6.

Khadir A, Tiss A, Abubaker J, Abu-Farha M, Al-Khairi I, Cherian P, John J, Kavalakatt S, Warsame S, Al-Madhoun A, Al-Ghimlas F, Elkum N,Behbehani K, Dermime S and Dehbi M (2015). MAP kinase phosphatase DUSP1 is overexpressed in obese humans and modulated by physical exercise. *American Journal of Physiology, Endocrinology and Metabolism.* 308(1):71-83.

Kjaergaard M, Nilsson C, Rosendal A, Nielsen MO and Raun K (2014). Maternal chocolate and sucrose soft drink intake induces hepatic steatosis in rat offspring associated with altered lipid gene expression profile. *Acta Physiologica.* 210(1):142-53.

Klein S, Burke LE, Bray GA, Blair S, Allison DB, Pi-Sunyer X, Hong Y and Eckel RH (2004). Clinical implications of obesity with specific focus on cardiovascular disease: a statement for professionals from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism: endorsed by the American College of Cardiology Found*. Circulation.* 110:2952–2967.

Kluding PM, Pasnoor M, Singh R, D'Silva LJ, Yoo M, Billinger SA, LeMaster JW, Dimachkie MM, Herbelin L and Wright DE (2015). Safety of aerobic exercise in people with diabetic peripheral neuropathy: single-group clinical trial. *Physical Therapy.* 95(2):223-34.

Knight SF, Quigley JE, Yuan J, Roy SS, Elmarakby A and Imig JD (2008). Endothelial dysfunction and the development of renal injury in spontaneously hypertensive rats fed a high-fat diet. *Hypertension.* 51(2):352-9.

Koizumi G, Kumai T, Egawa S, Yatomi K, Hayashi T, Oda G, Ohba K, Iwai S, Watanabe M, Matsumoto N and Oguchi K (2013). Gene expression in the vascular wall of the aortic arch in spontaneously hypertensive hyperlipidemic model rats using DNA microarray analysis. *Life Sciences.* 93(15):495-502.

Kong X, Zhang DY, Wu HB and Li FX (2011). Losartan and pioglitazone ameliorate nephropathy in experimental metabolic syndrome rats. *Biological & Pharmaceutical Bulletin.* 34(5):693-9.

Kong X, Ma MZ, Qin L, Zhang Y, Li XY, Wang GD, Su Q and Zhang DY (2014). Pioglitazone enhances the blood pressure-lowering effect of losartan via synergistic attenuation of angiotensin II-induced vasoconstriction. *Journal of the Renin-Angiotensin-Aldosterone System.* 15(15):259-70.

Koppo K, Larrouy D, Marques MA, Berlan M, Bajzova M, Polak J, Van de Voorde J, Bülow J, Lafontan M, Crampes F, Langin D, Stich V, de Glisezinski I (2010). Lipid mobilization in subcutaneous adipose tissue during exercise in lean and obese humans. Roles of insulinand natriuretic peptides. *The American journal of physiology and Endocrinology and metabolism.* 299(2):E258-65.

Kouamé N, Nadeau A, Lacourcière Y and Cléroux J (1995). Effects of different training intensities on the cardiopulmonary baroreflex control of forearm vascular resistance in hypertensive subjects. *Hypertension.* 25(3):391-8.

Králová V, Hanušová V, Staňková P, Knoppová K, Čáňová K, Skálová L (2013). Antiproliferative effect of benzimidazole anthelmintics albendazole, ricobendazole, and flubendazole in intestinal cancer cell lines. *Anti-cancer drugs*. 24(9):911-9.

Kraus WE, Houmard JA, Duscha BD, Knetzger KJ, Wharton MB, McCartney JS, Bales CW, Henes S, Samsa GP, Otvos JD, Kulkarni KR and Slentz CA (2002). Effects of the

amount and intensity of exercise on plasma lipoproteins. *The New England Journal of Medicine.* 347(19):1483-92.

Kumar P, Bhandari U, Jamadagni S (2014). Fenugreek seed extract inhibit fat accumulation and ameliorates dyslipidaemia in high fat diet-induced obese rats. *BioMed research international*. 2014; 2014:606021.

La Favor JD, Anderson EJ, Dawkins JT, Hickner RC and Wingard CJ (2013). Exercise prevents Western diet-associated erectile dysfunction and coronary artery endothelial dysfunction: response to acute apocynin and sepiapterin treatment. *American Journal of Physiology, Regulatory, Integrative and Comparative Physiology.* 305(4):423-34.

la Fleur SE, van Rozen AJ, Luijendijk MC, Groeneweg F and Adan RA (2010). A free-choice high-fat high-sugar diet induces changes in arcuate neuropeptide expression that support hyperphagia. *International Journal of Obesity.* 34(3):537-46.

Lambert E, Straznicky N, Sari CI, Eikelis N, Hering D, Head G, Dixon J, Esler M, Schlaich M, Lambert G (2013). Dyslipidaemia is associated with sympathetic nervous activation and impaired endothelial function in young females. *American journal of hypertension*. 26(2):250-6.

Lang HF, Chou CY, Sheu WH and Lin JY (2011). Weight loss increased serum adiponectin but decreased lipid levels in obese subjects whose body mass index was lower than 30 kg/m^2.Nutrition Research. (5):378-86.

Larsen S, Skaaby S, Helge JW, and Dela F (2014). Effects of exercise training on mitochondrial function in patients with type 2 diabetes. *World Journal of Diabetes.* 5(4):482-92.

Lao XQ, Ma WJ, Sobko T, Zhang YH, Xu YJ, Xu XJ, Yu DM, Nie SP, Cai QM, Wei XL, Xia L, Wong MC (2014). Dramatic escalation in metabolic syndrome and cardiovascular risk in a Chinese population experiencing rapid economic development. *BMC public health*. 20;14:983.

Levin BE and Dunn-Meynell AA (2002). Defense of body weight depends on dietary composition and palatability in rats with diet-induced obesity. *American Journal of Physiology-Regulatory, Integrative and Comparitive Physiology.* 282(1):R46-54.

Lewis TV, Dart AM, Chin-Dusting JP and Kingwell BA (1999). Exercise training increases basal nitric oxide production from the forearm in hyperchole sterolemic patients. *Arteriosclerosis, Thrombosis, and Vascular Biology.* 19(11):2782-7.

Li MQ, Shi ZX, Xu JY, Lu B, Li JQ, Xu YJ, Wang XM, Li SM, Mo X (2014). Hemodiafiltration combined with resin-mediated absorption as a therapy for hyperlipidemic acute pancreatitis. *Cell biochemistry and biophysics*. 69(3):699-702.

Liao Y, Kwon S, Shaughnessy S, Wallace P, Hutto A, Jenkins AJ, Klein RL, Garvey WT (2004). Critical evaluation of adult treatment panel III criteria in identifying insulin resistance with dyslipidaemia. *Diabetes care*. 27(4):978-83.

