A PATHOLOGIC ROLE FOR ANGIOTENSIN II AND ENDOTHELIN-1 IN CARDIAC REMODELLING AND ISCHAEMIA AND REPERFUSION INJURY IN A RAT MODEL OF THE METABOLIC SYNDROME.

Wayne Smith

Student number: 13453645-2000

Thesis presented in complete fulfillment of the requirements for the degree

Master of Science in Medical Sciences

Department of Medical Physiology and Biochemistry

University of Stellenbosch

Supervisor: Dr EF du Toit

Co-Supervisor: Prof JA Moolman

April 2006

Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously submitted it, in its entirety or in part, at any university for a degree.

Signature: …… **Date: ……**

ABSTRACT

Introduction: Obesity, which is implicated in the development of the metabolic syndrome (MS) is reaching epidemic proportions worldwide. MS significantly increases the risk of developing cardiovascular disease, which includes coronary artery disease. The current absence of animal models of diet induced obesity and the MS makes the investigation of the cardiovascular consequences of MS virtually impossible. As a result the effects of the MS on cardiac function, morphology and susceptibility to ischaemia are not well understood.

Aims: We set out to: 1) develop and characterize a rodent model of diet-induced obesity and the MS, 2) investigate the susceptibility of hearts from these animals to ischaemia/reperfusion induced injury and, 3) determine whether angiotensin II (Ang II) and endothelin-1 (ET-1) plays a role in cardiac remodelling and/or the severity of ischaemia and reperfusion injury in this model.

Methods: Male Wistar rats were fed a standard rat chow diet or cafeteria diet (CD) for 16 weeks. After the feeding period rats were sacrificed and blood and myocardial tissue samples were collected to document biochemical changes in these animals. Hearts were perfused on the isolated working rat heart perfusion apparatus to assess myocardial mechanical function before and after ischaemia. In a separate series of experiments, hearts underwent coronary artery ligation to determine the incidence and duration of ventricular arrhythmias during ischaemia and reperfusion, using electrocardiography. To assess a

possible link between myocardial remodelling and ischaemia/reperfusion injury and myocardial Ang II and ET-1 content, we also measured these peptides under basal conditions and during ischaemia. Two-dimensional targeted M-mode echocardiography was used to assess in vivo myocardial mechanical function in control and obese rats.

Results: After 16 weeks on the CD, obese rats satisfied the World Health Organization (WHO) criteria for the MS by having visceral obesity, insulin resistance, dyslipidaemia and an elevated systolic blood pressure, compared to control rats. Circulating Ang II levels, but not ET-1 levels, were elevated in CD fed rats. Obese rats had cardiac hypertrophy and ex vivo basal myocardial mechanical function was depressed in the CD fed rat hearts compared to control rat hearts. CD fed rat hearts had poorer aortic output (AO) recoveries compared to hearts from control rats. These hearts also had a higher incidence and duration of reperfusion arrhythmias. No such functional differences were seen in the in vivo experiments. No differences in basal or ischaemic myocardial Ang II and ET-1 levels were seen in either group.

Conclusion: We have developed and characterized a model of diet-induced obesity and the MS. Obesity is associated with cardiac hypertrophy and an increased myocardial susceptibility to ischaemia and reperfusion injury in our model. The hearts from obese rats were also more prone to reperfusion ventricular arrhythmias. As myocardial function was only poorer in the ex vivo obese animal experiments, our data suggests that the obesity associated changes in function observed in the ex vivo studies may be related to the

absence of circulating substrates or factors, which are essential for their normal mechanical function.

UITTREKSEL

Inleiding: Vetsug, wat as ‘n aanleidende faktor in die ontwikkeling van metaboliese sindroom (MS) geimpliseer word, bereik tans wêreldwyd epidemiese afmetings. MS verhoog die risiko vir die ontwikkeling van kardiovaskulêre siektes soos korenêre hartvatsiekte. Die huidige gebrek aan dier modelle van dieet geinduseerde vetsug en MS, maak die ondersoek na die kardiovaskulêre gevolge van MS feitlik onmoontlik. Die effek van MS op hart funksie, morfologie en vatbaarheid vir isgemiese skade is dus nog grootendeels onbekend

Doel: Ons het gepoog om: 1) ‘n model van dieet geinduseerde vetsug en MS te ontwikkel en karakteriseer, 2) die vatbaarheid van die harte van die diere vir isgemie/herperfusie skade te bepaal en, 3) te bepaal of angiotensien II (Ang II) en endotelien-1 (ET-1) ‘n rol in miokardiale hermodulering en/of isgemiese/herperfusie skade in hierdie model speel.

Metodes: Manlike Wistar rotte is vir 16 weke op ‘n standaard rotkos (kontrole) of kafeterie dieet (KD) geplaas. Aan die einde van die voerprogram is rotte geslag en bloed en miokardiale weefsel is versamel vir biochemiese bepalings. Harte is op die werkhart perfusie aparaat geperfuseer en meganiese funksie is voor, en na isgemie bepaal. In ‘n apparte reeks eksperimente is die koronêre arterie van harte afgebind en die insidensie van aritmieë gedurende isgemie en herperfusie is met behulp van elektrokardiografie bepaal. Om ‘n moontlike verband tussen miokardiale hermodulering en isgemie/reperfiesie skade te bepaal is hartspierweefsel versamel en basale en isgemiese Ang II en ET-1

vlakke bepaal. Twee dimensionele “M-mode” egokardiografie is gebruik om in vivo miokardiale funksie in kontrole en vetsugtige rotte te bepaal.

Resultate: Na 16 weke op die KD het die vet rotte aan die kriteria van die WGO vir MS voldoen. Hulle was viseraal vetsugtig, insulien weerstandig, en het abnormale lipied profiele en verhoogde sistoliese bloeddrukke gehad. Sirkulerende Ang II vlakke was verhoog in KD gevoerde rotte terwyl ET-1 vlakke onveranderd was. Vetsugtige rotte het hipertrofiese harte gehad en basale meganiese funksie was verlaag in vergelyking met kontrole rotte. Die KD gevoerde rotte het swakker aorta uitset herstelle getoon as die kontrole diere. Die harte het ook ‘n hoer insidensie van herperfusie aritmieë getoon. Daar was egter geen verskil in meganiese funksie in die in vivo eksperimente nie. Basale en isgemiese Ang II en ET-1 vlakke was vergelykbaar in die miokardium van die twee groepe diere.

Gevolgtrekkings: Ons het ‘n model van dieet geindieseerde vetsug en MS ontwikkel en gekarakteriseer. Vetsug word in ons model geassosieer met miokardiale hipertrofie en verhoogde vatbaarheid vir isgemie/herperfusie skade. Die harte van vetsugtige rotte was ook meer vatbaar vir herperfusie aritmieë. Die waarneeming dat meganiese funksie slegs in die ex vivo eksperimente in vetsugtige diere verlaag was, dui aan dat die verlaagde funksie dalk te wyte is aan die afwesigheid van sirkulerende substraat of faktore wat vir normale meganiese funksie van die hart noodsaaklik is.

ACKNOWLEDGMENTS

I would firstly like to thank Dr Joss du Toit for all his assistance and guidance throughout this study. Your dedication to this study and to helping me has really been exceptional.

Thank you also to Prof Amanda Lochner, Prof Johan Moolman, Prof Gavin Norton and Prof Angela Woodiwiss for their ideas, suggestions and input into this study.

Thank you to Dr Christo Muller for performing the cell size analysis.

For financial support I would like to thank the Department of Medical Physiology and the National Research Foundation.

Thanks to everyone in the department for all the encouragement and support I received from you. A special thanks to members of Lab 530 and 570 for all their assistance.

I would like to thank all my family and especially Candice as well as everyone at Family Harvest Church for all their love, support, encouragement and prayers during this time.

Most importantly I want to thank my Heavenly Father for strengthening me and for giving me the ability to write this thesis.

TABLE OF CONTENTS

Pectora roborant cultus recti

LIST OF ILLUSTRATIONS

Figures

Tables

LIST OF ABBREVIATIONS

Units of measurement

°C	degrees Celsius
cm	centimeter
g	gram
kg	kilogram
kJ	kilojoules
L	litre
M	molar
mg	milligram
ml	millilitre
mM	millimolar
mmol	minnimol
%	percentage
μ	micro
μ	microlitre
μm	micrometer
U	unit
v	volume

Chemical compounds

Ang II	angiotensin II
ATP	adenosine triphosphate
Ca^{2+}	calcium
CO_2	carbon dioxide

ET-1	endothelin-1
H^+	hydrogen
HCl	hydrochloric acid
HDL	high density lipoprotein
Hg	mercury
H_2O	water
K^+	potassium
PPAR	peroxisome proliferator-activated receptors
PI3K	phosphatidylinositol-3-kinase
MAPK	mitogen activated protein kinase
Na^+	sodium
NaOH	sodium hydroxide
NO	nitric oxide
O_2	oxygen
TFA	trifluoro-acetic acid
TG	triacylglycerol/triglyceride
VLDL	very low-density lipoprotein

Other abbreviations

AO	aortic output
CAL	coronary artery ligation
CD	cafeteria diet
CF	coronary flow
EDD	end-diastolic diameter
ESD	end-systolic diameter
FD	flow deprivation

FSend	endocardial fractional shortening
FSmid	midwall fractional shortening
i.p.	intraperitoneal
LD	Langendorff
min	minute
MS	metabolic syndrome
NCEPATP III	National Cholesterol Education Program Adult Treatment Panel III
PWT_{diast}	posterior wall thickness during diastole
PWT_{syst}	posterior wall thickness during systole
PWthick	posterior wall thickening
RAS	renin-angiotensin system
RIA	radioimmunoassay
SEM	standard error of the mean
SHR	spontaneous hypertensive rats
SRC	standard rat chow
T	time
VSMC	vascular smooth muscle cells
WKY	Wistar Kyoto
WH	working heart
WHO	World Health Organization
ZDF	zucker diabetic fatty

CHAPTER 1

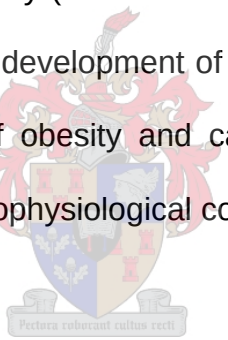
INTRODUCTION

The prevalence of obesity has increased and is reaching epidemic proportions worldwide (Pi-Sunyer, 2002). This is reflected in recent statistics where 30.5 % of Americans are thought to be obese (Flegal *et al*. 2002). Approximately 300 000 Americans die annually of obesity related causes (Allison *et al*. 1999). South Africa is not exempt of this epidemic, and current data indicate that 29.2 % of the men and 56.6 % of the women in South Africa are overweight or obese (Puoane *et al*. 2002). These statistics are concerning as it has been suggested that obesity leads to the development of a constellation of metabolic abnormalities associated with cardiovascular disease. This constellation of metabolic abnormalities is collectively termed the metabolic syndrome (MS) (NCEP ATP III, 2001; Reaven, 2005).

The MS consists of obesity, and particularly visceral obesity, diabetes mellitus or insulin resistance, dyslipidaemia, hypertension and microalbuminurea (Alberti and Zimmet, 1998). Each component of the MS is an individual risk factor for cardiovascular disease (Nakamura *et al*. 1994; Kenchaiah *et al.* 2002; Tagle *et al*. 2003; Glynn and Rosner, 2005; Caglayan *et al*. 2005). Of greater concern is that the co-occurrence of 2 or more of these components has been shown to increase the overall risk of developing cardiovascular disease (Klein *et al*. 2002). The cardiovascular risk associated with the MS has been confirmed in

longitudinal studies, where patients displaying characteristics of the MS were shown to have a fourfold increased risk of developing coronary heart disease, together with an increased risk for all cause mortality, mortality due to cardiovascular disease and type 2 diabetes, over a 9 to 14 year period (Lakka *et al*. 2002).

Besides the vascular effects associated with obesity, long term obesity may also result in various myocardial structural adaptations, due to the increase in preload associated with obesity. These obesity induced changes manifest in the development of cardiac hypertrophy (Paulson and Tahiliani, 1992). As obesity is an independent risk factor in the development of coronary artery disease (Rimm *et al*. 1995), the combination of obesity and cardiac hypertrophy could have serious medical concerns in pathophysiological conditions such as ischaemia.



The myocardial effects of ischaemia/reperfusion injury have previously been investigated in animals models of the MS, such as the fructose fed rat (FFR) (Morel *et al*. 2003) and the Zucker diabetic fat (ZDF) (Yue *et al*. 2005) rat. Both these models have shown increased myocardial susceptibility to ischaemia/reperfusion injury. Despite this, further research is required on the effect of obesity on the myocardial susceptibility to ischaemia/reperfusion injury in the MS as these animal models either have marked diabetes mellitus (ZDF) or insulin resistance without obesity (FFR).

Ischaemia/reperfusion injury may further manifest itself as decreased mechanical function after ischaemia, or as arrhythmias. Clinical studies suggest that obesity may be a risk factor for sudden death induced by arrhythmias (Aronson, 1981; Kopelman, 2000), and the presence of cardiac hypertrophy, an arrhythmogenic risk factor, may increase the risk for developing ventricular arrhythmias (Wolk, 2000). Eccentric cardiac hypertrophy was shown to be a risk factor for excessive ventricular ectopy in obese normotensive individuals, when compared with lean controls (Messerli *et al*. 1987). Despite this, no electrophysiological studies have been performed to show a direct link between obesity and fatal ventricular arrhythmias.

Obesity is also associated with elevated circulating levels of angiotensin II (Ang II) and endothelin-1 (ET-1). These peptides are well known for their growth promoting effects and their proposed role in the development of cardiac hypertrophy (Sadoshima and Izumo, 1993; Ito *et al*. 1993). They have also been implicated in increasing myocardial susceptibility to ischaemia and reperfusion injury (Yoshiyama *et al*. 1994; Brunner *et al*. 1997; Brunner and Opie, 1998; Frolkis *et al*. 2001). These peptides may also contribute to the development of cardiac arrhythmias (de Graeff *et al*. 1986; Brunner and Kukovetz, 1996). As both Ang II and ET-1 are associated with many of the abnormalities induced by obesity and possibly the MS, further investigations into their involvement in the MS are essential.

Despite the heightened cardiovascular risk associated with MS and the increasing prevalence of obesity, which is an initiator of the syndrome, it is concerning that there are, to our knowledge, no diet induced obesity models of the MS. Current established rodent models of the MS include the ZDF rat, which is a genetically obese model of the MS, however this model is one of marked diabetes mellitus which complicates the investigation of the impact of obesity on the cardiovascular system. Other MS models include the FFR (Hwang *et al*. 1987) and sucrose fed rat models (Baños *et al*. 1997), however these rodent models do not develop obesity. The development of such a diet induced obesity model of the MS with which to investigate the cardiovascular effects associated with the MS is therefore warranted.

CHAPTER 2

LITERATURE REVIEW

2.1 Metabolic syndrome and cardiovascular disease

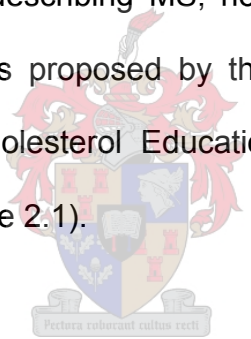
The MS is the term used to describe a host of metabolic abnormalities affecting an individual. MS was first comprehensively described in 1988 under the term “syndrome X”, and was said to encompass obesity, insulin resistance, hyperinsulinaemia, impaired carbohydrate metabolism or diabetes, hypertension, and dyslipidaemia, in the form of low high density lipoprotein (HDL) cholesterol and elevated triglyceride (TG) concentrations (Reaven *et al*. 1988).

The MS has major health implications as the various key components of the syndrome are associated with an increased risk for cardiovascular disease. Klein *et al*. (2002) showed that the risk of developing cardiovascular disease over a five year period increased with the number of components of the MS present. Furthermore, Lakka *et al*. (2002) showed that middle aged men with the MS had a fourfold increased risk for developing coronary heart disease, together with an increased risk for all cause mortality, mortality due to cardiovascular disease and type 2 diabetes, over a 9 to 14 year period. These findings were more significant since there was no evidence of cardiovascular diseases or diabetes when baseline measurements where performed at the beginning of the trial. Elsewhere it was shown that 46 % of the patients hospitalized with acute myocardial infarction, met the criteria for MS (under the NCEP ATPIII) (Zeller *et al*. 2005). Lastly, in a community based sample of

postmenopausal women, over a 8.5 ± 0.3 year period, the presence of an enlarged waist combined with elevated TG's (components of MS), was associated with a 4.7 fold increased risk for fatal cardiovascular events. These findings emphasize the increased risk for cardiovascular disease found in MS patients. The aggregation of the components of the MS was shown not to be by coincidence but that clustering does in fact occur (Aizawa *et al*. 2005). It is important to emphasize that it is the clustering of these individual components that make MS such an important risk factor for cardiovascular disease.

2.1.1 Definitions of the metabolic syndrome

There are a few definitions describing MS, however the two that are most commonly used are the ones proposed by the World Health Organization (WHO) and the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) (Table 2.1).



According to the WHO the MS includes impaired glucose tolerance, diabetes mellitus and/or insulin resistance together with two or more of the following abnormalities: raised arterial blood pressure (≥140/90 mmHg); dyslipidaemia, raised plasma TG concentration (≥1.7 mmol/l) and/or low HDL-cholesterol (males: <0.9 mmol/l; females: <1.0 mmol/l); central obesity (males: waist-to-hip ratio >0.90 cm; females: waist-to-hip ratio >0.85 cm) and/or a body mass index >30 kg/m^2; microalbuminuria (urinary albumin excretion rate ≥20 µg/min or albumin:creatinine ratio ≥30 mg/g) (Alberti and Zimmet, 1998).

The NCEP ATP III definition defines MS as a condition requiring three of the following to be present: abdominal obesity (males: waste circumference >102 cm; females: waste circumference >88 cm); elevated plasma TG's (≥1.69 mmol/l) and low HDL-cholesterol (males: <1.03 mmol/l; females: <1.29 mmol/l); elevated blood pressure (≥130/85 mmHg) and elevated fasting glucose ≥6.1 mmol/l (NCEP ATP III, 2001).

Other definitions of the MS also include systemic inflammation and prothrombotic disturbances (for a recent review see Caglayan *et al.* 2005).

The two main definitions (WHO and NCEP ATP III) agree on certain key components of the syndrome namely obesity, glucose intolerance, hypertension and dyslipidaemia. There are however differences with respects to some of the criteria for the diagnosis of this syndrome. The discrepancies between the two definitions could be attributed to the circumstances under which a diagnosis needed to be made. In a review by Eckel *et al*. (2005) it was stated that the WHO definition was better suited as a research tool whereas the NCEPATP III definition would be more useful in clinical practice as it requires simpler tools for assessment.

Table 2.1: Definitions of the metabolic syndrome

World Health Organisation			**National Cholesterol Education Program Adult Treatment Panel III**		
One of these	Impaired glucose tolerance Diabetes mellitus Insulin resistance *		**Any**	Abdominal obesity	M: waste c >102 cm F: waste c >88 cm
				Elevated plasma triglycerides	≥1.69 mmol/l
Any two	Raised arterial blood pressure	≥140/90 mmHg		Low HDL Cholesterol	M: <1.03 mmol/l F: <1.29 mmol/l
	Dyslipidemia				
	Raised plasma TG	≥1.7 mmol/l	**three**	Elevated blood pressure	≥130/85 mmHg
	Low HDL Cholesterol	M: <0.9 mmol/l F: <1.0 mmol/l		Elevated fasting glucose	≥6.1 mmol/l
	Central obesity	M: wh ratio >0.90 cm F: wh ratio >0.85 cm			
	High BMI	>30 kg/m^2			
	Microalbuminuria	UAE rate ≥20 µg/min			

***BMI*-body mass index; *F*-female; *M*-male; *TG*-triglyceride; *UAE*-urinary albumin excretion; *waste c*-waste circumference; *WH*-waste-to-hip;**

* Under hyperinsulinaemic, euglycaemic conditions, glucose uptake below lowest quartile for background population under investigation

2.1.2 The development of the MS

The resistance to insulin-mediated glucose uptake, or insulin resistance, is considered to be the central abnormality of the MS (NCEP ATP III, 2001). The question therefore arises as to the cause of the MS. The third report of the National Cholesterol Education Program (NCEP ATPIII, 2001) stated that, "The *root causes of the metabolic syndrome are overweight/obesity, physical inactivity, and genetic factors*." Reaven (2005) also mentioned that, " …*insulin resistance/hyperinsulinaemia does not cause obesity; obesity is a physiological variable that increases the likelihood that an individual will be resistant to insulin*." It has been shown that there is a decline in insulin's action with an increase in obesity, up to a body fat composition of 28-30 % (Bogardus *et al.* 1985). These observations reflect the susceptibility of obese individuals to developing insulin resistance. Thus it appears that lifestyle could also play a major role in the susceptibility of the individual to developing the MS. Obesity is therefore regarded as the starting point in the development of the MS, giving rise to insulin resistance, which, as will be discussed later, is speculated to be the driving force behind the MS (figure 2.1).

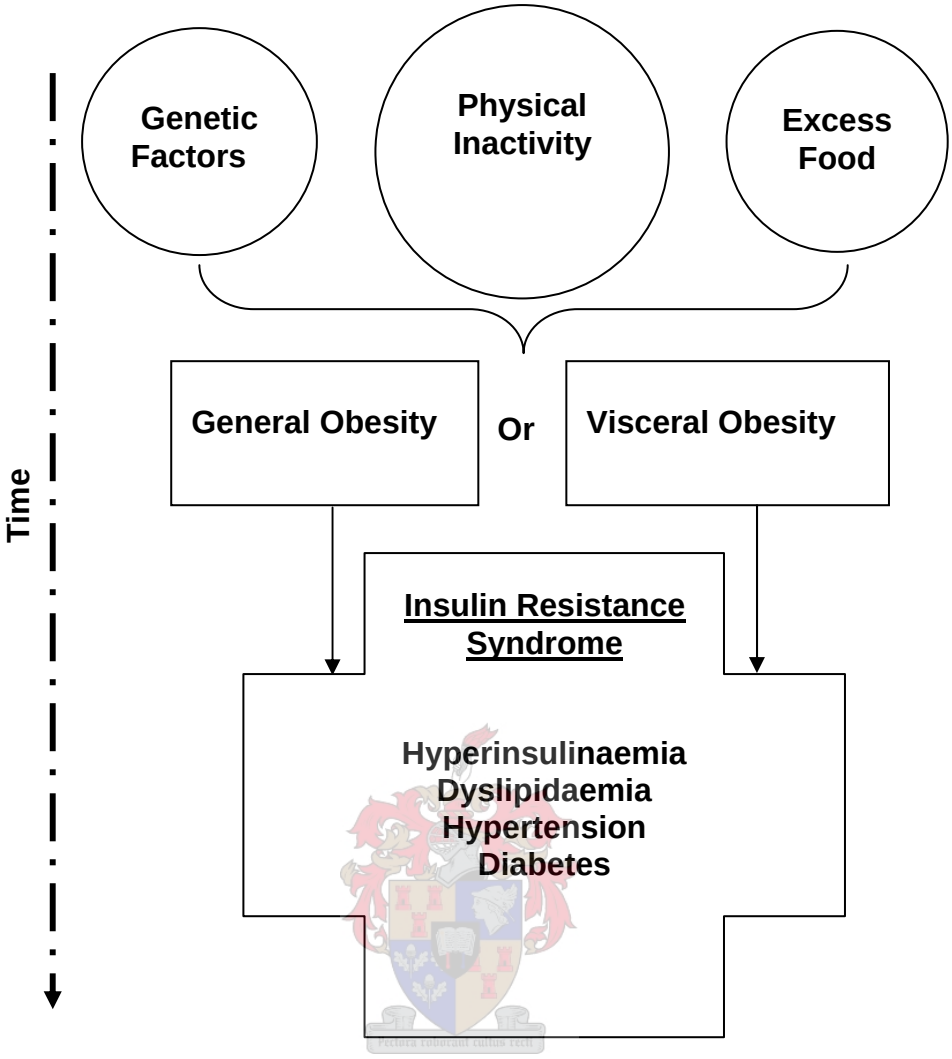


Figure 2.1: Development of the MS over time, after the development of obesity

2.1.3 Components of the metabolic syndrome and their impact on cardiovascular disease

2.1.3.1 Obesity

Obesity is essentially a consequence of excess white adipose tissue (Dizdar and Alyamac, 2004) and can be seen as an energy storage disorder, which occurs when there is an imbalance between calorie intake and utilization (Paulson and Tahiliani, 1992) over a period of time. Recent statistics indicate

that 29.2 % of the men and 56.6 % of the women in South Africa are overweight or obese. Within this group, 9.2 % of the men and 42 % of the women are viscerally obese (Puoane *et al.* 2002). These data are of particular concern since obesity has been found to be an independent risk factor for clinical heart failure (Kenchaiah *et al.* 2002).

2.1.3.1.1 Obesity and the significance of body fat distribution

When considering obesity and the MS it has become clear that more emphasis is being placed on the distribution of adipose tissue in the body, rather than just considering generalized obesity. It was recently shown in non-obese Japanese subjects that visceral fat accumulation contributes to the development of coronary artery disease and that it is possibly secondary to the development of insulin resistance (Kobayashi *et al*. 2001). Insulin resistance is defined as the ineffective glucose uptake by a tissue under the influence of normal physiological concentrations of circulating insulin (Reaven, 1988). Various other studies have corroborated the strong association between non-obese individuals with excess visceral fat accumulation, insulin resistance and cardiovascular disease (Evans *et al*. 1984; Nakamura *et al*. 1994). Excess visceral fat accumulation may therefore be more important in the development of insulin resistance or MS and the associated cardiovascular abnormalities than general obesity. The exact mechanism through which the presence of visceral fat induces insulin resistance is not known. However the literature suggests that the specific characteristics of the adipose tissue with respects to their release of free fatty acids (FFA) play an important role. Briefly, it is thought that elevated FFA as seen in obesity

(Golay *et al.* 1986), play an important role in the development of insulin resistance as elevated plasma FFA levels have been shown to suppress skeletal muscle glucose uptake in vivo (Kelly *et al*. 1993). Lipolysis is the hydrolysis of lipids stored in adipocytes by lipoprotein lipase, with resultant release of FFA and glycerol into the circulation (Korn, 1955). The rate of lipolysis as well as the responsiveness to various lipolytic hormones has been shown to vary in the different fat depots in the body, with low lipolysis rates seen in the subcutaneous fat depots (Reynisdottir *et al.* 1994) and higher rates of lipolysis seen in the visceral fat depots (Lönnqvist *et al*. 1995). This higher rate of visceral fat lipolysis could be due to the resistance of the visceral fat mass to insulin's suppression of lipolysis (Mittelamn *et al*. 2002). Furthermore, the lipolytic response to noradrenaline, as indicated by the amount of FFA released, was found to be greater in abdominal/visceral adipose tissue than gluteal or femoral adipose tissue in both men and women (Lönnqvist *et al*. 1995). It is speculated that the resultant increased release of FFA into the plasma may induce lipid accumulation and insulin insensitivity in skeletal muscle (Phillips *et al*. 1996) and liver (Mittelamn *et al*. 2002). Thus people that are viscerally obese are considered to have a higher risk for developing insulin resistance and the MS.

2.1.3.1.2 Obesity and lipogenic hormones

Obesity is a condition associated with excess adipose tissue (Forbes and Welle, 1983). It has been shown that adipose tissue, in addition to storing excess energy as fat, can act as an endocrine hormone (Rajala and Scherer, 2003). Some of the many factors released by the adipose tissue include

leptin, adiponectin, resistin, TNF-α and other cytokines, FFA, Ang II, PAI-1, CEPT and estrogens. Evidence exists that the secretory function of adipose tissue is impaired in obesity and this has a direct effect on the levels of these circulating factors (Hauner, 2004). In fact obesity is associated with systemic inflammation, which may be a direct result of inflammatory factors secreted by the adipose tissue. Moreover systemic inflammation is associated with an increased cardiovascular risk, and this may be influenced by these adipose tissue derived factors (Berg and Scherer, 2005). The secretory role of adipose tissue was not investigated in this study, but it is important to note that in an obese state, the secretory function of the adipose tissue is implicated in cardiovascular disease. The factors involved and the proposed mechanisms by which they influence the risk of cardiovascular disease are further reviewed by (Berg and Scherer, 2005).

2.1.3.1.3 Obesity and cardiac hypertrophy/remodelling

Cardiac hypertrophy is the adaptation of the heart to an increased workload due to biomechanical stress induced by an increased hemodynamic load. Growth factors and hormones also play a role in stimulating cardiac hypertrophy as will be discussed later. The heart adapts to the increased workload in order to maintain cardiac output but over time these changes can become maladaptive (Frey and Olson, 2003).

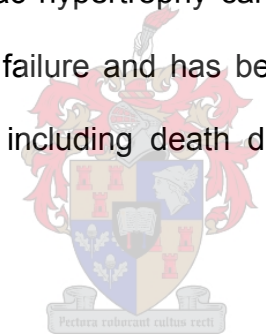
Obesity is associated with changes in several cardiovascular parameters responsible for increasing the stress on the heart and therefore contributes to the development of cardiac hypertrophy (figure 2.4). Investigations into

various cardiac parameters indicated that cardiac output, stroke volume, central blood volume, plasma and total blood volume were all increased in obese individuals (Messerli *et al.* 1983a).

Obesity has been associated with both eccentric and concentric hypertrophy, the latter occurring in the absence of hypertension (Messerli *et al*. 1983b; Opie, 1991; de la Maza *et al*. 1994). As the obese condition is associated with excessive weight gain due to an increase in adipose tissue and lean body mass (Forbes and Welle, 1983), it is not surprising that the overall metabolic demands (Carroll *et al.* 1995), especially the oxygen requirements by these tissues are increased. This increased oxygen demand has serious implications for the cardiovascular system, which compensates by increasing cardiac output (Alexander *et al*. 1953). There are a few discrepancies in the literature but the majority of the data indicates that an increase in stroke volume is responsible for the increased cardiac output found in obese individuals (Messerli *et al*. 1982). This is primarily due to the increased blood volume seen in obesity resulting in an increased end-diastolic volume and filling pressure (therefore increased preload). To compensate for these factors adaptive myocardial hypertrophy together with ventricular dilatation occurs resulting in eccentric cardiac hypertrophy. It needs to be emphasized that this is initially a compensatory mechanism aimed at maintaining cardiac output. With time, the continuously elevated wall stress exacerbates dilatation of the left ventricle without a concomitant increase in the ventricular wall thickness. A state of decompensated myocardial hypertrophy occurs which can ultimately result in heart failure (Opie. 1991; Paulson and Tahiliani, 1992).

Obesity combined with arterial hypertension can result in concentric hypertrophy. This form of hypertrophy is primarily due to the additional wall stress induced by the hypertensive state, which increases the dimensions of the left ventricular wall (posterior wall thickness) disproportionately to the ventricular chamber size (de la Maza *et al.* 1994). This may be of concern as in non-obese SHR's over a period of time, this compensatory mechanism of the left ventricle can over regress to dilatation of the left ventricle and eventually pump dysfunction (Tsotetsi *et al*. 2001).

Thus pathophysiologic cardiac hypertrophy can be regarded as a risk factor for the progression to heart failure and has been shown to predict a higher incidence of clinical events including death due to cardiovascular disease (Levy *et al*. 1990).



2.1.3.1.4 Obesity and cardiac function

Although obesity is an independent risk factor for coronary artery disease and heart failure, it seems as if obesity in the absence of any pathophysiological condition does not have a negative effect on normal cardiac function. Cardiac mechanical function seems to be normal if not improved in the obese state as reflected by an elevated cardiac output (Messerli *et al.* 1983a). In fact echocardiographic studies reveal that obese individuals have an augmented myocardial fractional shortening when compared to lean individuals (Berkalp *et al*. 1995 and Pascual *et al*. 2003). This implies that they have an improved systolic function.

Diastolic dysfunction may occur in obese individuals. Pascual *et al*. (2003) classified obese patients as slightly, moderately or severely obese based on BMI measurements. Left ventricular diastolic dysfunction was present in all grades of isolated obesity. In the presence of cardiac hypertrophy, ventricular diastolic dysfunction is however also a common finding. Patients with isolated septal hypertrophy or concentric or eccentric left ventricular hypertrophy all show signs of ventricular diastolic dysfunction (Corin *et al*. 1991; Nunez *et al*. 1994; Andren *et al*. 1996). In addition, in obese individuals, the severity of the diastolic dysfunction increases in the presence of hypertension (Lavie *et al*. 1987), as shown by indirect investigation of left atrial abnormalities using electrocardiogram and echocardiogram inspection.

It must be emphasized that diastolic dysfunction does not translate into mechanical dysfunction. The increased filling volumes as seen in obesity can compensate for the diastolic dysfunction. Therefore obese individuals can be seen to have a normal or slightly elevated cardiac mechanical function.

2.1.3.1.5 Obesity induced hypertrophy and myocardial susceptibility to ischaemia/reperfusion injury

We have previously shown that obesity can increase the susceptibility of the myocardium to ischaemia and reperfusion injury (Du Toit *et al*. 2005). These obese rats had a moderately elevated systolic blood pressure, when compared to controls, but were not hypertensive. Few other studies investigated the effect of uncomplicated obesity together with cardiac

hypertrophy, without other pathophysiologies, on ischaemia/reperfusion injury. This may be due to the availability of models displaying uncomplicated obesity. The Zucker diabetic fatty (ZDF) rat is a genetic model of obesity and diabetes. Hearts isolated from ZDF rats have been shown to have an improved post-ischaemic functional recovery during reperfusion (Wang *et al*. 2004). These findings are however complicated by the co-occurrence of diabetes in these animals. Therefore in addition to obese patients being at risk of developing heart failure, through the progression of cardiac hypertrophy (as mentioned above), their hearts may additionally be more susceptible to an ischaemic insult.

Possible role players responsible for inducing the greater mechanical dysfunction during reperfusion following an ischaemic insult in a hypertrophied myocardium include 1) increased Ca^{2+} overload during reperfusion compared to normal hearts (Allard *et al*. 1994). This has previously been shown to induce mechanical dysfunction (see myocardial stunning), and 2) alterations in myocardial energy metabolism. This is explained in more detail by Galiñanes and Fowler, (2004) and involves exaggerated uncoupling of glycolysis from glucose oxidation following the ischaemic insult in the hypertrophied myocardium (Wambolt *et al*. 1997).

Lastly, obesity is considered to be a risk factor for myocardial infarction (Yusuf *et al*. 2005). It is however uncertain whether or not in the absence of conventional cardiovascular risk factors, obesity affects clinical outcomes following a myocardial infarction. This is somewhat of a paradox as increased

BMI has been associated with both improved (Lopez-Jimenez *et al*. 2004; Kennedy *et al*. 2005; Eisenstein *et al*. 2005; Nikolsky *et al*. 2006) and worse (Rea *et al*. 2001; Rana *et al*. 2004; Kragelund *et al*. 2005) clinical outcomes following a myocardial infarction and reperfusion. This obesity paradox emphasises the need to develop animal models of obesity, without traditional cardiovascular risk factors, to clarify this issue.

2.1.3.1.6 Obesity, cardiac hypertrophy and myocardial arrhythmias

Obesity has been documented as an independent risk factor for sudden death. Obesity may predispose the individual to fatal ventricular arrhythmias by inducing cardiac hypertrophy, which is an arrhythmogenic risk factor (Wolk, 2000) (figure 2.2). Eccentric cardiac hypertrophy induced by obesity has been shown to be a risk factor for excessive ventricular ectopy in obese normotensive individuals, when compared with lean controls (Messerli *et al*. 1987). This study was done using echocardiographic techniques. No electrophysiological studies have however been performed to show a direct link between obesity and fatal ventricular arrhythmias. Bril *et al*. (1991) investigated the incidence of ischaemia and reperfusion induced ventricular arrhythmias in a rabbit model of chronic heart failure. Heart failure was induced by volume and pressure overload, which was characterized by marked cardiac hypertrophy (85%). The heart failure group had a higher incidence of both ischaemia and reperfusion induced arrhythmias. Taken together this data together with the findings of Messerli *et al*. (1987), suggest that a direct link between obesity and fatal ventricular arrhythmias may exist.

Mild to moderate forms of ventricular hypertrophy have been shown to predispose the myocardium to early afterdepolarizations (Aronson, 1981) and early afterdepolarization-induced triggered activity (Charpentier *et al*. 1991). It was recently proposed (Wolk, 2000) that constant abnormalities observed in these hypertrophied hearts were the prolongation of the action potential duration and refractoriness. Wolk (2000) proposes that these abnormalities may promote arrhythmias in the hypertrophied hearts. It is thought that changes in Ca^{2+} and Na^{+}-Ca^{2+} exchange currents play a role in promoting this phenomenon. Significantly, it was found that the density of the Ca^{2+}ATPase pumps in the sarcoplasmic reticulum are diminished, and this seems to be proportional to the degree of cardiac hypertrophy present (de la Bastie *et al*. 1990). This implies that the sarcoplasmic reticulum Ca^{2+}ATPase pumps are less capable of Ca^{2+} reuptake, which could possibly contribute to changes in the action potential. Although this was a pressure overload model of hypertrophy, the same may occur in situations where volume overload induces hypertrophy (Takahashi *et al*. 2000).

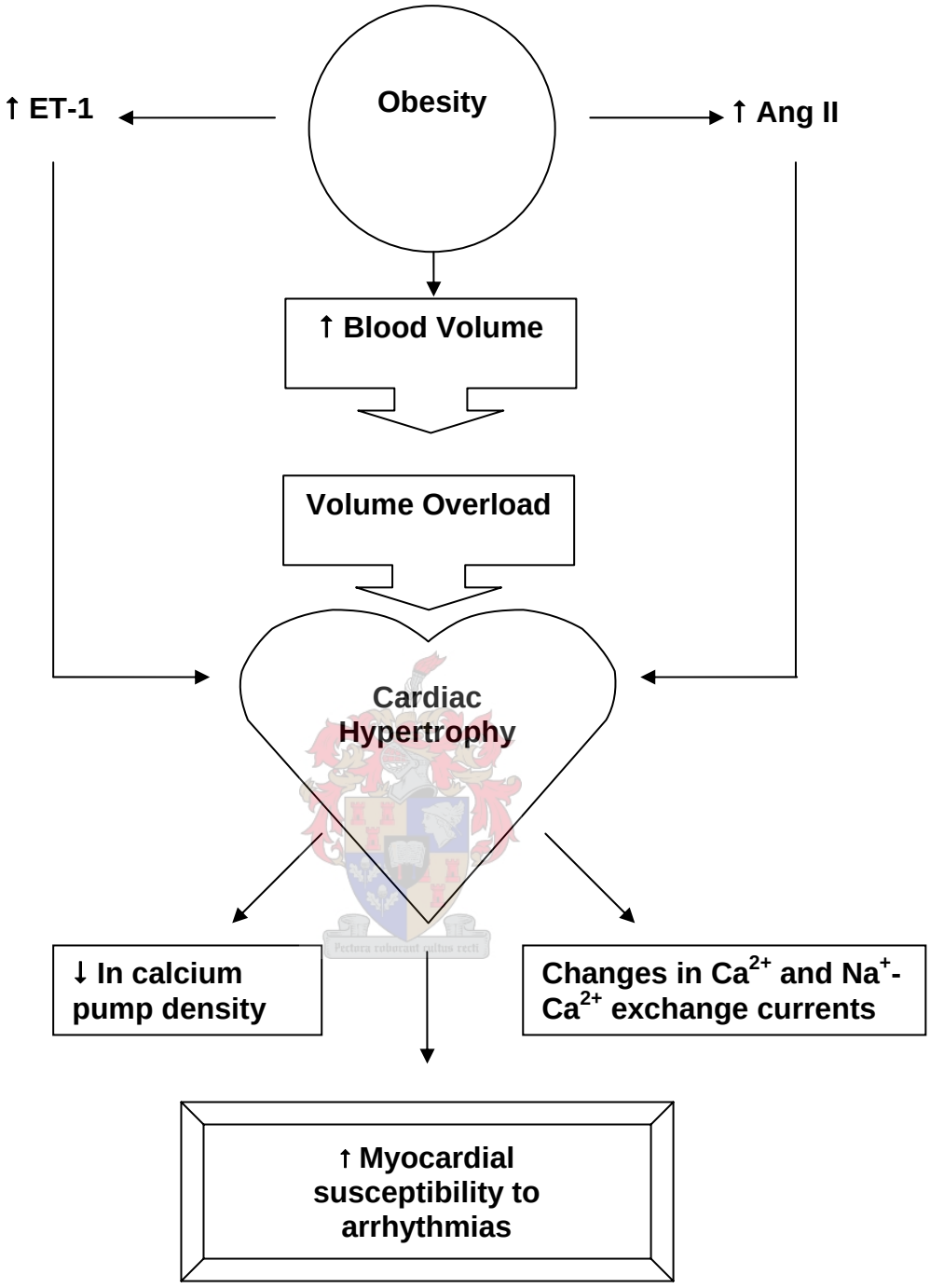


Figure 2.2: Obesity induced cardiac hypertrophy results in an increased risk of myocardial arrhythmias during ischaemia reperfusion. *Ang II*-angiotensin II; *ET-1*-endothelin-1.

2.1.3.2 Insulin resistance

In the 1987 Banting lecture, Reaven suggested that insulin resistance may be the underlying cause of the cluster of cardiovascular risk factors found in MS (Reaven, 1988). Together with this, insulin resistance is a common occurrence in both obesity and type 2 diabetes (Modan *et al*. 1985). There are however reports indicating that insulin resistance may be the underlying cause of many, but not all the cardiovascular risk factors associated with MS (Meigs *et al*. 1997; Hanley *et al*. 2002). Nevertheless, insulin resistance is seen as pivotal in the progression of MS.

Insulin resistance is the ineffective glucose uptake by a muscle under normal physiological concentrations of circulating insulin. To compensate for this the beta cells of the pancreas have to secrete more insulin, a state called hyperinsulinaemia. This compensation enables patients with insulin resistance to manage their blood glucose and prevents the onset of diabetes as long as the pancreas can maintain this compensation (Alberti and Zimmet, 1998). Despite not having frank diabetes, insulin resistant individuals have an increased risk of developing cardiovascular disease (Reaven, 1988).

2.1.3.2.1 Factors and mechanisms that contribute to the development of insulin resistance

2.1.3.2.1.1 Obesity and insulin resistance

Obesity, physical inactivity or a genetic predisposition is usually, but not always, the starting point for the development of the insulin resistant state (Bogardus *et al.* 1985; Rosenthal *et al.* 1983). It is however also possible to

be insulin resistant without being obese (Rosenthal *et al.* 1983). It has recently been shown that the distribution of body fat (central/visceral or peripheral obesity) plays an important role in the development of insulin resistance, despite the absence of generalized obesity. This was dealt with in the previous section on obesity. Obese individuals usually have elevated circulating FFA (Golay *et al.* 1986) and it is thought that the extra substrate availability together with the inhibitory effect of excess intramuscular lipids on glucose uptake (Krssak *et al*. 1999; Perseghin *et al*. 1999), leads to an insulin resistant state in skeletal muscle (Tenenbaum *et al*. 2004; Eckel *et al*. 2005).