Lima AH, Couto HE, Cardoso GA, Toscano LT, Silva AS and Mota MP (2012). Aerobic training does not alter blood pressure in menopausal women with metabolic syndrome. *Arquivos Brasileiros de Cardiologia.* 99(5):979-87.

Linkis P, Jørgensen LG, Olesen HL, Madsen PL, Lassen NA and Secher NH (1995). Dynamic exercise enhances regional cerebral artery mean flow velocity. *Journal of Applied Physiology.* 78(1):12-6.

Lionetti L, Mollica MP, Donizzetti I, Gifuni G, Sica R, Pignalosa A, Cavaliere G, Gaita M, De Filippo C, Zorzano A and Putti R (2014). High-lard and high-fish-oil diets differ in their effects on function and dynamic behaviour of rat hepatic mitochondria. *PLoS One*. 9(3):e92753.

Lithander FE, Herlihy LK, Walsh DM, Burke E, Crowley V, Mahmud A (2013). Postprandial effect of dietary fat quantity and quality on arterial stiffness and wave reflection: a randomised controlled trial. *Nutrition journal*. 10; 12:93.

Lomba A, Milagro FI, García-Díaz DF, Marti A, Campión J and Martínez JA (2010). Obesity induced by a pair-fed high fat sucrose diet: methylation and expression pattern of genes related to energy homeostasis. Lipids in Health and Disease. 9;9:60.

Lowenstein JA, Caniggia C, Rousse G, Amor M, Sánchez ME, Alasia D, Casso N, García A, Zambrana G, Lowenstein Haber DM and Darú V (2014). Coronary flow velocity reserve during pharmacologic stress echocardiography with normal contractility adds important prognostic value in diabetic and nondiabetic patients. *Journal of the American Society of Echocardiography.* 27(10):1113-9.

Lowndes J, Sinnett S, Pardo S, Nguyen VT, Melanson KJ, Yu Z, Lowther BE, Rippe JM (2014). The effect of normally consumed amounts of sucrose or high fructose corn syrup on lipid profiles, body composition and related parameters in overweight/obese subjects. *Nutrients*. 6(3):1128-44.

Macor C, Ruggeri A, Mazzonetto P, Federspil G, Cobelli C, Vettor R (1997). Visceral adipose tissue impairs insulin secretion and insulin sensitivity but not energy expenditure in obesity. *Metabolism: clinical and experimental*. 46(2):123-9.

Malin SK, Niemi N, Solomon TP, Haus JM, Kelly KR, Filion J, Rocco M, Kashyap SR, Barkoukis H and Kirwan JP (2012). Exercise training with weight loss and either a high- or low-glycemic index diet reduces metabolic syndrome severity in older adults. *Annals of Nutrition & Metabolism.* 61(2):135-41.

Malin SK, Huang H, Mulya A, Kashyap SR and Kirwan JP (2013). Lower dipeptidyl peptidase-4 following exercise training plus weight loss is related to increased insulin sensitivity in adults with metabolic syndrome. *Peptides.* 47:142-7.

Maloney SK, Fuller A, Mitchell D, Gordon C and Overton JM (2014).Translating animal model research: does it matter that our rodents are cold? *Physiology (Bethesda).* 29(6):413-20.

Malta A, de Oliveira JC, Ribeiro TA, Tófolo LP, Barella LF, Prates KV, Miranda RA, Elmhiri G, Franco CC, Agostinho AR, Trombini AB, Pavanello A, Gravena C,Abdennebi-

Najar L, Mathias PC (2014). Low-protein diet in adult male rats has long-term effects on metabolism. *The journal of endocrinology.* 221(2):285-95.

Mariotti F, Hermier D, Sarrat C, Magné J, Fénart E, Evrard J, Tomé D and Huneau JF (2008). Rapeseed protein inhibits the initiation of insulin resistance by a high-saturated fat, high-sucrose diet in rats. *The British Journal of Nutrition.* 100(5):984-91.

Martinez Calejman C, Di Gruccio JM, Mercau ME, Repetto EM, Astort F, Sanchez R, Pandolfi M, Berg G, Schreier L, Arias P and Cymeryng CB (2012). Insulin sensitization with a peroxisome proliferator-activated receptor γ agonist prevents adrenocortical lipid infiltration and secretory changes induced by a high-sucrose diet. *The Journal of Endocrinology.* 214(3):267-76.

Maslak E, Buczek E, Szumny A, Szczepnski W, Franczyk-Zarow M, Kopec A, Chlopicki S, Leszczynska T,Kostogrys RB (2015). Individual CLA Isomers, c9t11 and t10c12, Prevent Excess Liver Glycogen Storage and Inhibit Lipogenic Genes Expression Induced by High-Fructose Diet in Rats. *BioMed research international.* 2015:535982.

Mason C, Foster-Schubert KE, Imayama I, Kong A, Xiao L, Bain C, Campbell KL, Wang CY, Duggan CR, Ulrich CM, Alfano CM, Blackburn GL and McTiernan A (2011). Dietary weight loss and exercise effects on insulin resistance in postmenopausal women. *American Journal of Preventive Medicine.* 41(4):366-75.

Masset G, Scarborough P, Rayner M, Mishra G and Brunner EJ (2015). Can nutrient profiling help to identify foods which diet variety should be encouraged? Results from the Whitehall II cohort. *The British Journal of Nutrition.* 113(11):1800-9.

Mather KJ, Hunt AE, Steinberg HO, Paradisi G, Hook G, Katz A, Quon MJ and Baron AD (2001). Repeatability characteristics of simple indices of insulin resistance: implications for research applications. *The Journal of Clinical Endocrinology and Metabolism.* 86:5457-64.

Matsui H, Yokoyama T, Sekiguchi K, Iiima D, Sunaga H, Maniwa M, Ueno M, Iso T, Arai M and Kurabayashi M (2012). Stearoyl-CoA desaturase-1 (SCD1) augments saturated fatty acid-induced lipid accumulation and inhibits apoptosis in cardiac myocytes. *PLoS One.* 7(3):e33283.

Matsumoto K, Nakamura H, Ueki Y, Tominaga T and Miyake S (2001). Correction of hyperglycaemia reduces insulin resistance and serum soluble E-selectin levels in patients with Type 2 diabetes mellitus. *Diabetic Medicine.* 18:224-8.

Mattar LE, Mattar MA, Batal M, Mouneimne Y and Obeid OA (2010). Stimulation of postprandial in vivo glycogenesis and lipogenesis of rats fed high fructose diet with varied phosphate content. *Nutrition Research.* 30(2):151-5.

McNeilly AM, McClean C, Murphy M, McEneny J, Trinick T, Burke G, Duly E, McLaughlin J and Davison G (2012). Exercise training and impaired glucose tolerance in obese humans. *Journal of Sports Science.* 30(8):725-32.

Meex RC, Schrauwen-Hinderling VB, Moonen-Kornips E, Schaart G, Mensink M, Phielix E, van de Weijer T, Sels JP, Schrauwen P and Hesselink MK (2010). Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. *Diabetes.* 59(3):572-9.

Medford HM, Chatham JC and Marsh SA (2012). Chronic ingestion of a Western diet increases O-linked-β-N-acetylglucosamine (O-GlcNAc) protein modification in the rat heart. *Life Science.* 90(23-24):883-8.

Mendelsohn AR and Larrick JW(2013). Dietary modification of the microbiome affects risk for cardiovascular disease. *Rejuvenation Research.* 16(3):241-4.

Méndez L, Pazos M, Molinar-Toribio E, Sánchez-Martos V, Gallardo JM, Rosa Nogués M, Torres JL and Medina I (2014). Protein carbonylation associated to high-fat, high-

sucrose diet and its metabolic effects. *The Journal of Nutritional Biochemistry.* 25(12):1243-53.

Merino-Aguilar H, Arrieta-Baez D, Jiménez-Estrada M, Magos-Guerrero G, Hernández-Bautista RJ, Susunaga-Notario Adel C, Almanza-Pérez JC,Blancas-Flores G, Román-Ramos R, Alarcón-Aguilar FJ (2014). Effect of fructooligosaccharides fraction from Psacalium decompositum on inflammation and dyslipidaemia in rats with fructose-induced obesity. *Nutrients*. 6(2):591-604.

Millen AME, Norton GR, Avidon I and Woodiwiss AJ (2014). Effects of short-term exercise-training on tissue Doppler indices of left ventricular diastolic function in overweight and obese individuals. *Journal of Sport Science* 32(5):487-499.

Milton K and Bauman A (2015). A critical analysis of the cycles of physical activity policy in England. *The International Journal of Behavioural Nutrition Physical Activity.* 12(1):8.

Mohr M, Nordsborg NB, Lindenskov A, Steinholm H, Nielsen HP, Mortensen J, Weihe P and Krustrup P (2014). High-intensity intermittent swimming improves cardiovascular health status for women with mild hypertension. *BioMed Research International.* 2014:728289.

Morikawa M, Okamoto N, Kiuchi K, Tomioka K, Iwamoto J, Harano A, Saeki K, Fukusumi M, Hashimoto K, Amano N, Hazaki K, Yanagi M, Iki M, Yamada F,Kishimoto T, Kurumatani N (2013). Association between depressive symptoms and metabolic syndrome in Japanese community-dwelling older people: a cross-sectional analysis from the baseline results of the Fujiwara-kyo prospective cohort study. *International journal of geriatric psychiatry*. 28(12):1251-9.

Motahari-Tabari N, Ahmad Shirvani M, Shirzad-E-Ahoodashty M, Yousefi-Abdolmaleki E and Teimourzadeh M (2014). The effect of 8 weeks aerobic exercise on insulin resistance in type 2 diabetes: a randomized clinical trial. *Global Journal of Health Science.* 7(1):115-21.

Mousavi SE, Shahriari A, Ahangarpour A, Vatanpour H and Jolodar A (2012). Effects of Teucrium polium Ethyl acetate Extract on Serum, Liver and Muscle Triglyceride Content of Sucrose-Induced Insulin Resistance in Rat. *Iranian Journal of Pharmaceutical Research.* 11(1):347-55.

Mu L, Mukamal KJ, Naqvi AZ (2014). Erythrocyte saturated fatty acids and systemic inflammation in adults. *Nutrition*. 30(11-12):1404-8.

Munshi RP, Joshi SG, Rane BN (2014). Development of an experimental diet model in rats to study hyperlipidemia and insulin resistance, markers for coronary heart disease. *Indian Journal of Pharmacology.* 46(3):270-6.

Muraki E, Hayashi Y, Chiba H, Tsunoda N and Kasono K (2011). Dose-dependent effects, safety and tolerability of fenugreek in diet-induced metabolic disorders in rats. *Lipids in Health and Disease.* 10:240.

Murrell CJ, Cotter JD, Thomas KN, Lucas SJ, Williams MJ and Ainslie PN (2013). Cerebral blood flow and cerebrovascular reactivity at rest and during sub-maximal exercise: effect of age and 12-week exercise training. *Age.* 35(3):905-20.

Myles IA (2014). Fast food fever: reviewing the impacts of the Western diet on immunity. *Nutrition Journal.* 13:61.

Nasri R, Abdelhedi O, Jemil I, Daoued I, Hamden K, Kallel C, Elfeki A, Lamri-Senhadji M, Boualga A, Nasri M, Karra-Châabouni M (2015). Ameliorating effects of goby fish protein hydrolysates on high-fat-high-fructosediet-induced hyperglycaemia, oxidative stress and deterioration of kidney function in rats. *Chemico-biological Interactions*. 242:71-80.

Natsume T (1993). Therapeutic advances in the treatment of left ventricular hypertrophy. *European Heart Journal*. 14 Suppl D:33-7.

Navar-Boggan AM, Peterson ED, D'Agostino RB Sr, Neely B, Sniderman AD, Pencina MJ (2015). Hyperlipidemia in early adulthood increases long-term risk of coronary heart disease. *Circulation*. 131(5):451-8. 26.

Nelson R H. Hyperlipidemia as a risk factor for cardiovascular disease (2013*). Primary Care.* 40(1):195-211.

Newens KJ, Thompson AK, Jackson KG, Wright J, Williams CM (2011). DHA-rich fish oil reverses the detrimental effects of saturated fatty acids on postprandial vascular reactivity. *The American journal of clinical nutrition.* 94(3):742-8.

Nicholls SJ, Tuzcu EM, Brennan DM, Tardif JC, Nissen SE (2008). Cholesteryl ester transfer protein inhibition, high-density lipoprotein raising, and progression of coronary atherosclerosis: insights from ILLUSTRATE (Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Inhibition and HDL Elevation). *Circulation*. 118(24):2506-14.

Nielsen TS, Jessen N, Jørgensen JO, Møller N, Lund S (2014). Dissecting adipose tissue lipolysis: molecular regulation and implications for metabolic disease. *Journal of molecular endocrinology*. 52(3):R199-222.

Nishikawa K, Takahashi K, Okutani T, Yamada R, Kinaga T, Matsumoto M, Yamamoto M (2015).Risk of chronic kidney disease in non-obese individuals with clustering of metabolic factors: a longitudinal study. *Internal medicine.(Tokyo, Japan)*. 54(4):375-82.

Noborisaka Y, Ishizaki M, Yamada Y, Honda R, Yokoyama H, Miyao M and Tabata M (2013). Distribution of and factors contributing to chronic kidney disease in a middle-aged working population. *Environmental Health and Preventive Medicine.* 18(6):466-76.

Northcott CA, Fink GD, Garver H, Haywood JR, Laimon-Thomson EL, McClain JL, Pires PW, Rainey WE, Rigsby CS and Dorrance AM (2012). The development

of hypertension and hyperaldosteronism in a rodent model of life-long obesity. *Endocrinology.* 153(4):1764-73.