An additional cellular mechanism for insulin resistance is the impaired tyrosine phosphorylation of insulin receptor substrate protein-1 in skeletal muscle (Pratipanawatr *et al*. 2001). Tyrosine phosphorylation of insulin receptor substrate protein-1 is essential in the activation of insulin's mediator, PI3-kinase, which plays a role in insulin mediated glucose uptake by skeletal muscle (Peterson *et al*. 2002). The precise mechanism for the insulin resistance however remains unknown.

2.1.3.2.1.2 Insulin resistance and hypertension

In the context of the MS, hyperinsulinaemia is thought to play a fundamental role in the development of essential hypertension. A link was seen between hypertension, insulin resistance and hyperinsulinaemia as early as 1966 when Welborn *et al*. (1966) studied a group of non-diabetic patients with essential hypertension and discovered that they had significantly higher plasma insulin concentrations than the normotensive control group. The link between insulin

resistance, hyperinsulinaemia and essential hypertension has subsequently been studied extensively, but the exact nature of the relationship between these variables has been questioned (Reaven, 2003). Insulin resistance and hyperinsulinaemia, could possibly lead to hypertension through 1) stimulation of renal sodium reabsorption leading to volume expansion (DeFronzo *et al*. 1975; Bigazzi *et al*. 1996), 2) increased sympathetic nervous system activity (hypothesis) (Kendall *et al*. 2003) and 3) growth mediated structural changes of the vascular wall (Cruzado *et al*. 1998), all of which are seen in a hyperinsulinemic state.

2.1.3.2.1.3 Insulin resistance and cardiac hypertrophy

Insulin resistance and the resultant hyperinsulinaemia may play a role in the development of cardiac hypertrophy, which itself is a cardiovascular risk factor as patients with cardiac hypertrophy have been shown to have a lower survival rate in a five year follow-up study (Sullivan *et al*. 1993). An enlarged left ventricular mass has been shown to occur in non-diabetic obese individuals, where the increase in ventricular mass was associated with the degree of insulin resistance (Sasson *et al*. 1993). An enlarged left ventricular mass also occurs in endocrine diseases such as such as acromegaly (Lacka *et al*. 1988) and hyperthyroidism (Santos *et al*. 1980) all of which are conditions characterized by insulin resistance, hyperinsulinaemia or some degree of glucose intolerance. To further support the growth promoting properties of insulin, it has been shown that acute insulin administration in humans reduces myocardial protein degradation by 80 % (McNulty *et al*. 1995), thus disrupting the balance between the cellular anabolic and catabolic

processes and favouring cell growth. There are however conflicting reports in the literature as to whether or not insulin resistance and the consequent hyperinsulinaemia, could (Ilercil *et al*. 2002; Phillips *et al*. 1998; Lind *et al* 1995) or could not (Galvan *et al*. 2000; Avignon *et al*. 1997; Kupari *at al*. 1994) play a role in increasing left ventricular mass. The possible explanation for this discrepancy could be due to the inclusion, in many of the studies, of confounding variables such as the presence or absence of diabetes, hypertension, varying plasma glucose levels, age, sex or body mass index, which are known to influence left ventricular mass. However, Iacobellis *et al*. (2003) recently conducted a study where they carefully selected relatively healthy normotensive subjects with uncomplicated (non-diabetic and normotensive) obesity that were either insulin sensitive or insulin resistant and compared them to a group of lean healthy controls to investigate the relationship between insulin resistance and left ventricular mass in a setting free of all the confounding variables seen in previous studies. Left ventricular hypertrophy was defined as a left ventricular mass/body surface area of >134 g/m^2 for men and >110 g/m^2 for women, together with a left ventricular mass/$height^{2.7}$ ratio >51 $g/m^{2.7}$. Using echocardiography, it was shown that only the obese group with insulin resistance (still able to maintain fasting plasma glucose levels) had an increased left ventricular mass, with only some of this group displaying cardiac hypertrophy, while others showed signs of the development of eccentric hypertrophy (enlarged end diastolic and systolic diameters). Iacobellis *et al*. (2003) suggested that the increased left ventricular mass in the insulin resistant obese individuals may be due to volume overload in combination with effects due to the elevated insulin levels.

Possible mechanisms of how hyperinsulinaemia could promote cardiac hypertrophy could revolve around insulin's known growth promoting effects whereby insulin could directly or indirectly, via its interaction with insulin-like growth factor 1, stimulate growth (Banskota *et al*. 1989; Delaughter *et al*. 1999). Hyperinsulinaemia, can also facilitate sodium retention (DeFronzo *et al*. 1975), possibly due to its actions in the distal nephron, thus increasing the extracellular fluid volume (volume overload), which could contribute to cardiac hypertrophy by increasing the workload on the heart.

Insulin signalling in pressure-induced hypertrophied hearts, before and during ischaemia, may be defective. A hypertrophied myocardium is associated with both decreased phosphorylation of a key receptor in insulin-mediated glucose transport (insulin receptor substrate protein-1), and decreased activity of a mediator of the insulin signalling pathway (PI3K). Defective insulin signalling was shown to restrict insulin-mediated GLUT-4 translocation to the plasma membrane, and consequently reduce glucose uptake by the myocardium (Friehs *et al*. 2005). As glucose is the substrate for glycolysis, which is essential for ATP generation in the ischaemic myocardium (evidence for this is reviewed by Carvajal and Moreno-Sánchez, 2003), this finding may have serious implication for individuals with pressure induced cardiac hypertrophy when faced with an ischaemic challenge. Whether the same abnormalities in insulin signalling occur with obesity induced cardiac hypertrophy is uncertain.

2.1.3.2.1.4 Insulin resistance and dyslipidaemia

MS associated dyslipidaemia consists of elevated very low-density lipoprotein (VLDL) cholesterol particles, hypertriglyceridaemia, reduced HDL-cholesterol and elevated small dense low density lipoprotein (LDL) cholesterol (Medvedeva *et al*. 2003; Tkac, 2005).

Although experimental studies show clear evidence that insulin resistance plays a role in the development of the dyslipidaemic state, the exact mechanism by which insulin resistance does so remains unknown (Adeli *et al*. 2001). With an increase in FFA flux to the liver, as is seen in insulin resistance and obesity, there is an increased production of TG rich VLDL particles (Lewis *et al*. 1995). The proposed mechanism for this in the insulin resistant state is reviewed by Adeli *et al*. (2001), and involves an enhanced flux of FFA to the liver and decreased sensitivity to the inhibitory effects of insulin on VLDL secretion. The overproduction of VLDL-cholesterol is thought to result in the hypertriglyceridemic state seen in insulin resistance. TG rich VLDL-cholesterol particles exchange their core lipids with HDL cholesterol, a process which is enhanced in hypertriglyceridemic states (Rashid *et al.* 2002). Consequently the HDL cholesterol is TG enriched. Lamarche *et al.* (1999) provided direct evidence that the enrichment of HDL particles with TG's may play a role in the enhanced clearance of HDL from the circulation, thereby lowering its levels in the blood.

2.1.3.2.1.5 Insulin resistance and diabetes

Insulin resistance has been shown to precede and predict the development of type 2 diabetes (Charles *et al.* 1991). The currently favoured hypothesis in the development of type 2 diabetes is that in chronic insulin resistance, blood glucose homeostasis is managed by compensatory hyperinsulinaemia. After a period of time, as the insulin resistance continues, impaired glucose tolerance develops despite the hypersecretion of insulin by the pancreatic β cells. Diabetes develops with increased insulin resistance and reduced insulin secretion from the pancreas due to pancreatic β cells failure (Abate, 2000; Petersen *et al*. 2002). Possible mechanisms for pancreatic β cell dysfunction and failure are reviewed by Porte and Kahn (2001).

2.1.3.2.2 Signalling mechanism of insulin

Insulin mediates its various effects via two distinct pathways, the phosphatidylinositol-3-kinase (PI3K) pathway (Myers *et al*. 1992), which is involved in glucose transport (Haruta *et al*. 1995), glycogen synthesis (Sheperd *et al*. 1995), protein synthesis (Mèndez *et al*. 1996) and vasodilatation (Scherrer *et al*. 1994; Zeng *et al*. 1996). The second pathway, the mitogen activated protein kinase (MAPK) pathway (Skolnik *et al*. 1993), is associated with cell growth (Sale *et al*. 1995).

Significantly, it has been shown that the insulin induced stimulation of the PI3K pathway is reduced, while the sensitivity of the MAPK pathway is unchanged in skeletal muscle in the insulin resistant state (Cusi *et al*. 2000). This study was conducted on type 2 diabetic patients, obese non-diabetic

patients and lean controls. Obese non-diabetic patients showed reduced stimulation of the PI3K pathway, indicating the presence of insulin resistance, whereas with diabetic patients, insulin response of the PI3K pathway was virtually absent.

2.1.3.2.3 Insulin resistance and cardiac metabolism

In conditions of obesity and insulin resistance there is an increase in circulating FFA. It has been shown in animal models (Aasum *et al.* 2003) and recently in humans (Peterson *et al.* 2004) that this increase in plasma FFA results in increased fatty acid uptake by the myocardium. This in turn causes a change in substrate utilization with a shift from glucose to fatty acid utilization by the myocardium. In animal models, a shift to fatty acid utilization leads to an initial increase in the fatty acid oxidation and oxygen consumption of the myocardium. This may decrease the efficiency of the myocardium (Mjøs, 1971; Lopaschuk *et al*. 2003). In addition, it was found that over a period of time a mismatch occurs between fatty acid uptake and fatty acid oxidation, with a resultant accumulation of fatty acid intermediates in the myocardium. Research conducted by Zhou *et al*. (2000) on ZDF rats (fa/fa) showed a gradual time dependant increase in the TG concentration of the myocardium. Excess TG underwent non-oxidative fatty acid metabolism with the resultant accumulation of fatty acid intermediates and an increase in ceramide production. This group therefore demonstrated a lipotoxic effect of TG accumulation in the myocardium of the ZDF rats through the production of ceramide. They linked the increased ceramide production to cardiomyocytes apoptosis and thus impaired cardiac function.

2.1.3.2.4 Insulin resistance as a risk factor for coronary artery disease

Hyperinsulinaemia has been shown to be an independent risk factor for ischaemic heart disease (Després *et al*. 1996) although the mechanism thereof is unknown. It is however thought that the dyslipidaemic state associated with insulin resistance or an altered fibrinolysis, due to increased plasma concentrations of plasminogen-activator inhibitor type I may increase susceptibility to atherosclerosis and thrombosis (Juhan-Vague *et al*. 1991), and increase the risk of developing ischaemic heart disease.

2.1.3.2.5 Effects of insulin resistance on the susceptibility to ischaemia and reperfusion injury

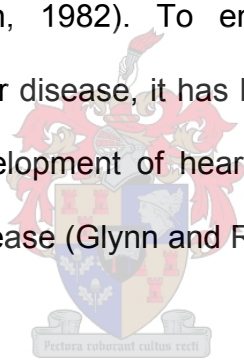
Many of the studies investigating insulin resistance and the susceptibility to ischaemia/reperfusion injury did so in a diabetic model. Limited information is available on the effects of insulin resistance on ischaemia and reperfusion induced injury. Recently it was shown that treatment of ZDF rats with rosiglitazone improved cardiac insulin resistance when compared to lean ZDF rats. The increase in cardiac insulin sensitivity was accompanied with an increased resistance to ischaemia/reperfusion injury (Yue *et al*. 2005).

In an animal model of the MS, fructose fed rats (FFR) (prediabetic with insulin resistance and hyperinsulinaemia) were more susceptible to in vivo ischaemia and reperfusion induced injury when compared to control littermates. This study used coronary artery ligation, with infarct size as an endpoint. The prediabetic state of the FFR was associated with an increased severity of

ischaemia induced arrhythmias (Morel *et al*. 2003). The ischaemic episode also produced significantly greater infarct sizes in the FFR, when compared to the control groups.

2.1.3.3 Hypertension

Two types of hypertension occur, primary or essential hypertension, which is characterized by a chronic increase in blood pressure, without known aetiology. The renin-angiotensin-aldosterone system also plays a role in this condition. The second form of hypertension, secondary hypertension, is characterized by high blood pressure, accompanied by a disorder, such as renal artery stenosis (Porth, 1982). To emphasize the importance of hypertension in cardiovascular disease, it has been shown to be a risk factor for amongst others, the development of heart failure (Kannel *et al*. 1972), stroke and coronary heart disease (Glynn and Rosner, 2005).



Essential hypertension is associated with vascular complications such as endothelial cell dysfunction (Sainani and Maru, 2004) and is therefore a risk for developing atherosclerosis. However, only myocardial effects of hypertension will be discussed in this literature review.

2.1.3.3.1 Hypertension and cardiac hypertrophy

The link between hypertension and cardiac hypertrophy is well established. Switzer and Osterman (1950) identified the presence of cardiac hypertrophy in hypertensive patients from myocardial tissue samples obtained from autopsies. Cardiac structural abnormalities associated with hypertension

include increased left ventricular mass index, thicker ventricular walls together with an increased ventricular mass/chamber diameter ratio (Laufer *et al*. 1989). This is in contrast to obesity, where cardiac hypertrophy is associated with a decreased ventricular mass/chamber diameter ratio (Lorell and Carabello, 2000) (figure 2.3). Hypertension related increases in cardiac hypertrophy together with the associated increase in fibrosis further result in decreased chamber compliance (Opie, 1991). Tsotetsi *et al*. (2001) investigated the progression of essential hypertension and the resulting left ventricular hypertrophy in spontaneous hypertensive rats (SHR). These rats were compared with age-matched Wistar Kyoto controls (WKY), while monitoring a separate group of SHR and WKY on the antihypertensive drug, hydralazine. The progression from left ventricular hypertrophy to left ventricular dilatation and the subsequent development of signs of heart failure were prevented in the SHR drug treated group. The outcomes of this study suggests that in essential hypertension the myocardium will progress from a state of left ventricular hypertrophy to left ventricular dilatation and heart failure.

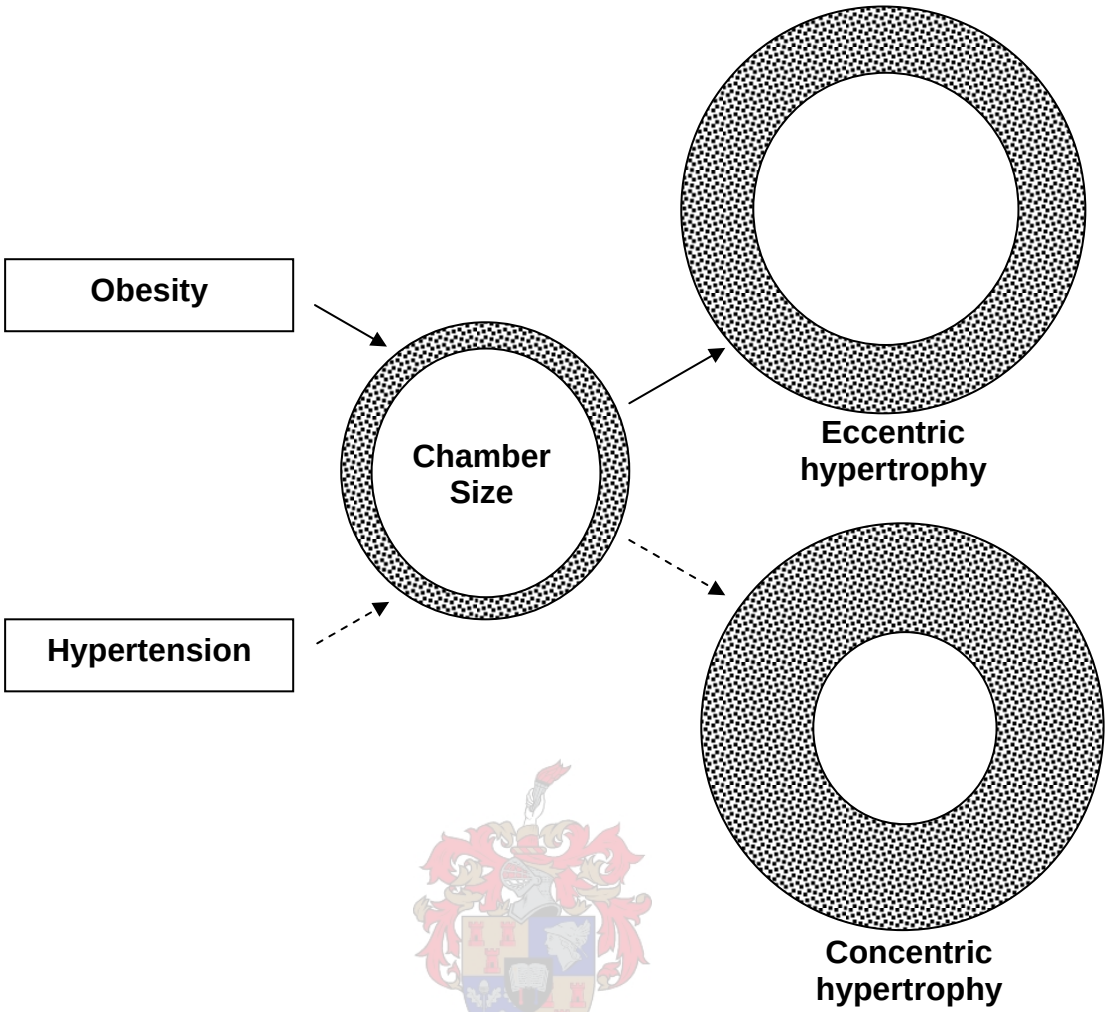


Figure 2.3: Effect of obesity and hypertension on myocardial chamber and wall morphology (Modified from Opie, 1991)

2.1.3.3.2 Effect of obesity and hypertension on systemic haemodynamics and the myocardium

A strong link between obesity and hypertension exists (Van Itallie, 1985). Obesity as stated earlier is associated with an increased blood volume and consequently increased preload to the myocardium. Hypertension is associated with an increased afterload (Messerli *et al*. 1981; Aneja *et al*. 2004). Messerli *et al*. (1981) assessed systemic hemodynamics in 135 lean, mildly obese, or distinctly overweight subjects that were normotensive or had

borderline or established essential hypertension. All measurements were taken at rest. Cardiac output was elevated and peripheral resistance lower in obese normotensive subjects, while hypertensive subjects had a higher peripheral resistance. These findings were later confirmed by Schmeider and Messerli (1993). Significantly, Schmeider and Messerli (1993) found that obese hypertensive subjects had a lower peripheral resistance compared to lean hypertensive subjects. Moreover the degree of left ventricular hypertrophy was more pronounced in the hypertensive groups than in the normotensive groups. It was however observed that the level of hypertrophy progressively increased with the presence of obesity. Lastly obese hypertensive patients were shown to have normal ventricular function (normal fractional shortening) indicating that the heart was able to compensate with the double burden placed on it. Schmeider and Messerli (1993) concluded that obesity mitigated systemic changes in the vascular bed imposed by hypertension, but the two conditions together exacerbated the degree of left ventricular hypertrophy.

2.1.3.3.3 Hypertension and ischaemia/reperfusion injury

As hypertension can result in cardiac hypertrophy, the majority of the studies investigate the effect of hypertension induced cardiac hypertrophy on myocardial susceptibility to ischaemia and reperfusion injury. Many studies make use of SHR's and compared them with WKY control rats. These studies show that SHR's rats are more susceptible to ischaemia and reperfusion injury compared to the WKY rats (Tisne Vesailles *et al*. 1983; Golden *et al*. 1994). Okamoto *et al*. (1993) treated SHR rats with hydralazine or captopril

from 19 to 26 weeks of age. Both drugs decreased systemic blood pressure but only captopril reduced left ventricular weight when compared to control SHR. At the end of the study, the hearts were removed and perfused on the isolated working rat heart perfusion apparatus. The susceptibility of the SHR hearts to ischaemia and reperfusion injury was reduced in rats that had a decrease in arterial blood pressure and a decrease in left ventricular weight (captopril treated group). Ischaemic tolerance did not however improve in rats with a decreased arterial blood pressure but increased heart weight (hydralazine treated group). This suggests that the cardiac hypertrophy, induced by hypertension, may be responsible for the increased ischaemia and reperfusion injury seen in hypertension.

Hypertension, as mentioned earlier, is associated with alterations in the renin-angiotensin system (RAS), as antagonists of the Ang II receptor leads to a drop in systemic blood pressure (Rossi *et al*. 2000). Data published by Schmieder *et al*. (1996) suggested a possible link between elevated blood Ang II, hypertension and left ventricular hypertrophy in patients with a high dietary sodium intake. Ang II mediates its effects via its AT_1 receptor (to be discussed later) and the expression of the AT_1 receptor has been seen to increase following ischaemia and reperfusion the rat heart (Sun and Weber, 1994; Yang *et al*. 1997). Activation by Ang II, and increased expression of the AT_1 receptor has been shown to play a role in ischaemia and reperfusion injury as antagonists of the AT_1 receptor have been shown to be cardioprotective by improving post-ischaemic left ventricular function (Yoshiyama *et al*. 1994), a finding that has been confirmed by Frolkis *et al*.

(2001). Inhibiting the effects of Ang II by using an Ang II converting enzyme inhibitor, lisinopril, or an Ang II antagonist, L-158,338 prior to an infarction, induced myocardial protection as reflected by an improved functional recovery after ischaemia (Werrmann and Cohen, 1994). Therefore it could be speculated that hypertensive individuals with elevated blood Ang II levels may be more susceptible to ischaemia and reperfusion injury than their normotensive counterparts.

2.1.3.4 Dyslipidaemia

The lipid profile of patients with MS is usually associated with elevated VLDL-cholesterol particles, hypertriglycerigemia, reduced HDL-cholesterol and elevated small dense LDL-cholesterol (Medvedeva *et al*. 2003; Tkac, 2005). Lowered levels of HDL cholesterol are undesirable as HDL-cholesterol is atheroprotective, by mediating reverse cholesterol transport from the peripheral tissue to the liver (Stein and Stein, 1999). Low levels of HDL-cholesterol especially with decreased amounts of apolipoprotein A-I content are consequently a strong independent risk factor for coronary heart disease (Luc *et al*. 2002).

Dyslipidaemia is associated with many vascular complications but for the purpose of this study only the myocardial consequences of this condition will be discussed further. It is important to remember that rats do not become hypercholesterolemic, but display a different lipid profile to other mammals, after consuming a high cholesterol diet (Horton *et al*. 1995; Roach *et al*. 1993).

2.1.3.4.1 Hypercholesterolaemia and cardiac function

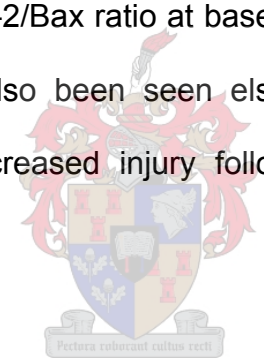
Schwemmer *et al*, (2000) demonstrated myocardial dysfunction in adult male guinea pigs, in a model of diet-induced hypercholesterolemia (1% cholesterol). These Langendorff perfused hearts had decreased basal left ventricular pressures, contractility and diastolic relaxation. These functional abnormalities were associated with enhanced oxidative stress, which was indirectly measured by monitoring plasma xanthine oxidase activity. When the AT_1 receptor blocker, losartan, or the angiotensin converting enzyme inhibitor, enalapril were added to the diet, myocardial systolic and diastolic function was effectively restored (Schwemmer *et al*. 2000). This finding was probably due to reduced oxidative stress following AT_1 receptor antagonism, as indicated by a lower plasma activity of xanthine oxidase. It has been shown that signalling via the AT_1 receptor favours the formation superoxide, through the activation of NAD(P)H, in the vasculature of rabbits fed a hypercholesterolemic diet. This effect was abolished upon AT_1 receptor blockade (Warnholtz *et al*. 1999). This suggests that elevated Ang II may indirectly increase the amount of oxidative stress placed on the heart. Several other studies also highlight the adverse consequences of upregulated components of the RAS as seen in dyslipidaemia (Nickenig *et al*. 1997; Daugherty *et al*. 2004).

Cardiac functional reserve may also be impaired in hypercholesterolemic states. Minipigs were fed a high cholesterol diet for 8 months. Cardiac functional reserve was calculated by measuring the magnitude of the inotropic response of the heart to an isoproterenol challenge. The isoproterenol elicited an increase in ventricular contractility and relaxation rate. The resultant

increase in cardiac output was lower in the high cholesterol fed group compared to control minipigs, demonstrating a decreased functional reserve due to hypercholesterolaemic state in the minipigs (Wang *et al*. 2002b).

2.1.3.4.2 Hypercholesterolaemia and ischaemia/reperfusion injury

Models of diet-induced hypercholesterolemia have demonstrated the adverse effects of dyslipidaemia on the myocardium following an ischaemic insult. Wang *et al*. (2002a) showed that rabbits fed a 10 % cholesterol supplemented diet, had increased infarct size and increased cardiomyocyte apoptosis after left circumflex artery occlusion followed by reperfusion. This was despite the fact that their myocardial Bcl-2/Bax ratio at baseline was higher than controls. Larger infarct sizes have also been seen elsewhere (Golino *et al*. 1987) emphasizing the risk of increased injury following an ischaemic insult in hypercholesterolaemia.



Controversially, there is one study in which diet-induced (2 % cholesterol) hypercholesterolemia resulted in an increased resistance to ischemia/reperfusion injury in a rabbit heart after 6 weeks of feeding (Le Grand *et al*. 1995). This same model has however previously been used to show that the rabbit myocardium is more susceptible to an ischaemia/reperfusion insult after 2-3 and 5 weeks on the diet (Tilton *et al*. 1987). Cardiac functional recovery did improve following an ischaemic insult from the 2-3 to the 5 week diet-fed rabbits, suggesting the development of resistance to ischaemia/reperfusion injury, with prolonged cholesterol feeding

2.1.3.4.3 Hypertriglyceridaemia and cardiac function

Hypertriglyceridemia, another component of the MS definition, also exerts an effect on myocardial function. In the normal functioning heart, nitric oxide (NO) derived from various NO synthases, plays an important role in cardiac function (Ashley *et al.* 2002; Massion and Ballingand, 2002). Experiments performed on male Wistar rats, consuming a 2 % cholesterol enriched diet to induce hyperlipidemia (elevated TG's), have demonstrated a decreased cardiac performance, as indicated by an increased left ventricular end-diastolic pressure, using a working heart perfusion model (Ònody *et al.* 2003). This finding illustrated an inability of these hearts to relax adequately in the dyslipidaemic state. This was attributed to decreased bioavailability of NO and an increased formation of superoxide and their reaction product, peroxynitrite. Peroxynitrite is thought to produce this cytotoxic effect on the heart by reducing the coupling between ATP production and mechanical work (Schulz *et al.* 1997).

2.1.3.4.4 Hypertriglyceridaemia and ischaemia/reperfusion injury

Monti *et al*, (2001) used a rat model displaying hyperglycaemia and hyperinsulinaemia, and perfused the hearts on the Langendorff perfusion system. Intralipid is a pool of different types of TG's which was added to the perfusion buffer to mimic various TG concentrations. Hearts were subjected to 60 minutes low-flow ischaemia. As TG concentrations were increased, the hearts were more susceptible to the ischaemia/reperfusion insult as seen in the progressively poorer myocardial functional recoveries during reperfusion. Of interest was the fact that increased TG concentrations resulted in a dose-

dependant increase in ET-1 release from the heart. This increase was proportional to the ischaemia/reperfusion induced mechanical dysfunction seen in the model. The mechanism of TG induced ischaemia/reperfusion induced injury may involve ET-1, although the exact mechanism is unknown.

2.1.3.5 Microalbuminuria

As microalbuminuria was not investigated in this study, it will only be dealt with briefly. Protein in the urine, even in small amounts, is considered to be a risk factor for kidney disease and cardiovascular disease in individuals with diabetes and/or hypertension (Tagle *et al*. 2003). A miniscule amount of albumin, the major plasma protein, is indeed excreted in the urine. The 90th percentile for the urinary albumin excretion rate in population-based studies is 20 µg/min (Tagle *et al*. 2003). Microalbuminuria is defined as the abnormally high urinary albumin excretion rate of 20-200µg/min (Parving, 1996). Microalbuminuria has been shown to occur in conjunction with other factors (Niskanen *et al*. 1990; Sengul *et al*. 2005) associated with the MS.

It was reported that the presence of microalbuminuria in otherwise healthy individuals is associated with high blood pressure, and low plasma concentrations of apolipoprotein A-1 and HDL cholesterol, thus increasing the risk of developing atherosclerosis (Jensen *et al*. 1995). Significantly, microalbuminuria has been shown to be an independent risk factor for ischaemic heart disease (Borch-Johnsen *et al*. 1999).

2.1.4 Metabolic syndrome and cardiac arrhythmias

Individuals with the MS may also be predisposed to ventricular arrhythmias. Sorrentino *et al*. (2003) compared normoglycaemic, normolipaemic, mild-moderate hypertensive patients (group A) to mild-moderate hypertensive patients displaying elevated serum cholesterol and/or TG's and/or glucose levels (group B). Patients with signs of ischaemia or hypokalaemia were excluded. Ventricular ectopic beats were measured elecrocardiographically. 66 % of group A and 100 % of group B displayed ventricular ectopic beats, which were less severe in group A. In group B, the most severe ventricular arrhythmias were seen in those patients with left ventricular hypertrophy (72.7 % of group B).

Elevated plasma FFA, which are characteristic in the MS, have also been proposed to increase the incidence of ventricular arrhythmias especially ventricular fibrillation (Oliver, 2002).

2.1.5 Cardiac hypertrophy as a unifying manifestation of the metabolic syndrome

After assessing the individual components of the MS, and their influence on the cardiovascular system, cardiac hypertrophy emerged as a unifying consequence of most of the components of this condition. Recently Chinali *et al*. (2004) performed a population based study, in which they investigated left ventricular geometry and function under the NCEP ATPIII criteria for the MS. Subjects with the MS (non-diabetic, obese, hypertensive and insulin resistant with compensatory hyperinsulinaemia) had significantly greater left ventricular

dimensions, relative wall thickness, mass and a higher prevalence of left ventricular hypertrophy compared to control subjects.

Cardiac hypertrophy thus seems to be a manifestation of the MS since its involvement in the syndrome has been described in other studies (Davis *et al*. 2002; Lind *et al*. 1995). The question arises as to whether there could be additional stimuli that are present in the MS, which could further contribute to the development of cardiac hypertrophy, besides the mechanical and hemodynamic stress placed on the myocardium as a result of obesity induced volume overload.

An additional factor that may play a role in the development of cardiac hypertrophy and the MS is Ang II. Substantial evidence is available implicating the involvement of Ang II in the development of cardiac hypertrophy. In earlier studies, cardiomyocytes isolated form Wistar-Kyoto rats were incubated with Ang II and Dulbecco's modified Eagle's medium (Neyses *et al*. 1993). The presence of Ang II was shown to upregulate Egr-1 and c-fos, which are growth and differentiation genes. Adding Ang II to both cultured neonatal rat cardiomyocytes and cardiac fibroblasts additionally increased the rate of protein synthesis in the cardiomyocyte, an effect that was abolished by AT_1 receptor blockade (Sadoshima and Izumo, 1993). Using an in vivo model Bendall *et al*. (2002) infused Ang II into mice with targeted disruption of the NADPH oxidase subunit gp91(phox). Ang II infusion caused no changes in systolic blood pressure between groups. Ang II increased heart/body weight ratio, atrial natriuretic factor and beta-myosin heavy chain mRNA expression,

myocyte area, and cardiac collagen content in wild-type but not in knock out mice. These data indicate that Ang II induces cardiac hypertrophy by the production of NADPH oxidases, which is an important source of ROS. In addition, following nontransmural myocardial infarction in rats, the expected cardiac remodelling was reduced after treatment with both an angiotensin converting enzyme inhibitor, or an AT_1 receptor antagonist indicating the involvement of Ang II in the remodelling process (Youn *et al*. 1999).

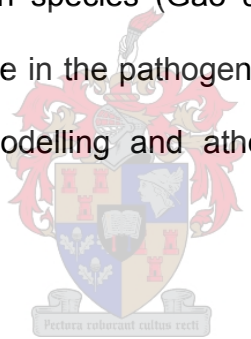
2.1.6 Angiotensin II

The RAS has traditionally been seen as a circulating endocrine system involving circulating peptides. During the nineties it became evident that the circulatory RAS may not be the only source of Ang II. The existence of local RAS systems has now been well established and well described in certain tissues such as the gonads (Speth *et al.* 1999), adipose tissue (Engeli *et al.* 2000), pancreas (Seria, 2001), placenta (Nielson *et al.* 2000), brain, vasculature, kidney and heart (Bader *et al.* 2001).

The octapeptide Ang II has diverse effects and can induce cell growth, stimulation of hormone synthesis, ion transport and vasoconstriction. These effects are carried out following the activation of the Ang II receptors. Ang II receptors have been isolated in the brain, pituitary gland, vascular endothelium, heart lungs, adrenal glands, kidney and liver (Timmermans *et al*. 1993). Two Ang II receptor subtypes have been identified in humans (Whitebread *et al*. 1989) namely the AT_1 and AT_2 receptors. Both receptors belong to the seven transmembrane, G-protein coupled receptor superfamily

(Inagami *et al.* 1994). The AT_1 receptor was subsequently found to have two subtypes, the AT_{1A} and AT_{1B} (Iwai and Inagami, 1992), however these have only been identified in the rat.

Signalling through the AT_1 receptor has been shown, via a diverse set of signalling pathways, to induce vasoconstriction (Silverthorn, 2004), sodium and water retention (Silverthorn, 2004), sympathetic facilitation (Stegbauer *et al*. 2003), positive inotropy (Moravec *et al*. 1990; Spinale *et al*. 1997), cellular growth and proliferation (Sadoshima and Izumo, 1993), extracellular matrix protein regulation (Brassard *et al*. 2005) and the generation of NAD(P)H oxidases and reactive oxygen species (Gao *et al*. 2005). AT_1 signalling is therefore thought to play a role in the pathogenesis of hypertension, vascular and cardiac hypertrophy/remodelling and atherosclerosis (Kaschina *et al*. 2003).



The effects of signalling through the AT_2 receptor are less well known. A possible reason for this could be the low distribution of AT_2 receptors relative to AT_1 receptors in the various organs or tissues (Zhuo *et al*. 1992). AT_2 signalling is generally thought to counteract that of AT_1 signalling, and is thought to play a role in vasodilatation (Tsutsumi *et al*. 1999), differentiation and regeneration (Reinecke *et al*. 2003), apoptosis (Dimmeler *et al*. 1997), and exert antigrowth or antiproliferative (Stoll *et al*. 1995) effects as seen by a reduction in cardiac and vascular hypertrophy upon AT_1 blockage in AT_2 null mice (Wu *et al*. 2002).

The AT_1 receptor is the predominant receptor in the rat heart (Yang *et al*. 1997), however in humans, it has been shown that the AT_2 receptor is the predominant subtype (Regitz-Zagrosek *et al*. 1995; Wharton *et al*. 1998). Ang II through the AT_1 receptor plays an important role in the development of cardiac hypertrophy. Following the development of cardiac hypertrophy in response to aortic banding, Lopez *et al*. (1994) saw that the AT_1 receptor density decreased with the AT_2 receptor becoming the predominant receptor subtype. This change in receptor density could however depend on the model used to induce cardiac hypertrophy. The FFR, a dietary model of MS, was shown to retain the AT_1 receptor in the left ventricle as the predominant receptor during cardiac hypertrophy (Iyer et al. 1996b).

2.1.6.1 Obesity, metabolic syndrome and angiotensin II

The RAS may play a role in the MS. In humans, therapeutic inhibition of components of the RAS has been used to combat the effects of the MS (Nalbantgil *et al*. 1998; Nashar *et al*. 2004).

Obesity, which is the potential starting point of the MS, is associated with increasing RAS activity (Barton *et al*. 2003) due to the presence of a local RAS in the adipocytes. Data presented by Harte *et al*. (2005) suggest that an increase in adipose tissue, as seen in obesity, together with a hyperinsulinemic state may upregulate RAS activity. Additionally they found a strong correlation between BMI and circulating Ang II levels.

Insulin could possibly be involved in modulating the RAS in the MS. Present data suggest a complex interplay between the actions of insulin and Ang II, although the molecular basis thereof requires further investigation. It may however be possible for the hyperinsulinemic state as seen in obesity and the MS to alter the RAS activity, especially in the vasculature. Doses of 100 and 1000, but not 10 μIU/mL of insulin, has been shown to increase angiotensinogen mRNA expression and production as well as stimulate the growth of cultured rat vascular smooth muscle cells (VSMC), similar to that seen with Ang II stimulated growth. These insulin induced growth effects were inhibited by inhibiting the production of Ang II as well as blockage of the AT_1 receptor (Kamide *et al*. 1998). Additionally, 139 μIU/mL insulin was shown to cause an upregulation of the AT_1 receptor expression, thus increasing the density of the AT_1 receptors in the VSMC. This increase in receptor density resulted in an enhanced functional response of the VSMC to Ang II stimulation (Nickenig *et al*. 1998). These concentrations of insulin are pathophysiological. Lastly insulin was shown to increase the Ang II induced DNA synthesis in a concentration dependent manner in VSMC (Ko *et al*. 1993). This has implications for Ang II induced growth in other tissues in hyperinsulinemic states. The effect of Ang II induced growth in response to various concentrations of insulin in cardiomyocytes has not yet been investigated.

Insulin and Ang II also share certain signalling pathways, as Ang II was shown to induce tyrosine phosphorylation of IRS-1 and its association with PI3K, insulin's major signalling molecule. This was performed in vivo in the rat heart

(Saad *et al*. 1995). The same group showed however that Ang II stimulation resulting in the association of IRS-1 and PI3K, results in an acute inhibition of basal as well as insulin stimulated PI3K activity (Velloso *et al*. 1996). This could have serious implications during insulin resistant states.

Nevertheless, regardless of the stimulus, the RAS can be considered to play an important role in MS.

2.1.6.2 Angiotensin II and ischaemia/reperfusion injury

Ang II has been implicated in ischaemia and reperfusion injury. Angiotensin converting enzyme inhibitors have been used in animal models to investigate ischaemia and reperfusion injury and have been shown to improve cardiac functional recovery as well as reducing reperfusion arrhythmias in the myocardium following an ischaemic insult (de Graeff *et al*. 1986). Inhibition of Ang II signalling through AT_1 receptor blockade has also been shown to reduce reperfusion arrhythmias (Yahiro *et al*. 2003).

Yang *et al*. (1997) clearly demonstrated the negative effects of the Ang II AT_1 receptor signalling during ischaemia and reperfusion in isolated perfused rat hearts. Cardiac mechanical function was predictably worse in hearts undergoing ischaemia and subsequent reperfusion compared to continuously perfused hearts. This drop in mechanical function in the ischaemia/reperfusion group occurred concurrently with a rise in myocardial AT_1 receptor expression. This rise in AT_1 expression did not occur in the continuously perfused group. Treatment with an AT_1 receptor antagonist,

losartan, before ischaemia/reperfusion and during the reperfusion period prevented both the decrease in reperfusion cardiac mechanical function and the increase in AT_1 expression.

2.1.6.3 Obesity/metabolic syndrome, angiotensin II and cardiac hypertrophy

As described in section 2.1.5, it is well established that Ang II is involved in the development of cardiac hypertrophy. The question arises as to whether there is any evidence that Ang II signalling is implicated in the development of cardiac hypertrophy in the context of obesity and the MS.

Obesity and specifically the presence of visceral obesity has been associated with an upregulated RAS (Barton *et al*. 2003). Endogenous over-expression of the RAS occurs in the visceral tissue of obese humans (Giacchetti *et al*. 2002) and in diet-induced obese rats (Boustany *et al*. 2004). Ang II may therefore play a role in mediating cardiac hypertrophy in the MS as a result of obesity. In fact, Ang II has previously been shown to contribute to the development of cardiac hypertrophy in our model of obesity-induced cardiac hypertrophy (Du Toit *et al*. 2005). Treatment of obese rats with losartan, an AT_1 receptor antagonist reduced the extent of the cardiac hypertrophy occurring in these rats.

The FFR is a prediabetic model used to investigate the pathophysiologic mechanisms of the various changes associated with MS. These rats develop insulin resistance, hyperinsulinaemia, hypertriglyceridaemia, hypertension and

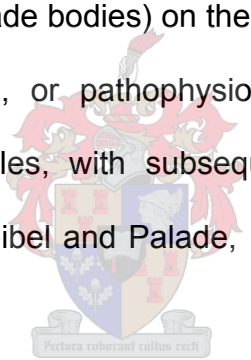
cardiac hypertrophy (Miatello *et al*. 2004). Significantly the FFR displays visceral obesity (Furuhashi *et al*. 2004), has elevated plasma Ang II concentrations and a high density of AT_1 receptors in the hypertrophied ventricles (Iyer *et al*. 1996b). In the FFR the development of cardiac hypertrophy was attenuated by AT_1 receptor antagonism (Kamide *et al.* 2002). AT_1 receptor blockers used in FFR improved insulin sensitivity together with glucose tolerance as well as ameliorating the overproduction of TG's (Iyer *et al*. 1996a; Okada *et al*. 2004). Hypertrophied FFR hearts have been shown to have higher levels of Ang II in the left ventricles (Kamide *et al.* 2002). Baker *et al.* (2004) demonstrated the significance of elevated Ang II in the cardiac tissue and provided evidence of an intracrine mechanism in Ang II induced cardiac hypertrophy. Briefly, plasmid vectors, constructed to express Ang II intracellularly, were injected into adult mice. These mice developed significant cardiac hypertrophy, without the intervention affecting blood pressure or circulating Ang II levels in the plasma. Blocking the AT_1 receptor with losartan did not prevent the growth effects, indicating that it was the Ang II inducing cardiac growth intracellularly. These studies suggest a role for the RAS in the pathogenesis of the MS

2.1.7 Endothelin-1

ET-1 is produced most abundantly by the vascular endothelial cells. The constitutive pathway in which ET is synthesized starts with the gene product of the ET-1 gene, preproET-1. A signal peptidase removes a short peptide sequence from preproET-1 to form pro-ET-1, which is converted to big-ET-1 by a maturing enzyme, furin (Blais *et al*. 2002). Big-ET-1 is released into the

peripheral circulation from the endothelial cell. Subsequent proteolytic cleavage of big-ET-1 by endothelin converting enzyme produces the mature ET-1 (Xu *et al.* 1994). The alternative pathway in the formation of ET-1 involves an enzyme called chymase, derived from mast cells, which converts big-ET-1 into ET-1 by cleavage at Tyr^{31}-gly^{32} (D'Orléans-Juste *et al*. 2003).

ET-1, which is considered to be the most potent vasoconstrictor, is continuously released from the endothelial cells into the circulation where they act upon the VSMC to maintain basal vascular tone (Davenport, 2002). ET-1 can also be released rapidly in the regulated pathway from cell specific storage granules (Weibel-Palade bodies) on the endothelium. It is thought that external physiological stimuli, or pathophysiological stimuli, can result in degranulization of the granules, with subsequent release of ET-1 and a vasoconstrictor response (Weibel and Palade, 1964; Russell and Davenport, 1999).