Norton GR, Veliotes DG, Osadchii O, Woodiwiss AJ, Thomas DP (2008). Susceptibility to systolic dysfunction in the myocardium from chronically infarcted spontaneously hypertensive rats. American Journal of Physiology Heart Circulation Physiolog. 294(1):H372-8.

Novak EM, Keller BO and Innis SM (2012). Metabolic development in the liver and the implications of (Mattar et al., 2010; Mousavi et al., 2012; the n-3 fatty acid supply. American Journal of Physiology. *Gastrointestinal and Liver Physiology.* 302(2):250-9.

Noubiap JJ, Nansseu JR, Bigna JJ, Jingi AM, Kengne AP (2015). Prevalence and incidence of dyslipidaemia among adults in Africa: a systematic review and meta-analysis protocol. *BMJ open*. 5(3):e007404.

Oberbach A, Kirsch K, Lehmann S, Schlichting N, Fasshauer M, Zarse K, Stumvoll M, Ristow M, Blüher M and Kovacs P (2010). Serum vaspin concentrations are decreased after exercise-induced oxidative stress. *Obesity Facts.* 3(5):328-31.

Ochiai M and Matsuo T (2013). Pioglitazone-induced increase in the stearoyl-CoA desaturation index and fataccumulation in rat muscles are not related to lipoprotein lipase activity. *Journal of Oleo Science.* 62(9):745-54.

Okere IC, Chess DJ, McElfresh TA, Johnson J, Rennison J, Ernsberger P, Hoit BD, Chandler MP and Stanley WC (2005). High-fat diet prevents cardiac hypertrophy and improves contractile function in the hypertensive dahl salt-sensitive rat. *Clinical and Experimental Pharmacology and Physiology*. 32(10): 825–831.

Oliveira Junior SA, Padovani CR, Rodrigues SA, Silva NR, Martinez PF, Campos DH, Okoshi MP, Okoshi K, Dal-Pai M and Cicogna AC (2013). Extensive impact of saturated fatty acids on metabolic and cardiovascular profile in rats with diet-induced obesity: a canonical analysis. *Cardiovascular Diabetology.* 12:65.

O'Neil PM, Smith SR, Weissman NJ, Fidler MC, Sanchez M, Zhang J, Raether B, Anderson CM and Shanahan WR (2012). Randomized placebo-controlled clinical trial of lorcaserin for weight loss in type 2 diabetes mellitus: the BLOOM-DM study. *Obesity.* 20(7):1426-36.

Ono K (2012). Current concept of reverse cholesterol transport and novel strategy for atheroprotection. *Journal of Cardiology.* 60(5):339-43.

Ortega JF, Fernández-Elías VE, Hamouti N, Mora-Rodriguez R (2013). Increased blood cholesterol after a high saturated fat diet is prevented by aerobic exercise training. *Applied physiology, nutrition, and metabolism.* 38(1):42-8.

Osabe M, Sugatani J, Fukuyama T, Ikushiro S, Ikari A, Miwa M (2008). Expression of hepatic UDP-glucuronosyltransferase 1A1 and 1A6 correlated with increased expression of thenuclear constitutive androstane receptor and peroxisome proliferator-activated receptor alpha in male rats fed ahigh-fat and high-sucrose diet. *Drug metabolism and disposition: the biological fate of chemicals*. 36(2):294-302.

Osakabe N and Shibata M (2012). Ingestion of cocoa ameliorates endothelial dysfunction in mesentery arterioles induced by high fat diet in rats: an in vivo intravital microscopy study. *Life Science.* 91(23-24):1196-200.

Osei-Hyiaman D, Liu J, Zhou L, Godlewski G, Harvey-White J, Jeong WI, Bátkai S, Marsicano G, Lutz B, Buettner C, Kunos G (2008). Hepatic CB1 receptor is required for development of diet-induced steatosis, dyslipidaemia, and insulin and leptin resistance in mice. *The Journal of clinical investigation.* 118(9):3160-9.

Pan QR, Ren YL, Zhu JJ, Hu YJ, Zheng JS, Fan H, Xu Y, Wang G and Liu WX (2014). Resveratrol increases nephrin and podocin expression and alleviates renal damage in rats fed a high-fat diet. *Nutrients.* 6(7):2619-31.

Pan X, Wang P, Luo J, Wang Z, Song Y, Ye J, Hou X (2015). Adipogenic changes of hepatocytes in a high-fat diet-induced fatty liver mice model and non-alcoholic fatty liver disease patients. *Endocrine*. 48(3):834-47.

Papanas N and Ziegler D (2013). Polyneuropathy in impaired glucose tolerance: is postprandial hyperglycaemia the main culprit? A mini-review. *Gerontology.* 59(3):193-8.

Parasuraman S, Raveendran R, Kesavan R (2010). Blood sample collection in small laboratory animals. *Journal of pharmacology & Pharmacotherapeutics*. 1(2):87-93.

Passoneau JV and Lauderdale VR (1974). A comparison of three methods of glycogen measurement in tissues. *Analytical Biochemistry* 60: 405-12.

Paulino EC, Ferreira JC, Bechara LR, Tsutsui JM, Mathias W Jr, Lima FB, Casarini DE, Cicogna AC, Brum PC and Negrão CE (2010). Exercise training and caloric restriction prevent reduction in cardiac Ca2+-handling protein profile in obese rats. *Hypertension.* 56(4):629-35.

Perez-Cornago A, Zulet MA, Martinez JA (2015). Association between mood and diet quality in subjects with metabolic syndrome participating in a behavioural weight-loss programme: a cross-sectional assessment. *Nutritional neuroscience*. 18(3):137-44.

Petkevičienė J, Klumbienė J, Ramažauskienė V, Kriaučionienė V, Sakytė E, Grabauskas V (2012). Diet and dyslipidaemias in a Lithuanian rural population aged 25-64: the CINDI survey. *Medicina (Kaunas, Lithuania)*. 48(4):211-7.

Pierine DT, Navarro ME, Minatel IO, Luvizotto RA, Nascimento AF, Ferreira AL, Yeum KJ and Corrêa CR (2014). Lycopene supplementation reduces TNF-α via RAGE in the kidney of obese rats. *Nutrition & Diabetes.* 4:142.

Pinhas-Hamiel O, Levek-Motola N, Kaidar K, Boyko V, Tisch E, Mazor-Aronovitch K, Graf-Barel C, Landau Z, Lerner-Geva L, Frumkin Ben-David R (2015). Prevalence of

overweight, obesity and metabolic syndrome components in children, adolescents and young adults with type 1 diabetes mellitus. *Diabetes/ metabolism research and reviews*. 31(1):76-84.

Pozzan R, Brandão AA, da Silva SL and Brandão AP (1997). Hyperglycaemia, hyperinsulinemia, overweight, and high blood pressure in young adults: the Rio de Janeiro Study. *Hypertension.* 30(3 Pt 2):650-3.

Pradhan A (2007). Obesity, metabolic syndrome, and type 2 diabetes: inflammatory basis of glucose metabolic disorders. *Nutrition Review*. 65(12 Pt 2):S152-6.

Pranprawit A, Wolber FM, Heyes JA, Molan AL and Kruger MC (2013). Short-term and long-term effects of excessive consumption of saturated fats and/orsucrose on metabolic variables in Sprague Dawley rats: a pilot study. *Journal of the Science of Food and Agriculture.* 93(13):3191-7.

Pratt WE and Connolly ME (2010). Contrasting effects of systemic and central sibutramine administration on the intake of a palatable diet in the rat. *Neuroscience Letters.* 484(1):30-4.

Prior SJ, Blumenthal JB, Katzel LI, Goldberg AP and Ryan AS (2014). Increased skeletal muscle capillarization after aerobic exercise training and weight loss improves insulin sensitivity in adults with IGT. *Diabetes Care.* 37(5):1469-75.

Ragab SM, Abd Elghaffar SKh, El-Metwally TH, Badr G, Mahmoud MH, Omar HM (2015). Effect of a high fat, high sucrose diet on the promotion of non-alcoholic fatty liver disease in male rats: the ameliorative role of three natural compounds. *Lipids in health and disease*. 31; 14:83.

Rasmussen BM, Vessby B, Uusitupa M, Berglund L, Pedersen E, Riccardi G, Rivellese AA, Tapsell L, Hermansen K; KANWU Study Group (2006). Effects of dietary saturated, monounsaturated, and n-3 fatty acids on blood pressure in healthy subjects. *The American journal of clinical nutrition.* 83(2):221-6.

Renna R, Pilato F, Profice P, Della Marca G, Broccolini A, Morosetti R, Frisullo G, Rossi E, De Stefano V, Di Lazzaro V (2014). Risk factor and etiology analysis of ischemic stroke in young adult patients. *Journal of stroke and cerebrovascular diseases*. 23(3):e221-7.

Resnick HE, Valsania P, Halter JB and Lin X (1998). Differential effects of BMI on diabetes risk among black and white Americans. *Diabetes Care.* 21(11):1828-35.

Rhee MY(2015). High sodium intake: review of recent issues on its association with cardiovascular events and measurement methods. *Korean circulation journal*. 45(3):175-83.

Riccio P and Rossano R (2015). Nutrition facts in multiple sclerosis. *ASN Neuro.* 18;7(1).

Rivera CA, Abrams SH, Tcharmtchi MH, Allman M, Ziba TT, Finegold MJ and Smith CW (2006). Feeding a corn oil/sucrose-enriched diet enhances steatohepatitis in sedentary rats. *American Journal of Physiology- Gastrointestinal and Liver Physiology.* 290(2):386-93.

Roberts CK, Vaziri ND and Barnard RJ (2001). Protective effects of estrogen on gender-specific development of diet-induced hypertension. *Journal of Applied Physiology.* 91(5):2005-9.

Roberts CK, Barnard RJ, Liang KH and Vaziri ND (2002). Effect of diet on adipose tissue and skeletal muscle VLDL receptor and LPL: implications for obesity and hyperlipidemia. *Atherosclerosis.* 161(1):133-41.

Roberts CK, Liang K, Barnard RJ, Kim CH and Vaziri ND (2004). HMG-CoA reductase, cholesterol 7alpha-hydroxylase, LDL receptor, SR-B1, and ACAT in diet-induced syndrome X. *Kidney International.* 66(4):1503-11.

Roberts CK, Barnard RJ, Sindhu RK, Jurczak M, Ehdaie A and Vaziri ND (2005). A high-fat, refined-carbohydrate diet induces endothelial dysfunction and oxidant/antioxidant

imbalance and depresses NOS protein expression. *Journal of Applied Physiology*. 98(1):203-10.

Roca B, Suárez C, Ceballos A, Varela JM, Nonell F, Montes J, Sobrino J, de la Peña A and CIFARC Group (2005). Control of hypertension in patients at high risk of cardiovascular disease. *QJM: Monthly Journal of the association of physicians.*98(8):581-8.

Rodrigues B, Lira FS, Consolim-Colombo FM, Rocha JA, Caperuto EC, De Angelis K and Irigoyen MC (2014). Role of exercise training on autonomic changes and inflammatory profile induced by myocardial infarction. *Mediators of Inflammation.* 2014(9):702473.

Rosety-Rodriguez M, Rosety I, Fornieles-Gonzalez G, Diaz-Ordonez AJ, Camacho A, Rosety MA, Pardo A, Rosety M, Alvero R and Ordonez FJ (2012). A 6-week training program increased muscle antioxidant system in elderly diabetic fatty rats*. Medical Science Monitor.* 18(9):BR346-50.

Rugg-Gunn AJ, Fletcher ES, Matthews JN, Hackett AF, Moynihan PJ, Kelly S, Adams J, Mathers JC, Adamson A (2007). Changes in consumption of sugars by English adolescents over 20 years. *Public health nutrition*. 10(4):354-63.

Ruggenenti P, Cattaneo D, Loriga G, Ledda F, Motterlini N, Gherardi G, Orisio S and Remuzzi G (2009). Ameliorating hypertension and insulin resistance in subjects at increased cardiovascular risk: effects of acetyl-L-carnitine therapy. *Hypertension.* 54(3):567-74.

Roussel M, Garnier S, Lemoine S, Gaubert I, Charbonnier L, Auneau G and Mauriège P (2009). Influence of a walking program on the metabolic risk profile of obese postmenopausal women. *Menopause.* 16(3):566-75.

Ruegsegger GN, Toedebusch RG, Braselton JF, Roberts CK, Booth FW (2015). Reduced metabolic disease risk profile by voluntary wheel running accompanying juvenile

Western diet in ratsbred for high and low voluntary exercise. *Physiology and behaviour.* 152(Pt A):47-55.

Ryan AS, Li G, Blumenthal JB and Ortmeyer HK (2013). Aerobic exercise + weight loss decreases skeletal muscle myostatin expression and improves insulin sensitivity in older adults. *Obesity.* 21(7):1350-6.

Saiki R, Okazaki M, Iwai S, Kumai T, Kobayashi S and Oguchi K (2007). Effects of pioglitazone on increases in visceral fat accumulation and oxidative stress in spontaneously hypertensive hyperlipidemic rats fed a high-fat diet and sucrose solution. *Journal of Pharmacological Science.* 105(2):157-67.

Sahlén A, Abdula G, Norman M, Manouras A, Brodin LÅ, Lund LH, Shahgaldi K and Winter R (2011). Arterial vasodilatory and ventricular diastolic reserves determine the stroke volume response to exercise in elderly female hypertensive patients. *American Journal of Physiology- Heart and Circulatory Physiology.* 301(6):H2433-41.