To date only 2 endothelin receptors have been isolated and cloned, they are ET_A (Adachi *et al*. 1991) and ET_B (Nakamuta *et al*. 1991). Since enothelin-1 is an endothelial derived vasoactive peptide, its receptors are expressed in all tissues. The function of the ET_A receptor is mainly to induce vasoconstriction, whereas the ET_B receptor may play a role in the release of endothelium derived relaxing factors (Warner *et al*. 1989; Davenport *et al*. 2002). Both receptors are present in the heart (Thibault *et al*. 1995)

2.1.7.1 Endothelin-1 and the metabolic syndrome

Besides the involvement of the RAS in the MS, it is possible for another vasoactive peptide, ET-1 to also play a role in the development of cardiac hypertrophy in the MS, due to its interaction with Ang II. In rats, Ang II has been shown to increase ET-1 protein expression and functional endothelin converting enzyme activity, in vascular smooth muscle and kidney. This occurs via the ET_A receptor in vivo (Barton *et al*. 1997). Additionally, the Ang II induced increased expression of ET-1 in the vasculature, was shown to mediate a major part of Ang II's growth effects on VSMC (Moreau *et al*. 1997). This process was abolished following ET_A inhibition in the preparation. In cultured neonatal cardiomyocytes, Ang II was shown to upregulate preproET-1 mRNA 3-fold and stimulate an increase in ET-1 release from the cardiomyocytes in a dose dependant manner. ET-1 subsequently acted in an autocrine/paracrine manner via its ET_A receptor to increase protein synthesis (Ito *et al*. 1993), implying a role for ET-1 in Ang II-induced cardiac hypertrophy. However in an in vivo study, using Sprague-Dawley rats, it was shown that ET-1 did not mediate the cardiac hypertrophic effect of Ang II through its ET_A receptor, implying a role for the ET_B receptor in the development of cardiac hypertrophy (De Smet *et al*. 2003). The information provided here suggests that in the MS, the RAS and ET-1 may play an important role in the development of cardiac hypertrophy.

Data which further support a role for ET-1 in the MS was presented by Piatti *et al*. (2000). They showed that ET-1 release could be upregulated in the MS. Multiple regression analysis conducted by this group showed that ET-1 levels

correlated with TG levels and the amount of glycosylated haemoglobin in the MS, suggesting that ET-1 may play a role in the MS. It was recently shown that adolescents with obesity, hypertension, or diabetes had higher plasma ET-1 levels. This group showed that there was a strong correlation between plasma ET-1 levels and body mass index, lipid parameters and systolic blood pressure, all of which are components of MS (Glowinska *et al*. 2004).

2.1.7.2 Endothelin-1 and ischaemia/reperfusion injury

ET-1 is though to play a role in ischaemia/reperfusion injury as pericardial fluid ET-1 levels are more elevated in patients with ischaemic heart disease, relative to those with non-ischaemic heart disease (Namiki *et al*. 2003). Experimental evidence supporting a role for ET-1 in ischaemia/reperfusion injury was seen when ET-1 induced cardiomyocyte necrosis during an episode of ischaemia and reperfusion (Brunner *et al*. 1997). Further studies have revealed that exogenous ET-1 added to the perfusion buffer may, via its ET_A receptor, worsen ventricular and coronary reperfusion dysfunction and exacerbate ischaemic contracture (Brunner and Opie, 1998).

An experiment by Brunner and Kukovetz (1996) revealed that ET-1 may also be proarrhythmic. As mentioned, angiotensin converting enzyme inhibitors have been shown to improve functional recovery as well as reducing reperfusion arrhythmias in the myocardium following an ischaemic insult (de Graeff *et al*. 1986). Brunner and Kukovetz (1996) perfused isolated hearts treated either with an endothelin receptor antagonist or an angiotensin converting enzyme inhibitor. Both drugs reduced the incidence of reperfusion

arrhythmias. This incidence was almost completely reduced when the drugs were used simultaneously. The angiotensin converting enzyme inhibitors effect on the incidence of arrhythmias was accompanied by a decrease in ET-1 release in the coronary effluent. Exogenous ET-1 added to the perfusion buffer was seen to be proarrhythmogenic, suggesting a role for ET-1 in reperfusion induced arrhythmias.

2.1.7.3 Endothelin-1, angiotensin II and cardiac hypertrophy

ET-1 possibly also plays a role in cardiac hypertrophy. Indirectly, it has been seen that plasma levels of ET-1 were significantly higher in hypertensive patients with left ventricular hypertrophy compared to hypertensive controls (Hua *et al*. 2000), implying involvement of ET-1 in the development of cardiac hypertrophy. Directly, ET-1 has been shown to stimulate growth in cultured ventricular cardiomyocytes by acting via both its ET_A and ET_B receptors (Ito *et al*. 1993; Cullen *et al*. 2001). When cardiac hypertrophy was induced by pressure overload in a rat model, using aortic banding, plasma and ventricular ET-1 levels and endothelin receptor density on the membrane was increased (Wang *et al*. 2001c). Finally, when SHR that develop cardiac hypertrophy were treated with the general endothelin receptor antagonist, bosentan, it had no effect on blood pressure, but decreased left ventricular hypertrophy and cardiac fibrosis compared to untreated SHR (Karam *et al*. 1996). ET-1 has been shown to increase cardiac contractility (Zerkowski *et al*. 1993), but it is not known whether or not this plays a role in the development of cardiac hypertrophy.

2.1.8 Obesity, metabolic syndrome and coronary artery disease

Obesity has been shown to be an independent risk factor for coronary artery disease (Rimm *et al*. 1995). Considering the various components associated with the MS, one would expect a strong association between MS and coronary artery disease. Longitudinal studies support this notion. Lakka *et al*. (2002) performed a 9 – 14 year follow up study on patients meeting the NCEP ATP III criteria of MS. At the beginning of the study these patients were without any form of cardiovascular disease or type 2 diabetes. Patients were reassessed at the end of the study term. The study concluded that patients meeting the criteria for the MS had 4 fold risk of developing coronary heart disease. Despite the high risk of developing coronary artery disease in obesity and the MS, there is a lack of obesity induced models of MS available to study the effects of the syndrome on the myocardial susceptibility to ischaemic disease.

2.2 Cardiac ischaemia and reperfusion

Ischaemia literally means inadequate blood flow, and was said to occur (Jennings, 1970) whenever the arterial blood flow through a diseased vessel is reduced to a volume below that required by the myocardium for adequate function. Metabolically, ischaemia occurs when the arterial blood flow is insufficient to provide enough oxygen for intracellular respiration by shifting from aerobic to anaerobic respiration (Jennings and Yellon, 1992). For the survival of an ischaemic myocardium, early reperfusion is essential. There is however a paradox surrounding reperfusion. Although it is absolutely necessary, there is sufficient evidence to suggest that reperfusion of the myocardium may itself result in cell death (Hearse, 1977; Becker and Ambrosio, 1987). Consequently there are 2 forms of injury that take place following an ischaemic insult. Injury induced by an ischaemic episode, and injury induced by the subsequent reperfusion of the myocardium. Both ischaemia and reperfusion induced injury are manifested in either reduced mechanical function of the myocardium, or cardiac arrhythmias, or both.

2.2.1 Ischaemia

From the work of Jennings *et al*. (1960) it became evident that two forms of ischaemic injury occur, namely reversible and irreversible ischaemic injury. The severity as well as the duration of the ischaemic insult plays an important role in the degree of injury to the myocardium. DeBoer *et al*. (1983) showed that the product of coronary flow deprivation (FD) (due to the ischaemic insult) and the duration of the ischaemic insult (T) (FD x T), correlated with the extent of myocardial necrosis and was used as an ischaemic index. This ischaemic

index was also able to predict the development of irreversible ischaemic damage. Reversible ischaemic injury is thus considered to be due to a brief ischaemic period, with a rapid recovery of the myocardium upon reperfusion, while irreversible ischaemic injury is defined as the consequence of a prolonged, severe ischaemic insult, causing cell necrosis (Jennings *et al*. 1960). The common viewpoint was that necrosis was the only mechanism of myocyte death, following a myocardial infarction (Nadal-Ginard *et al*. 2003). In contrast, evidence is available to suggest that apoptosis may also contribute to the cell death seen during ischaemia. Ischaemia, over a prolonged period of time (2.25 hours), was shown to induce a certain amount of apoptosis (Fliss and Gattinger, 1996). It is speculated that the extent of apoptosis may either be due to the severity and duration of the ischaemic insult or due to the infiltration of neutrophils. Immediately after the ischaemic event, it is thought that apoptosis affects more than 80 %, and necrosis less than 20 % of the myocytes in the ischaemic zone respectively (Bardales *et al*. 1996). However, the contribution of necrosis induced cell death increases with time, until the two types of cell death overlap (Nadal-Ginard *et al*. 2003). After coronary artery occlusion, single and double strand DNA breaks are found in the myocytes, especially in the region surrounding the dead tissue (Li *et al*. 1997). Double stranded breaks signify apoptosis (Nadal-Ginard *et al.* 2003). These data suggest that apoptosis may continue in the non-ischaemic region of the heart adjacent to the infarct.

2.2.2 Metabolic and ultrastructural changes associated with ischaemia

Ischaemia is associated with various metabolic and ultrastructural changes (Table 2.2). Puri *et al*. (1975) investigated the various metabolic and ultrastructural changes in the myocardium of dogs' in vivo, after the induction of ischaemia by coronary artery ligation for 30, 60 or 90 minutes, with subsequent reperfusion for 60 minutes. The changes seen during the ischaemic insult included ATP and creatine phosphate loss, swelling of the sarcoplasmic reticulum (after 60 and 90 minutes ischaemia), myofibril relaxation and mitochondrial damage (swelling, decreased matrix density and partial loss of cristae). Jennings and Ganote, (1976) and Schaper *et al*. (1979) observed similar ultrastructural changes in the myocardium during ischaemia. These ultrastructural changes are thought to be brought about by a combination of mechanisms occurring during ischaemia. These include depletion of energy stores (Puri *et al*. 1975; Jennings and Ganote, 1976; Schaper *et al*. 1979); accumulation of metabolic by-products (Neely *et al*. 1973) such as lipid metabolites (Corr *et al*. 1984); intracellular acidosis (Cobbe and Poole-Wilson, 1980; Neely *et al*. 1984); intracellular calcium (Ca^{2+}) accumulation (Clusin *et al*. 1983; Nayler *et al*. 1988); and reactive oxygen species (Hess and Manson, 1984; Kako, 1987). Additionally ischaemia is associated with an increase cytosolic Na^{+} (Shen and Jennings, 1972; Tani and Neely, 1989), which is largely responsible for the increased cytosolic Ca^{2+} (Murphy *et al*. 1990) (the mechanism is reviewed by Karmazyn and Moffat, 1993). Loss of K^{+} from the cell (Opie *et al*. 1969) also occurs during ischaemia. Lastly, Iwai *et al*. (2002) have recently presented data to suggest that elevated Na^{+} itself may induce deterioration of mitochondrial

function during an ischaemic insult, and that this damage may be responsible for the post ischaemic contractile dysfunction seen during reperfusion.

It is seen that in reversible ischaemic injury induced by 15 minutes coronary artery occlusion, the cellular changes (low high-energy phosphates and the adenine nucleotide pool levels, depletion of glycogen, with lactate and H^+ accumulation, and mild intracellular edema) normalize following reperfusion (Jennings *et al*. 1990).

Table 2.2: Changes associated with myocardial ischaemia
Swelling of the sarcoplasmic reticulum
Myofibril relaxation
Mitochondrial damage
Swelling
Decreased matrix density
Partial loss of cristae
Depletion of energy stores
Accumulation of metabolic byproducts
Intracellular acidosis
Accumulation of intracellular calcium
Accumulation of reactive oxygen species
Increased cytosolic sodium
Loss of intracellular potassium

These metabolic and ultrastructural abnormalities may play a role in affecting the myocardium on a functional level (table 2.3)

2.2.3 Ischaemia and contractile dysfunction

During an ischaemic insult functional changes such as depressed contractile activity are almost immediately detectable (Puri *et al*. 1975; Jennings and Ganote, 1976; Schaper *et al*. 1979; Jennings *et al*. 1990). It is thought that one of the most important mechanisms underlying ischaemia-reperfusion-

induced myocardial contractile dysfunction is the inability to produce energy (Jennings and Ganote, 1976; Takeo *et al*. 1988). Indeed, ATP levels are slightly lowered before the onset of contractile failure (Hearse, 1979). Whether ATP is the sole factor playing a role in contractile dysfunction is doubtful. The decrease in ATP may be responsible for the inactivity of channel pumps that are essential to maintain ion homeostasis. Disruption of ion homeostasis as seen in ischaemia plays an important role in ischaemia induced injury of the myocardium. Elevated cytosolic Ca^{2+} may also play a role in the activation of phospholipases, increase depolarizations and cause ischaemic contracture. Significantly this contracture can be prevented with the production of ATP from glucose (Owen *et al*. 1990). Na^{+} overload is also problematic since it indirectly results in Ca^{2+} overload upon activation of the Na^{+}/Ca^{2+} exchanger (Murphy *et al*. 1988).

The second possible reason for the myocardial contractile dysfunction seen during ischaemia may be the accumulation of inhibitory metabolites. Accumulation of protons, a by-product of glycolysis, during ischaemia and the resultant acidosis was shown to play a role in inducing irreversible damage to the myocardium during the ischaemic period (Neely *et al*. 1984). Acidosis may also hinder contractile function of the myocardium. Tennant (1935) noticed that contractile dysfunction was associated with lactic acid accumulation. This may be an indirect effect of protons displacing Ca^{2+} from their binding sites on the thin filament (Katz and Hecht, 1969).

2.2.4 Cardiac ventricular arrhythmias

The term arrhythmias or dysrhythmias, simply means to have an abnormal heart rhythm. Arrhythmias can originate ventricularly or supraventricularly. Cardiac arrhythmias are a form of reversible injury and have several causes including ischaemia/reperfusion (Opie, 1991). The presence a hypertrophied myocardium may additionally increase the prevalence of cardiac arrhythmias (Wolk, 2000). The heartbeat or rhythm is generated through the coordination of electrical signals by means of ion channels. Ion channels are pore-forming proteins that are incorporated and cross the lipid membrane of the cell. Their function is to conduct ions across this otherwise impermeable membrane. Ion channels can selectively move ions down their electrochemical gradient across the cell membrane. As a result of this, they can either depolarize a cell, by moving positively charged ions into the cytosol, or repolarize a cell, by allowing positively charged ions to move out of the cell (Kass, 2005). Precisely timed opening and closing of the cardiac ion channels results in cell excitation which is in turn coupled to the rhythmic contraction/relaxation of the heart (Clancy and Kass, 2005). Abnormalities occurring with the ion channel, or the ion homeostasis can disrupt the normal electrical rhythm of the heart predisposing the heart to the development of debilitating cardiac arrhythmias. (for a review see by Roden *et al*. 2002). Depending on the severity of the arrhythmias, they can disrupt the coordinated contraction of the heart and lead to the failure of the heart to develop sufficient pressure for normal blood circulation. Abnormalities in heart rhythm are of concern as it has been reported that as many as 300000 Americans die of sudden death annually as a result of arrhythmias (Zipes and Wellens, 1998). These figures also reflect

the reality of the situation in several other westernized countries including South Africa.

For the purpose of this discussion, only arrhythmias of a ventricular origin will be discussed. Secondly, emphasis will be placed on the contribution of cardiac hypertrophy to the initiation of ventricular arrhythmias, as the model we are presenting has been shown to have cardiac hypertrophy (Du Toit *et al*. 2005). Ventricular hypertrophy has been shown to predispose the myocardium to early afterdepolarization induced triggered activity (Aronson, 1981; Charpentier *et al*. 1991). Because we observed arrhythmias after an ischaemic insult in our study, the development of re-entry circuits and ventricular automaticity will also be discussed as potential arrhythmogenic initiators.

2.2.5 Ischaemic ventricular arrhythmias

It is thought that there are 3 mechanisms responsible for the development of ischaemic ventricular arrhythmias; 1) ventricular automaticity 2) ventricular re-entry circuits 3) triggered activity.

2.2.5.1 Automaticity

Automaticity describes the development of action potentials from a new site in non-nodal tissue. Ventricular automaticity usually develops in Purkinje fibers. Such a site is able to induce an ectopic beat with the possibility of inducing ventricular tachycardia or ventricular fibrillation (Opie. 1991). Problems in K^+ ion homeostasis have been implicated in the development of ventricular

automaticity. Dyckner *et al*. (1975) found that 15 % of patients with acute myocardial infarction were hypokalemic, which was related to a greater incidence of ventricular arrhythmias. In a review by Motte (1984) it was stated that hypokalemia might result in automaticity-induced arrhythmias by increasing the slope of diastolic depolarization of the myocardial cell (phase 4 of the contractile cardiomyocyte action potential).

2.2.5.2 Ventricular re-entry circuits

Re-entry circuits are regarded as the most probable cause of clinical arrhythmias and can give rise to single premature depolarization's or sustained tachycardia. Re-entry circuits occur 1) in the presence of anatomical obstacles to the conduction or localized slow conduction, with or without a unidirectional block, of the electric impulse or 2) abnormal dispersion of refractoriness in areas of adjacent tissue, resulting in localized areas of slow conduction or block (West, 1990). An ischaemic insult is therefore problematic as it decreases the duration of the generated action potentials in the ischaemic cells (Victor and Zipes, 1977). The resultant variation of the action potential duration through ischaemic and non-ischaemic cells and between sites of different severities of ischaemia can generate differences in the refractory state of the myocardium (Opie, 1998), and increase the risk for the development of re-entry circuits. Complicating matters in the hypertrophied myocardium is the fact that the action potential duration is much longer than in a normal myocardium, thus increasing the magnitude of the variation of action potential duration between the ischaemic and the nonischaemic zone (Opie, 1998)

A model often used to study re-entry circuit induced ventricular arrhythmias is the branched Purkinje fiber preparation attached to ventricular myocardium (Figure 2.4)

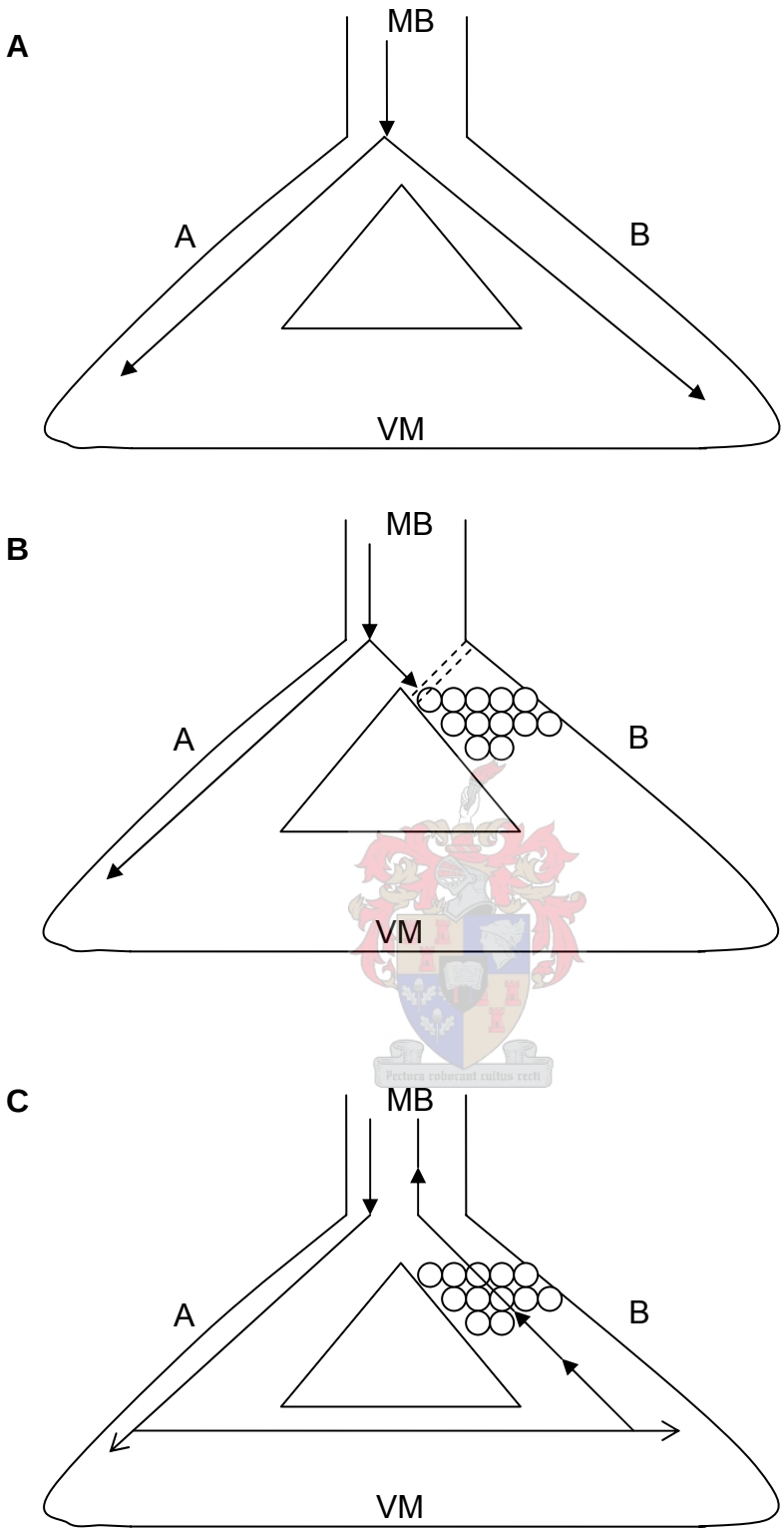


Figure 2.4: The re-entry phenomenon as recorded in a loop of Purkinje fiber bundles (A and B) and ventricular muscle (VM). The circles in branch B represent a unidirectional block. (Adapted from Wit *et al*. 1974) *MB;* main bundle

An impulse would normally travel along both Purkinje fiber bundles A and B from the main bundle (MB) in order to rapidly depolarize the ventricular muscle (VM) simultaneously (Figure 2.4 A). Action potentials in the His-Purkinje system are of a longer duration than that in the ventricular muscle, implying that re-entry into the bundles would not be able to occur as they would still be in the refractory period. The circles in figure 2.4 B represent an area of abnormal conduction in branch B, which is refractory to the antigrade impulse (represented by the double lines). The effects of this are that this region in branch B would markedly slow down the impulse or block it totally. The same can be said if we were considering an ischaemic zone in ventricular myocytes (Victor and Zipes, 1977). An impulse would therefore only travel through bundle A antegradely to depolarize the ventricular muscle. However, as the impulse travels through the ventricle it finds branch B is not refractory (branch B exhibits unidirectional block) and will therefore enter the branch retrogradely (figure 2.4 C). The impulse thus causes early re-excitation (re-entry) of the main bundle as it travelled through the circular area in a retrograde manner. Furthermore the impulse may re-enter branch A in a circular movement. These early re-entry circuits may also be rapid and repetitive and may dominate the ventricular rhythm (Wit and Cranefield 1977; West, 1990)

The slow conduction through the areas of the myocardium is achieved during ischaemic events due to dead cells or inhibition of fast ion channels. Early K^+ loss and Ca^{2+} influx into the cell after coronary artery occlusion, have been

implicated in early alterations in action potentials leading to re-entry circuits (Harris *et al*. 1954; Opie and Clusin, 1990) Intracellular acidosis may also block action potential conduction (De Mello, 1982). This may be due decreased free Ca^{2+} during intracellular acidosis (Stowe *et al*. 1986).

2.2.5.3 Triggered activity

Triggered activity is the result of early or late afterpotentials following an action potential. These early or late afterpotentials have the ability to cause a further action potential or series of action potentials. It may occur in the presence of elevated catecholamine levels and electrolyte disturbances (hypokalemia) (West, 1990).

An ischaemic insult is of major concern due to all these possible outcomes on the myocardial functional capacity (table 2.3).

Table 2.3: Consequences of ishaemia in the isolated perfused heart
Cell Death
Depressed contractile function
Ventricular arrhythmias
ventricular automaticity
reentry circuits
triggered activity

2.2.6 Reperfusion

Reperfusion is the full restoration of blood flow to an ischaemic area. Although it is absolutely necessary, Jennings *et al*. (1960) was one of the first to report evidence suggesting that reperfusion of the myocardium, may increase the amount of cardiomyocyte necrosis occurring in an ischaemic heart.

2.2.7 Reperfusion injury

Reperfusion injury in the clinical setting usually manifests itself as; 1) myocyte death and necrosis, 2) myocardial stunning, 3) reperfusion arrhythmias, 4) and endothelial or microvascular dysfunction, after the removal of an ischaemic stimulus (Moens *et al*. 2005). Attention will however only be given to cardiomyocyte death and necrosis, myocardial stunning and reperfusion arrhythmias.

2.2.7.1 Cardiomyocyte death

Reperfusion is thought to induce a certain amount of cellular death. The initial work of Jennings *et al*. (1960) revealed the development of contraction band necrosis upon reperfusion. These changes could however be delayed by postponing the reperfusion phase. The development of cardiomyocyte contracture is thought to be the primary method for necrosis induced cell injury upon reperfusion (Moens *et al*. 2005). Reperfusion may also induce cardiomyocyte apoptosis. Reperfusion, although it has a protective effect by decreasing the amount of apoptosis due to ischaemia, has a paradoxical effect as it was shown to accelerate apoptosis in cells, previously predestined to die (Fliss and Gattinger, 1996). Additional evidence supporting reperfusion induced apoptosis involves the upregulatation of p53 (Xie *et al*. 2000) and Bax (Zhao *et al*. 2000), which are promoters of apoptosis.

2.2.7.2 Myocardial stunning

In a review by Braunwald and Kloner (1982) myocardial stunning was defined as, “the prolonged post ischaemic mechanical dysfunction that continues after reperfusion of previously ischaemic tissue, in the absence of irreversible damage, which includes myocardial necrosis”. Ferrari (1995) emphasizes 3 points concerning stunning: 1) stunning in temporary and is fully reversible, provided sufficient time is given for the myocardium to recover; 2) stunning is a mild injury that is separate from injury seen in myocardial infarction; 3) the stunned myocardium has a normal or close to normal coronary flow.

Experimental evidence for stunning has been seen in animals for a number of years. Charlat *et al*. (1989) induced a 15-minute occlusion of the left anterior descending coronary artery followed by reperfusion, in an in vivo canine model, while monitoring systolic and diastolic functional parameters. These functional parameters only normalized after a few days. Humans have also been shown to be prone to myocardial stunning after acute myocardial infarction, with normal mechanical function only returning after a few months (Sakata *et al*. 1994). Stunning has also been seen to occur following unstable angina. Jeroudi *et al*. (1994) echocardiographically assessed regional left ventricular wall motion in six subjects immediately after angina, at rest, and at several time points thereafter. The results obtained demonstrated that angina at rest, was followed by a prolonged depression of cardiac contractile function, which was seen to persist for a 24 hours or longer.

Experiments by Heyndrickx *et al*. (1975) indicated that in order to achieve complete reversible stunning soon after reperfusion, the average duration of the ischaemic insult would have to be between 5 and 20 minutes. Ischaemic insults of longer duration could possibly result in adverse effects, such that the myocardium may not recover. This could be seen when Lavallee *et al*. (1983) induced coronary artery occlusion in an in vivo canine model for a period of 2 or 3 hours, with subsequent reperfusion. 4 weeks of reperfusion did not completely restore mechanical function. This results in what has become known as the “maimed myocardium” (Boden *et al*. 1995).

The proposed mechanisms responsible for myocardial stunning are thought to involve the generation of reactive oxygen species (Bolli *et al*. 1988) and cytosolic calcium overload (Du Toit and Opie, 1992), conditions, which are exacerbated during reperfusion. The cellular targets for reactive oxygen species are not very well know. Reactive oxygen species have recently been shown to oxidize cardiac proteins, in the ventricle and also in the mitochondria (Tatarkova *et al*. 2005) and possibly contribute to the reperfusion injury seen. They may also act by damaging mitochondrial phospholipids and induce lipid peroxidation of the mitochondrial membrane (Paradies *et al*. 1999). Cytosolic calcium overload occurring during postischaemic reperfusion is proposed to damage the contractile apparatus (Meissner and Morgan, 1995) thus hindering contractile function. The mechanisms by which these factors may further induce myocardial stunning are reviewed by Bolli (1990).

2.2.7.3 Reperfusion induced cardiac arrhythmias

For more than 2 decades, it has been known that reperfusion of an ischaemic heart may predispose the myocardium to potentially lethal arrhythmias (Manning *et al*. 1984) as electrical activity is fully restored upon reperfusion (Pogwizd and Corr, 1987). In this regard, ventricular tachycardia and ventricular fibrillation are associated with an increased risk of mortality following reperfusion (He *et al*. 1992). Whether or not ventricular arrhythmias occur upon reperfusion of the ischaemic myocardium may depend on the degree of reversible ischaemic injury as no reperfusion arrhythmias are seen to occur in dead cardiomyocytes (Corr and Witkowski, 1983).

Ca^{2+} is proposed to play a central role in the development of reperfusion arrhythmias. Brooks *et al*. (1995) using an isolated rat heart perfusion model, showed that upon reperfusion, the development of ventricular tachycardia and fibrillation were preceded by large increases in the amplitude of the calcium transient. Most significantly, hearts that were not arrhythmogenic did not have increased systolic Ca^{2+} transients.

Direct Ca^{2+} measurements performed on normal isolated rat hearts reveal large cytosolic Ca^{2+} oscillations during reperfusion (Meissner and Morgan, 1995). This may be due to the provision of ATP upon reperfusion, thus allowing the excess cytosolic cycling of Ca^{2+}, which could predispose the myocardium to delayed afterdepolarizations (a form of triggered activity) (Opie and Coetzee, 1988). This continuous over-cycling of Ca^{2+} could induce a

transient inward current that could predispose the myocardium to ventricular automaticity (Coetzee and Opie, 1987).

In cardiac hypertrophy, the myocardium has been shown to be predisposed to early afterdepolarizations (Aronson, 1981) and early afterdepolarization-induced trigger activity (Charpentier *et al*. 1991). Certain abnormalities occurring in the hypertrophied myocardium contribute to this form of arrhythmia. These abnormalities will be further discussed in section 2.1.3.1.6 relating to cardiac hypertrophy

2.3 Objectives of this study

In view of the above we set out to develop a model of diet induced obesity and the MS and:

1) Characterize our model of obesity in terms of the working definition of MS proposed by the WHO.
2) Investigate the susceptibility of the hearts from obese rats to ischaemia and reperfusion induced injury.
3) Determine whether Ang II and ET-1 plays a role in cardiac remodelling and/or the severity of ischaemia and reperfusion injury in this model.

CHAPTER 3

MATERIALS AND METHODS

3.1 Animals

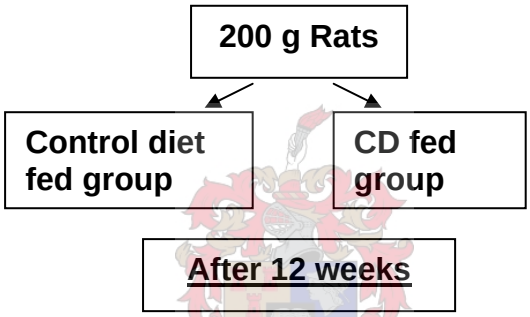
Age and weight matched male Wistar rats were used for this study. Rats were weaned at 4 weeks of age and were allowed free access to food and water. Rats were housed in the University of Stellenbosch Central Research Facility, with a 12-hour artificial day-night cycle where a constant temperature (22°C) and humidity (40%) were maintained. The South African Medical Research Councils guide for the humane use of laboratory animals was followed throughout the study. The Ethics committee of the Faculty of Health Sciences, University of Stellenbosch, approved the study.

3.2 Study design (figure 2.1)

Upon reaching 200 ± 20g, rats were randomly assigned to either a control or a cafeteria diet (CD) group. Control rats were fed a standard rat chow diet (SRC) while the experimental group received a special diet (cafeteria diet). Obesity in this model was induced primarily by hyperphagia and not by changes in the dietary composition (Pickavance *et al*. 1999) of the food. Rats were fed for a period of either 12 or 16 weeks. This was done in order to document the progression of obesity in our rat model with respect to certain biometric parameters as well as cardiac function before and after an ischaemic insult. Evaluation of these parameters allowed us to determine the length of time rats would have to be on the diet to develop the characteristics of the MS. At the appropriate time the rats were weighed; sacrificed; their

hearts removed and perfused on the isolated working rat heart perfusion apparatus for the documentation of mechanical function (protocol 1) and arrhythmias (protocol 2). Following the experimental protocol, certain hearts were weighed while others were freeze clamped (protocol 3 and 4). Freeze clamped hearts, together with blood samples obtained from the rats were stored at -80 ºC and -20 ºC respectively, and were used for biochemical analysis at a later stage. A separate series of rats were used for each protocol (see protocol 1-4).

A)



Biometric determinations

- Body weight
- Body weight/ ventricular weight
- Tibia length/ventricular weight

Biochemical determinations

Serum

- Insulin
- Ang II
- ET-1

Tissue

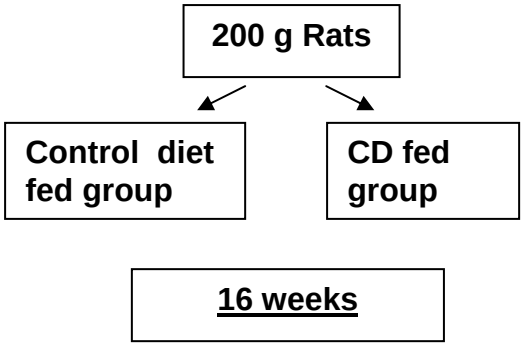
- Ang II
- ET-1

Mechanical function determinations

Global ischaemia

- Aortic output
- CF
- ADP & ASP
- Heart rate
- % AO recovery

B)



Biometric determinations

- Body weight
- Body weight/ ventricular weight
- Tibia length/ventricular weight
- Visceral fat mass
- Systolic blood pressure

Cell isolation

- Myocyte size

Metabolic determinations

Serum

- Insulin
- Lipids
- Ang II
- ET-1

Tissue

- Ang II
- ET-1

Blood

- Glucose
- HbA1c

Mechanical function determinations

Global ischaemia (ex vivo)

- Aortic output
- CF
- ADP & ASP
- Heart rate
- % AO recovery

Regional ischaemia

- Incidence & duration of arrhythmias

Echocardiography (in vivo)

- FSend & FSmid
- PWthick
- EDD & ESD
- PWTsyst & PWTdiast

Figure 3.1 A & B: Study design for rats after 12 (A) and 16 (B) weeks on the respective diets. *Ang II*-angiotensin II; *AO*-aortic output; *ADP*-aortic diastolic pressure; *ASP*-aortic systolic pressure; *CF*-coronary flow; *EDD*-end diastolic diameter; *ESD*-end systolic diameter; *ET-1*-endothelin-1; *FSend*-endocardial fractional shortening; *FSmid*- midwall fractional shortening; *PWthick*-posterior wall thickness; *PWTdiast*-posterior wall thickening during diastole; *PWTsyst*-posterior wall thickening during systole

3.3 Special Diet

The CD, or high caloric diet, (65 % carbohydrate, 19 % protein and 16 % fat) contained a similar macronutrient composition to the SRC (60 % carbohydrate, 30 % protein and 10 % fat). The CD consisted of 33 % SRC, 33% sweetened full cream condensed milk (Clover), 7 % sucrose and 27 % water (Pickavance *et al*. 1999). Food was replaced daily to avoid fermentation of wet food. Control rats consumed 379.9 kJ of energy per day compared to the 575.7 kJ taken in by the CD fed rats.

Calculation of the energy consumption of each group:

	Control diet	CD
Food consumption:	29 g/day	57 g/day
Energy content	13.1 kJ/g	10.1 kJ/g
Energy consumption per day (food consumption x energy content)	379.9 kJ	575.7 kJ

3.4 Experimental procedures

3.4.1 Isolated working rat heart perfusions

Rats from the 12 week or 16 week feeding groups were anaesthetized with an intraperitoneal injection of pentobarbitone sodium (12 mg/kg). Once anaesthetized, the rat heart was quickly excised and immediately placed in 4°C Krebs-Henseleit solution. Within 60 seconds the excised heart was mounted and perfused via the aortic cannula of the isolated working rat heart perfusion apparatus. The heart was initially retrogradely perfused in the Langendorff mode for a 10 minute stabilization period. All excess tissue was

trimmed off the heart. The left atrium was subsequently cannulated through the pulmonary vein, to allow perfusion of the left atrium, in working heart (WH) mode at the appropriate time. This was according to the working heart model of Neely (1967) as modified by Opie and coworkers (1971). Cardiac mechanical function was documented at predetermined time. Hearts were freeze clamped with Wollenburger tongs, pre-cooled in liquid nitrogen, at the appropriate times and stored at -80 ºC (see 3.5.1 and 3.10.2).

Myocardial temperature was monitored throughout the experiment with a thermistor probe, which was inserted into the right coronary sinus. This was done by making a small incision in the coronary sinus, and passing the probe through the incision. A waterbath (Grant instruments, Cambridge, England) maintained perfusate temperature by circulating warm water through water-jacketed glassware throughout the system. A separate waterbath (Grant instruments, Cambridge, England) was used to maintain the temperature of the water-jacket surrounding the perfused heart. The heart was perfused at a constant pressure of 100 cmH_2O during Langendorff mode and at a constant pressure during working heart mode. The preload was 15 cmH_2O and the afterload was 100 cmH_2O. Circulation of the buffer through the perfusion system was maintained by a peristaltic pump (Watson Marlow Ltd, UK).

The perfusion solution (Krebs-Henseleit solution) contained 118.46 mM NaCl; 24,995 mM $NaHCO_3^-$; 4.748 mM KCl; 1.185 mM $KH_2PO_4^-$; 1.19 mM $MgSO_4.7H_2O$; 1.25 mM $CaCl_2.2H_2O$ and 10 mM glucose (Merck Pty.Ltd., Darmstadt Germany). Fresh perfusion buffer was made up on each

experimental day and was gassed with a mixture of 95% O_2, 5% CO_2 before being filtered through a 0.45 µm mixed cellulose ester pore (Advantec MFS Inc, Pleasanton USA).

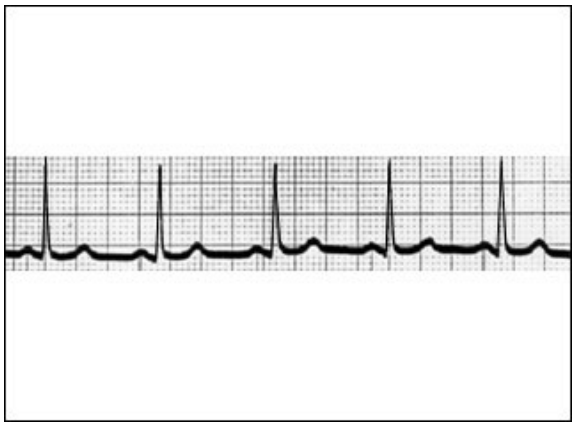
3.4.2 Perfusion to assess ventricular arrhythmias

To monitor the incidence and duration of ventricular arrhythmias during regional ischaemia and the subsequent reperfusion period, electrocardiographic techniques were implemented. Rats were anaesthetized as described above. Once the heart was mounted on the aortic cannula of the isolated working rat heart perfusion apparatus, and perfused (Krebs-Henseleit solution), electrocardiograph electrodes were attached to the aorta and the apex of the heart. The electrodes were connected to a Lectromed Multitrace 2 chart recorder (Rue-Fondon, Channel Islands), which produced an electrocardiogram trace. To induce regional ischaemia the left anterior descending coronary artery was ligated high up with a 3/0 silk suture (Johnson & Johnson, SA), for 35 minutes. Each ligation was performed by the same researcher and made in the same place for each rat heart. Reperfusion was induced by releasing the suture which restored coronary blood flow and initiated the reperfusion arrhythmias. Only rats from the 16-week groups were assessed for vulnerability to ischaemic and reperfusion arrhythmias.

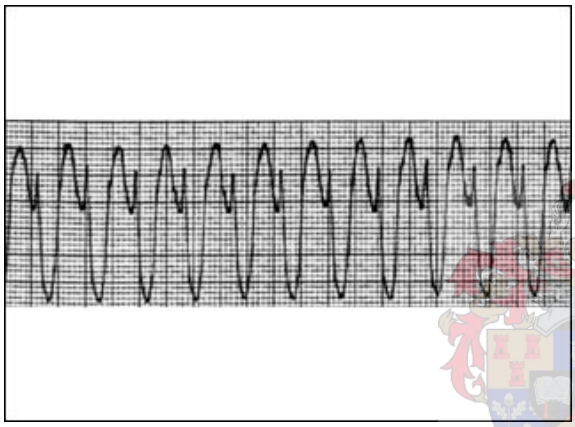
Ectopic beats were defined as premature beats in relation to the expected impulse of the basic sinus rhythm (Surawicz and Knilans, 2001). Ventricular arrhythmias were classified as ventricular tachycardia and ventricular fibrillation (Figure 3.2). Ventricular tachycardia was defined as three or more

consecutive, morphologically similar, rapid ventricular extrasystoles. Ventricular fibrillation was defined as more than six consecutive ventricular complexes showing complete morphological irregularity (Du Toit and Opie, 1993).

A)



B)



C)

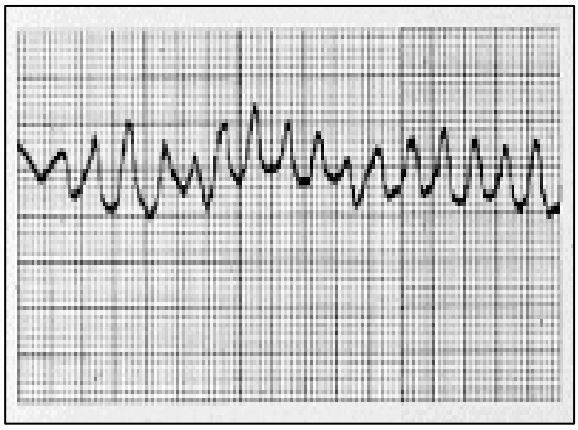


Figure 3.2: Electrocardiograph representations of normal sinus rhythm (A), ventricular tachycardia (B) and ventricular fibrillation (C).

3.5 Experimental protocols

For a brief summary of the various experimental perfusion protocols see figures 3.3-3.4.

3.5.1 Determination of myocardial function

Perfused hearts of the 12 week and 16 week fed rats were subjected to the following protocol (protocol 1) on the isolated working rat heart perfusion apparatus (figure 3.3):

- *Protocol 1*: Hearts were stabilized by a 10-minute perfusion in the Langendorff mode. This was followed by a further 20-minute perfusion in working heart mode, during which mechanical function (aortic output (AO), coronary flow (CF), heart rate, aorta peak diastolic and systolic pressure) was monitored and documented. Following WH mode, the heart was subjected to 15 minutes of total global ischaemia. Hearts were reperfused for 30 minutes after the 15 minute global ischaemic insult. Reperfusion consisted of a 10-minute stabilization phase in Langendorff mode, followed by 20 minutes in WH mode. Myocardial functional parameters were documented during the reperfusion phase. Myocardial temperature was maintained at 34 ºC – 35 ºC during Langendorff mode, 36 ºC - 36.5 ºC during WH mode and at 36 ºC - 36.5 ºC during ischaemia.