Santuré M, Pitre M, Marette A, Deshaies Y, Lemieux C, Larivière R, Nadeau A and Bachelard H (2002). Induction of insulin resistance by high-sucrose feeding does not raise mean arterial blood pressure but impairs haemodynamic responses to insulin in rats. *British Journal of Pharmacology.* 137(2):185-96.

Scherer T, O'Hare J, Diggs-Andrews K, Schweiger M, Cheng B, Lindtner C, Zielinski E, Vempati P, Su K, Dighe S, Milsom T, Puchowicz M, Scheja L, Zechner R, Fisher SJ, Previs SF, Buettner C (2011). Brain insulin controls adipose tissue lipolysis and lipogenesis. *Cell metabolism*. 13(2):183-94.

Schneider M, Dunn A and Cooper D (2009). Affect, exercise, and physical activity among healthy adolescents. *Journal of Sport and Exercise Psychology.* 31(6):706-23.

Schonfeld G, Pfleger B (1971). Utilization of exogenous free fatty acids for the production of very low density lipoprotein triglyceride by livers ofcarbohydrate-fed rats. *Journal of lipid research*. 12(5):614-21.

Schrauwen-Hinderling VB, Meex RC, Hesselink MK, van de Weijer T, Leiner T, Schär M, Lamb HJ, Wildberger JE, Glatz JF, Schrauwen P and Kooi ME (2011). Cardiac lipid content is unresponsive to a physical activity training intervention in type 2 diabetic patients, despite improved ejection fraction. *Cardiovascular Diabetology.* 10:47.

Schwarz JM, Neese RA, Turner S, Dare D, Hellerstein MK (1995). Short-term alterations in carbohydrate energy intake in humans. Striking effects on hepatic glucose production, de novo lipogenesis, lipolysis, and whole-body fuel selection. *The Journal of clinical investigation.* 96(6):2735-43.

Serné EH, de Jongh RT, Eringa EC, IJzerman RG and Stehouwer CD (2007). Microvascular dysfunction: a potential pathophysiological role in the metabolic syndrome. *Hypertension.* 50(1):204-11.

Seymour AM, Giles L, Ball V, Miller JJ, Clarke K, Carr CA and Tyler DJ (2015). In vivo assessment of cardiac metabolism and function in the abdominal aortic banding model of compensated cardiac hypertrophy. *Cardiovascular Research.* 106:249-60.

Sixt S, Beer S, Blüher M, Korff N, Peschel T, Sonnabend M, Teupser D, Thiery J, Adams V, Schuler G and Niebauer J (2010). Long- but not short-term multifactorial intervention with focus on exercise training improves coronary endothelial dysfunction in diabetes mellitus type 2 and coronary artery disease. *European Heart Journal.* 31(1):112-9.

Slentz CA, Aiken LB, Houmard JA, Bales CW, Johnson JL, Tanner CJ, Duscha BD and Kraus WE (2005). Inactivity, exercise, and visceral fat. STRRIDE: a randomized, controlled study of exercise intensity and amount. *Journal of Applied Physiology*. 99(4):1613-8.

Solomon TP, Malin SK, Karstoft K, Kashyap SR, Haus JM and Kirwan JP (2013). Pancreatic β-cell function is a stronger predictor of changes in glycemic control after an aerobic

exercise intervention than insulin sensitivity. The Journal of Clinical Endocrinology and *Metabolism.* 98(10):4176-86.

Sørensen LB, Vasilaras TH, Astrup A, Raben A (2014). Sucrose compared with artificial sweeteners: a clinical intervention study of effects on energy intake, appetite, and energy expenditure after 10 wk of supplementation in overweight subjects. *The American journal of clinical nutrition.* 100(1):36-45.

Soria A, D'Alessandro ME and Lombardo YB (2001). Duration of feeding on a sucrose-rich diet determines metabolic and morphological changes in rat adipocytes. *Journal of Applied Physiology (1985).* 91(5):2109-16.

Souza CG, Moreira JD, Siqueira IR, Pereira AG, Rieger DK, Souza DO, Souza TM, Portela LV and Perry ML (2007). Highly palatable diet consumption increases protein oxidation in rat frontal cortex and anxiety-like behavior. *Life Science.* 81(3):198-203.

Stamler J, Caggiula A, Grandits GA, Kjelsberg M, Cutler JA (1996). Relationship to blood pressure of combinations of dietary macronutrients. Findings of the Multiple Risk Factor Intervention Trial (MRFIT). *Circulation*. 15; 94(10):2417-23.

Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G and Baron AD (1996). Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. *The Journal of Clinical Investigation.* 97(11):2601-10.

Stephenson EJ, Camera DM, Jenkins TA, Kosari S, Lee JS, Hawley JA and Stepto NK (2012). Skeletal muscle respiratory capacity is enhanced in rats consuming an obesogenic Western diet. *American Journal of Physiology-Endocrinology and Metabolism.* 302(12):E1541-9.

Stepto NK, Benziane B, Wadley GD, Chibalin AV, Canny BJ, Eynon N and McConell GK (2012). Short-term intensified cycle training alters acute and chronic responses of PGC1α and Cytochrome C oxidase IV to exercise in human skeletal muscle. *PLoS One.* 7(12):e53080.

Stevens J, Cai J, Evenson KR and Thomas R (2002). Fitness and fatness as predictors of mortality from all causes and from cardiovascular disease in men and women in the lipid research clinics study. *American Journal of Epidemiology.* 156(9):832-41.

Stewart KJ, Bacher AC, Turner K, Lim JG, Hees PS, Shapiro EP and Tayback M, Ouyang P (2005). Exercise and risk factors associated with metabolic syndrome in older adults. *American Journal of Preventive Medicine.* 28(1):9-18.

Stolk RP, Pols HA, Lamberts SW, de Jong PT, Hofman A and Grobbee DE (1997). Diabetes mellitus, impaired glucose tolerance, and hyperinsulinemia in an elderly population. The Rotterdam Study. *American Journal of Epidemiology.*45(1):24-32.

Strable MS and Ntambi JM (2010). Genetic control of de novo lipogenesis: role in diet-induced obesity. *Critical Review in Biochemistry and Molecular Biology.* 45(3):199-214.

Stanley WC, Dabkowski ER, Ribeiro RF Jr and O'Connell KA (2012). Dietary fat and heart failure: moving from lipotoxicity to lipoprotection. *Circulation Research*. 110(5):764-76.

Stuart CA, South MA, Lee ML, McCurry MP, Howell ME, Ramsey MW and Stone MH (2013). Insulin responsiveness in metabolic syndrome after eight weeks of cycle training. *Medicine and Science in Sports and Exercise.* 45(11):2021-9.

Sugatani J, Wada T, Osabe M, Yamakawa K, Yoshinari K and Miwa M (2006). Dietary inulin alleviates hepatic steatosis and xenobiotics-induced liver injury in rats fed a high-fat and high-sucrose diet: association with the suppression of hepatic cytochrome P450 and hepatocyte nuclear factor 4alpha expression. *Drug Metabolism and disposition: the biological fate of chemicals.* 34(10):1677-87.

Sugatani J, Osabe M, Wada T, Yamakawa K, Yamazaki Y, Takahashi T, Ikari A and Miwa M (2008). Comparison of enzymatically synthesized inulin, resistant maltodextrin and clofibrate effects on biomarkers of metabolic disease in rats fed a high-fat and high-sucrose (cafeteria) diet. *European Journal of Nutrition.* 47(4):192-200.

Sun H, Jiang T, Wang S, He B, Zhang Y, Piao D, Yu C, Wu N and Han P (2013). The effect of LXRα, ChREBP and Elovl6 in liver and white adipose tissue on medium- and long-chain fatty acid diet-induced insulin resistance. *Diabetes Research and Clinical Practice.* 102(3):183-92.

Sung K and Bae S (2012). Effects of a regular walking exercise program on behavioral and biochemical aspects in elderly people with type II diabetes. *Nursing & Health Science.* 14(4):438-45.

Teixeira de Lemos E, Pinto R, Oliveira J, Garrido P, Sereno J, Mascarenhas-Melo F, Páscoa-Pinheiro J, Teixeira F and Reis F (2011). Differential effects of acute (extenuating) and chronic (training) exercise on inflammation and oxidative stress status in an animal model of type 2 diabetes mellitus. *Mediators of Inflammation.* 2011:253061.

Teramoto T, Kawamori R, Miyazaki S, Teramukai S, Sato Y, Okuda Y and Shirayama M (2015). Lipid and blood pressure control for the prevention of cardiovascular disease in hypertensive patients: a subanalysis of the OMEGA study. *Journal of Atherosclerosis and Thrombosis*. 22(1):62-75.

Tierney AC, McMonagle J, Shaw DI, Gulseth HL, Helal O, Saris WH, Paniagua JA, Gołąbek-Leszczyñska I, Defoort C, Williams CM, Karsltröm B, Vessby B,Dembinska-Kiec A, López-Miranda J, Blaak EE, Drevon CA, Gibney MJ, Lovegrove JA, Roche HM (2011). Effects of dietary fat modification on insulin sensitivity and on other risk factors of the metabolic syndrome--LIPGENE: a European randomized dietary intervention study. *International journal of obesity*. 35(6):800-9.

Tighe P, Duthie G, Vaughan N, Brittenden J, Simpson WG, Duthie S, Mutch W, Wahle K, Horgan G and Thies F (2010). Effect of increased consumption of whole-grain foods on blood pressure and other cardiovascular risk markers in healthy middle-aged

persons: a randomized controlled trial. *The American Journal of Clinical Nutrition.* 92(4):733-40.

Thijssen DHJ, Maiorana AJ, O'Driscoll G, Cable NT, Hopman MTE and Green DJ (2010). Impact of inactivity and exercise on the vasculature in humans. *European Journal of Applied Physiology.* 108:845–875.

Tovar J, Nilsson A, Johansson M and Björck I (2014). Combining functional features of whole-grain barley and legumes for dietary reduction of cardiometabolic risk: a randomised cross-over intervention in mature women. *The British Journal of Nutrition.* 111(4):706-14.

Trachta P, Drápalová J, Kaválková P, Toušková V, Cinkajzlová A, Lacinová Z, Matoulek M, Zelinka T, Widimský J Jr, Mráz M and Haluzík M (2014). 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.* 2:299-308.

Tremblay AJ, Lamarche B, Guay V, Charest A, Lemelin V and Couture P (2013). Short-term, high-fat diet increases the expression of key intestinal genes involved in lipoprotein metabolism in healthy men. *The American Journal of Clinical Nutrition.* 98(1):32-41.

Tunar M, Ozen S, Goksen D, Asar G, Bediz CS and Darcan S (2012). The effects of Pilates on metabolic control and physical performance in adolescents with type 1 diabetes mellitus. *Journal of Diabetes and its Complications.* 26(4):348-51.

Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V and Uusitupa M; Finnish Diabetes Prevention Study Group (2001). Prevention of type

2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *The New England Journal of Medicine.* 344(18):1343-50.

Tur JJ, Escudero AJ, Romaguera D, Burguera B (2013). How can we predict which morbidly obese patients will adhere to weight-loss programs based on life stylechanges? *Endocrinologica y nutricion.* 60(6):297-302.

Turcotte LP, Rovner AS, Roark RR and Brooks GA (1990). Glucose kinetics in gluconeogenesis-inhibited rats during rest and exercise. *The American Journal of Physiology.* 258(1 Pt 1):203-11.

Turkieh A, Caubère C, Barutaut M, Desmoulin F, Harmancey R, Galinier M, Berry M, Dambrin C, Polidori C, Casteilla L, Koukoui F, Rouet P, Smih F (2014). Apolipoprotein O is mitochondrial and promotes lipotoxicity in heart. *The journal of clinical investigation.* 124(5):2277-86.

Tzeng TF, Lu HJ, Liou SS, Chang CJ and Liu IM (2012). Emodin, a Naturally Occurring Anthraquinone Derivative, Ameliorates Dyslipidaemia by Activating AMP-Activated Protein Kinase in High-Fat-Diet-Fed Rats. *Evidence Based Complementary and Alternative Medicine.* 2012:781812. doi: 10.1155/2012/781812.

Uriarte G, Paternain L, Milagro FI, Martínez JA and Campion J (2013). Shifting to a control diet after a high-fat, high-sucrose diet intake induces epigenetic changes in retroperitoneal adipocytes of Wistar rats. *Journal of Physiology and Biochemistry.* 69(3):601-11.

Utimura R, Fujihara CK, Mattar AL, Malheiros DM, Noronha IL and Zatz R (2003). Mycophenolate mofetil prevents the development of glomerular injury in experimental diabetes. *Kidney International.* 63(1):209-16.

Vaccaro O, Ruffa G, Imperatore G, Iovino V, Rivellese AA and Riccardi G (1999). Risk of diabetes in the new diagnostic category of impaired fasting glucose: a prospective analysis. *Diabetes Care.* 22(9):1490-3.

Vafeiadou K, Weech M, Altowaijri H, Todd S, Yaqoob P, Jackson KG, Lovegrove JA (2015). Replacement of saturated with unsaturated fats had no impact on vascular function but beneficial effects on lipid biomarkers, E-selectin, and blood pressure: results from the randomized, controlled Dietary Intervention and VAScular function (DIVAS) study. *The American journal of clinical nutrition.* 102(1):40-8.

Vancea DM, Vancea JN, Pires MI, Reis MA, Moura RB and Dib SA (2009). Effect of frequency of physical exercise on glycemic control and body composition in type 2 diabetic patients. *Arquivos Brasileiros de Cardiologia.* 92(1):23-30.

Virtanen JK, Mursu J, Tuomainen TP, Voutilainen S (2014). Dietary fatty acids and risk of coronary heart disease in men: the Kuopio Ischemic Heart Disease Risk Factor Study. *Arteriosclerosis, thrombosis, and vascular biology*. 34(12):2679-87.