3.5.2 Protocol for investigating ventricular arrhythmias

Protocol 2 was used to investigate ventricular arrhythmias (figure 3.4). Briefly:

- *Protocol 2:* Hearts were perfused on the isolated working rat heart perfusion apparatus for 10 minutes in Langendorff mode, to stabilize the heart, and 20 minutes in WH mode to document cardiac function (protocol 4). This was followed by 35-minutes of regional ischaemia. To induce ischaemia the left anterior descending coronary artery was ligated. Hearts were subsequently reperfused, by removing the ligation, for 20 minutes in Langendorff mode. Elecrocardiographic measurements were documented during regional ischaemia and for the first 5 minutes of reperfusion. Myocardial temperatures were maintained at 36 – 37 °C throughout the experiments.

Protocol 1:

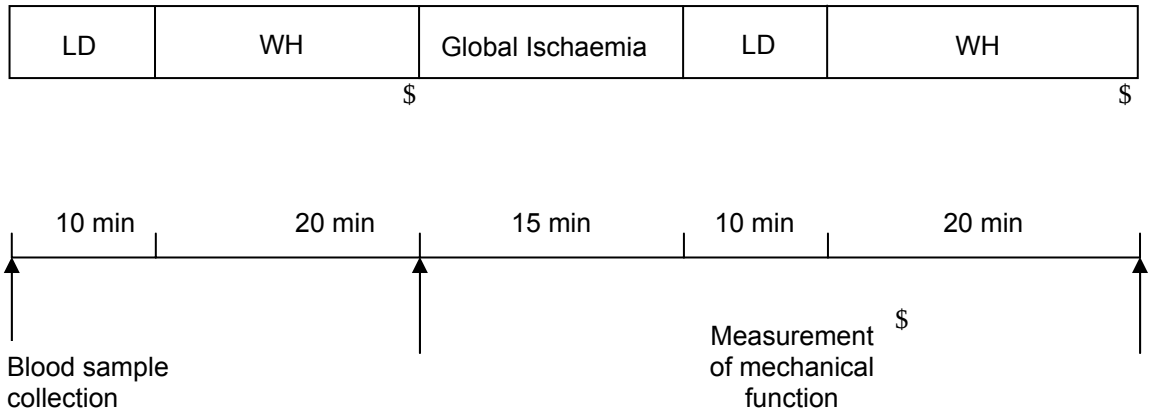


Figure 3.3: Experimental protocol used for the determination of mechanical function ($^{\$}$) of control and obese rat hearts on the isolated working rat heart perfusion apparatus. LD = Langendorff mode and WH = Working heart mode.

Protocol 2:

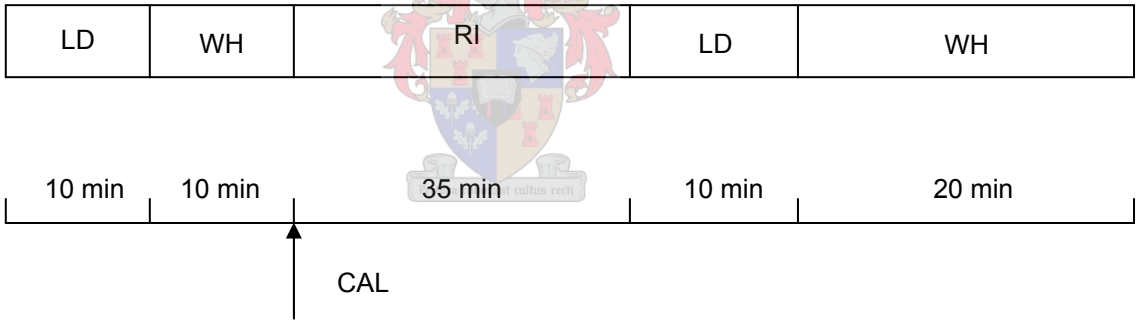


Figure 3.4: Experimental protocol followed for quantification of myocardial arrhythmias. Incidence of arrhythmias was measured during the ventricular RI and the subsequent reperfusion period. CAL was performed on the left anterior descending coronary artery to induce the ischaemic insult. LD = Langendorff mode; WH = Working heart mode, RI = regional ischaemia and CAL = coronary artery ligation.

3.6 Determination of visceral fat content

After rats were anaesthetized and their hearts removed, further incisions were made exposing the fat masses in the viscera. The peritoneal and retroperitoneal fat was removed. The visceral fat was weighted and expressed as a percentage of the rat's body weight.

3.7 Blood pressure determinations

Systolic blood pressure of the 12-week and 16-week fed groups were determined using a standard tail-cuff method (Bunag, 1973). Briefly, rats were anaesthetized with pentobarbitone sodium (12 mg/kg i.p.). A tail-cuff and pneumatic pulse sensor were fitted on the base of the tail. The tail-cuff was connected to a sphygmomanometer and the pulse sensor to a Lectromed Multitrace 2 chart recorder (Rue Fondon, Channel Islands). To determine systolic blood pressure, the tail cuff was carefully inflated until the pressure curve on the chart recorder disappeared. The sphygmomanometer, which was linked to the tail-cuff, was gradually deflated till the reappearance of the pulse pressure on the chart recorder. The pressure on the sphygmomanometer that corresponded with the reappearance of the pulse pressure curve was taken a systolic blood pressure reading. The final systolic blood pressure was determined by repeating the procedure twice and the mean systolic blood pressure was determined.

3.8 Indices of cardiac hypertrophy

3.8.1 Ventricular weight to body weight ratio

Rats were weighed before being sacrificed. At the end of each perfusion, the rat heart was rapidly removed form the perfusion apparatus and placed in a 4 °C Krebs-Henseleit solution to arrest it. While still in the solution, both atria were removed along with any other excess non-cardiac tissue, leaving only the ventricles. Once the heart had been arrested it was blotted on an absorbent paper towel, weighed and subsequently freeze clamped. Hypertrophy was indicated by an increase in ventricular weight normalized for body weight (ventricular weight/body weight). The method has recently been used in other publications (Skoumal *et al*. 2004; Asai *et al*. 2005)

3.8.2 Ventricular weight to tibia length ratio

Once animals had been anaesthetized and the heart excised, the right hind limb was amputated. This was done close to the hip joint, so as not to damage the tibia. The hind limb was placed in a 1 M NaOH solution for a few hours to remove most of the flesh, after which it was removed from the solution and cleaned. The tibia was removed, the length of the tibia measured with a caliper and expressed as a ratio to heart weight. This approach was found to be a more accurate index for cardiac hypertrophy than heart weight to body weight ratios in conditions with body weight fluctuations (Yin *et al*. 1982; Brede *et al*. 2003 and Saupe *et al*. 2003)

3.8.3 Cell size determination

Rat hearts were perfused on the isolated working rat heart perfusion apparatus for a period of 10 minutes in Langendorff mode. The perfusion buffer was then switched and the heart was perfused for 5 minutes with a fixing solution containing: 4 % formaldehyde in a phosphate buffer (pH 7.4). The heart was subsequently removed from the perfusion apparatus. Excess tissue was removed, and the heart was placed in a sealed tube containing the fixing buffer. Hearts were analyzed within a week.

Image analysis of the interventricular septum was performed on a Zeiss image analysis system, consisting of a Zeiss Axioskop 2 (Carl Zeiss, Jena, Germany) microscope fitted with a Zeiss Axiocam (Carl Zeiss, Jena, Germany) digital camera and connected to a pentium III 750 MHz computer (Mecer, Johannesburg, South Africa). Image digitalization, enhancement and analysis were performed using the KS 300 Image processing software (Carl Zeiss, Jena, Germany). Images for image analysis were captured using a Zeiss AcroPlan x 20 objective (K300 users guide, 1997).

The system was calibrated with a 1mm slide objective micrometer (Nikon Corp, Tokyo, Japan) with 0.01mm (10 μm) divisions using the x 20 Zeiss Acroplan objectives.

Average myocyte size was determined by dividing the myocyte nuclear count by the total myocyte cytoplasm area within the myocardium of the septum.

Myocyte nuclei were identified by colour hue thresholding and size to distinguish them from other nuclei. Total myocyte cytoplasm area per field was determined by colour thresholding (True, 1996).

The data was stored in the KS 300 database and exported into Excel 97 (Microsoft Corp, USA) for calculation and basic statistical evaluation.

3.8.4 Echocardiography

After 16 weeks on the feeding program, rats were taken for echocardiography to determine cardiac function and dimensions. Rats were anaesthetized intramuscularly with 0.5 mg/kg Domitor® (Novartis, South Africa) and 75 mg/kg Anaket-V (Centaur Labs, South Africa). Once anaesthetized, their chest area was sterilized with a solution of hibicol and shaved in preparation for echocardiography. Rats were placed in a box which had been cut so that the rat could lie with the chest region exposed to the probe.

Two-dimensional targeted M-mode echocardiography with Doppler color flow mapping was performed with a Hewlett Packard Sonos 5500 echocardiograph using a 7.5 MHz transducer. All studies were performed and interpreted by the same operator. This was a blinded study. Left ventricular dimensions were measured according to the American Society of Echocardiography guidelines (Sahn *et al*. 1978). When a suitable image was found, the recording was frozen and the image printed for subsequent analysis. After echocardiography, the rats were placed in a cage and the anaesthetic reversed with Antisedan (0.5 mg/kg).

3.8.4.1 Investigated parameters

For morphological characterization of the hearts, the following echocardiographic parameters were compared between control and CD fed rats:

- End-diastolic diameter (EDD)
- End-systolic diameter (ESD)
- Posterior wall thickness during systole (PWT_{syst})
- Posterior wall thickness during diastole (PWT_{diast})

From these parameters, the following could be calculated:

- Endocardial fractional shortening (FSend) was calculated from ((EDD - ESD) / EDD)) x 100.
- Midwall fractional shortening (FSmid) was calculated from (((EDD + PWT_{diast}) – (ESD + PWT_{syst})) / (EDD + PWT_{diast})) x 100
- Posterior wall thickening was calculated from ((PWT_{syst} - PWT_{diast}) / (PWT_{diast})) x 100.

3.8.4.2 Calculation of various parameters

Figure 3.5 represents a typical echocardiograph in which the dimensions were labeled A-E.

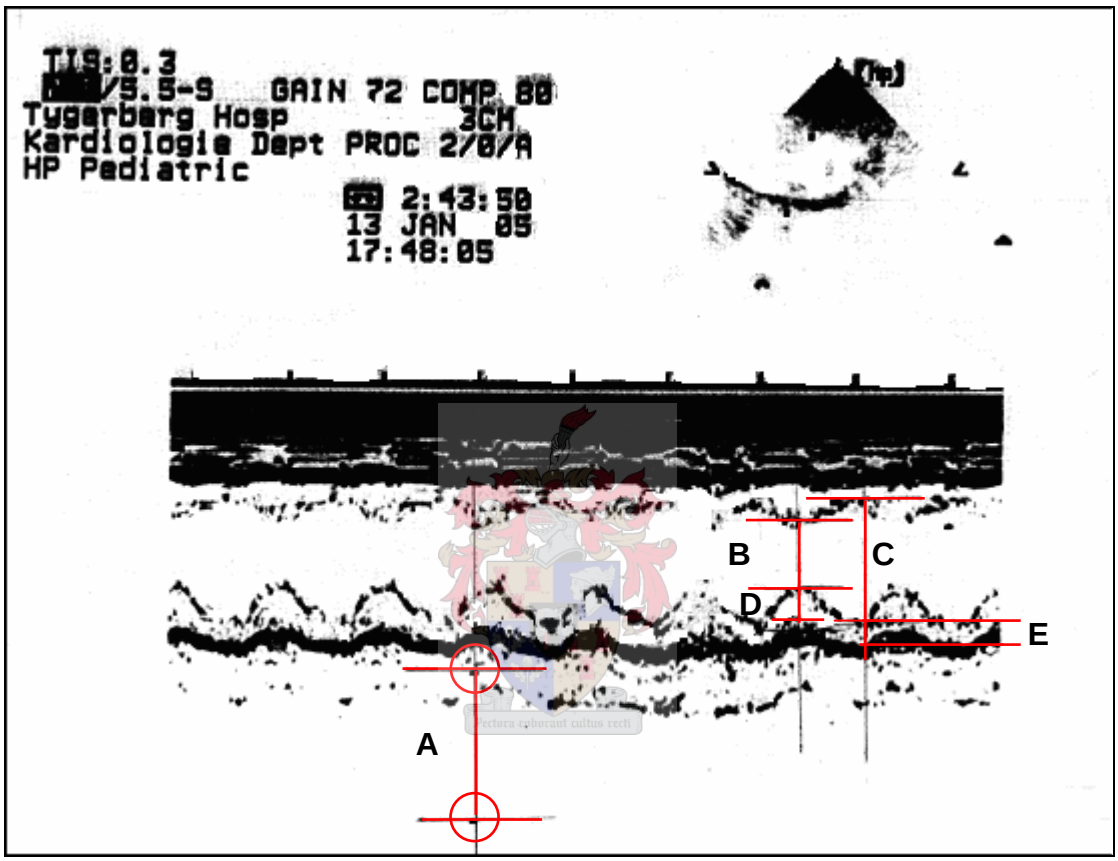


Figure 3.5. A typical echocardiograph indicating the dimensions of the rat heart that were measured

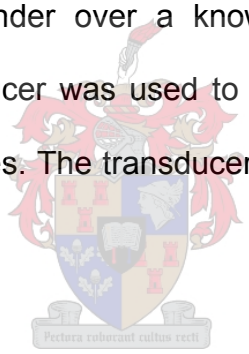
- A: The scale given on the original print out was 3 cm between the markings (circles). However since the image had been enlarged, the scale had to be readjusted accordingly.
- B: indicates the ESD
- C: indicates the EDD
- D: indicates the PWT_{syst}
- E: indicates the PWT_{diast}

3.9 Functional parameters measured on the perfusion apparatus

The functional parameters investigated were documented every 5 minutes during perfusions in WH mode on the isolated working rat heart perfusion apparatus. Parameters investigated were:

- AO
- CF
- Heart rate
- Aorta peak systolic and diastolic pressure

AO and CF were measured manually by collection of the AO and the coronary effluent in a measuring cylinder over a known period of time. A Viggo-Spectramed pressure transducer was used to record heart rate, aortic peak systolic and diastolic pressures. The transducer was connected to a side arm of the aortic cannula.



3.10 Indirect assessment of ischaemic/reperfusion damage: myocardial function

In order to investigate the susceptibility of our model to ischaemia/reperfusion injury, we compared basal and post-ischaemic AO values. Reperfusion AO values were expressed as a percentage of the pre-ischaemic value (reperfusion AO/pre-ischaemic x 100) to determine the percentage recovery between experimental groups.

3.11 Biochemical analysis

3.11.1 Blood Sample collection

Unless specified, blood samples were taken from the thoracic cavity of the rat and transferred into BD Vacutainer tubes, following the excision of the heart. The tubes were centrifuged at 3000 rpm at 4ºC for 10 minutes. The serum was isolated and stored at -20 ºC until Ang II, ET-1 and insulin assays were performed.

3.11.2 Myocardial tissue sample collection

Hearts perfused according to protocol 3 and 4 on the isolated working rat heart perfusion apparatus were used for biochemical analysis (figure 3.6). Briefly:

- *Protocol 3*: Hearts were stabilized by a 10-minute perfusion in the Langendorff mode. This was followed by a further 20-minute perfusion in working heart mode, during which mechanical function (AO, CF, heart rate, aorta peak diastolic and systolic pressure) was monitored and documented to ensure that the hearts were functioning properly. After WH mode the hearts were freeze clamped.

- *Protocol 4*: Hearts were perfused similarly to protocol 3. Following WH mode, hearts were subjected to 15 minutes of total global ischaemia. Ischaemic temperature was maintained between 36ºC and 36.5ºC. At the end of the ischaemic period, hearts were freeze clamped without being reperfused.

After being freeze clamped hearts were stored at – 80 ºC until assays were performed. Tissue samples were used to determine 1) basal and 2) ischaemic myocardial Ang II and ET-1 tissue levels. These samples were collected before ischaemia or at the end of ischaemia.

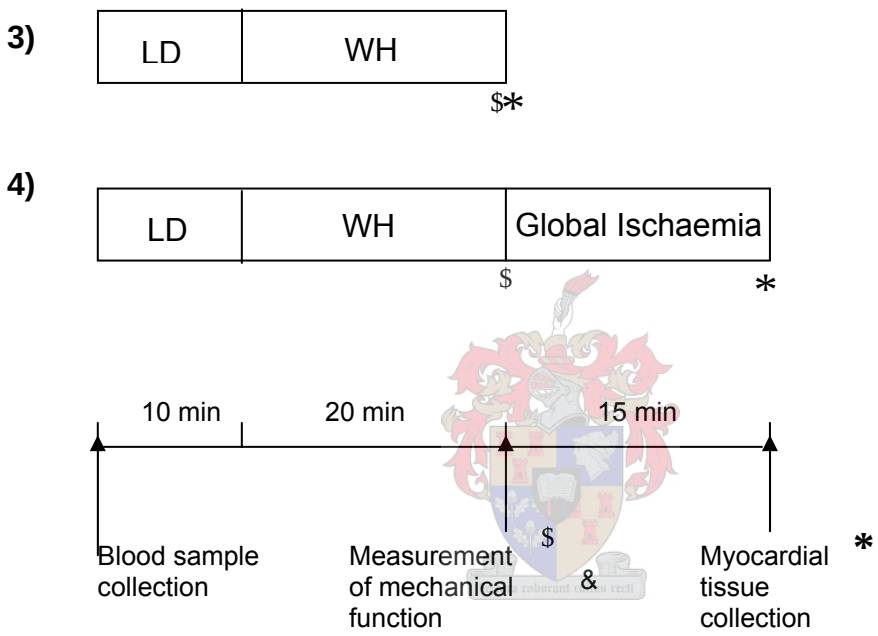


Figure 3.6: Experimental protocols 3 & 4 used for tissue collection of control and obese rat hearts after being perfused on the isolated working rat heart perfusion apparatus. Hearts were freeze clamped at the end of the various protocols to be used for biochemical analysis (*). LD = Langendorff mode and WH = Working heart mode.

3.11.3 Blood glucose determination

Blood glucose management was determined by two methods 1) directly using a blood glucose meter and 2) indirectly by measuring the percentage glycosylation of the hemoglobin (HbA1c testing).

3.11.3.1 Blood glucose meter – Principle

Glucose in the blood sample gets converted to gluconolactone by glucose dehydrogenase. The electrons that are liberated from this reaction reacts with a coenzyme electron acceptor, the mediator cyanoferrate III (oxidized form), to produce the reduced form of this mediator, hexacyanoferrate II. The test strip used for the glucose meter employs the principle of biamperometry. Briefly, the meter applies a voltage between two identical electrodes. This results in the reduced form of the mediator, formed in the incubation period, to be reconverted to the oxidized mediator. In the process a small electrical current is generated, which is read by the meter, and converted to the appropriate glucose concentration.

3.11.3.2 Blood glucose meter - Procedure

Overnight fasting blood samples were collected from the tail vein. For post prandeal blood glucose levels, blood was collected from the rats when they were killed for the removal of the heart. A drop of blood was placed on the absorbent film of an Accu-check advantage II slip (Roche Diagnostics, USA). The slip was subsequently inserted into an Accu-Check glucometer (Roche diagnostics, USA) to determine blood glucose of the experimental groups.

3.11.3.3 HbA1c testing – Principle

HbA1c is a specific subtype of hemoglobin A. Glucose binds slowly to hemoglobin in the erythrocyte to form the A1c subtype (glycosylation). The more blood glucose present, the more the glycosylation of HbA1c. Due to the fact that the decomposition of HbA1c proceeds very slowly, its levels can be strongly correlated with the average blood glucose levels during the preceding 4 weeks. The test therefore measures the extent of glycosylation of the hemoglobin over a period of up to 3 months. When the % glycosylation of the haemoglobin is below 7, one may assume that the blood glucose levels of the animal was maintained at normal levels for the preceding 3 months.

3.11.3.4 HbA1c testing – Procedure

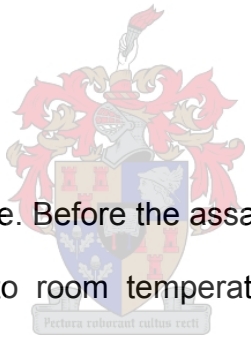
Once the rat had been anaesthetized, an incision was made in its hind limb to locate the femoral vein, and animals were heparinized. A period of 30 seconds was given for the heparin to circulate in the body, after which the rat was sacrificed. Blood removed from the thoracic cavity was stored at 4 °C until analyzed.

Stored blood samples were sent to the National Health Laboratory Service at Tygerberg Hospital where the test was performed.

3.11.4 Serum insulin determination

3.11.4.1 Assay principle

Non fasting blood samples were collected as described above. The assay used, was a competitive radioimmunoassay (RIA) (Coat-A-Count® Insulin, Diagnostic Products Corporation, LA, USA). A fixed amount of ^{125}I-labeled insulin would compete with insulin, present in the blood sample, for binding sites on an insulin specific antibody. Because the antibody is immobilized in the wall of the polypropylene tube, simply decanting the tube isolates the antibody bound fraction of the radiolabeled insulin. Radioactivity could then be measured by using a gamma scintillation counter (Cobra II Auto Gamma, A.D.P, South Africa).



3.11.4.2 Assay procedure

All tests were done in duplicate. Before the assay was started, all components of the assay were brought to room temperature, as by instruction of the manufactures. Four uncoated polypropylene tubes were labeled accordingly: Total counts (1-2) and non-specific binding (3-4). Fourteen insulin antibody-coated tubes were labeled (A-G) for the standards. Additional tubes for the controls and samples were also labeled.

200 µl of the zero calibrator was added to tube A as well as to the non-specific binding (NSB) tube. Furthermore 200 µl of the remaining calibrator, control and sampled was pipetted to their respective prepared tubes. 1.0 ml of ^{125}I insulin was added to each tube and subsequently vortexed. Samples were incubated for 18-24 hours at room temperature. Following the incubation

period, samples were decanted. This was done by placing each tube (except the total count tube) in a foam decanting rack and allowing the tubes to drain for 2 to 3 minutes. Following this, each tube was struck on an absorbent paper. Removal of the excess moisture in this manner would enhance the precision of the assay. The radioactivity of each tube was then measured in a gamma counter (Cobra II Auto Gamma, A.D.P, South Africa) for 1 minute per tube.

3.11.5 Determination of serum lipid levels

Total cholesterol, TG and HDL-cholesterol levels were measured in the serum by means of spectrophotometric analysis (Technicon RA 1000 Auto Analyser). Serum LDL cholesterol was not measured as this is not possible using this assay.

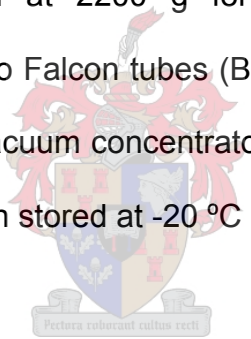
3.11.6 Determination of serum and myocardial angiotensin II

3.11.6.1 Extraction procedure for serum

1 ml of serum was added to 4 ml chilled ethanol and was mixed and vortexed for 2 minutes. The samples were centrifuged at 2000 g for 10 minutes at 4 ºC. The supernatant was decanted into Falcon tubes, freeze-dried and stored at -20ºC. This was done in accordance with the directions from the manufacturers of the Ang II RIA kit.

3.11.6.2 Acidified ethanol tissue extraction

Approximately 0.279 ± 0.0175 g wet weight of frozen tissue was extracted in 20 volumes of ice-cooled 0.18 M HCl:ethanol (1:3 v/v). The tissue was homogenized with a Polytron PT10 (Switzerland) for two cycles of ten seconds each. The homogenate was centrifuged (Sorvall RC-5B, Du Point Instruments) at 32500 x g for 20 minutes at 4 ºC. Following centrifugation, the supernatant was decanted, and its pH adjusted to between 5.5 and 6.0 with 1 M NaOH, and subsequently kept on ice for 1 hour. The supernatant was centrifuged a second time at 2200 g for 10 minutes at 4 ºC, after which pH was adjusted with 1 M NaOH and/or 0.18 M HCl where necessary. Again the supernatant was centrifuged at 2200 g for 20 minutes at 4 ºC. The supernatant was decanted into Falcon tubes (Becton Dickinson Labware, NJ, USA) and freeze-dried in a vacuum concentrator (Freeze Mobile 6, The Virtis Company, New York) and then stored at -20 ºC (Naik *et al*, 2001).



3.11.6.3 Solid-phase extraction

The freeze-dried tissue extracts were reconstituted (2 x 500 µl of 1 M formic acid) and underwent solid-phase extraction using Sep Pak C_{18} columns (Waters Corporation, Massachusetts, USA). Each column was conditioned with 6 ml of methanol and deionized water. The reconstituted supernatants were added to the pre-conditioned C18 Sep Pak columns, and allowed to pass through. The loaded Sep Pak column was washed with 6 ml of 10 % methanol in 1 M formic acid. The Sep Pak column was then eluted with 8 ml of 80 % methanol in 1 M formic acid, and the Ang II with its fragments were

collected in falcon tubes. The elluent collected was then freeze-dried and stored at -20 ºC.

The columns were conditioned and washed at a flow rate of 5 to 10 ml/min. For loading and eluting the samples, a flow rate of 2 to 10 ml/min was used. Flow rates used were as instructed by the manufacturer.

3.11.6.4 Angiotensin II radioimmunoassay (RIA)

3.11.6.4.1 Assay principle

Ang II was assayed by a competitive RIA (Euria-angiotensin II form Euro-Diagnostica, Malmoe, Sweden). Ang II in the samples would compete with ^{125}I-labeled Ang II for binding to the antibodies. The amount of bound ^{125}I-Ang II is inversely proportional to the concentration of Ang II in the samples and standards. Bound and unbound phases are separated by a second antibody bound to solid phase particles, which is followed by a centrifugation step. The radioactivity of the bound fraction could then be measured using a gamma counter (Cobra II Auto Gamma, A.D.P, South Africa).

3.11.6.4.2 Assay procedure

All freeze dried samples in the falcon tubes were reconstituted with 1 ml assay buffer and thoroughly vortexed. Samples were placed on ice and for the duration of the RIA, all pipetting steps were done on ice. Polystyrene tubes were labeled in duplicate for the RIA as follows: Total counts (1-2), NSB (3-4), standards (5-18), controls (19-22) and unknown samples (23-102).

400 µl of each standard, control and extracted sample was pipetted in duplicate to their corresponding tube. 400 µl and 600 µl of assay buffer was added to the 0 standard and NSB respectively. Finally, 200 µl of the AngII antiserum was pipetted into every tube except the NSB and total counts tube. All tubes were vortexed and incubated at 4 ºC for 6 hours.

After the incubation the radiolabeled ^{125}I-Ang II tracer was added to all tubes. Tubes were vortexed and incubated at 4 ºC for 18-22 hours. Following the incubation period, 100 µl of the double antibody was pipetted to all tubes, except total counts, vortexed and incubated for a further 30 – 60 minutes. Finally all tubes were centrifuged at 1700 g for 15 minutes at 4 ºC. Supernatants were decanted and the residues counted for 2 minutes each in the gamma counter (Cobra II Auto Gamma, A.D.P, South Africa).

3.11.7 Determination of serum and myocardial endothelin-1

3.11.7.1 Tissue preparation

Approximately 0.262 ± 0.00248g wet weight, of tissue was transferred into a polystyrene tube containing 4 ml of a 1 M acetic acid-20 mM HCl-9 mM benzamidine solution, at 4 ºC. A Polytron PT10 (Switzerland) was used to homogenize the samples for two cycles of ten seconds each, after which the homogenates were incubated for 10 minutes at 4 ºC. Homogenates were then centrifuged (Sorvall RC-5B, Du Point Instruments) at 27000 g for 20 minutes at 4 ºC, and the supernatants collected were stored at –20 ºC and later used for the extraction process (Motte *et al*, 2003).

3.11.7.2 Serum and tissue extraction

Serum and tissue (freeze dried homogenates) samples were extracted in the same manner as described by Motte *et al*, (2003). Again sep-pak C-18 columns (Waters Corporation, Massachusetts, USA) were used for solid phase extraction of rat serum and tissue samples.

Serum and tissue samples that were stored at –20 ºC were allowed to thaw. 2.50 ml of each sample was transferred to appropriately labeled polystyrene tubes and subsequently placed on ice. 25 µl of trifluoro-acetic acid (TFA): distilled water (1:10) was added to each sample and was vortexed for 10 seconds. After samples were centrifuged at 1500 g for 10 minutes at 8 ºC, they were placed on ice untill further use.

The sep pak C-18 columns were placed in an appropriate rack, suspended in glass test tubes to collect wash-out, and labeled accordingly. Each column had to be preconditioned as follows:

- 10 ml of TFA, distilled water, methanol (0.50, 99.5, 400) at a flow rate of 10-20 ml/min.
- 10 ml of TFA:distilled water (0.5:500) at a flow rate of 10-20 ml/min.

The centrifuged samples were quantitatively transferred to their respective labeled column and allowed to pass through. Columns were washed with 4 ml TFA:distilled water (0.50, 500), which was allowed to pass through. Finally endothelin was eluted from the columns by adding 4ml of TFA: distilled water:

methanol (0.50, 99.5, 400) at a flow rate of 4-8 ml/min. Eluate was collected in falcon tubes, freeze dried and stored at -20 ºC.

After each step, the columns were allowed to drain before the next step commenced. Columns were not reused for further extractions.

3.11.7.3 Endothelin-1 RIA

3.11.7.3.1 Assay principle

Endothelin was assayed by a competitive RIA (Euria-endothelin form Euro-Diagnostica, Malmoe, Sweden) using a rabbit antiserum raised against an ET-1 albumin conjugate. Briefly, endothelin competed with ^{125}I-labelled endothelin for binding to the antibodies. The binding of ^{125}I is inversely proportional to the concentration of the endothelin in the samples and standards. A double antibody solid phase is used to separate the antibody-bound ^{125}I-endothelin from the unbound fraction, with the aid of a centrifugation step. The radioactivity of the bound fraction could then be measured using a gamma counter (Cobra II Auto Gamma, A.D.P, South Africa).

3.11.7.3.2 Assay procedure

Freeze dried samples were reconstituted with 0.25 ml assay buffer and placed on a mechanical shaker for 10 minutes. Endothelin working standards were prepared by diluting the endothelin standard with the assay buffer as directed by the manufactures of the kit. All tests (standards, controls samples etc.) were done in duplicate.

Polystyrene tubes were labeled accordingly: Total counts (1-2), NSB (3-4), standards (5-18), Controls (upper and lower) (19-26) and unknown samples (27-106). 100 µl of the standards, controls and unknown samples were pipetted into their respective tubes, together with 500 µl anti-endothelin (except for the non specific binding and total count tubes). 100 µl zero standard and 500 µl assay buffer was added to the non specific binding tubes. All tubes were vortexed and incubated for 18-24 hours at 4 ºC. After the incubation period, 500 µl ^{125}I-endothelin was added to each tube and incubated for a further 18-24 hours at 4 ºC. This was followed by adding 100 µl of the solid phase double antibody to all tubes except total count tubes. Samples were vortexed and incubated for 60 minutes at 4 ºC. Finally samples were centrifuged at 1700 g for 15 minutes at 4 ºC. Supernatants were immediately decanted and the radioactivity counted in a gamma counter (Cobra II Auto Gamma, A.D.P, South Africa) at a counting time of 2 minutes.

3.12 Statistics

For comparative studies, significance was measured with either a paired or unpaired Students t-test between CD and control fed rats. To test for significance when analyzing the incidence of arrhythmias, the Fishers Exact test was used. All data are presented as mean ± SEM. A $p < 0.05$ was considered significant.

CHAPTER 4

RESULTS

4.1 12 Week data

4.1.1 Biometric and metabolic data

4.1.1.1 Biometric data

Table 4.1: Biometric data of 12 week control and CD fed rats

	12 Weeks	
	Control	**CD**
Body weight (g)	413.87 ± 6.76	464.84 ± 6.35*
VW/BW (mg/g)	3.06 ± 0.03	3.01 ± 0.04
VW/TL mg/mm	0.031 ± 0.0005	0.035 ± 0.0005*

***BW*-body weight; *CD*-cafeteria diet; *TL*-tibia length; *VW*-ventricular weight.**

***p<0.001 Control vs. CD within feeding group**

n = 31-35.

After 12 weeks on the respective diets, CD fed rats were significantly heavier than their age-matched control diet fed rats (table 4.1). Although there were no differences between CD and control fed rats in the VW/BW ratio, the VW/TL ratio was significantly different between these groups.

4.1.1.2 Serum insulin levels after 12 weeks on the diets

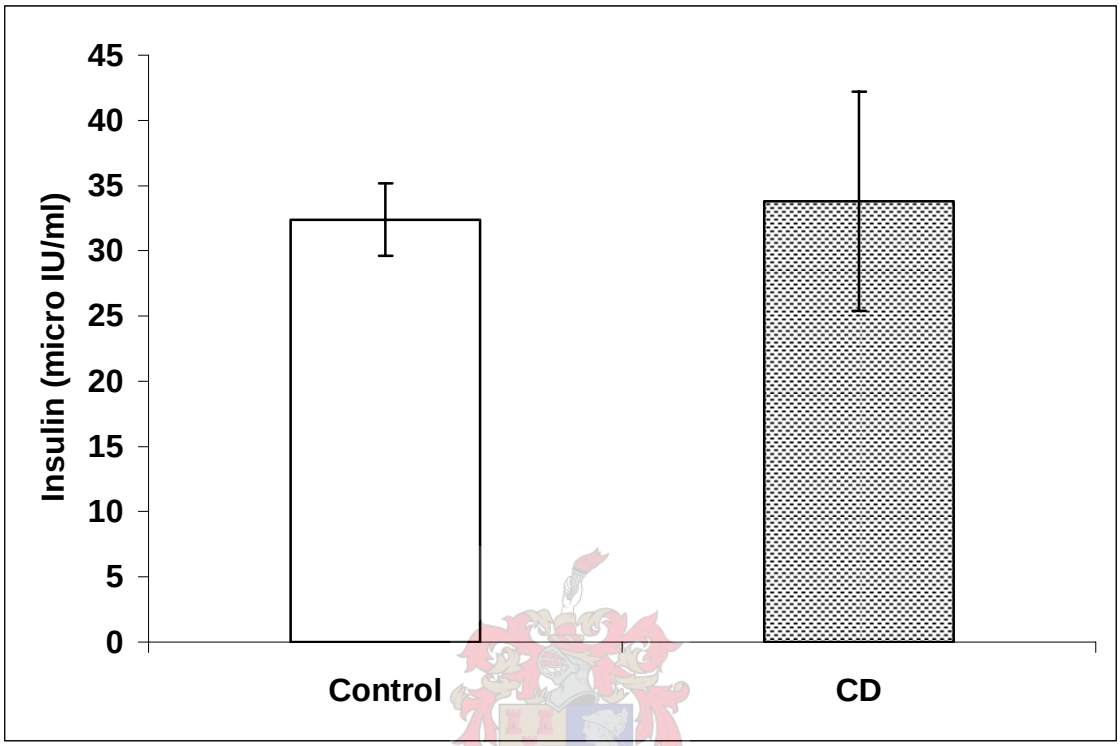


Figure 4.1: Non-fasting serum insulin levels of 12 week control and CD fed rats. All values expressed as mean ± SEM. *CD*-cafeteria diet.

There were no differences (Figure 4.1) between non-fasting serum insulin levels from CD and control diet fed rats (33.81 ± 8.40 IU/ml vs. 32.38 ± 2.77 IU/ml). This finding indicated that after 12 weeks on the diet, CD fed rats would not meet the WHO criteria for MS.

4.1.2 Ex vivo functional data

4.1.2.1 Myocardial function

Table 4.2: Myocardial mechanical function of ex vivo hearts from 12 week control and CD fed rats. Baseline myocardial parameters were measured after 20 minutes working heart perfusion. Reperfusion parameters were measured during the post-ischaemic working heart mode after 20 minutes reperfusion.

	12 weeks	
	Control	**CD**
	Baseline	
AO (ml / min)	42.37 ± 1.89	27.81 ± 1.96$^{\$}$
CF (ml/min)	19.44 ± 1.41	17.13 ± 0.51
ADP (mmHg)	65.74 ± 1.82	67.40 ± 0.57
ASP (mmHg)	101.64 ±1.71	96.66 ± 1.52*
HR (beats/min)	239.73 ± 5.72	231.43 ± 5.88
	Reperfusion	
AO (ml / min)	27.19 ± 2.96$^{\#}$	15.13 ± 1.76*$^{\#}$
CF (ml/min)	16.63 ± 1.19$^{\#}$	15.45 ± 0.79
ADP (mmHg)	68.47 ± 0.76	67.63 ± 0.94
ASP (mmHg)	93.41 ± 2.47$^{\#}$	90.37 ± 1.52$^{\wedge}$
HR (beats/min)	254.18 ± 9.59	214.23 ± 14.68*

***ADP*-aorta diastolic pressure; *ASP*-aorta systolic pressure; *AO*-aortic output; *CD*-cafeteria diet; *CF*-coronary flow; *HR*-heart rate.**

All values expressed as means ± SEM.

***p<0.05, $^{\$}$p<0.0001 for control vs. CD**

$^{\wedge}$p<0.05, $^{\#}$p<0.001, for baseline vs. reperfusion within respective groups

n = 5-8.

Myocardial function (control vs. CD rats).

After 12 weeks of feeding CD fed rats had significantly poorer baseline and reperfusion AO's when compared to their controls ($p<0.05$) (table 4.2). CD fed rat hearts also showed a decrease in baseline ASP and reperfusion HR compared to hearts from control diet fed ($p<0.05$).

Myocardial function (pre-ischaemia vs. reperfusion)

After the 12 week feeding period both groups displayed decreased reperfusion AO's after ischaemia (Table 4.2). The ischaemic insult led to an exacerbated drop in ASP in the hearts of CD fed rats ($p<0.05$). There was a decrease in the coronary flow (CF) and ASP of the control hearts ($p<0.001$).

4.1.2.2 Percentage aortic output recovery

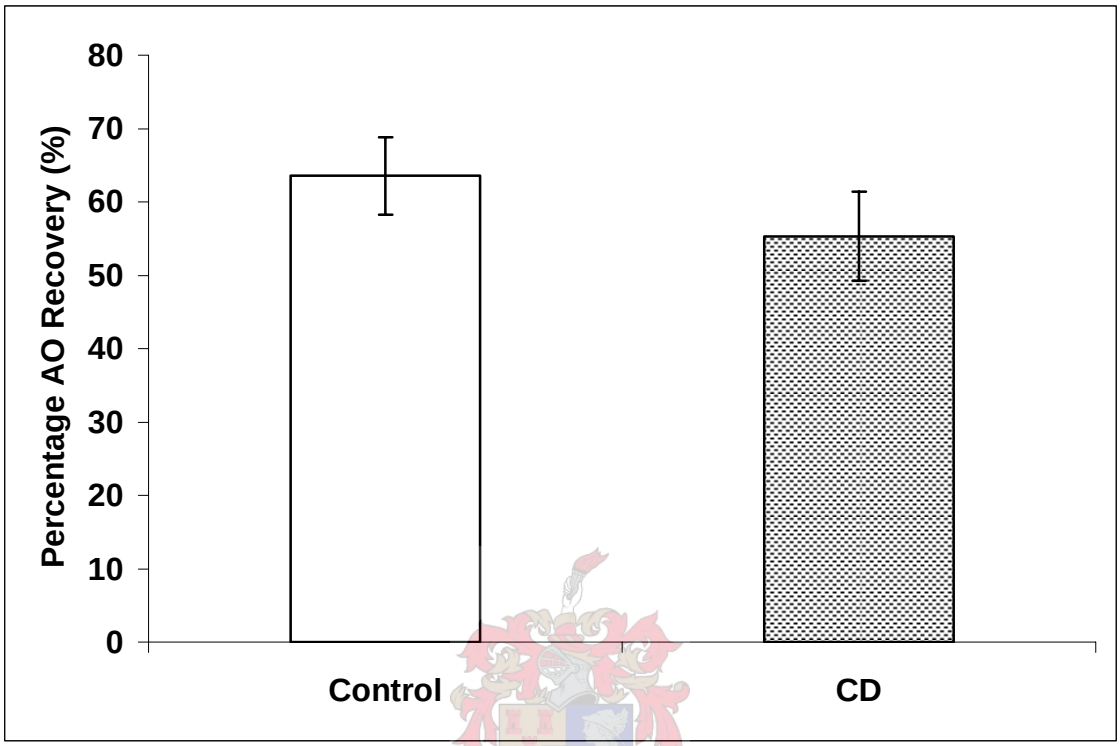


Figure 4.2: Percentage AO recoveries for 12 week control and CD fed rats. Baseline and reperfusion AO's are expressed as mean percentages ± SEM. *AO*-aortic output; *CD*-cafeteria diet

***p<0.05; n = 8-9.**

After being subjected to 15 minutes of global ischaemia and 20 minutes reperfusion, the percentage aortic output recovery was not different between hearts from 12 week CD and control diet fed rats (55.31 ± 6.09 % vs. 63.55 ± 5.31 %)(figure 4.2).

4.1.3 Biochemical data

4.1.3.1 Serum angiotensin II levels after 12 weeks on the diets

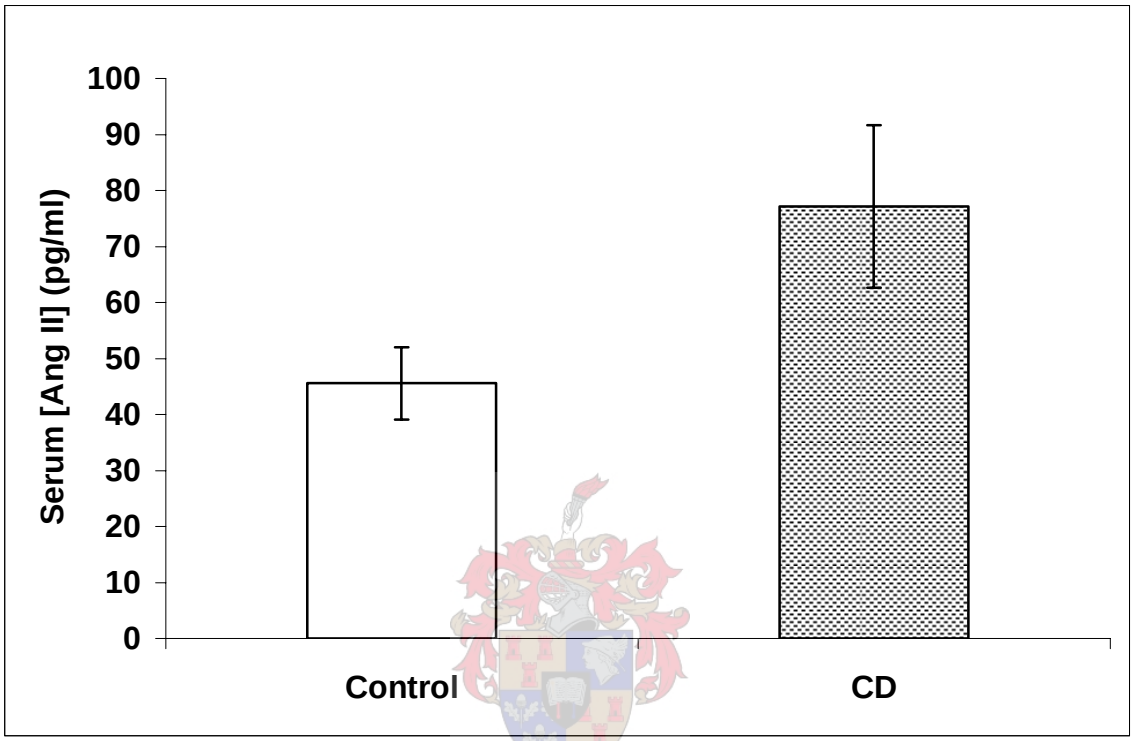


Figure 4.3: Serum Ang II levels after 12 weeks on the control and CD. Values presented as means ± SEM. *Ang II*-angiotensin II; *CD*-cafeteria diet. n = 8.