Walther G, Obert P, Dutheil F, Chapier R, Lesourd B, Naughton G, Courteix D and Vinet A (2015). Metabolic syndrome individuals with and without type 2 diabetes mellitus present generalized vascular dysfunction: cross-sectional study. *Arteriosclerosis, Thrombosis and Vascular Biology.* 35(4):1022-9.

Walton RG, Zhu B, Unal R, Spencer M, Sunkara M, Morris AJ, Charnigo R, Katz WS, Daugherty A, Howatt DA, Kern PA, Finlin BS (2015). Increasing adipocyte lipoprotein lipase improves glucose metabolism in high fat diet-induced obesity. *The journal of biological chemistry*. 290(18):11547-56.

Watkins LL, Sherwood A, Feinglos M, Hinderliter A, Babyak M, Gullette E, Waugh R and Blumenthal JA (2003). Effects of exercise and weight loss on cardiac risk factors associated with syndrome X. *Archives of Internal Medicine.* 163(16):1889-95.

Watt EW, Wiley J and Fletcher GF (1976). Effect of dietary control and exercise training on daily food intake and serum lipids in postmyocardial infarction patients. *The American Journal of Clinical Nutrition.* 29(8):900-4.

Weech M, Vafeiadou K, Hasaj M, Todd S, Yaqoob P, Jackson KG, Lovegrove JA (2014). Development of a food-exchange model to replace saturated fat with MUFAs and n-6 PUFAs in adults at moderate cardiovascular risk. *The journal of nutrition*. 144(6):846-55.Westman EC, Feinman RD, Mavropoulos JC, Vernon MC, Volek JS, Wortman JA, Yancy WS, Phinney SD (2007). Low-carbohydrate nutrition and metabolism. The American journal of clinical nutrition. 86(2):276-84.

Whelton PK, He J, Appel LJ, Cutler JA, Havas S, Kotchen TA, Roccella EJ, Stout R, Vallbona C, Winston MC, Karimbakas J and National High Blood Pressure Education Program Coordinating Committee (2002). Primary prevention of hypertension: clinical and public health advisory from The National High Blood Pressure Education Program. *JAMA*. 288(15):1882-8.

Wietlisbach V, Marques-Vidal P, Kuulasmaa K, Karvanen J, Paccaud F and WHO MONICA Project (2013). The relation of body mass index and abdominal adiposity with dyslipidaemia in 27 general populations of the WHO MONICA Project. *Nutrition, Metabolism, Cardiovascular Diseases.* 23(5):432-42.

Williams PT (2013). Walking and running produce similar reductions in cause-specific disease mortality in hypertensives. *Hypertension.* 62(3):485-91.

Wilson PW, D'Agostino RB, Parise H, Sullivan L and Meigs JB (2005). Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. *Circulation.* 112:3066-72.

Wilson DP, McNeal C, Blackett P (2015).Pediatric dyslipidaemia: recommendations for clinical management. *Southern medical journal*. 108(1):7-14.

Woodiwiss AJ, Trifunovic B, Philippides M and Norton GR (2000). Effects of an androgenic steroid on exercise-induced cardiac remodeling in rats. *Journal of Applied Physiology*. 88(2):409-15.

Woodiwiss AJ TO, Sprott S, Lancaster EJ, Mela T, Chung ES, Meyer TE and Norton GR (2001). Reduction in myocardial collagen cross-linking parallels left ventricular dilatation in rat models of systolic chamber dysfunction. *Circulation.* 1:155-60.

Xie F, Fu H, Hou JF, Jiao K, Costigan M and Chen J (2013). High energy diets-induced metabolic and prediabetic painful polyneuropathy in rats. *PLoS One.* 8(2):e57427.

Yamamoto Y, Oue E (2006). Antihypertensive effect of quercetin in rats fed with a high-fat high-sucrose diet. *Bioscience, Biotechnology, and Biochemistry.* 7(4):933-9.

Yamamoto Y and Yasuoka A (2010). Welsh onion attenuates hyperlipidemia in rats fed on high-fat high-sucrose diet. *Bioscience, Biotechnology and Biochemistry.* 74(2):402-4.

Yavari A, Hajiyev AM and Naghizadeh F (2010). The effect of aerobic exercise on glycosylated hemoglobin values in type 2 diabetes patients. *The Journal of Sports Medicine and Physical Fitness.* 50(4):501-5.

Yoo YK, Kim SK and Song MS (2013). Effects of muscular and aqua aerobic combined exercise on metabolic indices in elderly women with metabolic syndrome. *Journal of Exercise Nutrition and Biochemistry.* 17(4):133-41.

Yoshida H, Ishikawa T, Suto M, Kurosawa H, Hirowatari Y, Ito K, Yanai H, Tada N and Suzuki M (2010). Effects of supervised aerobic exercise training on serum adiponectin and parameters of lipid and glucose metabolism in subjects with moderate dyslipidaemia. *Journal of Atherosclerosis and Thrombosis.* 17(11):1160-6.

Young DR, Coleman KJ, Ngor E, Reynolds K, Sidell M and Sallis RE (2014). Associations between physical activity and cardiometabolic risk factors assessed in a Southern California health care system, 2010-2012. *Preventing Chronic Disease.* 11:219.

Zhang CY, Baffy G, Perret P, Krauss S, Peroni O, Grujic D, Hagen T, Vidal-Puig AJ, Boss O, Kim YB, Zheng XX, Wheeler MB, Shulman GI, Chan CB and Lowell BB (2001).

Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, beta cell dysfunction, and type 2 diabetes. *Cell.* 105(6):745-55.

Zhang L, Song H, Ge Y, Ji G and Yao Z (2015). Temporal relationship between diet-induced steatosis and onset of insulin/leptin resistance in male wistar rats. *PLoS* One. 10(2):e0117008.

Zernicke RF, Salem GJ, Barnard RJ and Schramm E (1995). Long-term, high-fat-sucrose diet alters rat femoral neck and vertebral morphology, bone mineral content, and mechanical properties. *Bone.* 16(1):25-31.

Zhou Y, Lin S, Chang HH, Du J, Dong Z, Dorrance AM, Brands MW and Wang MH (2005). Gender differences of renal CYP-derived eicosanoid synthesis in rats fed a high-fat diet. *American Journal of Hypertension.* 4(4 Pt 1):530-7.

Zhou X, Han D, Xu R, Li S, Wu H, Qu C, Wang F, Wang X and Zhao Y (2014). A model of metabolic syndrome and related diseases with intestinal endotoxemia in rats fed a high fat d high sucrose diet. *PLoS One.* 9(12):e115148.

Zhukova NV, Novgorodtseva TP and Denisenko YK (2014). Effect of the prolonged high-fat diet on the fatty acid metabolism in rat blood and liver. *Lipids in Health and Disease.* 13:49.