After 12 weeks of feeding, there was no difference in serum Ang II levels between CD and control diet fed rats (77.16 ± 14.49 pg/ml vs. 45.48 ± 6.44 pg/ml) (figure 4.3).

4.1.3.2 Myocardial angiotensin II levels after 12 weeks on the diets

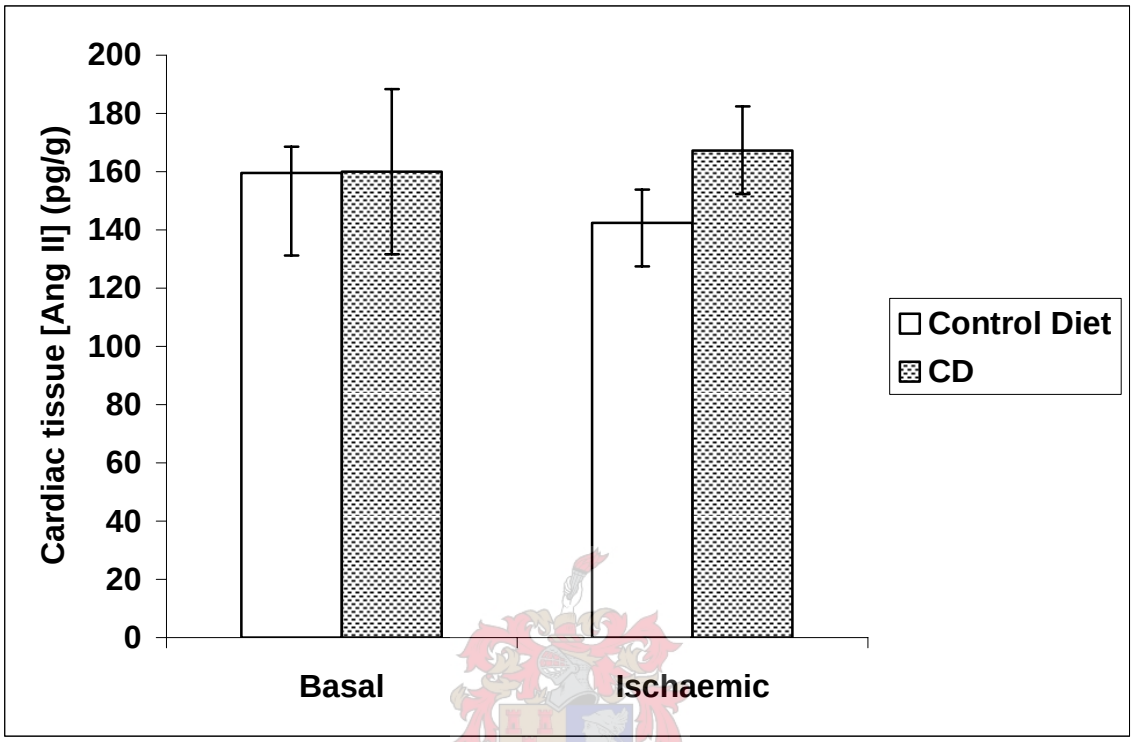


Figure 4.4: Myocardial Ang II levels before and at the end of 15 minutes of global ischaemia in hearts from 12 week control and CD fed rats. *Ang II*-angiotensin II; *CD*-cafeteria diet.

n = 6-7

Analysis of Ang II levels in cardiac tissue from 12 week fed rats revealed no differences between CD and control groups under both basal and ischaemic conditions (figure 4.4). In addition, there was no difference between basal and ischaemic myocardial Ang II content within CD and control groups.

4.1.3.3 Serum endothelin-1 levels after 12 weeks on the diets

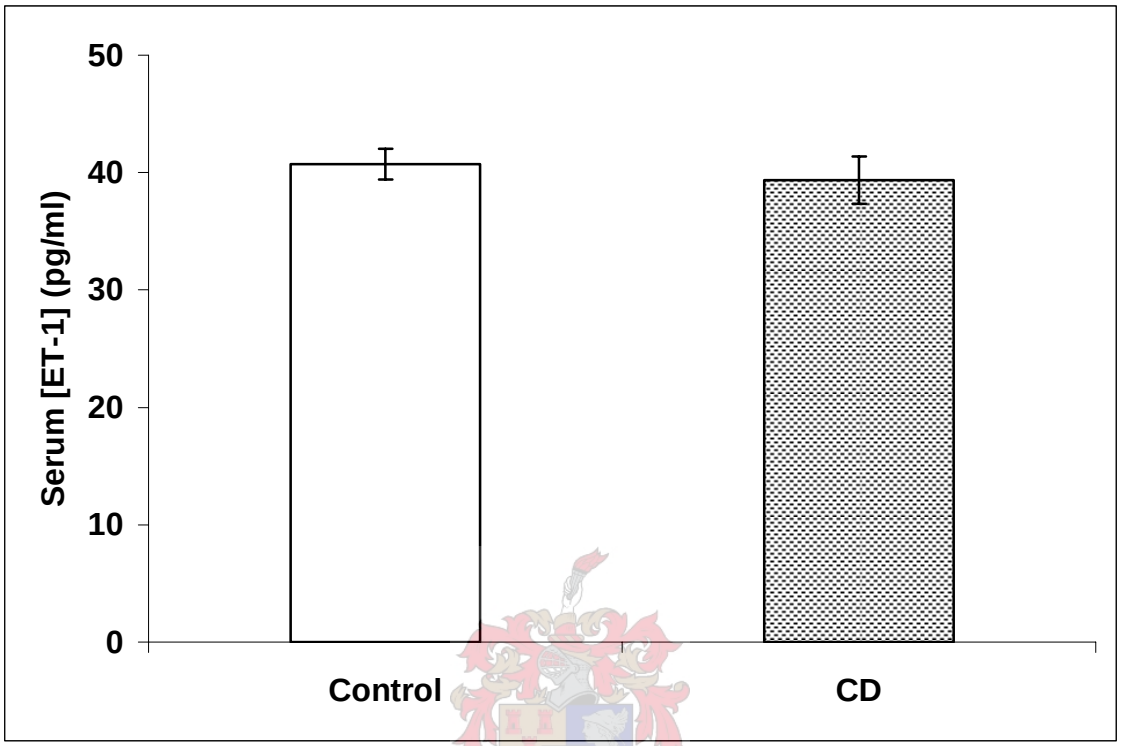


Figure 4.5: Serum ET-1 levels of 12 week control and CD fed rats. Values presented as means ± SEM. *CD*-cafeteria diet; *ET-1*-endothelin-1.

n = 8.

After 12 weeks of feeding, serum ET-1 levels in CD and control rats were comparable (39.36 ± 2.04 pg/ml vs. 40.70 ± 1.31 pg/ml)(figure 4.5).

4.1.3.4 Myocardial endothelin-1 levels after 12 weeks on the diets

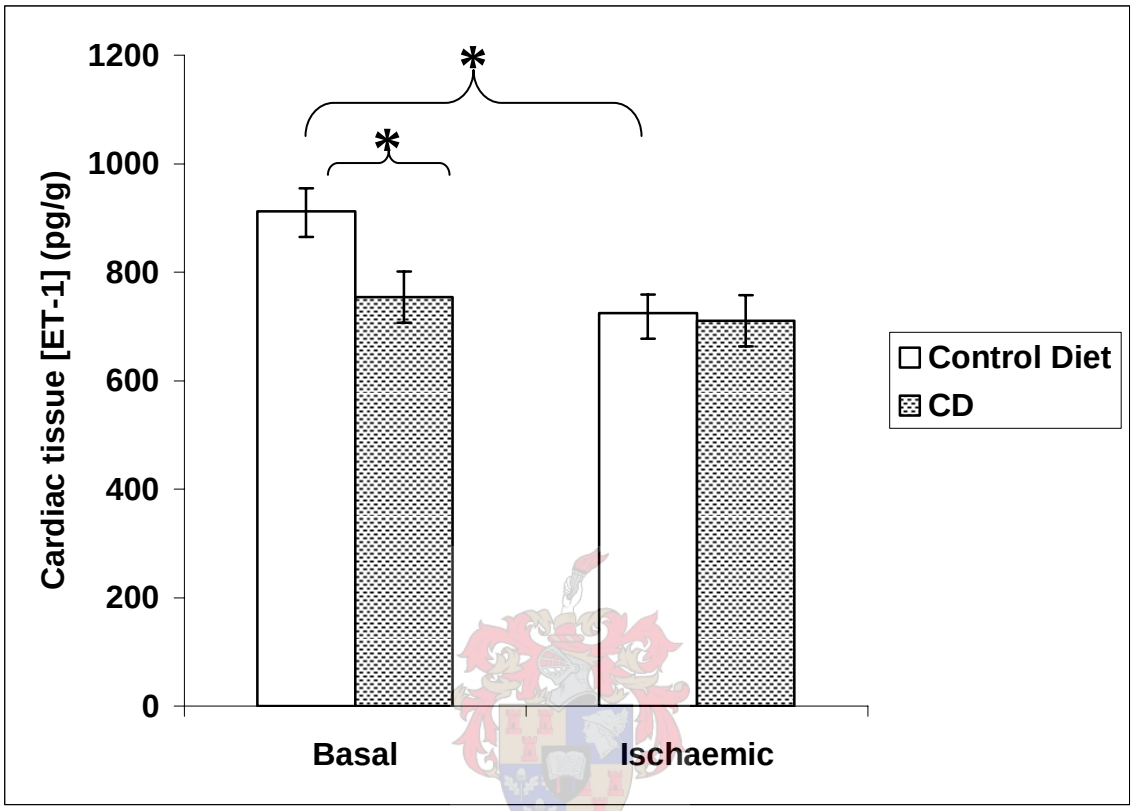


Figure 4.6: Myocardial ET-1 levels before and at the end of 15 minutes of global ischaemia in hearts from 12 week control and CD fed rats. *CD*-cafeteria diet; *ET-1*-endothelin-1.

n = 6

Basal myocardial ET-1 levels were lower (figure 4.6) in CD fed rats than their control littermates after 12 weeks on the diet (753.65 ± 47.37 pg/g vs. 912.17 ± 41.96 pg/g). This difference in cardiac tissue ET-1 content between CD and control rat hearts was however not seen after the ischaemic period. There was also a significant decrease in cardiac tissue ET-1 content in control rat heart at

the end of the ischaemic episode (912.17 ± 41.96 pg/g vs. 724.67 ± 34.47 pg/g; $p<0.05$). No such change was seen in cardiac tissue ET-1 content of hearts from the CD fed group ($p>0.05$).

4.2 Characterization of the model after 16 weeks on the CD

After 12 weeks on the diet, CD fed rats where significantly heavier, however there was no difference in serum Ang II levels nor in the susceptibility of their hearts to an ischaemic insult. In addition, non-fasting insulin levels where not elevated in the CD fed rats after 12 weeks of feeding. In order to have a model of MS, it would be essential for serum insulin or glucose levels to be different between the groups to satisfy the characteristics of the MS. In addition there was no difference in myocardial susceptibility to an ischaemic insult between the groups. We consequently decided to fully characterize this model after 16 weeks of feeding on the respective diets.

4.2.1 Biometric and metabolic data

4.2.1.1 Biometric data

Table 4.3: Biometric data of 16 week control and CD fed rats

	16 Weeks	
	Control	**CD**
Body weight (g)	456.24 ± 5.74$^{\#}$	542.56 ± 5.57*$^{\#}$
VW/BW (mg/g)	2.90 ± 0.50$^{\#}$	2.82 ± 0.51$^{\#}$
VW/TL mg/mm	0.031 ± 0.0005	0.036 ± 0.0004*

***BW*-body weight; *CD*-cafeteria diet; *TL*-tibia length; *VW*-ventricular weight.**

***p<0.001 Control vs. CD within feeding group**

$^{\#}$p<0.001 12 vs. 16 week feeding period

n = 31-35.

After 16 weeks of feeding, the CD rats were significantly heavier and had significantly greater VW/TL ratio's (table 4.3). There was no difference in the VW/BW ratio between CD rats and their control littermates.

Interestingly, there was a significant drop in VW/BW ratio between the 12 and 16 week feeding periods between both the CD and control diet fed groups. As expected, there was also a significant increase in body weight between 12 and 16 week fed CD rats and control fed rats. (table 4.1 and table 4.3)

4.2.1.2 Fasting blood glucose levels

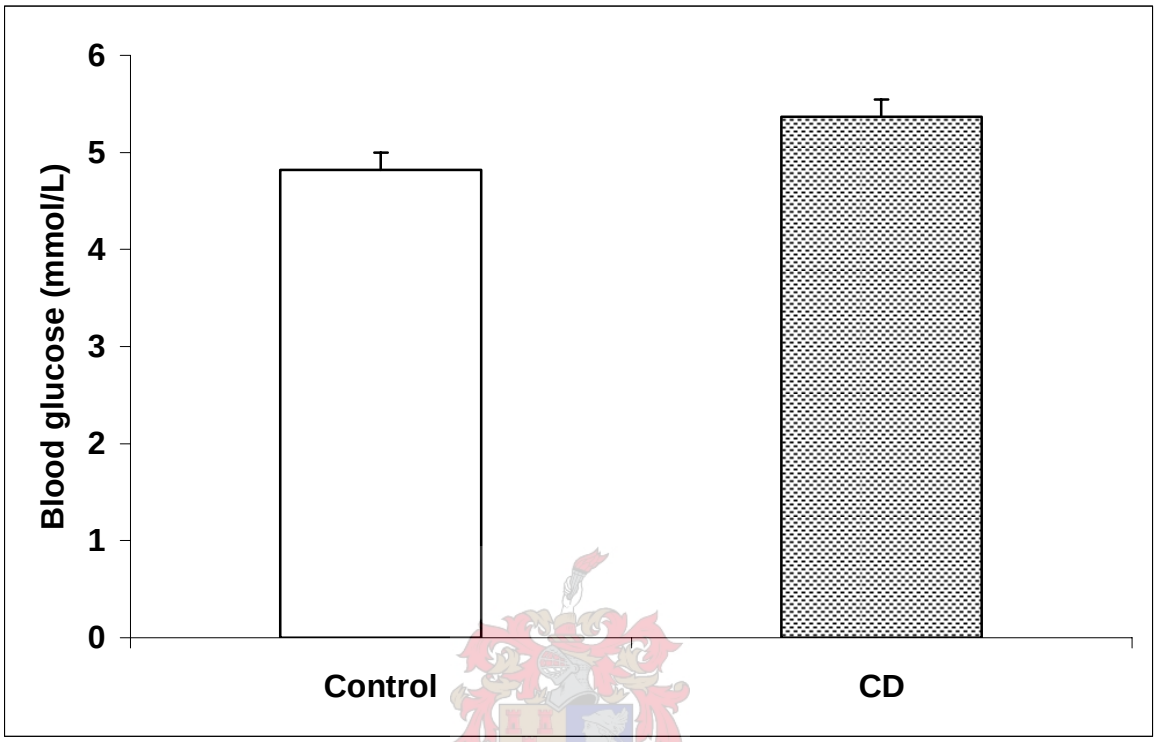


Figure 4.7: Fasting blood glucose levels of 16 week control and CD fed rats. All values expressed as mean ± SEM. *CD*-cafeteria diet

n = 6

After 16 weeks on the diet, CD fed rats had similar fasting blood glucose levels when compared to their control littermates (figure 4.7).

4.2.1.3: Non-fasting blood glucose levels

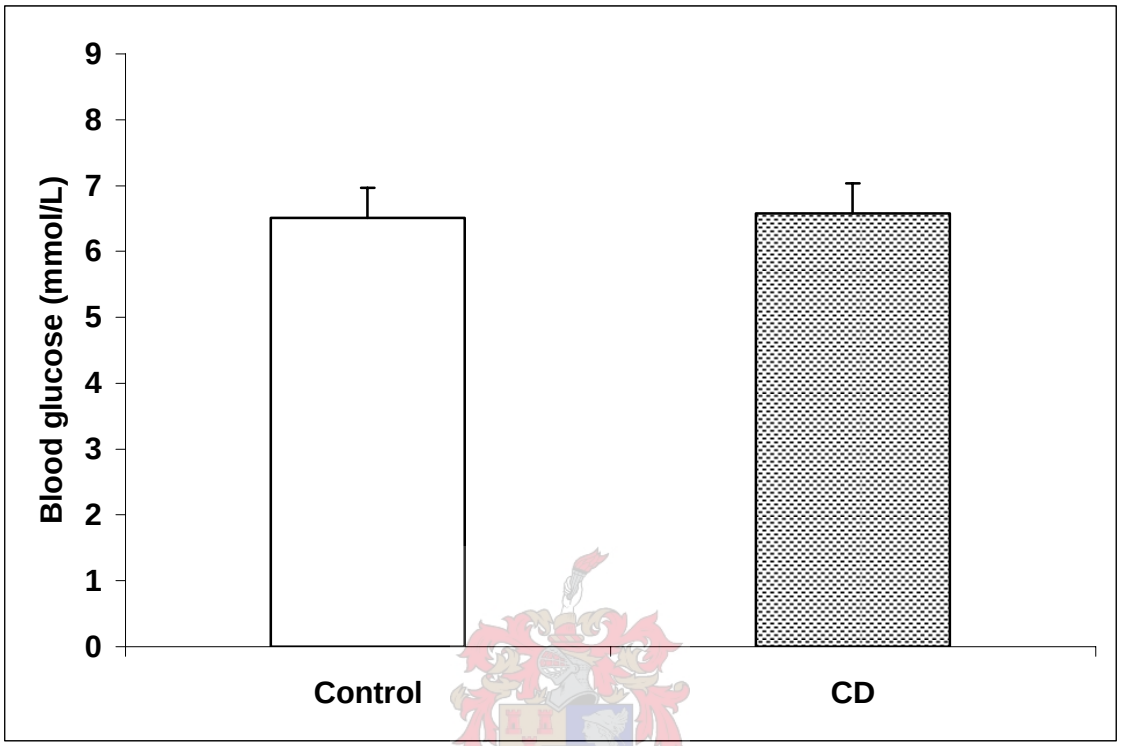


Figure 4.8: Non-fasting blood glucose levels of 16 week control and CD fed rats. All values expressed as mean ± SEM. *CD*-cafeteria diet

n = 8

Following 16 weeks of feeding there were no differences in the non-fasting blood glucose levels between CD and control fed rats (figure 4.8)

4.2.1.4 HbA1c levels

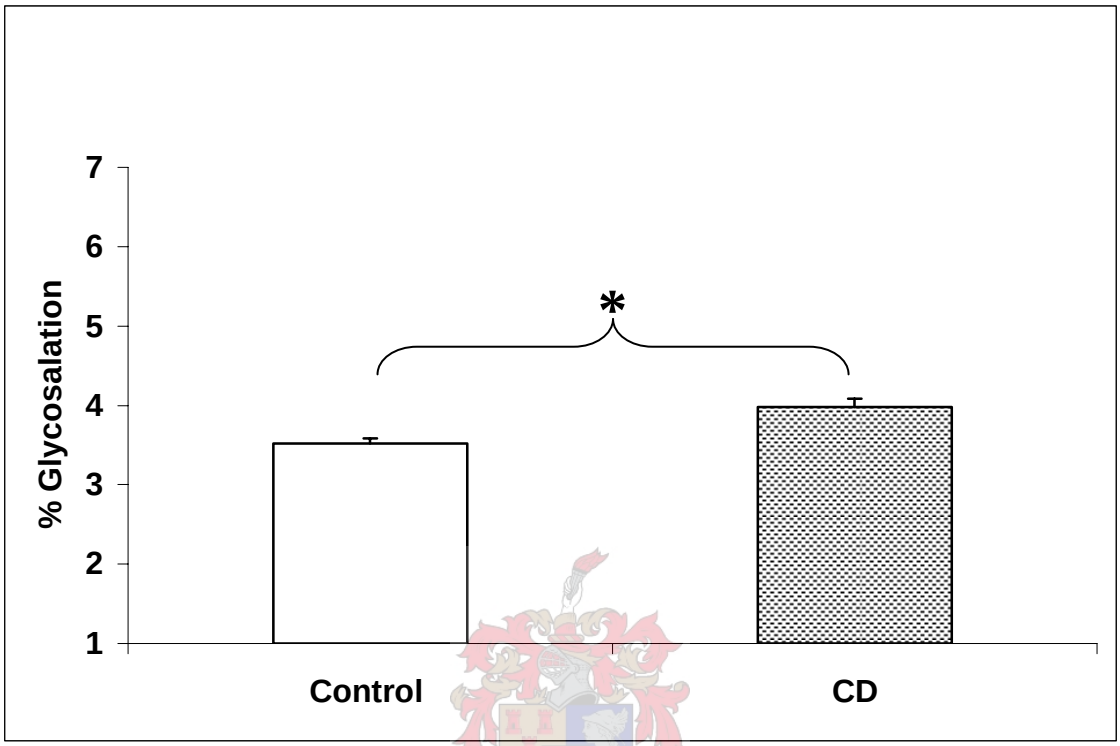


Figure 4.9: % Glycosylated hemoglobin in 16 week control and CD fed rats. All values expressed as mean ± SEM. *CD*-cafeteria diet

*** p<0.05; n = 8-9**

The HbA1c levels, indicative of the % glycosylated hemoglobin, were significantly elevated in the CD fed group when compared to their controls after 16 weeks on the diet (3.98 ± 0.11 % vs. 3.52 ± 0.06 %) (figure 4.9). The HbA1c levels of less than 7 are however indicative of normal blood glucose management over the previous three months. These data suggest that the animals maintained normal glucose levels prior to the HbA1c determinations.

4.2.1.5 Non-fasting serum insulin levels

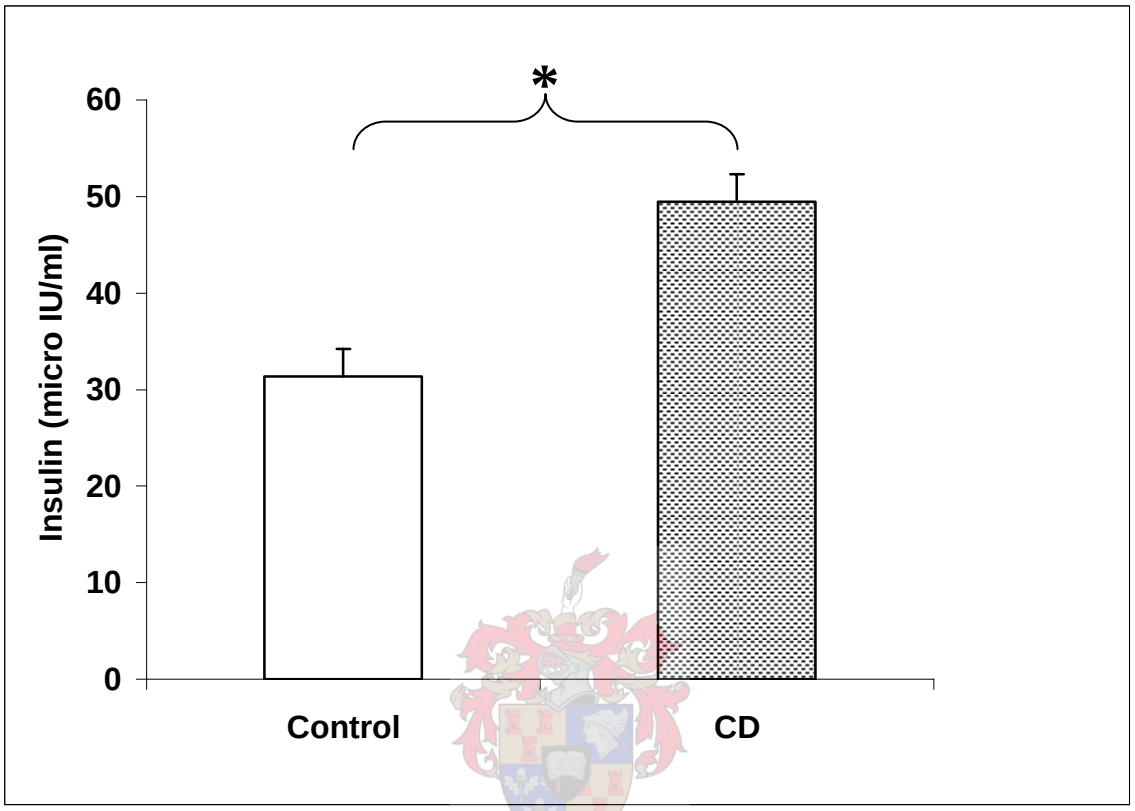


Figure 4.10: Non-fasting serum insulin levels of 16 week control and CD fed rats. All values expressed as mean ± SEM. *CD*-cafeteria diet

***p<0.05; n = 11**

After 16 weeks on the diet, CD rats had significantly elevated serum insulin levels (figure 4.10) compared to their control littermates (49.48 ± 6.18 micro IU/ml vs. 31.38 ± 2.85 micro IU/ml; $p<0.05$). Although these were non-fasting insulin values, they are indicative of a hyperinsulinaemic state.

4.2.1.6 Non-fasting serum lipid levels

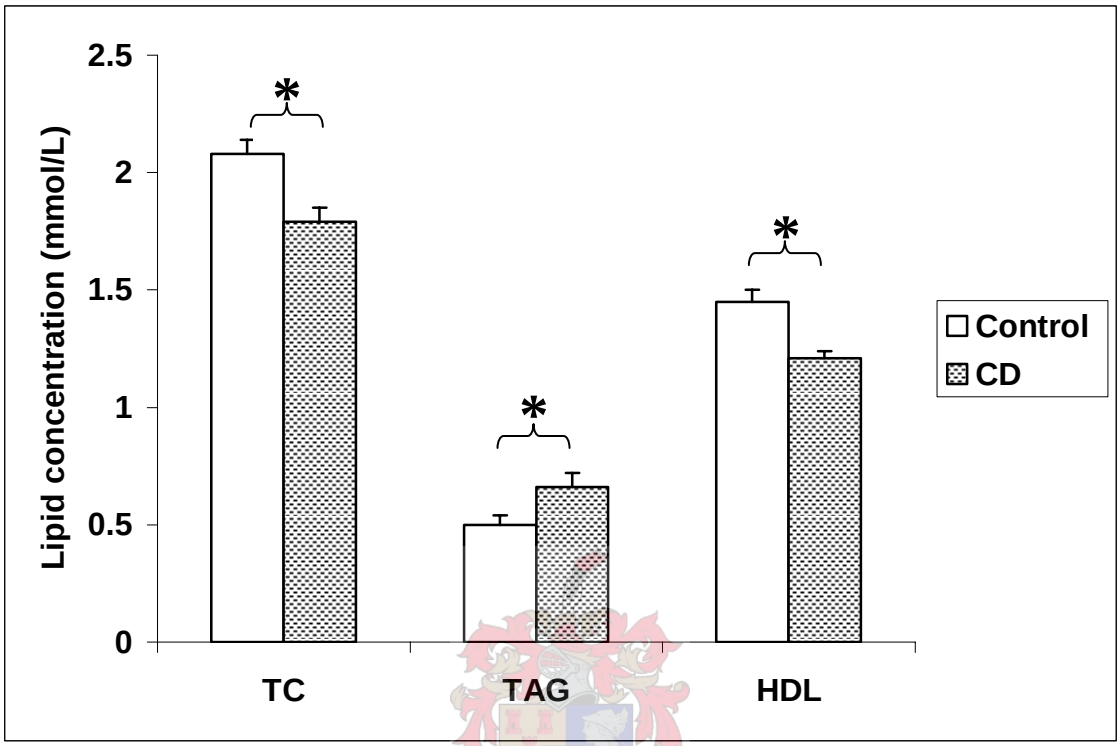


Figure 4.11: Serum TC, TAG and HDL-cholesterol levels of 16 week control and CD fed rats. All values expressed as mean ± SEM. *CD*-cafeteria diet; *HDL*-high density lipoprotein cholesterol; *TAG*-triacylglycerol; *TC*-total cholesterol.

***p<0.05; n = 10**

After 16 weeks of feeding, CD rats had significantly altered non-fasting serum lipid levels, when compared to their controls (figure 4.11). Serum TG levels was elevated, while both total cholesterol and HDL-cholesterol levels were lowered in CD fed rats (TG: 0.66 ± 0.06 mmol/L vs. 0.50 ± 0.04 mmol/L, $p<0.05$; Total cholesterol: 1.79 ± 0.06 mmol/L vs. 2.08 ± 0.06 mmol/L, $p<0.05$; HDL-cholesterol:

1.21 ± 0.03 vs. 1.45 ± 0.05; $p<0.05$).

4.2.1.7 Percentage visceral fat

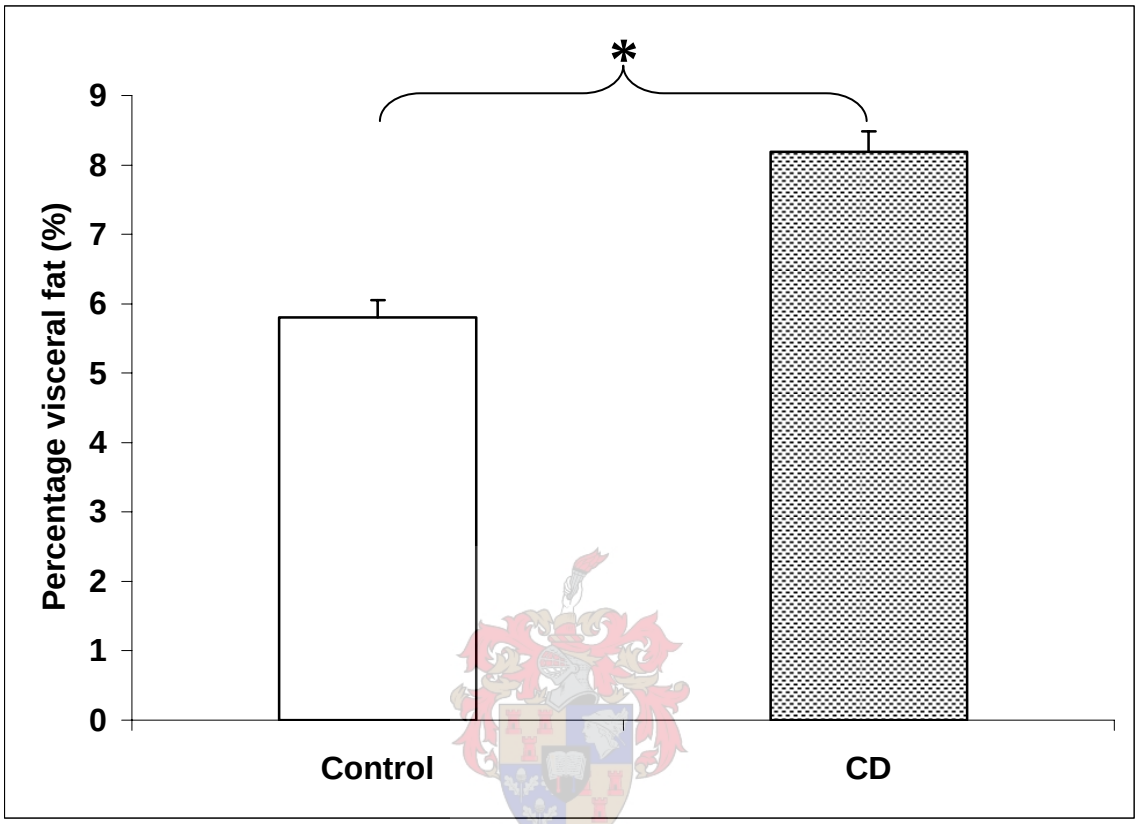


Figure 4.12: Percentage visceral fat of 16 week control and CD fed rats. All values expressed as mean ± SEM. *CD*-cafeteria diet

***p<0.0001; n = 10-11**

After 16 weeks on the diet, CD fed rats had greater amounts of visceral fat than their control counterparts (8.19 ± 0.30 % vs. 5.80 ± 0.25 %; p<0.0001) (figure 4.12). These data indicate that the CD fed rats had visceral/central obesity.

4.2.1.8: Systolic blood pressure

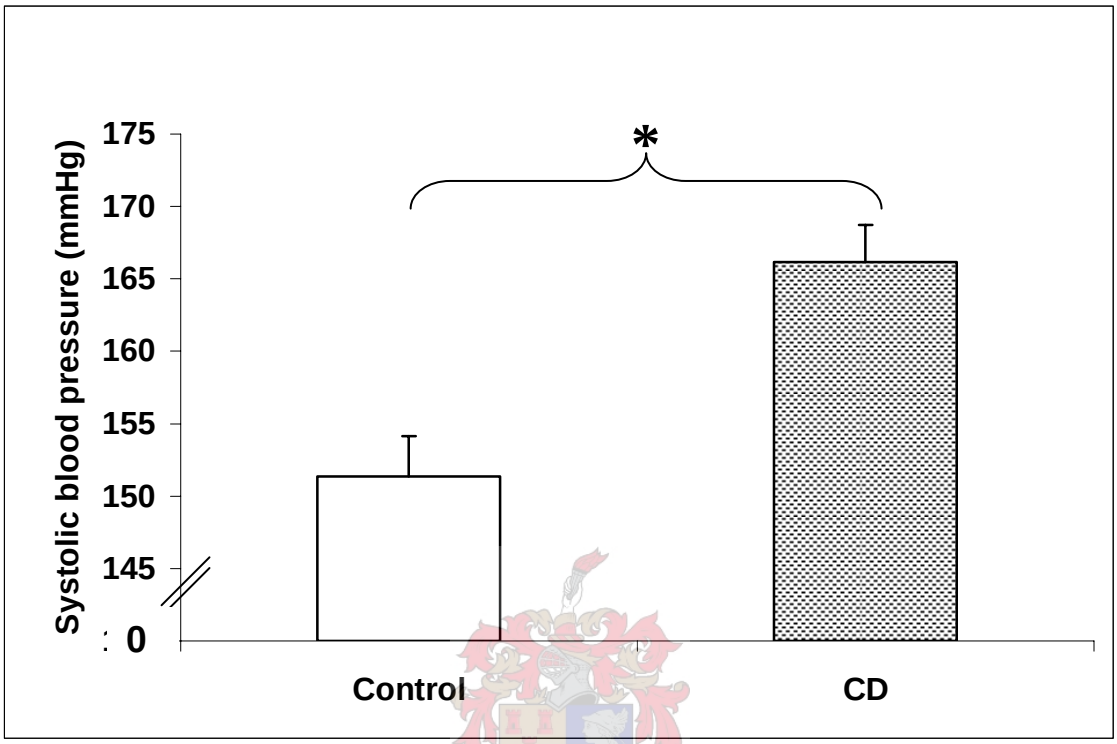


Figure 4.13: Systolic blood pressure of 16 week control and CD fed rats, as determined by the tail-cuff method. All values expressed as mean ± SEM. *CD*-cafeteria diet.

***p<0.001; n = 14-19**

Systolic blood pressure was significantly elevated in the 16 week CD fed rats compared to their control littermates (166.16 ± 2.55 mmHg vs. 151.36 ± 2.77 mmHg; $p<0.05$) (figure 4.13). Although the blood pressure was elevated, the absence of a definition for hypertension in the rat prevents us from determining whether they are indeed hypertensive.

4.2.1.9 Ventricular Morphology

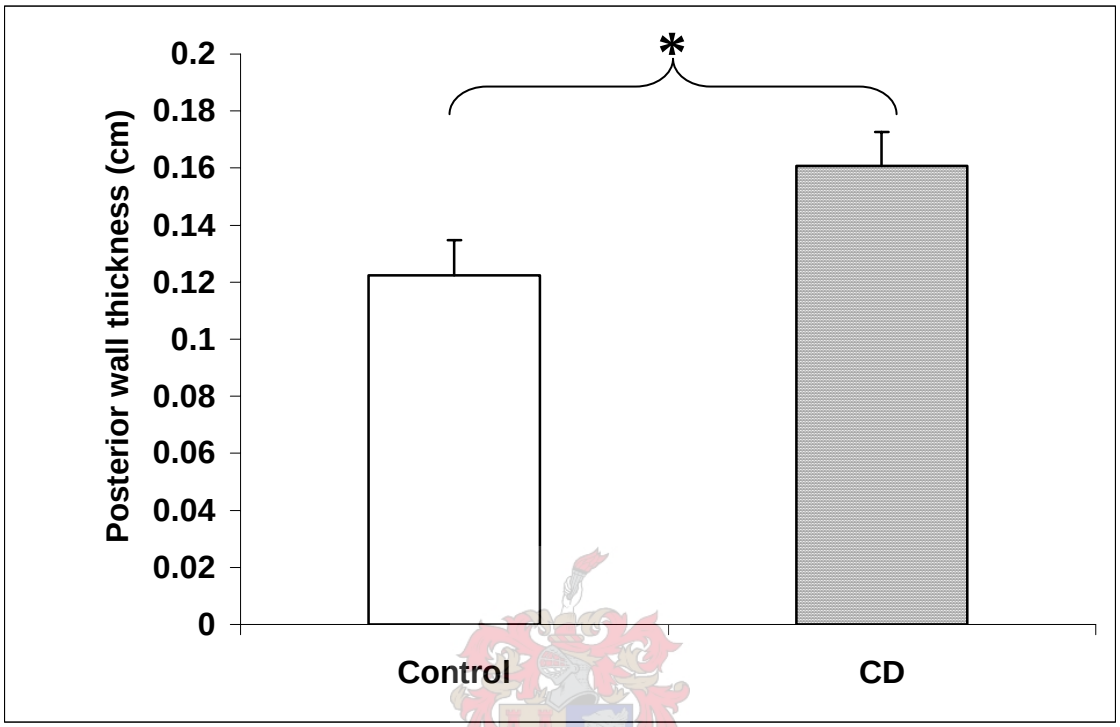


Figure 4.14: Diastolic ventricular posterior wall thickness of control and CD fed rat hearts after 16 weeks feeding, as determined by echocardiography. All values expressed as mean ± SEM. *CD*-cafeteria diet

***p<0.05; n = 8**

After 16 week on the respective diets, left ventricular morphology was assessed using echocardiography (figure 4.14). CD fed rats had a significantly thicker ventricular posterior wall during diastole, compared to the control rats (0.16 ± 0.01 cm vs. 0.12 ± 0.01 cm; $p<0.05$). This comparison provided direct evidence to support left ventricular wall hypertrophy in the CD fed rats.

4.2.1.10 Myocyte size

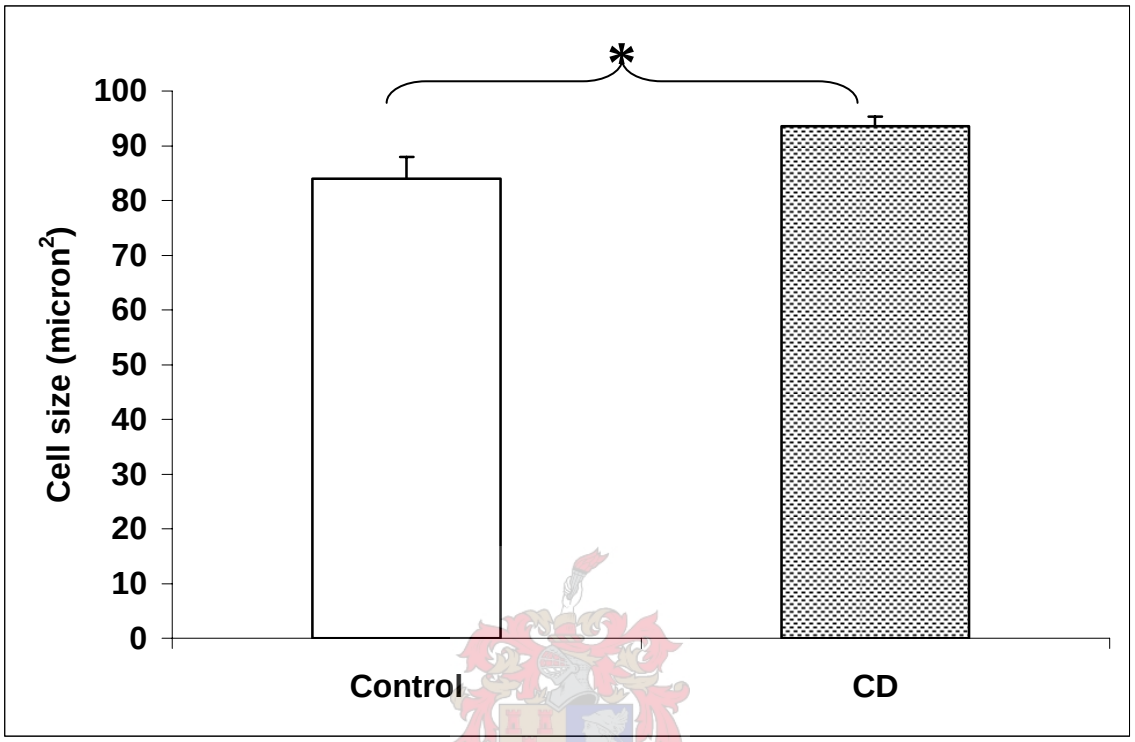


Figure 4.15: Myocyte size of 16 week control and CD fed rats as determined by using light microscopy. Values presented as means ± SEM. *CD*-cafeteria diet

***p<0.05; n = 8.**

Interventricular septum samples were collected after 16 weeks on the diets. Interventricular myocytes were significantly larger in the CD hearts than myocytes from the control group (93.53 ± 1.78 micron^2 vs 84.03 ± 3.96 micron^2; p<0.05) (figure 4.15).

4.2.2: Functional Data

4.2.2.1: Ex vivo functional data

4.2.2.1.1: Myocardial function

Table 4.4: Myocardial mechanical function of ex vivo hearts from the 16 week, control and CD fed rats. Baseline myocardial parameters were measured after 20 minutes working heart perfusion. Reperfusion parameters were measured during the post-ischaemic working heart mode after 20 minutes reperfusion.

	16 weeks	
	Control	**CD**
	Baseline	
AO (ml / min)	41.29 ± 1.15	30.57 ± 1.75$^{\&}$
CF (ml/min)	18.36 ±0.64	18.00 ± 0.59
ADP (mmHg)	66.68 ± 0.93	65.85 ± 0.57
ASP (mmHg)	101.07 ± 1.21	97.85 ± 1.75
HR (beats/min)	234.722 ± 6.45	226.00 ± 2.90
	Reperfusion	
AO (ml / min)	29.43 ± 1.79$^{\#}$	14.97 ± 2.07$^{\&@}$
CF (ml/min)	15.93 ± 0.77$^{\#}$	18.86 ± 1.36
ADP (mmHg)	65.43 ± 0.73	68.19 ± 0.30*$^{\wedge}$
ASP (mmHg)	95.63 ± 1.36$^{\wedge}$	89.91 ± 2.23$^{\#}$
HR (beats/min)	215.44 ± 11.91	227.67 ± 4.36

***ADP*-aorta diastolic pressure; *AO*-aortic output; *ASP*-aorta systolic pressure; *CD*-cafeteria diet; *CF*-coronary flow; *HR*-heart rate.**

All values expressed as means ± SEM.

***p<0.05, $^{\&}$p<0.001, for control vs. CD**

$^{\wedge}$p<0.05, $^{\#}$p<0.001, $^{@}$p<0.0001 for baseline vs reperfusion within respective groups

n = 5-8.

Myocardial function (control vs. CD rats).

CD fed rats had poorer baseline and reperfusion AO's when compared to their controls ($p<0.05$) after 16 weeks of feeding (table 4.4). Only reperfusion ADP was significantly elevated in the CD group when compared with the control group.

There were no differences in basal and reperfusion myocardial mechanical function between the 12 and 16 week CD and control diet fed rats.

Myocardial function (pre-ischaemia vs. reperfusion)

After the 16 week feeding period there were decreases in AO's during the reperfusion phase of the experiment (Table 4.4). The ischaemic insult brought about a decrease in the ASP of hearts from both the control and CD groups ($p<0.05$ and $p<0.001$ respectively). Following ischaemia, a drop in the control hearts CF was seen during reperfusion ($p<0.001$). Lastly, there was an increase in the ADP in hearts from CD fed rats ($p<0.05$) following ischaemia and reperfusion.

4.2.2.1.2 Percentage aortic output recovery

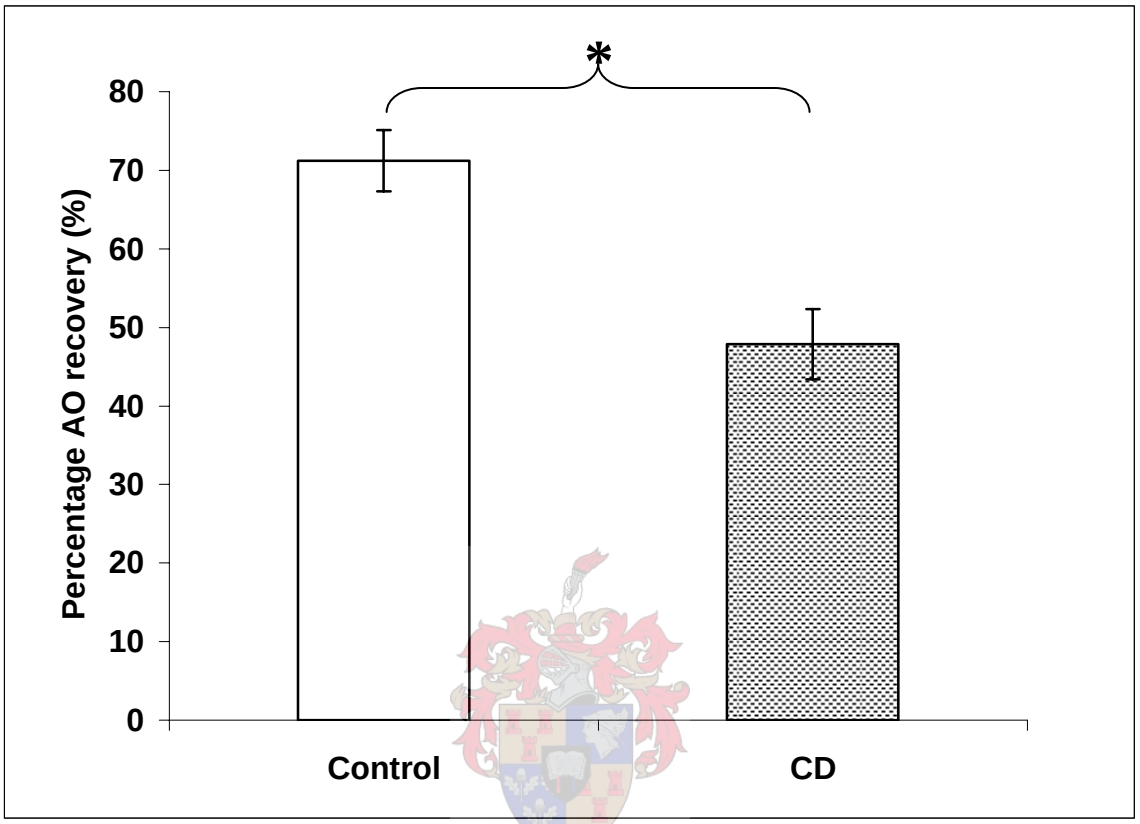


Figure 4.16: Percentage AO recoveries for the 16 week control and CD fed rats. Baseline and reperfusion AO's are expressed as mean percentages ± SEM. *AO*-aortic output; *CD*-cafeteria diet

***p<0.05; n = 8-9.**

After being subjected to 15 minutes of global ischaemia and 20 minutes reperfusion, hearts from CD fed rats recovered poorly after an ischaemic insult when compared with their respective controls (51.24 ± 5.15 % vs. 71.20 ± 3.89 %; $p<0.05$) (table 4.16).

There were no differences in the percentage aortic output recoveries when comparing the 12 and 16 week CD and 12 and 16 week control diet fed rats.

4.2.2.1.3 Ventricular Arrhythmias

4.2.2.1.3.1 Incidence of ventricular arrhythmias during ischaemia

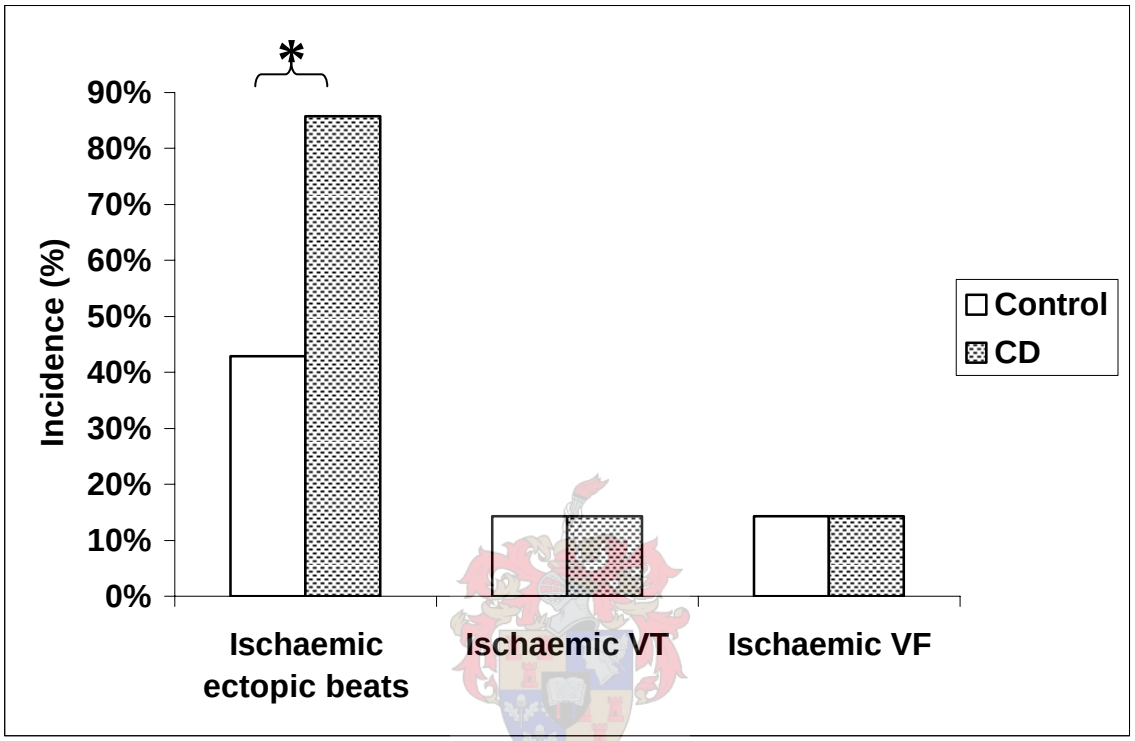


Figure 4.17: Incidence of ischaemic ectopic beats, VT and VF of hearts from 16 week control and CD fed rats. Ischaemic arrhythmias are presented as a percentage incidence during the 35 minutes of coronary artery occlusion. *CD*-control diet; *VF*-ventricular fibrillation; *VT*-ventricular tachycardia. *p<0.05; n = 7.

During the 35 minutes of coronary artery occlusion, there was a significant increase in the incidence of ectopic beats in hearts from CD fed rats compared to their controls (85.71 % vs. 42.86 %)(figure 4.17). No differences in the incidence of VT and VF were seen when comparing CD and control hearts.

4.2.2.1.3.2 Duration of ischaemic ventricular arrhythmias

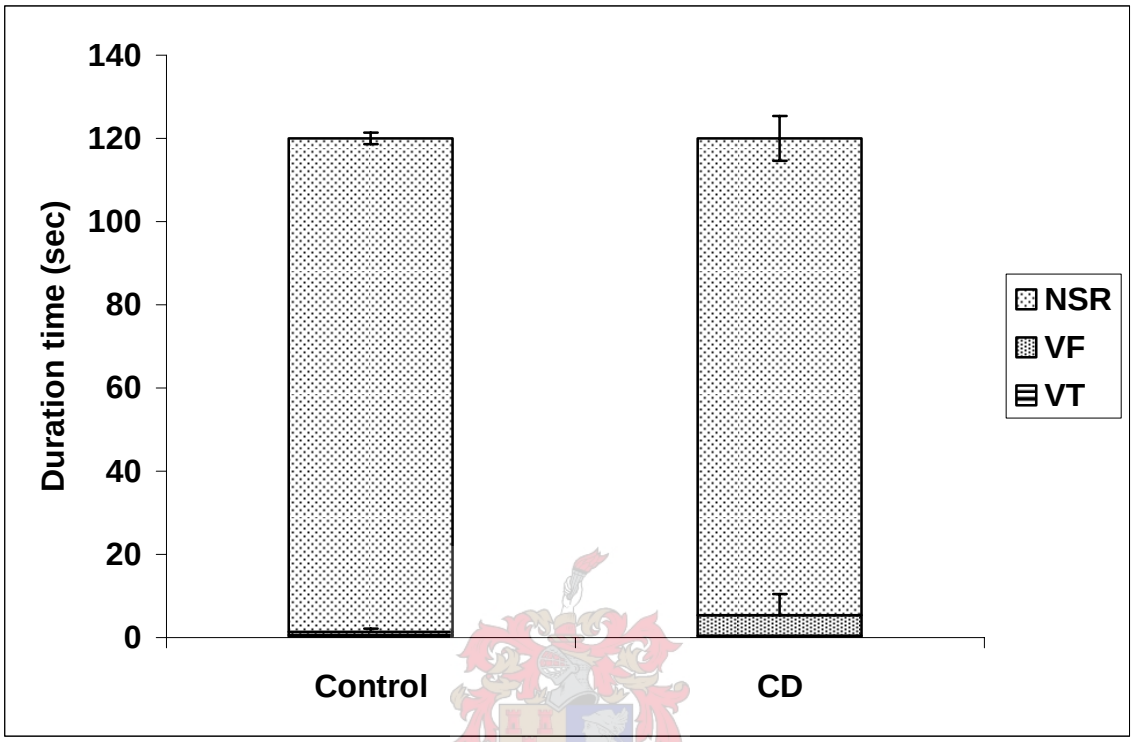


Figure 4.18: Duration of ischaemic normal sinus rhythm, VT and VF of hearts from 16 week control and CD fed rats. Values presented as means ± SEM. *CD*-control diet; *NSR*-normal sinus rhythm; *VF*-ventricular fibrillation; *VT*-ventricular tachycardia.

n = 7.

There was no difference in the duration of normal sinus rhythm or ischaemic ventricular VT and VF when comparing hearts from 16 week CD fed rats to their controls (figure 4.18).

4.2.2.1.3.3 Incidence of ventricular arrhythmias during reperfusion

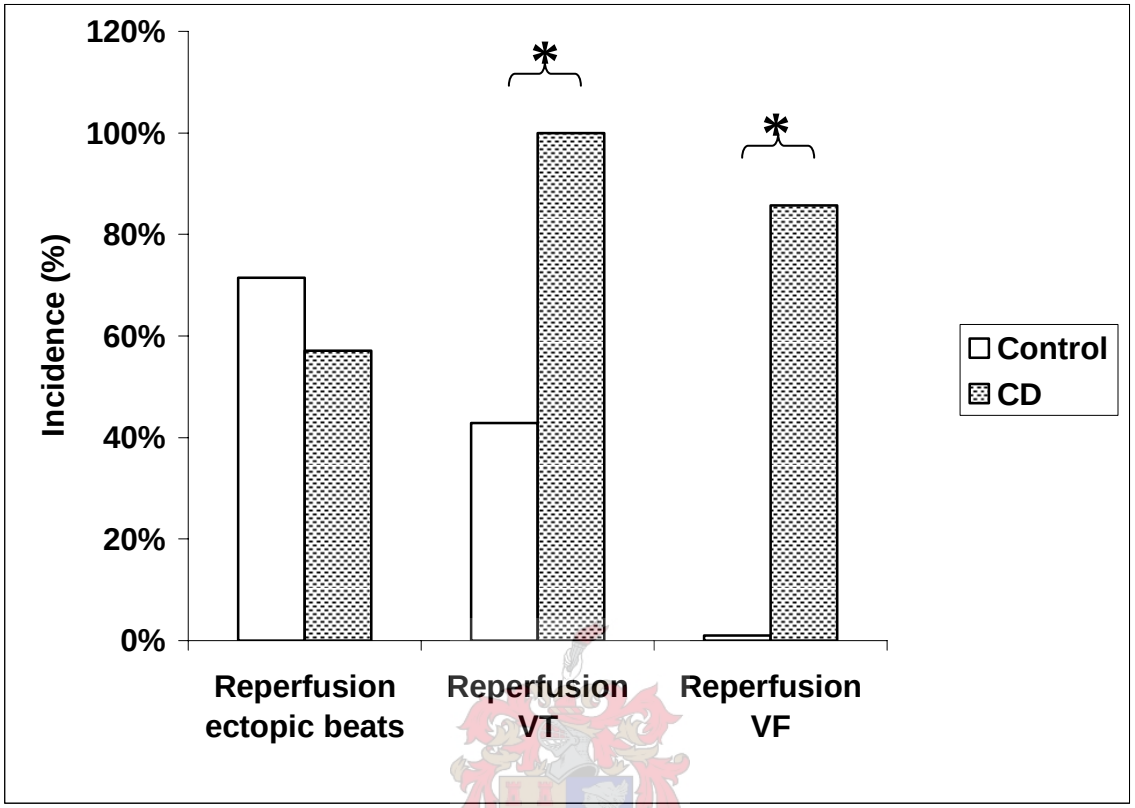


Figure 4.19: Incidence of reperfusion ectopic beats, VT and VF of hearts from 16 week control and CD fed rats. Reperfusion arrhythmias are presented as a percentage incidence during the first 5 minutes of reperfusion. *CD*-control diet; *VF*-ventricular fibrillation; *VT*-ventricular tachycardia.

***p<0.05; n = 7.**

During the first 5 minutes of reperfusion, following coronary artery occlusion, there were no differences in the incidence of ectopic beats between 16 week CD and control diet fed rats (figure 4.19). The incidence of reperfusion ventricular VT and VF was however greater during reperfusion in hearts from CD fed rats when

compared to their controls (VT: 100 % vs. 43 %, $p<0.05$; VF: 85.71 % vs. 0 %, $p<0.05$).

4.2.2.1.3.4 Duration of reperfusion ventricular arrhythmias

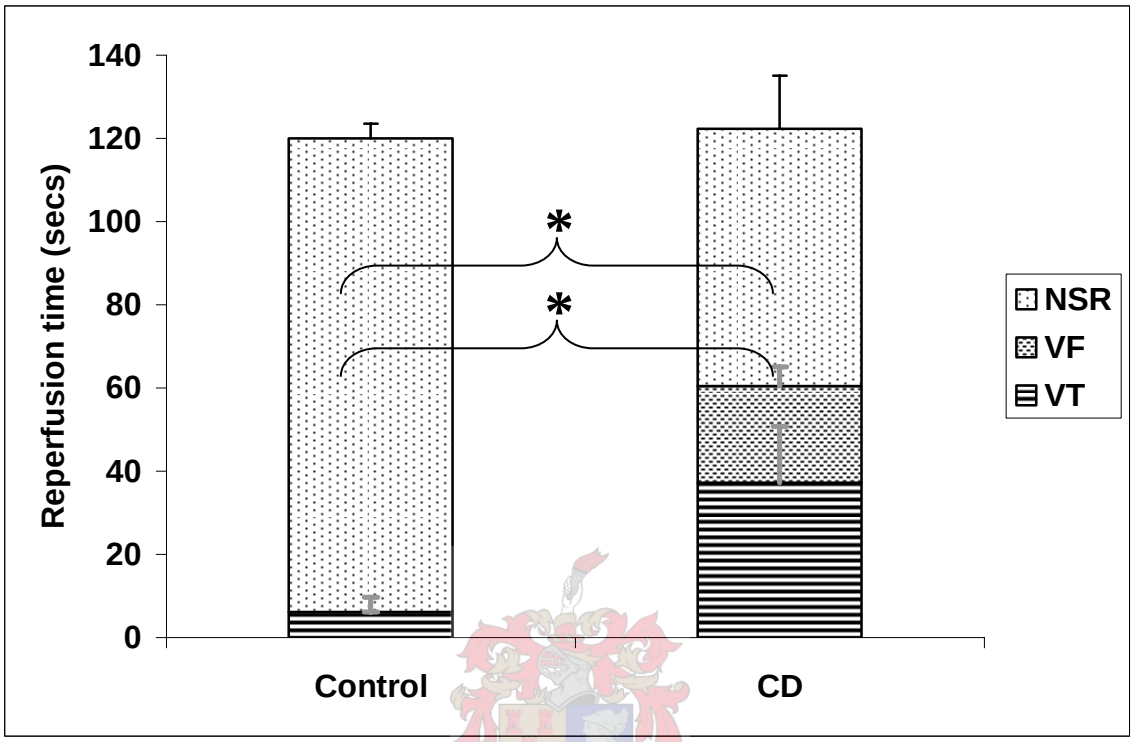


Figure 4.20: Duration of reperfusion normal sinus rhythm, VF and VT of hearts from 16 week control and CD fed rats. Values presented as means ± SEM. *CD*-control diet; *NSR*-normal sinus rhythm; *VF*-ventricular fibrillation; *VT*-ventricular tachycardia.

***p<0.05; n = 7.**

During the first 2 minutes of reperfusion the duration of reperfusion induced ventricular arrhythmias of hearts from CD fed rats was increased compared to hearts from control rats (figure 4.20). This was indicated by the significantly increased duration of VT and VF in CD fed rat hearts in comparison to their

controls (VT: 37.29 ± 13.53 sec vs. 6.14 ± 3.60 sec, $p<0.05$; VF: 23.14 ± 4.62 sec vs. 0 ± 0 sec, $p<0.05$).

4.2.2.2 In vivo myocardial function and morphology

Table 4.5: Myocardial function and morphology for 16 week control and CD fed rats as determined by echocardiography.

	16 weeks	
	Control	**CD**
FSend (%)	41.74 ± 2.21	39.86 ± 2.06
FSmid (%)	21.32 ± 4.82	24.14 ± 2.03
PWthick (%)	154.48 ± 31.02	76.60 ± 10.41*
EDD (cm)	0.82 ± 0.02	0.89 ± 0.03*
ESD (cm)	0.48 ± 0.02	0.53 ± 0.01*
PWTdiast (cm)	0.12 ± 0.01	0.16 ± 0.01*
PWTsyst (cm)	0.26 ± 0.02	0.26 ± 0.01

***CD*-cafeteria diet; *EDD*-end diastolic-diameter; *ESD*-end systolic diameter; *FSend*-endocardial fractional shortening; *FSmid*-mid wall fractional shortening; *PWTdias*-posterior wall thickness during diastole; *PWTsyst*-posterior wall thickness during systole; *PWthick*-posterior wall thickening.**

***p<0.05; n = 6-8**

Assessment of the in vivo myocardial function revealed no differences in the percentage FSend and FSmid between 16 week CD and control fed rats (table 4.5). However the percentage PWthick was significantly lower in the CD fed rats ($p<0.05$). Despite this, the in vivo mechanical function can be considered to be normal in the CD fed rats.

Myocardial morphology was altered in the CD fed rats. EDD, ESD and PWTdias was increased in the CD fed rats when compared to hearts from control diet fed rats ($p<0.05$) (table 4.5).

4.2.3 Biochemical data

4.2.3.1 Serum angiotensin II levels after 16 weeks on the diets

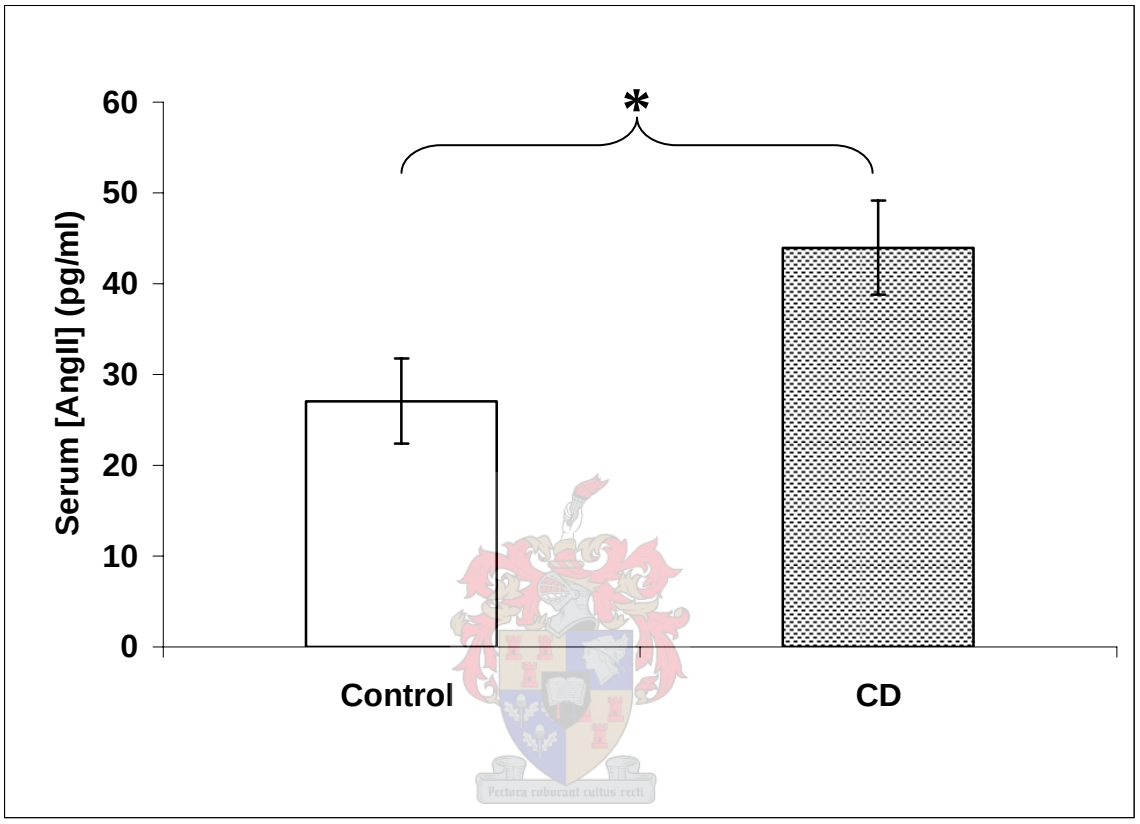


Figure 4.21: Serum Ang II levels after 16 weeks on the control and CD. Values presented as means ± SEM. *Ang II*-angiotensin II; *CD*-cafeteria diet. *p<0.05; n = 10

Serum levels of Ang II were elevated in 16 week CD fed rats when compared to their control littermates (43.96 ± 5.18 pg/ml vs. 27.07 ± 4.66 pg/ml, $p<0.05$) (figure 4.21).

4.2.3.2 Myocardial angiotensin II levels tissue after 16 weeks on the diets

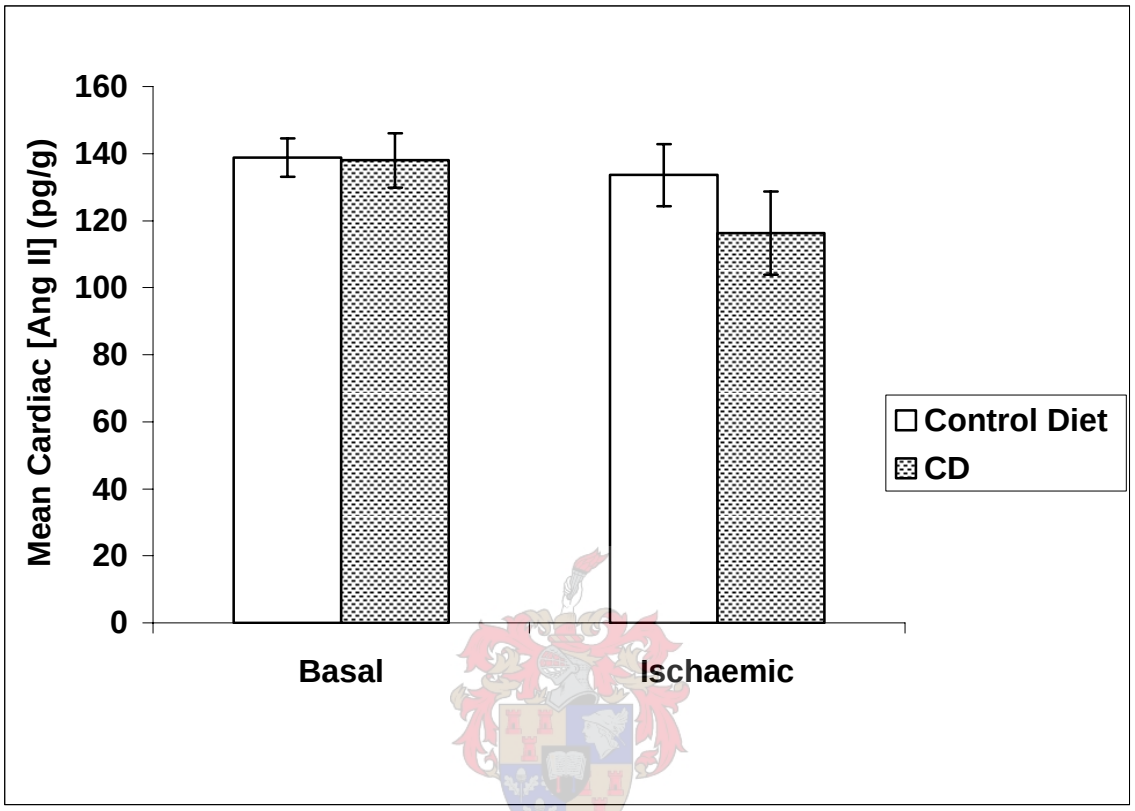


Figure 4.22: Myocardial Ang II levels before and at the end of 15 minutes of global ischaemia in hearts from 16 week control and CD fed rats. *Ang II* angiotensin II; *CD*-cafeteria diet.

n = 6-8

There was no difference in the basal myocardial Ang II content from 16 week CD and control fed rats (figure 4.22). Similarly ischaemia had no effect on myocardial Ang II content of either CD or control diet fed rats.

4.2.3.3 Serum endothelin-1 levels after 16 weeks on the diets

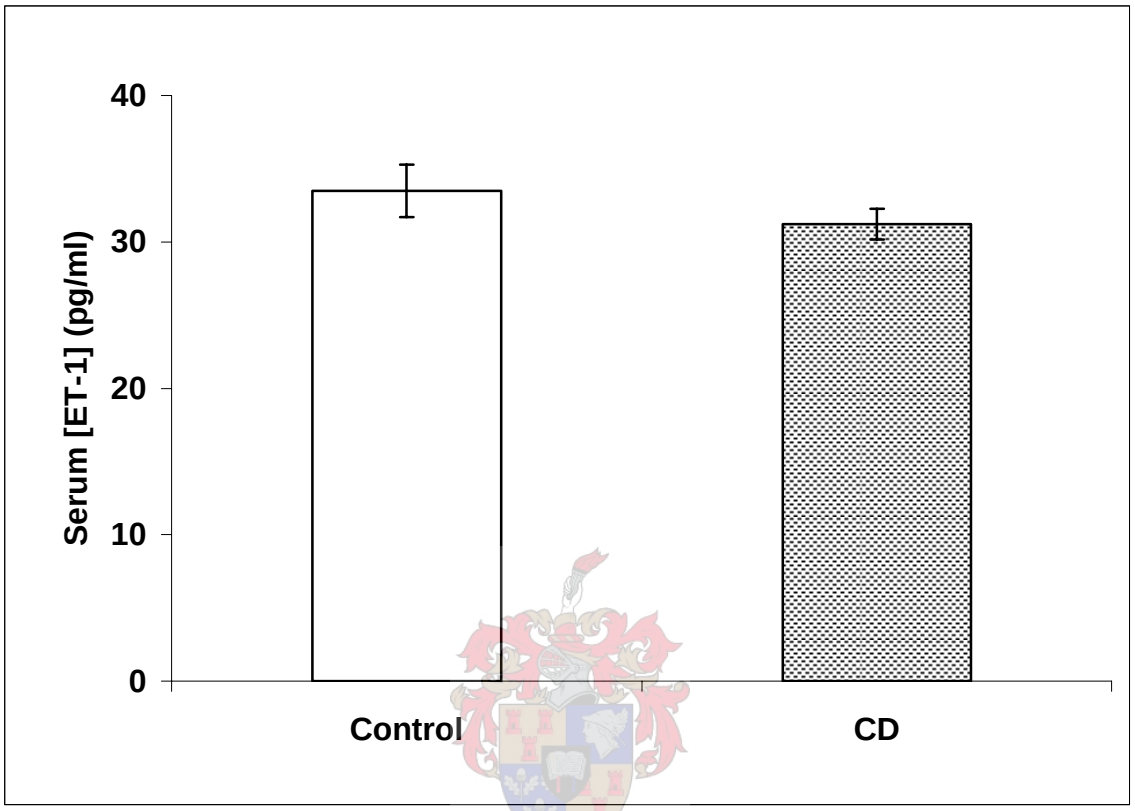


Figure 4.23: Serum ET-1 levels of 16 week control and CD fed rats. Values presented as means ± SEM. *CD*-cafeteria diet; *ET-1*-endothelin-1. n = 9-10.

After 16 weeks of feeding, serum ET-1 levels were still comparable between CD rats and their control counterparts (31.21 ± 1.04 pg/ml vs. 33.49 ± 1.79 pg/ml) (figure 4.23).

4.2.3.4 Myocardial endothelin-1 levels after 16 weeks on the diets

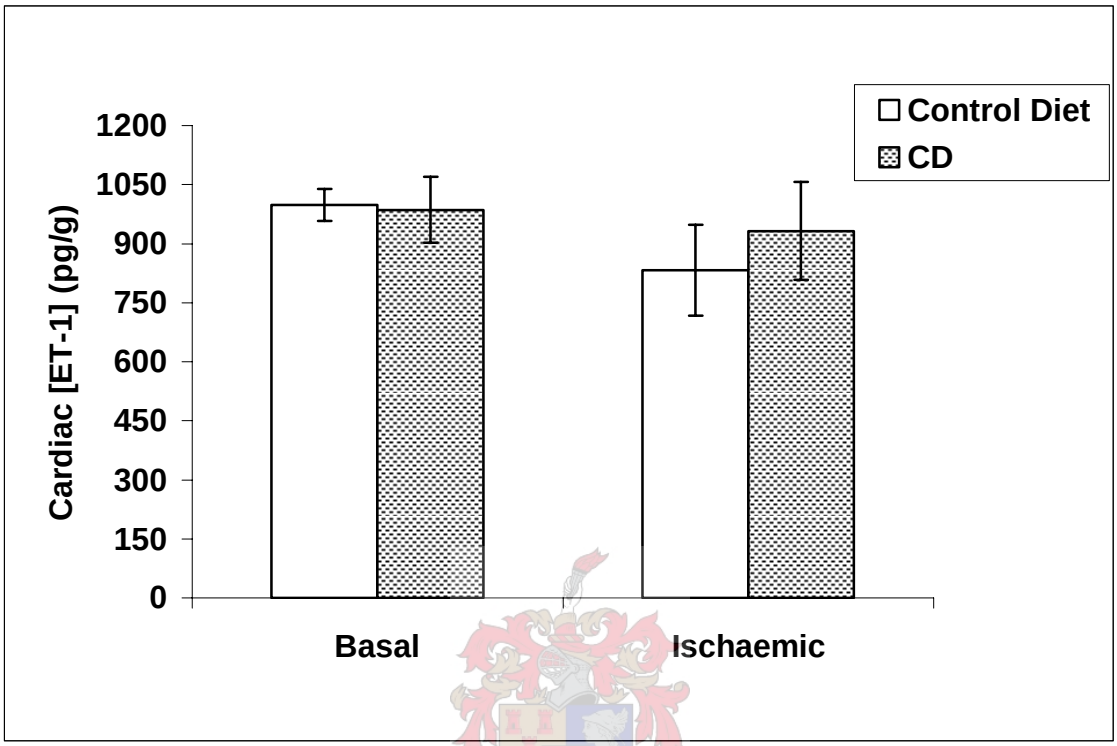


Figure 4.24: Myocardial ET-1 levels before and at the end of 15 minutes of global ischaemia in hearts from 16 week control and CD fed rats. *CD*-cafeteria diet; *ET-1*-endothelin-1.

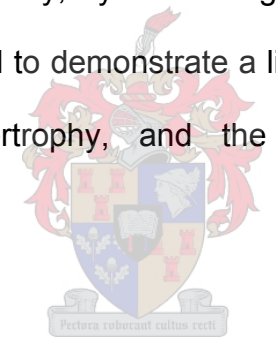
n = 5-6.

Cardiac tissue ET-1 content was not different in hearts from 16 week fed CD rats when compared to hearts from their control littermates before and after ischaemia (figure 4.24). Similarly there were no differences in cardiac tissue ET-1 content between hearts of both CD groups and control groups after the ischaemic insult.

CHAPTER 5

DISCUSSION

We set out to develop a rodent model of diet induced obesity and the MS. After determining the optimum feeding period on a CD, we characterized our model of obesity in terms of the working definition of the MS described by the WHO (Alberti and Zimmet, 1998). Once we had established a suitable model of the MS, we investigated how obesity and the abnormalities associated with the MS, affected myocardial morphology, function, and susceptibility to ischaemia and reperfusion induced injury. Finally, by measuring serum and myocardial Ang II and ET-1 levels, we attempted to demonstrate a link between the levels of these peptides and cardiac hypertrophy, and the myocardial susceptibility to ischaemia/reperfusion injury.



In this study, we successfully developed and characterized a model of diet induced obesity and the MS. Our model displayed visceral obesity, insulin resistance, hyperinsulinaemia, dyslipidaemia, elevated systolic blood pressure and cardiac hypertrophy. Obesity and the associated MS increased myocardial susceptibility to ischaemia/reperfusion injury as reflected by a poorer AO recovery and a higher incidence and duration of reperfusion ventricular arrhythmias. Based on our findings we also propose that elevated serum Ang II levels may contribute to the myocardial hypertrophy in our model of obesity.

Obesity is reaching epidemic proportions world wide, and South Africa is no exception. Recently it was shown that 29.2 % of men and 56.6 % of women in South Africa are overweight or obese (Puoane *et al.* 2002). The clinical significance of obesity was illustrated by Kenchaiah *et al.* (2002) who showed that it is an independent risk factor for heart failure. Additionally, obesity is thought to play a crucial role in the development of the MS, and is considered to be one of the potential starting points of the syndrome (NCEP ATPIII, 2001; Reaven, 2005). Due to the high prevalence of obesity in South Africa there is a risk that obese South Africans may develop the MS. The MS can be seen as a cluster of cardiovascular risk factors which aggregate together. Middle aged men with the MS have a fourfold increased risk for developing coronary heart disease, together with an increased risk for all cause mortality, mortality due to cardiovascular disease and type 2 diabetes (Lakka *et al*. 2002). The risk of cardiovascular disease increases proportionately with the number of components of the MS present in the patient (Klein *et al*. 2002). Although the MS is associated with these cardiovascular abnormalities more information concerning the effects of this syndrome on the heart during both normal and pathophysiological conditions such as myocardial ischaemia is required.

5.1 Characterization of a rodent model of the metabolic syndrome

The MS is multifactorial. We characterized our model according to the definition proposed by the WHO, and not the NCEP ATPIII definition. This definition was chosen as the WHO definition is thought to be more suitable as a research tool (Eckel *et al*. 2005).

Briefly the WHO requires that the patient has any one of the following (Alberti and Zimmet, 1998):

- Impaired glucose tolerance, or diabetes mellitus or insulin resistance (under hyperinsulinaemic, euglycaemic conditions, with glucose uptake below the lowest quartile for the population under investigation).

Together with any two of the following:

- High BMI
- Central obesity
- Dyslipidaemia
  - Raised serum TG's
  - Low HDL cholesterol
- Raised arterial blood pressure
- Microalbuminurea

Although the ZDF rat is a prominent rodent model of obesity, it is genetically manipulated and does not represent a true model of hyperphagia induced obesity

and the MS. Due to the growing epidemic of obesity in South Africa we chose a diet to induce obesity by hyperphagia, as this would be more appropriate to the current global scenario where overeating and inactivity is the main cause of obesity and the MS. Obesity in our model was induced by the consumption of a CD, which consisted of 65 % carbohydrates (which includes 7 % sucrose), 19 % fat and 16 % protein. One of the primary objectives of the present study was to establish whether obesity, would lead to the metabolic abnormalities characteristic of the MS.

5.1.1 12-week model

After 12 weeks of feeding CD fed rats were obese, but were not insulin resistant, one of the key characteristics of the MS. In addition, hearts from the 12 week CD fed rats were not more susceptible to an ischaemic insult than hearts from their control littermates. We therefore decided to characterize our model of diet induced obesity after 16 weeks of feeding to confirm that they had suitably elevated body weights and had adopted other characteristics of the MS.

5.1.2 16 week model

By feeding male Wistar rats a CD for 16 weeks, we successfully generated and characterized a model meeting the WHO criteria of the MS.

Rats on the CD displayed the following characteristics:

- Normoglycaemia as illustrated by:
    - Normal fasting and non-fasting blood glucose levels
    - Normal HbA1c values
- Insulin resistance as illustrated by:
    - Elevated non-fasting serum insulin levels
    - Reduced myocardial glucose uptake (unpublished data being prepared for publication)
- Visceral obesity
- Dyslipidaemia as illustrated by:
    - Elevated TG's
    - Low HDL cholesterol
- Elevated systolic blood pressure

CD fed rats also develop cardiac hypertrophy. Our model can therefore be considered to be a prediabetic model of the MS after 16 weeks of feeding. MS in our model was in all likelihood induced by the obesity, as 12 weeks on the CD led to obesity but not insulin resistance. Insulin resistance was only noted after

16 weeks of feeding in the CD fed rats. These data suggest that the insulin resistance was secondary to obesity.

5.2 How does this model compare with others?

Previously, the consumption of high carbohydrate diets has been associated with many metabolic disturbances. High sucrose containing diets administered to animals have been associated with a variety of effects depending on the model. These include visceral obesity, insulin resistance, hyperinsulinaemia, hyperglycaemia, hypertriglyceridaemia, elevated FFA, high myocardial TG content, lowered HDL cholesterol, and hypertension (Reaven *et al*. 1979; Lombardo *et al.* 1983; Chicco *et al*. 1991; Pagliassotti *et al*. 1996; Baños *et al*. 1997; Soria *et al*. 2001; Fukuchi *et al*. 2004; Kamgang *et al*. 2005). Rats fed primarily a fructose diet, develop similar characteristics (Hwang *et al*. 1987; Kobayashi *et al*. 1993; Bezerra *et al*. 2000; Kamide *et al*. 2002; Ryu and Cha, 2003; Morel *et al*. 2005). Obesity may also develop in these models, but only manifests after an extended feeding period, long after the development of insulin resistance which would suggest that obesity is not the primary cause of the other abnormalities (Chicco *et al*. 2003; Fukuchi *et al*. 2004).

The metabolic features described in our model are comparable with findings from previous studies making use of high carbohydrate diets (Reaven *et al*. 1979; Lombardo *et al.* 1983; Hwang *et al*. 1987; Chicco *et al*. 1991; Kobayashi *et al*. 1993; Pagliassotti *et al*. 1996; Baños *et al*. 1997; Bezerra *et al*. 2000; Kamide *et*

al. 2002; Ryu and Cha, 2003; Fukuchi *et al*. 2004; Morel *et al*. 2005). Certain studies using the fructose (66 % fructose enriched diet) or sucrose (30 % sucrose in drinking water) fed rat model, meet the WHO criteria for MS (Hwang *et al*. 1987; Baños *et al*. 1997). None of these studies are however relevant or comparable to ours as the metabolic changes occur in the absence of obesity (Reaven *et al*. 1979; Thresher *et al.* 2000; Chicco *et al*. 2005).

Studies employing the same CD model as ours have also reported most of the metabolic disturbances described in this study. With the exception of Naderali *et al*. (2001a), none of the other studies making use of the CD rat model have investigated the model in the context of the MS (Pickavance *et al*. 1999; Naderali *et al*. 2001b; Holemans *et al*. 2004; Du Toit *et al*. 2005). Rats from the one study of Naderali *et al*. (2001a) did not meet the WHO criteria for MS. This could possibly be attributed to the feeding program being too short, which extended for only 12 weeks. Data from our study show that 12 weeks of feeding would be insufficient to produce all the metabolic abnormalities required to satisfy the WHO definition of the MS. CD fed rats in the study by Holemans *et al.* (2004) were hyperinsulinaemic and hyperglycaemic after 4 weeks of feeding. These data are interesting as the experiments were performed on female Wistar rats, and although it is not known whether gender affects susceptibility to diabetes or glucose intolerance, the changes occurred earlier than in our study with male rats.

There are some discrepancies between our findings and those of others using the same model with respects to metabolic abnormalities and blood pressure. These will be dealt with individually.

5.2.1 Insulin resistance and diabetes

The mechanism of the development of insulin resistance is poorly understood, but may be initiated by obesity (Bogardus *et al.* 1985). Insulin resistance over a period of time may progress to type 2 diabetes (Charles *et al.* 1991).

Obesity in our study probably lead to the development of insulin resistance after 16 weeks as after 12 weeks, CD fed rats were obese in the absence of hyperinsulinaemia. Studies supplementing the diet with vast amounts of carbohydrates have been able to induce insulin resistance in rats (Lombardo *et al*. 1983; Pagliassotti *et al*. 1996). These diets however produced insulin resistance in the absence of obesity and are therefore not comparable to our study. This insulin resistance may be due to direct adverse effects of the diet as rats developed insulin resistance within 3-5 weeks in these studies (Reaven *et al*. 1979; Thresher *et al.* 2000; Chicco *et al*. 2005). A sucrose dose dependant effect was shown by Reiser *et al*. (1981). Carbohydrate sensitive individuals consumed diets providing either 5, 18, and 33% of the calories as sucrose for six weeks. Fasting insulin levels increased proportionally with the sucrose content of the diet, and subjects consuming the largest amounts of sucrose developed signs of glucose intolerance. The findings of Reiser *et al*. (1981) and data from animal

studies (Reaven *et al*. 1979; Thresher *et al.* 2000; Chicco *et al*. 2005) suggest that high levels of simple sugars elicit an increased pancreatic insulin secretion, which over time may develop into insulin resistance. In fact in a recent review, Bessesen (2001) stated that the adverse effects of sucrose and fructose diets are due to a combination of the dose and the exposure to the diet.

In contrast, studies making use of the same CD rodent model, were able to induce obesity, however these CD fed rats did not display elevated fasting insulin levels (Pickavance *et al*. 1998; Naderali *et al*. 2001a; 2000b; Du Toit *et al.* 2005). An age related effect may explain these different observations. The combined effect of diet and age on certain metabolic parameters was investigated by Pagliassotti *et al*. (2000). Rats of different ages (5, 10, 18, or 58 weeks) were placed on a high sucrose diet for 5 weeks. The data obtained from this study showed that the age at which the diet was introduced significantly affected the metabolic parameters, especially fasting serum insulin levels, after the 5 week feeding period. There was a significant step wise increase in fasting serum insulin levels with age (except in the 58 week old group, which was slightly less than the 18 week old group). The findings of Pagliassotti *et al*. (2000) suggest that differences in age at the onset of the diet may account for the discrepancies seen in serum insulin levels between our study and others using the same model.

Caution should be taken when interpreting our findings as we measured non-fasting insulin levels. The method used to measure serum insulin levels also differed between the various studies described in this section.

Both fasting and non-fasting blood glucose measurements revealed that CD fed rats in our study, were normoglycaemic. Other studies have also confirmed our findings that the CD model does not affect fasting blood glucose levels (Pickavance *et al*. 1999; Naderali *et al*. 2001a, 2001b).

Data from our lab using the same model (Du Toit *et al*. 2005) found an increase in blood glucose levels in the CD fed rats. This is contrary to the current study and that of other studies making use of the CD rodent model. These differences in fasting blood glucose levels may once again be due to the early initiation of the diet in that particular study as rats were placed on the diet directly after being weaned.

To confirm that our rats were not diabetic we determined the HbA1c in rats from both groups. HbA1c is a clinical test used to assess blood glucose management 3 months prior to the test. Although there were subtle differences in the percentage glycosylation of the hemoglobin between CD and control diet fed rats, these values remained in the normal range, which is below 6 %.

5.2.2 Lipid profiles

As expected with obesity, CD fed rats in our study developed dyslipidaemia characterized by elevated TG's and decreased total cholesterol and HDL-cholesterol in the serum. Du Toit *et al*. (2005) found similar alterations in serum lipid levels with comparable decreases in serum total cholesterol and HDL-cholesterol, together with elevated serum TG levels in the CD fed rats. Pickavance *et al*. (1999) and Naderali *et al*. (2001a, 2000b) only measured serum TG levels in their studies, but observed similar increases in serum TG levels in their CD fed rats. Pickavance *et al*. (1999) and Naderali *et al*. (2001a, 2000b) also showed that the CD rat model is associated with elevated serum FFA levels which are thought to contribute to the progression of the MS.

Dyslipidaemia can occur as a direct result of elevated serum FFA, as seen in obesity. With an increased FFA flux to the liver there is an increased production of TG rich VLDL particles (Lewis *et al*. 1995). The elevated serum TG's may be due to accelerated VLDL-TG secretion into the serum (Mittendorfer and Sidossis, 2001) as seen after the consumption of high carbohydrate diets. Elevated serum VLDL-cholesterol has a direct effect on serum HDL-cholesterol levels. TG rich VLDL-cholesterol particles can exchange their core lipids with HDL-cholesterol, a process which is enhanced in hypertriglyceridaemic states (Rashid *et al.* 2002). Consequently the HDL-cholesterol is TG enriched, which has been shown to enhance the clearance of HDL-cholesterol from the circulation (Lamarche *et al.*

1999). Lowered levels of HDL-cholesterol are problematic as HDL-cholesterol is atheroprotective, by mediating reverse cholesterol transport from the peripheral tissue to the liver (Stein *et al*. 1999).

Recently, peroxisome proliferator-activated receptor (PPAR) agonists have shown potential in treating animal models with dyslipidaemia (Ljung *et al*. 2002; Velliquette *et al*. 2005). PPAR's are ligand activated transcription factors that belong to the nuclear receptor superfamily. One such PPAR, PPAR-α, stimulates the expression of genes that are involved in lipoprotein and fatty acid metabolism (Tenenbaum, 2004). PPAR-α has been shown to be pharmacologically activated by fibrates. This results in increased fatty acid oxidation and the attenuation of TG synthesis and VLDL secretion. Additionally PPAR-α agonists increase the production of apolipoprotein A-I, which increases HDL-cholesterol levels (Vitarius, 2005). PPAR-α agonists would thus be useful in normalizing lipid levels and preventing other pathologies such as coronary artery disease.

5.2.3 Visceral obesity

The presence of visceral obesity in our model may play a significant role in the development of the dyslipidaemic profile seen in CD fed rats from our study. Visceral adipocytes have been shown to be more susceptible to lipolytic stimuli compared to other adipocytes (Lönnqvist *et al*. 1995; Phillips *et al*. 1996). Consequently, elevated visceral adiposity would result in greater amounts of FFA release into the serum and thus affect the lipid profile.

Besides its effects on the lipid profile of a patient, visceral obesity is also thought to play an important role in the development of insulin resistance, and possibly the resulting cardiovascular complications (Evans *et al*. 1984; Nakamura *et al*. 1994; Kobayashi *et al*. 2001).

CD fed rats in our study were viscerally obese which is comparable to the findings of Naderali *et al*. (2001a, 2000b). Naderali *et al*. (2001a, 2001b) also demonstrated the presence of larger amounts of gonadal and epididymal fat masses. This increase in fat pad mass would therefore contribute significantly to the progression of the syndrome by promoting the development of dyslipidaemia and insulin resistance.

5.2.4 Systolic blood pressure

Systolic blood pressure was elevated in CD fed rats in our study when compared to control littermates. The mean systolic blood pressure for our control diet fed rats was 151.36 ± 2.77 mmHg compared to the 166.15 ± 2.55 mmHg of the CD fed rats. Du Toit *et al*. (2005) saw a similar elevation in systolic blood pressure in obese rats using the CD rat model. These are to our knowledge the first studies to demonstrate elevations in blood pressure in response to diet induced obesity in the rat.

The sucrose fed rat has been described by Baños *et al*. (1997) as being hypertensive after 12 to 17 weeks of feeding. These diet fed rats had a systolic blood pressure of 142 mmHg, which was significantly different to control rat's values. Similarly, the FFR also develops elevated systolic blood pressures after five weeks on the diet (Erlich and Rosenthal, 1995). These findings suggest that diets containing large amounts of simple sugars, may elevate systolic blood pressures in rodents.

5.3. Consequences of the metabolic syndrome: cardiac hypertrophy

The increased blood volume associated with obesity, increases both the preload and the stress on the heart. The former in turn increases cardiac output (Messerli *et al*. 1983). These obesity induced changes ultimately affect cardiac structure and function and manifest as diastolic dysfunction (Pascual *et al*. 2003) and/or cardiac hypertrophy (Opie, 1991; Paulson and Tahiliani, 1992). The heart compensates for the increased stress placed on it, so that obesity is usually associated with an increase in left ventricular mass (Messerli *et al*. 1983b). Indices recently used to quantify cardiac hypertrophy include the VW/BW (Skoumal *et al*. 2004; Asai *et al*. 2005) and the VW/TL ratio (Brede *et al*. 2003; Saupe *et al*. 2003). In recent years, researchers have also used posterior wall thickness, as determined by echocardiography, to assess cardiac morphology and dimensions (Swarz *et al*. 1998).

Using the VW/TL ratio in this study, hearts from CD fed rats displayed signs of cardiac hypertrophy (table 4.1) at both 12 and 16 weeks. Cardiac hypertrophy as assessed by the VW/TL ratio was more marked after 16 weeks on the diet. In contrast the VW/BW ratio revealed no differences between control and diet fed groups. Previous data from our lab using the VW/BW ratio support the presence of cardiac hypertrophy in the CD fed rat after 16 weeks (Du Toit *et al*. 2005). Similarly the FFR model of MS also displays cardiac hypertrophy as demonstrated by an increased VW/BW ratio when compared to control rats (Iyer *et al*. 1996a).

The VW/BW ratio in our study possibly showed no differences between the groups due to the exponential increase in relative body weight seen in the CD fed rats compared to control diet fed rats. Yin *et al.* (1982) concluded that due to age related fluctuations in weight gain, the VW/TL ratio would be a more accurate and stable index for quantifying cardiac hypertrophy in the rodent. The VW/TL ratio would therefore be a more accurate index of cardiac hypertrophy in our study as it is not influenced by changes in body weight.

Transthoracic echocardiography provides repeatable quantitative and qualitative information to assess cardiac structure and function (Schwarz *et al*. 1998). Transthoracic echocardiography was used in our study to confirm the presence of cardiac hypertrophy in CD fed rats. This was shown in the form of thicker posterior walls and larger EDD's in the CD fed rats. This suggests that thickening

of the ventricular wall in addition to left ventricular dilatation had occurred in CD fed rats. Due to the novelty of this model there are no known obese rodent models with which to compare these findings.

Echocardiography in rats is a relatively new technique, and consequently few diet studies have used this technique to investigate cardiac structure and morphology. To our knowledge this is the first study using echocardiography to determine myocardial morphometry and geometry in a diet induced obese rat model of the MS. Echocardiographic evaluations have indeed previously been used in spontaneously hypertensive rats to study left ventricular remodeling (Veliotes *et al*. 2005). In a comparative study, obese (diabetic and hypertensive) rats had significantly thicker posterior walls and larger EDD's compared to control rats (Schwarz *et al*. 1998). Although the echocardiographic results obtained by Schwarz *et al*. (1998) yielded similar findings to our study, the obese rats used by Schwarz *et al*. (1998) had diabetes and hypertension. The study by Schwarz *et al*. (1998) does provide valuable information, and suggest that cardiac hypertrophy in our model may be due to a combination of obesity and the elevated systolic blood pressure. In fact Messerli *et al*. (1983b) conducted echocardiographic analysis on lean and obese patients that were either hypertensive or normotensive. Obesity was seen to be associated with an increased left ventricular diameter which can be considered to be indicative of eccentric left ventricular hypertrophy, while hypertension was associated with an

increased posterior wall thickness, which can be considered to be indicative of concentric left ventricular hypertrophy.

As CD fed rats in our study have thicker posterior walls and larger EDD's, evidence from the literature (Messerli *et al*. 1983b; Schwarz *et al*. (1998) suggests that cardiac hypertrophy in our CD fed rats was induced by a combination of obesity and an elevated systolic blood pressure.

5.4 The involvement of Angiotensin II and Endothelin-1 in the development cardiac hypertrophy

Circulating Ang II and ET-1, which are elevated in obesity (Giacchetti *et al*. 2002; Glowinska *et al*. 2004), are well known for their growth promoting effects in cardiomyocytes and are considered to play a role in the development of cardiac hypertrophy (Sadoshima and Izumo, 1993; Ito *et al*. 1993). Elevated levels of these peptides in the myocardium are also associated with cardiac hypertrophy (Baba *et al*. 2000; Baker *et al.* 2004). It is thought that in conditions in which these growth promoting peptides are upregulated, there is a possibility that they would increase the chances of developing cardiac hypertrophy. The growth promoting effects of Ang II may further be enhanced by the fact that Ang II can upregulate the formation of ET-1, which is also thought to promote growth (Ito *et al*. 1993).

5.4.1 Angiotensin II

The present study was the first to investigate myocardial Ang II levels in a dietary obese model of the MS. We did not find an increase in basal myocardial Ang II levels in hypertrophied hearts from CD fed rats after either 12 or 16 weeks on the diet. Serum Ang II levels of CD fed rats were elevated after 16 weeks on the diet. Our myocardial and serum Ang II data however support data from a previous study performed by our group (Du Toit *et al.* 2005). None of the other studies using the CD rat model to induced obesity have investigated serum and tissue Ang II content (Pickavance *et al*. 1999; Naderali *et al*. 2001a, 2001b).

Despite the absence of differences in myocardial Ang II content, Du Toit *et al*. (2005) indicated that the peptide might play a role in the development of cardiac hypertrophy in the CD rat model. Treatment of the CD fed rats with the AT_1 receptor antagonist, losartan was shown to significantly reduce the degree of cardiac hypertrophy in these rats when compared to untreated CD fed rats.

We did however find that hearts from the 12 week CD fed rats were hypertrophic but that their serum Ang II levels were not yet elevated. These data suggest that there may be additional stimuli besides the elevated Ang II that contribute to the development of cardiac hypertrophy. Ang II is however a potent vasoconstrictor and plays a role in the development of hypertension (Silverthorn, 2004). Hypertension itself is a stimulus for cardiac hypertrophy (Messerli *et al*. 1983b;

de la Maza *et al*. 1994). Ang II may therefore contribute to the elevated systolic blood pressure seen in our model.

The FFR, a model of MS, is also characterized by elevated serum Ang II levels, cardiac hypertrophy and a high density of AT_1 receptors on the myocardial membrane (Iyer *et al*. 1996b). Treatment of the FFR with olmesartan, an AT_1 receptor antagonist, attenuated the development of cardiac hypertrophy in these rats, suggesting a role for elevated serum Ang II in the development of cardiac hypertrophy (Kamide *et al.* 2002).

Significantly, in the studies of Kamide *et al*. (2002) and Du Toit *et al*. (2005) the attenuation in cardiac hypertrophy following drug treatment was accompanied with the normalization of systolic blood pressure in their experimental models. This suggests that both blood pressure and direct Ang II signaling on the myocardium played a role in the reduction of cardiac hypertrophy. These findings suggest that cardiac hypertrophy in our model may be due to a combined effect of elevated blood pressure and obesity.

5.4.2 Endothelin-1

CD fed rats in our study did not have elevated serum or myocardial ET-1 levels. None of the other studies using the CD rat model have attempted to measure ET-1 levels in either the serum or the myocardium. Our data suggests that ET-1 did not play a role in the development of cardiac hypertrophy in our model.

The FFR model of MS, which displays cardiac hypertrophy has however been shown to have elevated serum ET-1 levels (Juan *et al*. 1998), but the influence of ET-1 antagonism in the development of cardiac hypertrophy has not been investigated in the FFR model. Therefore due to the lack of intervention studies, the involvement of ET-1 in the development of cardiac hypertrophy in animals or subjects with the MS remains unknown.

5.5 Obesity and basal cardiac function

Myocardial mechanical function may be elevated in obese individuals as obese individuals have a higher cardiac output when compared to non-obese individuals (Messerli *et al.* 1983a). Obesity and the compensated cardiac hypertrophy is however associated with diastolic filling abnormalities, (Corin *et al*. 1991; Nunez *et al*. 1994; Andren *et al*. 1996). Despite this, systolic function may to be augmented in obese individuals (Berkalp *et al*. 1995; Pascual *et al*. 2003), however this view has recently been challenged with the development of more sensitive techniques with which to measure myocardial function (Wong *et al*. 2004; Peterson *et al*. 2004)

When isolated rat hearts were perfused in our study, hearts from CD fed rats generated a significantly lower AO compared to control hearts. These findings support previous data from our lab (Du Toit *et al*. 2005). Because our CD model induces obesity and the MS, few comparisons can be made with our data. The

data obtained from the isolated perfused hearts are interesting as they are contrary to what would be expected. The lowered AO's seen in the CD fed rats may be due to a defect in myocardial functioning induced by the obese state, or it may be due to the absence of factors in the perfusate that are usually present in vivo. The preload on the heart also remained constant on the perfusion apparatus. This is not the case in obesity. The volume increase in obesity causes an increase in preload on the heart and this may also increase in vivo function.

The isolated perfused heart contracts in the absence of any other substance that may influence in vivo contractility. Obesity is marked by an increased sympathetic drive (Egan *et al*. 1989). Obese CD fed rats would therefore be expected to have a higher sympathetic drive, and consequently larger amounts of catecholamines release into the circulation. Catecholamines help modulate myocardial contraction (Opie, 1991) and thus increase cardiac output. The absence of elevated catecholamines in the perfusate of the hearts from CD fed rats may account for the drop in AO seen in these hearts.

Since CD fed rats are hyperinsulinaemic, the absence of elevated insulin levels in the perfusion buffer may also contribute to the depressed basal mechanical function observed in the ex vivo experiments. Increased insulin levels, induced by insulin infusion, were shown to increases cardiac output together with heart rate in sheep fetuses (Milley, 1987). Moreover insulin is a positive inotrope (Vetter *et al*. 1988), and can increase myocardial contractility and consequently

cardiac output. These studies suggest a beneficial role for the hyperinsulinaemic condition in modulating cardiac mechanical function. Due to insulin effects on myocardial contractility, the absence of insulin from the perfusate in our study may contribute to the decrease in AO observed in the hearts from CD fed rats.

Insulin is also known to be responsible for controlling glucose uptake by a muscle. Hypertrophied hearts have been shown to have defective insulin signaling with a resultant decreased myocardial glucose uptake (Friehs *et al*. 2005). A decreased glucose uptake in the isolated perfused hearts from CD fed rats may also affect myocardial contractility, although there are currently no studies on obesity supporting this hypothesis.

CD fed rats from our study showed no myocardial mechanical functional abnormalities when compared to control littermates as assessed by echocardiography (Table 4.5). This was reflected by normal FSend and FSmid.

Echocardiographic studies of obese individuals and animals suggest that obesity either augments myocardial fractional shortening or has no effect on it. Both Berkalp *et al*. (1995) and Pascual *et al*. (2003) found that obese individuals have an augmented myocardial fractional shortening when compared to lean individuals. Similarly, obese rats were shown to have an increased myocardial fractional shortening when compared to lean control rats (Swarz *et al*. 1998). The experiment was however complicated by the co-occurrence of hypertension and

diabetes in these obese rats. Thus systolic function seems to be normal or improved in obese individuals.

These echocardiographic data confirm our in vivo data and suggest that obesity does not adversely affect myocardial mechanical function. This further strengthens our hypothesis that the absence of circulating catecholamines and/or insulin may contribute to the decreased mechanical function seen in the isolated perfused hearts of CD fed rats. Moreover the increased preload, associated with obesity, was not controlled for in the ex vivo experiments. It may be that under similar preload conditions, CD fed rats may have decreased in vivo mechanical function compared to control diet fed rats.

Recently it has however been shown that obese individuals may indeed have adversely altered systolic function. This was shown in studies on obese individuals using updated echocardiography techniques which are pre-load sensitive (Wong *et al*. 2004; Peterson *et al*. 2004). Acquiring these techniques would be useful in the investigation of the cardiovascular effects of obesity in our model.

5.6 Ischemia/reperfusion injury

5.6.1 The role of obesity and cardiac hypertrophy in ischaemia/reperfusion injury

Obesity is a risk factor for the development of coronary artery disease (Rimm *et al*. 1995). Hearts from obese individuals may therefore be more prone to ischaemia/reperfusion injury and this could be indirectly due to abnormalities associated with obesity related cardiac hypertrophy (Galiñanes and Flower, 2004).

In our study, obesity induced hypertrophied hearts from CD fed rats were more susceptible to ischaemia/reperfusion injury when compared to hearts from control diet fed rats after 16 weeks on the diet. This was evident from the decreased functional recovery of the CD fed rat hearts during the reperfusion period. The exact mechanism for the increased susceptibility of hearts from CD fed rats to ischaemia/reperfusion injury was not established in this study. Previous findings from our laboratory indicated that treatment of CD fed rats with losartan, an AT_1 receptor antagonist, was able to reduce the extent of cardiac hypertrophy in these rats. This reduction in cardiac hypertrophy attenuated the susceptibility of the CD fed rat myocardium to ischaemia/reperfusion-induced injury (Du Toit *et al*. 2005) and would suggest that the hypertrophy may contribute to the increased susceptibility of these hearts to ischaemia and reperfusion injury.

Hypertrophied hearts have previously been shown to have exacerbated left ventricular dysfunction following ischaemia (Hearse *et al.* 1978; Gaash *et al*. 1990). Allard *et al*. (1994) also found an increased susceptibility of hypertrophied hearts to ischaemia/reperfusion injury reflected by reduced functional recovery during the reperfusion period. Morphological analyses of these hypertrophied hearts revealed no differences in irreversible ischaemic damage (necrosis) between hypertrophied and control hearts. The calcium overload that occurred during reperfusion in the hypertrophied myocardium was however 2.5 fold greater than in the control rats' myocardium. Cardiac hypertrophy may therefore predispose the myocardium to calcium overload and calcium overload may in turn induce myocardial stunning (Du Toit and Opie, 1992).

Work from our laboratory has recently shown that CD fed rat hearts have lowered basal myocardial glucose uptake (data unpublished). It has also been hypothesized that hypertrophied hearts show altered glucose energy metabolism during an ischaemic insult. Experimental evidence to support this hypothesis was presented in the form of exaggerated uncoupling of glycolysis from glucose oxidation following an ischaemic insult in a hypertrophied myocardium (Schonekess *et al*. 1996). This was in turn associated with an increased myocardial susceptibility to ischaemia/reperfusion injury (Schonekess *et al*. 1996). The hypertrophied myocardium is also associated with defective insulin signaling, which restricts insulin-mediated GLUT-4 translocation to the cell membrane, and consequently reduces glucose uptake by the myocardium

(Friehs *et al*. 2005). Obesity is also associated with ineffective insulin signaling in skeletal muscle in the absence of diabetes (Cusi *et al*. 2000). Therefore metabolic abnormalities due to obesity/MS together with cardiac hypertrophy may have an additive effect on the glucose metabolism of the heart, which would become problematic during ischaemia and reperfusion and contribute to enhanced injury.

The presence of cardiac hypertrophy in CD fed rats from our study is though to be responsible for the increased susceptibility of CD fed rat hearts to ischaemia/reperfusion injury. However coupled with cardiac hypertrophy are the various metabolic abnormalities of the MS affecting the metabolism of the heart. Although the impact of these metabolic abnormalities in ischaemia/reperfusion injury was not investigated, they cannot be excluded from contributing to the increased susceptibility to injury as seen in our study.

5.6.2 The role of angiotensin II and endothelin-1 in ischaemia/reperfusion injury

Both Ang II (Yoshiyama *et al*. 1994; Frolkis *et al*. 2001) and ET-1 (Brunner and Opie, 1998) are known to play a role in exacerbating myocardial ischaemia/reperfusion induced injury. Data from this study showed no differences in myocardial Ang II content between the control and obese hearts during baseline perfusion or during ischaemia. Similarly, our group (Du Toit *et al*. 2005) found no differences in myocardial Ang II content of hearts from CD and control

diet fed rats. Inhibiting Ang II signaling, in a model with elevated Ang II, led to an improvement in mechanical function following reperfusion compared to untreated animals (Du Toit *et al*. 2005). Due to this, the contribution of Ang II to ischaemia/reperfusion induced injury in our study cannot be ruled out despite the absence of elevated levels of the peptide in the myocardium. The effect of losartan treatment in the perfusate before ischaemia was not tested in the present study but would provide valuable insight into the possible role of Ang II in ischaemia/reperfusion injury.

Current data suggest that ET-1 does not influence the susceptibility of this model to ischaemia/reperfusion injury as both circulating and myocardial ET-1 levels were comparable in the control and CD fed rats. Despite this, in normal rat hearts, bosentan, an endothelin A and B receptor antagonist, was able to provide protection against ischaemia/reperfusion injury in isolated rat hearts, when added to the perfusate during reperfusion (Xia *et al*. 2005). To our knowledge, there are no diet-induced obese rodent models investigating the effect of ET-1 on ischaemia/reperfusion injury. More studies investigating the involvement of ET-1 in myocardial ischaemia/reperfusion injury in obese rodent models are therefore required.

Hearts from CD fed rats in our study may therefore be more prone to ischaemia/reperfusion injury due to the presence of cardiac hypertrophy,

metabolic alterations associated with the MS and possibly a direct effect of Ang II.

5.6.3 Obesity, cardiac hypertrophy and ventricular arrhythmias

Obesity is considered to be a risk factor for cardiac arrhythmias and sudden cardiac death (Aronson, 1981; Kopelman, 2000). Electrocardiographic studies have revealed many electrical abnormalities in the hearts of obese individuals, many of which serve as markers for sudden death (Fraley *et al*. 2005). Obesity induced cardiac hypertrophy has been shown to be a risk factor for excessive ventricular ectopy in obese normotensive individuals, when compared with lean controls (Messerli *et al*. 1987). Indeed the susceptibility of the hypertrophied myocardium to cardiac arrhythmias has been confirmed experimentally in pressure overload models (Shimada and Avkiran, 2003). Moreover, Ang II, which is elevated in the blood in obesity, may also enhance myocardial susceptibility to ventricular arrhythmias, as the addition of compounds inhibiting Ang II signaling to the perfusate of isolated perfused hearts, has been shown to reduce the incidence of ventricular arrhythmias (de Graeff *et al*. 1986)

The mechanism for the increased susceptibility to arrhythmias is not fully elucidated. Cardiac hypertrophy however has been shown to predispose the myocardium to early afterdepolarizations (Aronson, 1981) and early afterdepolarization-induced triggered activity (Charpentier *et al*. 1991). This is thought to occur as the hypertrophied myocardium is more prone to calcium

overload (Opie and Coetzee, 1988; Brooks *et al*. 1995), which is a stimulus for ventricular arrhythmias (Opie and Coetzee, 1988). Calcium overload occurs directly due to the lower density of Ca^{2+} ATPase pumps found in the sarcoplasmic reticulum, of the hypertrophied myocardium (de la Bastie *et al*. 1990). These changes in the cytosolic Ca^{2+} concentrations add to the alterations in Ca^{2+} and Na^{+}-Ca^{2+} exchange currents seen in hypertrophied hearts. This contributes to the prolongation of the action potentials in the hypertrophied myocardium and predisposes the myocardium to early afterdepolarizations and early afterdepolarization-induced triggered activity.

To our knowledge no studies have set out to investigate the susceptibility of the heart of an obese animal to ventricular arrhythmias during ischaemia and reperfusion. Finding clarity on this issue is important, as obesity is an independent risk factor for developing coronary artery disease (Rimm *et al*. 1995) and there may therefore be a higher risk of obese individuals developing fatal cardiac arrhythmias.

Reperfusion in our study was associated with a higher incidence and duration of ventricular tachycardia and fibrillation in the hearts from obese animals. The mechanisms of these arrhythmias were not however investigated, but may be due to the presence of cardiac hypertrophy in these rats. Metabolic effects, due the MS, on myocardial susceptibility to ischaemia/reperfusion injury cannot be excluded. Our findings that the MS may increase the susceptibility to ventricular

arrhythmias during ischaemia/reperfusion are in agreement with Morel *et al* (2003) who used the FFR. The FFR, a model of the MS, which develop cardiac hypertrophy, was shown to display a higher incidence of ventricular arrhythmias during ischaemia following an in vivo myocardial infarction (Morel *et al*. 2003). This study differs to ours in that the FFR were not obese and arrhythmias were not monitored during reperfusion.

Another model of the MS is the sucrose fed rat. It has not been shown whether these rats develop cardiac hypertrophy. Carvajal and Banos (2002) subjected isolated hearts from sucrose fed rats to global ischaemia for 30 minutes, followed by reperfusion. Hearts form the sucrose fed rats were more susceptible to ventricular arrhythmias during reperfusion compared to their control littermates. Together with our study, these data would suggest that the mechanisms for increased myocardial susceptibility to ventricular arrhythmias may relate to changes in myocardial metabolism due to the MS, or because of the presence of cardiac hypertrophy.

The FFR model has previously been shown to have an upregulated RAS, with elevated circulating Ang II (Kamide *et al*. 2002). It is not known whether Ang II contributes to the incidence of arrhythmias seen in the FFR model. However when normal rat hearts were treated with an AT_1 antagonist in the perfusate, prior to coronary artery ligation, the incidence of reperfusion arrhythmias decreased significantly compared to untreated hearts (de Graeff *et al*. 1986).

Data from our study do not shed much light of the involvement of Ang II on the myocardial susceptibility to ventricular arrhythmias in our model as no drug interventions were used.

We would speculate that the combination of the metabolic abnormalities associated with the MS, together with the presence of cardiac hypertrophy in our model of obesity/MS is responsible for the increased susceptibility to ventricular arrhythmias. This study is however the first study to provide evidence showing a direct link between obesity and fatal arrhythmias. Due to the lack of differences in myocardial Ang II levels between groups seen in our study, the results suggest that Ang II did not influence the myocardial susceptibility to ventricular arrhythmias in our ex vivo model.

5.7 Limitations of this study

The possibility exists that blood pressure values obtained in this study, may not reflect normal blood pressures for these animals. This may be due to the use of anesthetic in combination with a non-invasive method of determining blood pressure. Both groups underwent the same procedure which would be expected to alter blood pressure similarly in both groups.

We proposed that the increased susceptibility of hearts from obese rats to ischaemia/reperfusion injury may be due to the presence of cardiac hypertrophy. The possibility however exists that hearts from these obese rats may be more

prone to necrosis during reperfusion, which would also contribute to a decreased functional recovery. Measuring myocardial infarct size would have provided valuable information as to the nature of the increased susceptibility of these hearts to ischaemia/reperfusion injury.

It must be emphasized that the ex vivo data obtained need to be interpreted with caution. Factors such as elevated insulin and catecholamines which are present in obese rats may increase myocardial function and tolerance to ischaemia/reperfusion injury in vivo. In future, perfusion buffers for this type of experiments should contain both insulin and fatty acids in varying amounts for control and obese rats to try and mimic in vivo conditions.

A clearer understanding of the involvement of Ang II and ET-1 in the development of cardiac hypertrophy and the mechanism of ischaemia/reperfusion-induced injury could have been obtained had we investigated these peptides at a molecular level. Angiotensinogen and preproendothelin mRNA quantification would have added value to this study as our assay may not have been sensitive enough to detect subtle variations in myocardial Ang II and ET-1 peptide content. The assays used are intended for measuring plasma Ang II and ET-1. We however also used the assays to determine tissue protein levels. The tissue extraction methods may have contributed to the absence of significant differences in the levels of these proteins. In addition, mRNA quantification would reflect an accurate functional

expression of these peptides by the myocardium, as the peptide may have been released from the myocardium, and lost in the perfusate.

Further understanding of the involvement of these peptides in cardiac function could be investigated by the addition of AT_1 or an ET_A receptor antagonist to the perfusate. This would eliminate locally produced Ang II and ET-1 from activating their signaling pathways and may therefore clarify the role of endogenous Ang II and ET-1 in ischaemia/reperfusion injury.

5.8 Future directions

A significant outcome of this study was that in vivo basal myocardial function appeared to be normal in the obese rats when assessed using echocardiography. When isolated hearts from the respective groups were however perfused, hearts from CD diet fed rats displayed poorer basal mechanical function, compared to hearts form their control littermates. It was recently shown in a hypertriglyceridaemic, hyperinsulinaemic rat model (sucrose fed rat) that the infusion of insulin in the perfusate of Langendorff perfused hearts, restored basal myocardial mechanical function (mechanical work and +d*p*/d*t* max) to normal values (Cárdenas *et al*. 2005). The beneficial effects of insulin administration were again seen, as the functional recovery between the groups was comparable. The decreased incidence of fatal ventricular arrhythmias seen in these sucrose fed rats disappeared following insulin administration to the perfusate. It has not been documented whether or not

cardiac hypertrophy exists in this model. These findings suggest a vital cardioprotective role of insulin in the MS. Future studies would aim to establish whether the same cadioprotective effect of insulin, both pre and post-ischaemic, would be evident in our obese model of MS. The mechanism behind this cardioprotective phenomenon is also incompletely understood and further investigations are required.

Lastly as basal in vivo myocardial mechanical function is comparable between CD and control diet fed groups, it would be worth investigating whether the in vivo heart responds differently to an infarct. This could be investigated by inducing an in vivo myocardial infarction and monitoring myocardial function, infarct size and cardiac remodeling. Hearts from CD fed rats would thus be exposed to all components of the MS. In fact, such studies have already been carried out in both the sucrose and FFR, however the findings from these studies are contradictory (Morel *et al*. 2003; Jordan *et al*. 2003). Further investigations are therefore warranted to establish whether or not these findings are model specific, or whether the presence of excess insulin, in the hyperinsulinaemic state is indeed cardioprotective.

CHAPTER 6

CONCLUSION

We have developed a model of diet-induced obesity that fulfills the WHO criteria of the MS. Our model is characterized by the presence of visceral obesity, insulin resistance, normoglycaemia, dyslipidaemia, elevated systolic blood pressure and cardiac hypertrophy.

Obesity in our model was associated with decreased ex vivo basal mechanical function and increased myocardial susceptibility to ischaemia and reperfusion injury, which may be related to the presence of cardiac hypertrophy or metabolic abnormalities in these hearts. Ex vivo myocardial mechanical function was decreased in the obese rats. The increased susceptibility to ischaemia/reperfusion injury was reflected in both decreased reperfusion function and increased incidence of reperfusion arrhythmias in these hearts from obese rats. As in vivo basal myocardial mechanical function was comparable between groups this data suggest that the differences may be related to the perfusion model being used.

The role of Ang II in the development of cardiac hypertrophy is not completely understood, and present data suggest that elevated circulating Ang II may contribute to the development of cardiac hypertrophy in our model. Ang II may contribute to the increased ischaemia and reperfusion injury seen, although

further studies would have to be conducted to further elucidate the involvement of Ang II in our model. ET-1 is unlikely to contribute to the development of cardiac hypertrophy or to myocardial ischaemia/reperfusion injury in this model as both circulating and myocardial levels of the peptide were unchanged in obese rats.

Although we developed this model for cardiovascular research purposes, the CD rodent model could provide valuable information as to how the MS affects other organ systems. This novel model could also provide new insight into the development of the MS and could therefore be used as a model to test pharmacological interventions aimed at preventing the MS.

REFERENCES

Aasum E, Hafstad AD, Severson DL, Larson TS. Age-dependent changes in metabolism, contractile function, and ischemic sensitivity in hearts from db/db mice. Diabetes. 2003; 52:434-441.

Abate N. Obesity and cardiovascular disease: Pathogenic role of the metabolic syndrome and therapeutic implications. J Diabetes Complications. 2000; 14:154-174.

Adachi M, Yang YY, Furuichi Y, Miyamoto C. Cloning and characterization of cDNA encoding human A-type endothelin receptor. Biochem Biophys Res Commun. 1991; 180:1265-1272.

Adeli K, Taghibiglou C, Van Iderstine, SC, Lewis GF. Mechanisms of hepatic very low-density lipoprotein overproduction in insulin resistance. TCM. 2001; 11:170-176.

Aizawa Y, Kamimura N, Watanabe H, Aizawa Y, Makiyama Y, Usuda Y, Watanabe T, Kurashina Y. Cardiovascular risk factors are really linked in the metabolic syndrome: this phenomenon suggests clustering rather than coincidence. Int J Cardiol. 2005; [Epub ahead of print]

Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998; 15:539-53.

Alexander SK, Dennis EW, Smith WG. Blood volume, cardiac output and distribution of systemic blood flow in extreme obesity. Cardiovasc Res Cent Bull. 1953; 1:39-44.

Allard MF, Flint JD, English JC, Henning SL, Salamanca MC, Kamimura CT, English DR. Calcium overload during reperfusion is accelerated in isolated hypertrophied rat hearts. J Mol Cell Cardiol. 1994; 26:1551-163.

Allison DB, Fontaine KR, Manson JE, Stevens J, VanItallie TB. Annual deaths attributable to obesity in the United States. JAMA. 1999; 282:1530-1538.

Andren B, Lind L, Hedenstierna G, Lithell H. Left ventricular hypertrophy and geometry in a population sample of elderly males. Eur Heart J. 1996; 17:1800-1807.

Aneja A, El-Atat F, McFarlane SI, Sowers JR. Hypertension and obesity. Recent Prog Horm Res. 2004; 59:169-205.

Aronson RS. Afterpotentials and triggered activity in hypertrophied myocardium from rats with renal hypertension. Circ Res. 1981; 48:720-727.

Asai T, Kushiro T, Fujita H, Kanmatsuse K. Different effects on inhibition of cardiac hypertrophy in spontaneously hypertensive rats by monotherapy and combination therapy of adrenergic receptor antagonists and/or the angiotensin II type 1 receptor blocker under comparable blood pressure reduction. Hypertens Res. 2005; 28:79-87.

Ashley EA, Sears CE, Bryant SM, Watkins HC, Casadei B. Cardiac nitric oxide synthase 1 regulates basal and β-adrenergic contractility in murine ventricular myocytes. Circulation. 2002; 105:3011-3016.

Avignon A, du Cailar G, Ribstein J, Monnier L, Mimran A. Determinants of the left ventricular mass in obese patients. Influence of lean body mass. Arch Mal Coeur Vaiss. 1997 Aug;90(8):1043-1046.

Bader M, Peters J, Baltatu O, Müller D.N, Luft F.C, Ganten D. Tissue rennin-angiotensin systems: new insights from experimental animal models in hypertension research. J Mol Med. 2001; 79:76-102.

Baker KM, Chernin MI, Schreiber T, Sanghi S, Haiderzaidi S, Booz GW, Dostal DE, Kumar R. Evidence of a novel intracrine mechanism in angiotensin II-induced cardiac hypertrophy. Regul Pept. 2004; 120:5-13.

Banos G, Carvajal K, Cardoso G, Zamora J, Franco M. Vascular reactivity and effect of serum in a rat model of hypertriglyceridemia and hypertension. Am J Hypertens. 1997; 10:379-388.

Banskota NK, Taub R, Zellner K, Olsen P, King GL. Characterization of induction of protooncogene c-myc and cellular growth in human vascular smooth muscle cells by insulin and IGF-I. Diabetes. 1989; 38:123-129.

Bardales RH, Hailey LS, Xie SS, Schaefer RF, Hsu SM. In situ apoptosis assay for the detection of early acute myocardial infarction. Am J Pathol. 1996; 149:821-829.

Barton M, Carmona R, Ortmann J, Krieger JE, Traupe T. Obesity-associated activation of angiotensin and endothelin in the cardiovascular system. Int J Biochem Cell Biol. 2003; 35:826-837.

Barton M, Shaw S, d'Uscio LV, Moreau P, Lüscher TF. Angiotensin II increases vascular and renal endothelin-1 and functional endothelin converting enzyme activity in vivo: role of ET_A receptors for endothelin regulation. Biochem Biophys Res Commun. 1997; 238:861-865.

Becker LC, Ambrosio G. Myocardial consequences of reperfusion. Prog Cardiovasc Dis. 1987; 30:23-44.

Bendall JK, Cave AC, Heymes C, Gall N, Shah AM. Pivotal role of a gp91(phox)-containing NADPH oxidase in angiotensin II-induced cardiac hypertrophy in mice. Circulation. 2002; 105:293-296.

Berg AH, Scherer PE. Adipose tissue, inflammation and cardiovascular disease. Circ Res. 2005; 96:939-949.

Berkalp B, Cesur V, Corapcioglu D, Erol C, Baskal N. Obesity and left ventricular diastolic dysfunction. Int J Cardiol. 1995; 52:23-26.

Bessesen DH. The role of carbohydrates in insulin resistance. J Nutr. 2001; 131:S2782-S2786.

Bezerra RM, Ueno M, Silva MS, Tavares DQ, Carvalho CR, Saad MJ. A high fructose diet affects the early steps of insulin action in muscle and liver of rats. J Nutr. 2000; 130:1531-1535.

Bigazzi R, Bianchi S, Baldari G, Campese VM. Clustering of cardiovascular risk factors in salt-sensitive patients with essential hypertension: role of insulin. Am J Hypertens. 1996; 9:24-32.

Blais V, Fugere M, Denault JB, Klarskov K, Day R, Leduc R. Processing of proendothelin-1 by members of the subtilisin-like pro-protein convertase family. FEBS Lett. 2002; 524:43-48.

Boden WE, Brooks WW, Conrad CH, Bing OH, Hood WB Jr. Incomplete, delayed functional recovery late after reperfusion following acute myocardial infarction: "maimed myocardium". Am Heart J. 1995; 130:922-932.

Bogardus C, Lillioja S, Mott DM, Hollenbeck C, Reaven G. Relationship between degree of obesity and in vivo insulin action in man. Am J Physiol. 1985; 248: E286-291.

Bolli R. Mechanism of myocardial "stunning". Circulation. 1990; 82:723-738.

Bolli R, Patel BS, Jeroudi MO, Lai EK, McCay PB. Demonstration of free radical generation in "stunned" myocardium of intact dogs with the use of the spin trap alpha-phenyl N-tert-butyl nitrone. J Clin Invest. 1988; 82:476-485.

Borch-Johnsen K, Feldt-Rasmussen B, Strandgaard S, Scholl M, Jensen JS. Urinary albumin excretion: an independent predictor of ischaemic heart disease. Thromb Vasc Biol. 1999; 19:1992-1997.

Boustany CM, Bharadwaj K, Daugherty A, Brown DR, Randall DC, Cassis LA. Activation of the systemic and adipose renin-angiotensin system in rats with diet-induced obesity and hypertension. Am J Physiol Regul Integr Comp Physiol. 2004; 287:R943-R949.

Brassard P, Amiri F, Schiffrin EL. Combined angiotensin II type 1 and 2 receptor blockade on vascular remodeling and matrix metalloproteinases in resistance arteries. Hypertension. 2005; 46:598-606.

Braunwald E, Kloner RA. The stunned myocardium: prolonged, postischemic ventricular dysfunction. Circulation. 1982; 66:1146-1149.

Brede M, Roell W, Ritter O, Wiesmann F, Jahns R, Haase A, Fleischmann BK, Hein L. Cardiac hypertrophy is associated with decreased eNOS expression in angiotensin AT_2 receptor-deficient mice. Hypertension. 2003; 42:1177-1182.

Bril A, Forest MC, Gout B. Ischaemia and reperfusion-induced arrhythmias in rabbits with chronic heart failure. Am J Physiol. 1991; 261:H301-H307.

Brooks WW, Conrad CH, Morgan JP. Reperfusion induced arrhythmias following ischaemia in intact rat heart: role of intracellular calcium. Cardiovasc Res. 1995; 29:536-542.

Bunag RD. Validation in awake rats of a tail-cuff method for measuring systolic pressure. J Appl Physiol. 1973; 34:279-282.

Brunner F, Kukovetz WR. Postischemic antiarrhythmic effects of angiotensin-converting enzyme inhibitors. Role of suppression of endogenous endothelin secretion. Circulation. 1996; 94:1752-1761.

Brunner F, Leonhard B, Kukovetz WR, Mayer B. Role of endothelin, nitric oxide and L-arginine release in ischaemia/reperfusion injury of rat heart. Cardiovasc Res. 1997; 36:60-66.

Brunner F, Opie LH. Role of endothelin-A receptors in ischemic contracture and reperfusion injury. Circulation. 1998; 97:391-398.

Caglayan E, Blaschke F, Takata Y, Hsueh WA. Metabolic syndrome-interdependence of the cardiovascular and metabolic pathways. Curr Opin Pharmacol. 2005; 5:135-142.

Carroll JF, Huang M, Hester RL, Cockrell K, Mizelle HL. Hemodynamic alterations in hypertensive obese rabbits. Hypertension. 1995; 26:465-470.

Cardenas G, Torres JC, Zamora J, Banos G. Isolated heart function during ischemia and reperfusion in sucrose-fed rats: effect of insulin infusion. Cardiovasc Pathol. 2005; 14:256-264.

Carvajal K, Banos G. Myocardial function and effect of serum in isolated heart from hypertriglyceridemic and hypertensive rats. Clin Exp Hypertens. 2002; 24:235-248.

Carvajal K, Moreno-Sanchez R. Heart metabolic disturbances in cardiovascular diseases. Arch Med Res. 2003; 34:89-99.

Charlat ML, O'Neill PG, Hartley CJ, Roberts R, Bolli R. Prolonged abnormalities of left ventricular diastolic wall thinning in the "stunned" myocardium in conscious dogs: time course and relation to systolic function. J Am Coll Cardiol. 1989; 13:185-194.

Charles MA, Fontbonne A, Thibult N, Warnet JM, Rosselin GE, Eschwege E. Risk factors for NIDDM in white population. Paris prospective study. Diabetes. 1991; 40:796-799.

Charpentier F, Baudet S, Le Marec H. Triggered activity as a possible mechanism for arrhythmias in ventricular hypertrophy. Pacing Clin Electrophysiol. 1991; 14:1735-1741.

Chicco A, D'Alessandro ME, Karabatas L, Pastorale C, Basabe JC, Lombardo YB. Muscle lipid metabolism and insulin secretion are altered in insulin resistant rats fed a high sucrose diet. J Nutr. 2003; 133:127-133.

Chicco A, Gutman R, Lombardo YB. Biochemical abnormalities in the heart of rats fed a sucrose-rich diet: is the low activity of the pyruvate dehydrogenase complex a result of increased fatty acid oxidation? Metabolism. 1991; 40:15-21.

Chinali M, Devereux RB, Howard BV, Roman MJ, Bella JN, Liu JE, Resnick HE, Lee ET, Best LG, de Simone G. Comparison of cardiac structure and function in American Indians with and without the metabolic syndrome (the Strong Heart Study). Am J Cardiol. 2004; 93:40-44.

Clancy CE, Klass RS. Inherited and acquired vulnerability to ventricular arrhythmias: cardiac Na^+ and K^+ channels. Physiol Rev. 2005; 85:33-47

Clusin WT, Buchbinder M, Harrison DC. Calcium overload, "injury" current, and early ischaemic cardiac arrhythmias--a direct connection. Lancet. 1983; 1:272-274.

Cobbe SM, Poole-Wilson PA. The time of onset and severity of acidosis in myocardial ischaemia. J Mol Cell Cardiol. 1980; 12:745-760.

Coetzee WA, Opie LH. Effects of components of ischemia and metabolic inhibition on delayed afterdepolarizations in guinea pig papillary muscle. Circ Res. 1987; 61:157-165.

Corin WJ, Murakami T, Monrad ES, Hess OM, Krayenbuehl HP. Left ventricular passive diastolic properties in chronic mitral regurgitation. Circulation. 1991; 83:797-807.

Corr PB, Gross RW, Sobel BE. Amphipathic metabolites and membrane dysfunction in ischemic myocardium. Circ Res. 1984; 55:135-154.

Corr PB, Witkowski FX. Potential electrophysiologic mechanisms responsible for dysrhythmias associated with reperfusion of ischemic myocardium. Circulation. 1983; 68:I16-I24.

Cruzado M, Risler N, Castro C, Ortiz A, Ruttler ME. Proliferative effect of insulin on cultured smooth muscle cells from rat mesenteric resistance vessels. Am J Hypertens. 1998; 11:54-58.

Cullen JP, Bell D, Kelso EJ, McDermott BJ. Use of A-192621 to provide evidence for involvement of endothelin ET(B)-receptors in endothelin-1-mediated cardiomyocyte hypertrophy. Eur J Pharmacol. 2001; 417:157-168.

Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, DeFronzo RA, Kahn CR, Mandarino LJ. Insulin resistance differentially affects the PI3-kinase- and MAP kinase–mediated signaling in human muscle. J Clin Invest. 2000; 105:311-320.

Datta K, Podolin DA, Davidson MB, Davidoff AJ. Cardiomyocyte dysfunction in sucrose-fed rats is associated with insulin resistance. Diabetes. 2001; 50:1186-1192.

Daugherty A, Rateri DL, Lu H, Inagami T, Cassis LA. Hypercholesterolemia stimulates angiotensin peptide synthesis and contributes to atherosclerosis through the AT1A receptor. Circulation. 2004; 110:3849-3857.

Davis CL, Kapuku G, Snieder H, Kumar M, Treiber FA. Insulin resistance syndrome and left ventricular mass in healthy young people. Am J Med Sci. 2002; 324:72-75.

Davenport AP. International union of pharmacology. XXIX. Update on endothelin receptor nomenclature. Pharmacol Rev. 2002; 54:219-226.

DeBoer LW, Rude RE, Kloner RA, Ingwall JS, Maroko PR, Davis MA, Braunwald E A flow- and time-dependent index of ischemic injury after experimental coronary occlusion and reperfusion. Proc Natl Acad Sci U S A. 1983; 80:5784-5788.

DeFronzo RA, Cooke CR, Andres R, Faloona GR, Davis PJ. The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. J Clin Invest. 1975; 55:845-55.

De Graeff PA, Van Gilst WH, De Langen CD, Kingma JH, Wesseling H. Concentration-dependent protection by captopril against ischemia-reperfusion injury in the isolated rat heart. Arch Int Pharmacodyn Ther. 1986; 280:181-193.

De la Bastie D, Levitsky D, Rappaport L, Mercadier JJ, Marotte F, Wisnewsky C, Brovkovich V, Schwartz K, Lompré AM. Function of the sarcoplasmic reticulum and expression of its Ca^{2+}-ATPase gene in pressure overload-induced cardiac hypertrophy in the rat. Circ Res. 1990; 66:554-564.

De la Maza MP, Estevez A, Bunout D, Klenner C, Oyonarte M, Hirsch S. Ventricular mass in hypertensive and normotensive obese subjects. Int J Obes Relat Metab Disord. 1994;18:193-197.

Delaughter MC, Taffet GE. Fiorotto ML, Entman ML, Schwartz RJ. Local insulin-like growth factor I expression induced physiologic, then pathologic, cardiac hypertrophy in transgenic mice. FASEB. 1999; 13:1923-1929.

De Mello WC. Intercellular communication in cardiac muscle. Circ Res. 1982; 51:1-9.

De Smet HR, Menadue MF, Oliver JR, Phillips PA. Endothelin ET_A receptor antagonism does not attenuate angiotensin II-induced cardiac hypertrophy in vivo in rats. Clin Exp Pharmacol Physiol. 2003; 30:278-83.

Després JP, Lamarche B, Mauriege P, Cantin B, Dagenais GR, Moorjani S, Lupien PJ. Hyperinsulinemia as an independent risk factor for ischaemic heart disease. N Engl J Med. 1996; 334:952-957.

Dimmeler S, Rippmann V, Weiland U, Haendeler J, Zeiher AM. Angiotensin II induces apoptosis of human endothelial cells. Protective effect of nitric oxide. Circ Res. 1997; 81:970-976.

Dizdar O, Alyamac E. Obesity: an endocrine tumor. Med Hypotheses. 2004; 63:790-792.

D'Orléans-Juste P, Plante M, Honoré JC, Carrier E, Labonté J. Synthesis and degradation of endothelin-1. Can J Pharmacol. 2003; 81:503-510.

Du Toit EF, Nabben M, Lochner A. A potential role for angiotensin II in obesity induced cardiac hypertrophy and ischaemia/reperfusion injury. Basic Res Cardiol. 2005; 100:1-9.

Du Toit EF, Opie LH. Modulation of severity of reperfusion stunning in the isolated rat heart by agents altering calcium flux at onset of reperfusion. Circ Res. 1992; 70:960-967.

Du Toit, Opie LH. Role for the Na^+/H^+ exchanger in reperfusion stunning in isolated perfused rat heart. J Cardiovasc Pharmacol. 1993; 22:877-883.

Dyckner T, Helmers C, Lundman T, Wester PO. Initial serum potassium level in relation to early complications and prognosis in patients with acute myocardial infarction. Acta Med Scand. 1975; 197:207-210.

Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005; 365:1415-1428.

Egan BM, Schork NJ, Weder AB. Regional hemodynamic abnormalities in overweight men. Focus on alpha-adrenergic vascular responses. Am J Hypertens. 1989; 2:428-434.

Eisenstein EL, McGuire DK, Bhapkar MV, Kristinsson A, Hochman JS, Kong DF, Califf RM, Van de Werf F, Yancy WS Jr, Newby LK. Elevated body mass

index and intermediate-term clinical outcomes after acute coronary syndromes. Am J Med. 2005; 118:981-990.

Engeli S, Negrel R, Sharma A.M. Physiology and pathophysiology of the adipose tissue renin-angiotensin system. Hypertension. 2000; 35:1270-1277.

Erlich Y, Rosenthal T. Effect of angiotensin-converting enzyme inhibitors on fructose induced hypertension and hyperinsulinaemia in rats. Clin Exp Pharmacol Physiol Suppl. 1995; 22:S347-S349.

Evans DJ, Hoffmann RG, Kalkhoff RK, Kissebah AH. Relationship of body fat topography to insulin sensitivity and metabolic profiles in premenopausal women. Metabolism. 1984; 33:68-75.

Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults, Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP). Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA. 2001; 285:2486-2497.

Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999-2000. JAMA. 2002; 288:1723-1727.

Ferrannini E, Buzzigoli G, Bonadonna R, Giorico MA, Oleggini M, Graziadei L, Pedrinelli R, Brandi L, Bevilacqua S. Insulin resistance in essential hypertension. N Engl J Med. 1987; 317:350-357.

Ferrari R. Metabolic disturbances during myocardial ischaemia and reperfusion. Am J Cardiol. 1995; 76:17B-24B.

Fliss H, Gattinger D. Apoptosis in ischaemic and reperfused rat myocardium. Circ Res. 1996; 79:949-956.

Forbes GB, Welle SL. Lean body mass in obesity. Int J Obes. 1983; 7:99-107.

Fraley MA, Birchem JA, Senkottaiyan N, Alpert MA. Obesity and the electrocardiogram. Obes Rev. 2005; 6:275-281.

Frey N, Olson EN. Cardiac hypertrophy: the good, the bad, and the ugly. Annu Rev Physiol. 2003; 65:45-79.

Friehs I, Cao-Danh H, Nathan M, McGowan FX, del Nido PJ. Impaired insulin-signalling in hypertrophied hearts contributes to ischemic injury. Biochem Biophys Res Commun. 2005; 331:15-22.

Frolkis I, Gurevitch J, Yuhas Y, Iaina A, Wollman Y, Chernichovski T, Paz Y, Matsa M, Pevni D, Kramer A, Shapira I, Mohr R. Interaction between paracrine tumor necrosis factor-alpha and paracrine angiotensin II during myocardial ischemia. J Am Coll Cardiol. 2001; 37:316-322.

Fukuchi S, Hamaguchi K, Seike M, Himeno K, Sakata T, Yoshimatsu H. Role of fatty acid composition in the development of metabolic disorders in sucrose-induced obese rats. Exp Biol Med (Maywood). 2004; 229:486-493

Furuhashi M, Ura N, Takizawa H, Yoshida D, Moniwa N, Murakami H, Higashiura K, Shimamoto K. Blockade of the renin-angiotensin system decreases adipocyte size with improvement in insulin sensitivity. J Hypertens. 2004; 22:1977-1982.

Gaasch WH, Zile MR, Hoshino PK, Weinberg EO, Rhodes DR, Apstein CS. Tolerance of the hypertrophic heart to ischemia. Studies in compensated and failing dog hearts with pressure overload hypertrophy. Circulation. 1990; 81:1644-1653.

Galiñanes M, Fowler AG. Role of pathologies in myocardial injury following ischaemia and reperfusion. Circ Res. 2004; 61:512-521.

Galvan AQ, Galetta F, Natali A, Muscelli E, Sironi AM, Cini G, Camastra S, Ferrannini E. Insulin resistance and hyperinsulinemia: no independent relation to left ventricular mass in humans. Circulation. 2000; 102:2233-2238

Gao L, Wang W, Li YL, Schultz HD, Liu D, Cornish KG, Zucker IH. Sympathoexcitation by central ANG II: roles for AT1 receptor upregulation and NAD(P)H oxidase in RVLM. Am J Physiol Heart Circ Physiol. 2005; 288:H2271-2279.

Giacchetti G, Faloia E, Mariniello B, Sardu C, Gatti C, Camilloni MA, Guerrieri M, Mantero F. Overexpression of the renin-angiotensin system in human visceral adipose tissue in normal and overweight subjects. Am J Hypertens. 2002; 15:381-388.

Glowinska B, Urban M, Hryniewicz A, Peczynska J, Florys B, Al-Hwish M. Endothelin-1 plasma concentration in children and adolescents with atherogenic risk factors. Kardiol Pol. 2004; 61:329-338.

Glynn RJ, Rosner B. Comparison of risk factors for the competing risks of coronary heart disease, stroke, and venous thromboembolism. Am J Epidemiol. 2005; [Epub ahead of print]

Golay A, Swislocki AL, Chen YD, Jaspan JB, Reaven GM. Effect of obesity on ambient plasma glucose, free fatty acid, insulin, growth hormone, and glucagon concentrations. J Clin Endocrinol Metab. 1986; 63:481-484.

Golden AL, Bright JM, Pohost GM, Pike MM. Ischaemic dysfunction and impaired recovery in hypertensive hypertrophied hearts is associated with exaggerated intracellular sodium accumulation. Am J Hypertens. 1994; 7:745-754.

Golino P, Maroko PR, Carew TE. The effect of acute hypercholesterolemia on myocardial infarct size and the no re-flow phenomenon during coronary occlusion reperfusion. Circulation. 1987; 75:292-298.

Hanley AJG, Karter AJ, Festa A, D'Agostino R, Jr., Wagenknecht LE, Savage P, Tracy RP, Saad MF, Haffner S. Factor analysis of metabolic syndrome using directly measured insulin sensitivity: The Insulin Resistance Atherosclerosis Study. Diabetes. 2002; 51:2642-2647.

Harris AS, Bisteni A, Russell RA, Brigham JC, Firestone JE. Excitatory factors in ventricular tachycardia resulting from myocardial ischemia; potassium a major excitant. Science. 1954; 119:200-203.

Harte A, McTernan P, Chetty R, Coppack S, Katz J, Smith S, Kumar S. Insulin-mediated upregulation of the renin angiotensin system in human subcutaneous adipocytes is reduced by rosiglitazone. Circulation. 2005; 111:1954-1961.

Haruta T, Morris AJ, Rose DW, Nelson JG, Mueckler M, Olefsky JM. Insulin-stimulated GLUT4 translocation is mediated by a divergent intracellular signaling pathway. J Biol Chem. 1995; 270:27991-27994.

He ZS, Komori S, Tamura K, Hashimoto K. Inhibitory effect of moricizine on reperfusion induced tachyarrhythmias in rats--a comparison study with disopyramide and mexiletine. Jpn Circ J. 1992; 56:861-865.

Hearse DJ. Oxygen deprivation and early myocardial contractile failure: a reassessment of the possible role of adenosine triphosphate. Am J Cardiol. 1979; 44:1115-1121.

Hearse DJ. Reperfusion of the ischaemic myocardium. J Mol Cell Cardiol. 1977; 9:605-616.

Hearse DJ, Stewart DA, Green DG. Myocardial susceptibility to ischemic damage: a comparative study of disease models in the rat. Eur J Cardiol. 1978; 7:437-450.

Hess ML, Manson NH. Molecular oxygen: friend and foe. The role of the oxygen free radical system in the calcium paradox, the oxygen paradox and ischemia/reperfusion injury. J Mol Cell Cardiol. 1984; 16:969-985.

Heyndrickx GR, Millard RW, McRitchie RJ, Maroko PR, Vatner SF. Regional myocardial functional and electrophysiological alterations after brief coronary artery occlusion in conscious dogs. J Clin Invest. 1975; 56:978-985.

Holemans K, Cauwaerts S, Poston L, Van Assche FA. Diet-induced obesity in the rat: A model for gestational diabetes mellitus. Am J Obstet Gynecol. 2004; 190:858-865.

Horton JD, Cuthbert JA, Spady DK. Regulation of hepatic 7 alpha-hydroxylase expression and response to dietary cholesterol in the rat and hamster. J Biol Chem. 1995; 270:5381-5387.

Hua L, Li C, Xia D, Qu P, Li Z, Zhang W, Feng X. Relationship between hypertensive left ventricular hypertrophy and levels of endothelin and nitric oxide. Hypertens Res. 2000; 23:377-380.

Hwang IS, Ho H, Hoffman BB, Reaven GM. Fructose-induced insulin resistance and hypertension in rats. Hypertension. 1987; 10:512-516.

Iacobellis G, Ribaudo MC, Zappaterreno A, Vecci E, Tiberti C, Di Mario U, Leonetti. Relationship of insulin sensitivity and left ventricular mass in uncomplicated obesity. Obes Res. 2003; 11:518-524.

Ilercil A, Devereux RB, Roman MJ, Paranicas M, O'Grady MJ, Lee ET, Welty TK, Fabsitz RR, Howard B. Associations of insulin levels with left ventricular structure and function in American Indians: the strong heart study. Diabetes. 2002; 51:1543-1547.

Inagami T, Guo DF, Kitami Y. Molecular biology of angiotensin II receptors: an overview. J Hypertens Suppl. 1994; 12:S83-S94.

Ito H, Hirata Y, Adachi S, Tanaka M, Tsujino M, Koike A, Nogami A, Murumo F, Hiroe M. Endothelin-1 is an autocrine/paracrine factor in the mechanism of angiotensin II-induced hypertrophy in cultured rat cardiomyocytes. J Clin Invest. 1993; 92:398-403.

Iwai N, Inagami T. Identification of two subtypes in the rat type I angiotensin II receptor. FEBS Lett. 1992; 298:257-260.

Iwai T, Tanonaka K, Inoue R, Kasahara S, Motegi K, Nagaya S, Takeo S. Sodium accumulation during ischemia induces mitochondrial damage in perfused rat hearts. Cardiovasc Res. 2002; 55:141-149.

Iyer SN, Katovich MJ. Effect of acute and chronic losartan treatment on glucose tolerance and insulin sensitivity in fructose-fed rats. Am J Hypertens. 1996a; 9:662-668.

Iyer SN, Raizada MK, Katovich MJ. AT1 receptor density changes during development of hypertension in hyperinsulinemic rats. Clin Exp Hypertens. 1996b; 18:793-810.

Jennings RB. Myocardial ischaemia – observations, definitions and speculations. J Mol Cell Cardiol. 1970; 2:345-349.

Jennings RB, Ganote CE. Mitochondrial structure and function in acute myocardial ischaemic injury. Circ Res. 1976; 38:I80-I91.

Jennings RB, Murry CE, Steenbergen C Jr, Reimer KA. Development of cell injury in sustained acute ischemia. Circulation. 1990; 82:II2-II12.

Jennings RB, Sommers HM, Smyth GA, Flack HA, Linn H. Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. Arch Pathol. 1960; 70:68-78.

Jennings RB, Yellon DM. Reperfusion injury: definitions and historical background. In: Myocardial protection: The pathophysiology of reperfusion and reperfusion injury. Raven press Ltd. New York. 1992:1-11.

Jensen JS, Borch-Johnsen K, Jensen G, Feldt-Rasmussen B. Atherosclerotic risk factors are increased in clinically healthy subjects with microalbuminuria. Atherosclerosis. 1995; 112:245-252.

Jeroudi MO, Cheirif J, Habib G, Bolli R. Prolonged wall motion abnormalities after chest pain at rest in patients with unstable angina: a possible manifestation of myocardial stunning. Am Heart J. 1994; 127:1241-1250.

Jordan JE, Simandle SA, Tulbert CD, Busija DW, Miller AW. Fructose-fed rats are protected against ischemia/reperfusion injury. J Pharmacol Exp Ther. 2003; 307:1007-1011.

Juan CC, Fang VS, Hsu YP, Huang YJ, Hsia DB, Yu PC, Kwok CF, Ho LT. Overexpression of vascular endothelin-1 and endothelin-A receptors in a fructose-induced hypertensive rat model. J Hypertens. 1998; 16:1775-1782.

Juhan-Vague I, Alessi MC, Vague P. Increased plasma plasminogen activator inhibitor 1 levels. A possible link between insulin resistance and atherothrombosis. Diabetologia. 1991; 34:457-462.

Kako KJ. Free radical effects on membrane protein in myocardial ischemia/reperfusion injury. J Mol Cell Cardiol. 1987; 19:209-211.

Kamgang R, Mboumi RY, N'dille GP, Yonkeu JN. Cameroon local diet-induced glucose intolerance and dyslipidemia in adult Wistar rat. Diabetes Res Clin Pract. 2005; 69:224-230.

Kamide K, Hori MT, Zhu J-H, Barrett JD, Eggena P, Tuck ML. Insulin-mediated growth in aortic smooth muscle and the vascular renin-angiotensin system. Hypertension. 1998; 32:482-487.

Kamide K, Rakugi H, Higaki J, Okamura A, Nagai M, Moriguchi K, Ohishi M, Satoh N, Tuck ML, Ogihara T. The renin-angiotensin and adrenergic nervous system in cardiac hypertrophy in fructose-fed rats. Am J Hypertens. 2002; 15:66-71.

Kannel WB, Castelli WP, McNamara PM, McKee PA, Feinleib M. Role of blood pressure in the development of congestive heart failure. The Framingham study. N Engl J Med. 1972; 287:781-787.

Karam H, Heudes D, Gonzales M, Loffler BM, Clozel M, and Clozel JP. Endothelin antagonism in end organ damage of spontaneously hypertensive rats. Hypertension. 1996; 28:379-385.

Karmazyn M, Moffat MP. Role of Na^+/H^+ exchange in cardiac physiology and pathophysiology: mediation of myocardiac reperfusion injury by the pH paradox. Cardiovasc Res. 1993; 27:915-924.

Kaschina E, Unger T. Angiotensin AT1/AT2 receptors: regulation, signalling and function. Blood Press. 2003; 12:70-88.

Kass RS. The channelopathies: novel insights into molecular and genetic mechanisms of human disease. J Clin Investig. 2005; 115:1986-1989.

Katz AM, Hecht HH. The early “pump” failure of the ischaemic heart. Am J Med. 1969; 47:497-502.

Kelly DE, Mokan M, Simineau JA, Mandarino LJ. Interaction between glucose and free fatty acid metabolism in human skeletal muscle. J Clin Invest. 1993; 92:91-98.

Kenchaiah S, Evans JC, Levy D, Wilson PW, Benjamin EJ, Larson MG, Kannel WB, Vasan RS. Obesity and the risk of heart failure. N Engl J Med. 2002; 347:305-313.

Kendall DM, Sobel BE, Coulston AM, Peters Harmel AL, McLean BK, Peragallo-Dittko V, Buse JB, Fonseca VA, Hill JO, Nesto RW, Sunyer FXP, The Partners Against Insulin Resistance Advisory Panel. The insulin resistance syndrome and coronary artery disease. Coron Artery Dis. 2003; 14:335-348.

Kennedy LM, Dickstein K, Anker SD, Kristianson K, Willenheimer R; OPTIMAAL Study Group. The prognostic importance of body mass index after complicated myocardial infarction. J Am Coll Cardiol. 2005; 45:156-158.

Klein BE, Klein R, Lee KE. Components of the metabolic syndrome and risk of cardiovascular disease and diabetes in Beaver Dam. Diabetes Care. 2002; 25:1790-1794.

Ko Y, Sachinidis A, Wieczorek AJ, Appenheimer M, Dusing R, Vetter H. Insulin enhances angiotensin II induced DNA synthesis in vascular smooth muscle cells of the rat. Clin Investig. 1993; 71:379-382.

Kobayashi H, Nakamura T, Miyaoka K, Nishida M, Funahashi T, Yamashita S, Matsuzawa Y. Visceral fat accumulation contributes to insulin resistance, small-sized low-density lipoprotein, and progression of coronary artery disease in middle-aged non-obese Japanese men. Jpn Circ J. 2001; 65:193-199.

Kobayashi R, Nagano M, Nakamura F, Higaki J, Fujioka Y, Ikegami H, Mikami H, Kawaguchi N, Onishi S, Ogihara T. Role of angiotensin II in high fructose-induced left ventricular hypertrophy in rats. Hypertension. 1993; 21:1051-1055.

Kopelman PG. Obesity as a medical problem. Nature. 2000; 404:635-643.

Korn ED. Clearing factor, a heparin-activated lipoprotein lipase. II. Substrate specificity and activation of coconut oil. J Biol Chem. 1955; 215:15-26.

Kragelund C, Hassager C, Hildebrandt P, Torp-Pedersen C, Kober L; TRACE study group. Impact of obesity on long-term prognosis following acute myocardial infarction. Int J Cardiol. 2005; 98:123-131.

Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M, Shulman GI. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. Diabetologia 1999; 42:113-116.

KS 300 user guide. Carl Zeiss Vission GMBH. 1997; 2:505, 521, 547-552.

Kupari M, Koskinen P, Virolainen J. Correlates of left ventricular mass in a population sample aged 36 to 37 years. Focus on lifestyle and salt intake. Circulation. 1994; 89:1041-1050.

Lacka K, Piszczek I, Kosowicz J, Gembicki M. Echocardiographic abnormalities in acromegalic patients. Exp Clin Endocrinol. 1988; 91:212-216.

Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA. 2002; 2709-2716.

Lamarche B, Uffelman KD, Carpentier A, Cohn JS, Steiner G, Barrett PH, Lewis GF. Triglyceride enrichment of HDL enhances in vivo metabolic clearance of HDL apo A-I in healthy men. J Clin Invest. 1999; 103:1191-1199.

Laufer E, Jennings GL, Korner PI, Dewar E. Prevalence of cardiac structural and functional abnormalities in untreated primary hypertension. Hypertension. 1989; 13:151-162.

Lavallee M, Cox D, Patrick TA, Vatner SF. Salvage of myocardial function by coronary artery reperfusion 1, 2, and 3 hours after occlusion in conscious dogs. Circ Res. 1983; 53:235-247.

Lavie CJ, Amodeo C, Ventura HO, Messerli FH. Left atrial abnormalities indicating diastolic ventricular dysfunction in cardiopathy of obesity. Chest. 1987; 92:1042-1046.

Le Grand B, Vie B, Faure P, Degryse AD, Mouillard P, John GW. Increased resistance to ischaemic injury in the isolated perfused atherosclerotic heart of the cholesterol-fed rabbit. Cardiovasc Res. 1995; 30:689-696.

Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. N Engl J Med. 1990; 322:1561-1566.

Lewis GF, Uffelman KD, Szeto LW, Weller B, Steiner G. Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. J Clin Invest. 1995; 95(1):158-166.

Li Q, Li B, Wang X, Leri A, Jana KP, Liu Y, Kajstura J, Baserga R, Anversa P. Overexpression of insulin-like growth factor-1 in mice protects from myocyte death after infarction, attenuating ventricular dilation, wall stress, and cardiac hypertrophy. J Clin Invest. 1997; 100:1991-1999.

Lind L, Andersson PE, Andren B, Hanni A, Lithell HO. Left ventricular hypertrophy in hypertension is associated with the insulin resistance metabolic syndrome. J Hypertens. 1995; 13:433-438.

Ljung B, Bamberg K, Dahllof B, Kjellstedt A, Oakes ND, Ostling J, Svensson L, Camejo G. AZ 242, a novel PPARalpha/gamma agonist with beneficial effects on insulin resistance and carbohydrate and lipid metabolism in ob/ob mice and obese Zucker rats. J Lipid Res. 2002; 43:1855-1863.

Lombardo YB, Chicco A, Mocchiutti N, de Rodi MA, Nusimovich B, Gutman R. Effect of sucrose diet on insulin secretion in vivo and in vitro and on triglyceride storage and mobilisation of the heart of rats. Horm Metab Res. 1983;15:69-76.

Lönnqvist F, Thörne A, Nilsell K, Hoffstedt J, Arner P. A pathogenic role of visceral fat ß$_3$-adrenoceptors in obesity. J Clin Invest. 1995; 95:1109-1116.

Lopaschuk GD, Barr R, Thomas PD, Dyck JRB. Beneficial effects of trimetazidine in ex vivo working ischaemic hearts are due to a stimulation of glucose oxidation secondary to inhibition of long-chain 3-ketoacyl coenzyme A thioase. Circ Res. 2003; 93:e33-e37.

Lopez JJ, Lorell BH, Ingelfinger JR, Weinberg EO, Schunkert H, Diamant D, Tang SS. Distribution and function of cardiac angiotensin AT_1- and AT_2-receptor subtypes in hypertrophied rat hearts. Am J Physiol. 1994; 267:H844-H852.

Lopez-Jimenez F, Jacobsen SJ, Reeder GS, Weston SA, Meverden RA, Roger VL. Prevalence and secular trends of excess body weight and impact on outcomes after myocardial infarction in the community. Chest. 2004; 125:1205-1212.

Lorell BH, Carabello BA. Left ventricular hypertrophy: Pathogenesis, detection and prognosis. Circulation. 2000; 102:470-479.

Luc G, Bard JM, Ferrieres J, Evans A, Amouyel P, Arveiler D, Fruchart JC, Ducimetiere P. Value of HDL cholesterol, apolipoprotein A-I, lipoprotein A-I, and lipoprotein A-I/A-II in prediction of coronary heart disease: the PRIME Study. Prospective Epidemiological Study of Myocardial Infarction. Arterioscler Thromb Vasc Biol. 2002; 22:1155-1161.

Manning AS, Coltart DJ, Hearse DJ. Ischemia and reperfusion-induced arrhythmias in the rat. Effects of xanthine oxidase inhibition with allopurinol. Circ Res. 1984; 55:545-548.

Massion PB, Balligand JL. Modulation of cariac contaction, relaxation and rate by the endothelial nitric oxide synthase (eNOS): lessons from genetically modified mice. J Physiol. 2003; 546:63-75.

McNulty PH, Louard RJ, Deckelbaum LI, Zaret BL, Young LH. Hyperinsulinemia inhibits myocardial protein degradation in patients with cardiovascular disease and insulin resistance. Circulation. 1995; 92:2151-2156.

Medvedeva IV, Dorodneva EF, Pugacheva TA, Bel'chikova LN, Zhuravleva TD. Characteristics of lipid profile in blood plasma of patients with metabolic syndrome and manifest disorder of carbohydrate metabolism. Ter Arkh. 2003; 75:21-24.

Meigs JB, D' Agostino RB, Sr., Wilson PWF, Cupples LA, Nathan DM, Singer DE. Risk variable clustering in the insulin resistance syndrome. Diabetes. 1997; 46:1594-1600.

Meissner A, Morgan JP. Contractile dysfunction and abnormal Ca^{2+} modulation during postischemic reperfusion in rat heart. Am J Physiol. 1995; 268:H100-H111.

Mèndez R, Myers MG, White MF, Rhoads RE. Stimulation of protein synthesis, eukaryotic translation initiation factor 4E phosphorylation, and PHAS-I phosphorylation by insulin requires insulin receptor substrate 1 and phosphatidylinositol 3-kinase. Mol Cell Biol. 1996; 16:2857–2864.

Messerli FH, Christie B, DeCarvalho JG, Aristimuno GG, Suarez DH, Dreslinski GR, Frohlich ED. Obesity and essential hypertension. Hemodynamics, intravascular volume, sodium excretion, and plasma renin activity. Arch Intern Med. 1981; 141:81-85.

Messerli FH, Nunez BD, Ventura HO, Snyder DW. Overweight and sudden death. Increased ventricular ectopy in cardiopathy of obesity. Arch Intern Med. 1987 Oct; 147:1725-17258.

Messerli FH, Sundgaard-Riise K, Reisin E, Dreslinski G, Dunn FG, Frohlich E. Disparate cardiovascular effects of obesity and arterial hypertension. Am J Med. 1983a; 74:808-12.

Messerli FH, Sundgaard-Riise K, Reisin ED, Dreslinski GR, Ventura HO, Oigman W, Frohlich ED, Dunn FG. Dimorphic cardiac adaptation to obesity and arterial hypertension. Ann Intern Med. 1983b; 99:757-761.

Messerli FH, Ventura HO, Reisin E, Dreslinski GR, Dunn FG, MacPhee AA, Frohlich ED. Borderline hypertension and obesity: two prehypertensive states with elevated cardiac output. Circulation. 1982 Jul;66(1):55-60.

Miatello R, Cruzado M, Risler N. Mechanisms of cardiovascular changes in an experimental model of syndrome X and pharmacological intervention on the renin-angiotensin-system. Curr Vasc Pharmacol. 2004; 2:371-377.

Milley JR. Effect of insulin on the distribution of cardiac output in the fetal lamb. Pediatr Res. 1987; 22:168-172.

Mittelamn SD, Van Citters GW, kirkman EL, Bergman RN. Extreme insulin resistance of central adipose depot in vivo. Diabetes. 2002; 51:755-761.

Mittendorfer B, Sidossis LS. Mechanism for the increase in plasma triacylglycerol concentrations after consumption of short-term high-carbohydrate diets. Am J Clin Nutr. 2001; 73:892-899.

Mjøs OD. Effect of free fatty acids on myocardial function and oxygen consumption in intact dog. J Clin Invest. 1971; 50:1386-1389.

Modan M, Halkin H, Almog S, Lusky A, Eshkol A, Shefi M, Shitrit A, Fuchs Z. Hyperinsulinemia. A link between hypertension obesity and glucose intolerance. J Clin Invest. 1985; 75:809-817.

Moens AL, Claeys MJ, Timmermans JP, Vrints CJ. Myocardial ischaemia/reperfusion injury, a clinical view on a complex pathophysiological process. Int J Cardiol. 2005; 100:179-190

Monti LD, Allibardi S, Piatti PM, Valsecchi G, Costa S, Pozza G, Chierchia S, Samaja M. Triglycerides impair postischemic recovery in isolated hearts: roles of endothelin-1 and trimetazidine. Am J Physiol Heart Circ Physiol. 2001; 281:H1122-H1130.

Moravec CS, Schluchter MD, Paranandi L, Czerska B, Stewart RW, Rosenkranz E, Bond M. Inotropic effects of angiotensin II on human cardiac muscle in vitro. Circulation. 1990; 82:1973-1984.

Moreau P, d'Uscio LV, Shaw S, Takase H, Barton M, Lüscher TF. Angiotensin II increases tissue endothelin and induces vascular hypertrophy: reversal by ET_A – receptor antagonist. Circulation. 1997; 96:1593-1597.

Morel S, Berthonneche C, Tanguy S, Toufektsian MC, Foulon T, de Lorgeril M, de Leiris J, Boucher F. Insulin resistance modifies plasma fatty acid distribution and decreases cardiac tolerance to in vivo ischaemia/reperfusion in rats. Clin Exp Pharmacol Physiol. 2003; 30:446-451.

Morel S, Berthonneche C, Tanguy S, Toufektsian MC, Perret P, Ghezzi C, de Leiris J, Boucher F. Early prediabetic state alters adaptation of myocardial glucose metabolism during ischaemia in rats. 2005; 272:9-17.

Motte G. Arrhythmia caused by potassium deficiency. Arch Mal Coeur Vaiss. 1984; 77:17-22.

Murphy E, Levy L, Raju B, Steenbergen C, Gerig JT, Singh P, London RE. Measurement of cytosolic calcium using 19F NMR. Environ Health Perspect. 1990; 84:95-98.

Murphy JG, Smith TW, Marsh JD. Mechanisms of reoxygenation-induced calcium overload in cultured chick embryo heart cells. Am J Physiol. 1988; 254:H1133-H1141.

Myers G, Backer JM, Sun XJ, Shoelson S, Hu P, Schlessinger J, Yoakim M, Schaffhausen B, White MF. IRS-1 activates phosphatidylinositol 3'-kinase by associating with src homology 2 domains of p85. Proc Natl Acad Sci U S A. 1992; 89:10350–10354.

Nadal-Ginard B, Kajstura J, Leri A, Anversa P. Myocyte death, growth and regeneration in cardiac hypertrophy and failure. Circ Res. 2003; 92:139-150.

Naderali EK, Pickavance LC, Wilding JPH, Williams G. Diet-induced endothelial dysfunction in the rat is independent of the degree of increase in total body weight. Clin Sci. 2001a; 100:635-641.

Naderali EK, Brown MJ, Pickavance LC, Wilding JPH, Doyle PJ, Williams G. Dietary obesity in the rat induces endothelial dysfunction without causing insulin resistance: a possible role for triacylglycerols.. 2001b; 101: 499-506.

Nakamura T, Tokunaga K, Shimomura I, Nishida M, Yoshida S, Kotani K, Islam AH, Keno Y, Kobatake T, Nagai Y. Contribution of visceral fat accumulation to the development of coronary artery disease in non-obese men. Atherosclerosis. 1994; 107:239-246.

Nakamuta M, Takayanagi R, Sakai Y, Sakamoto S, Hagiwara H, Mizuno T, Saito Y, Hirose S, Yamamoto M, Nawata H. Cloning and sequence analysis of a cDNA encoding human non-selective type of endothelin receptor. Biochem Biophys Res Commun. 1991; 177:34-39.

Nalbantgil I, Önder R, Altintig A, Nalbantgil S, Kiliççioglu B, Boydak B, Yilmaz H. Therapeutic benefits of cilazapril in patients with syndrome X. Cardiology 1998; 89:130-133.

Namiki A, Kubota T, Fukazawa M, Ishikawa M, Moroi M, Aikawa J, Ebine K, Yamaguchi T. Endothelin-1 concentrations in pericardial fluid are more elevated in patients with ischemic heart disease than in patients with nonischemic heart disease. Jpn Heart J. 2003; 44:633-644.

Nashar K, Nguyen JP, Jesri A, Morrow JD, Egan BM. Angiotensin receptor blockade improves arterial distensibility and reduces exercise-induced pressor responses in obese hypertensive patients with the metabolic syndrome. Am J Hypertens. 2004; 17:477-482.

Nayler WG, Panagiotopoulos S, Elz JS, Daly MJ. Calcium-mediated damage during post-ischaemic reperfusion. J Mol Cell Cardiol. 1988; 20:41-54.

Neely JR, Grotyohann LW. Role of glycolytic products in damage to ischemic myocardium. Dissociation of adenosine triphosphate levels and recovery of function of reperfused ischemic hearts. Circ Res. 1984; 55:816-824.

Neely JR, Liebermeister H, Battersby EJ, Morgan HE. Effect of pressure development on oxygen consumption by isolated rat heart. Am J Physiol. 1967; 212:804-814.

Neely JR, Rovetto MJ, Whitmer JT, Morgan HE. Effects of ischemia on function and metabolism of the isolated working rat heart. Am J Physiol. 1973; 225:651-658.

Neyses L, Nouskas J, Luyken J, Fronhoffs S, Oberdorf S, Pfeifer U, Williams RS, Sukhatme VP, Vetter H. Induction of immediate-early genes by angiotensin II and endothelin-1 in adult rat cardiomyocytes. J Hypertens. 1993; 11:927-934.

Nickenig G, Jung O, Strehlow K, Zolk O, Linz W, Scholkens BA, Bohm M. Hypercholesterolemia is associated with enhanced angiotensin AT_1-receptor expression. Am J Physiol. 1997; 272:H2701-H2707.

Nickenig G, Röling J, Strehlow K, Schnabel P, Böhm. Insulin induces upregulation of vascular AT_1 receptor gene expression by posttranscriptional mechanisms. Circulation. 1998; 98:2453-2460.

Nielsen A.H, Schauser K.H, Poulsen K. Current topic: the uteroplacental renin-angiotensin system. Placenta. 2000; 21:468-477.

Nikolsky E, Stone GW, Grines CL, Cox DA, Garcia E, Tcheng JE, Griffin JJ, Guagliumi G, Stuckey T, Turco M, Negoita M, Lansky AJ, Mehran R. Impact of body mass index on outcomes after primary angioplasty in acute myocardial infarction. Am Heart J. 2006; 151:168-175.

Niskanen L, Uusitupa M, Sarlund H, Siitonen O, Voutilainen E, Penttila I, Pyorala K. Microalbuminuria predicts the development of serum lipoprotein abnormalities favouring atherogenesis in newly diagnosed type 2 (non-insulin-dependent) diabetic patients. Diabetologia. 1990; 33:237-243.

Nunez BD, Lavie CJ, Messerli FH, Schmieder RE, Garavaglia GE, Nunez M. Comparison of diastolic left ventricular filling and cardiac dysrhythmias in hypertensive patients with and without isolated septal hypertrophy. Am J Cardiol. 1994; 74:585-589.

Okada K, Hirano T, Ran J, Adachi M. Olmesartan medoxomil, an angiotensin II receptor blocker ameliorates insulin resistance and decreases triglyceride overproduction. Hypertens Res. 2004; 27:293-299.

Okamoto K, Abe M, Haneda T. Effect of regression of cardiac hypertrophy on ischaemic myocardial damage in spontaneously hypertensive rats. Jpn Circ J. 1993; 57:147-160.

Oliver MF. Metabolic causes and prevention of ventricular fibrillation during acute coronary syndromes. Am J Med. 2002; 112:305-311.

Ònody A, Csonka C, Giricz Z, Ferdinandy P. Hyperlipidemia induced by a cholesterol-rich diet leads o enhanced peroxynitrate formation in rat hearts. Cardiovasc Res. 2003; 58:663-670.

Opie LH. The heart: Physiology and metabolism. Second edition. New York. Raven Press. 1991; p184-p185, p396-p400, p484, p489.

Opie LH. The heart: Physiology from cell to circulation. Third edition. Philadelphia, New York. Lippincott-Raven Publishers. 1998; P593-p594

Opie LH, Clusin WT. Cellular mechanism for ischemic ventricular arrhythmias. Annu Rev Med. 1990; 41:231-238.

Opie LH, Coetzee WA. Role of calcium ions in reperfusion arrhythmias: relevance to pharmacologic intervention. Cardiovasc Drugs Ther. 1988; 2:623-636.

Opie LH, Owen P, Thomas M, Young V. Potassium loss, metabolic changes and ventricular arrhythmias following acute experimental coronary occlusion. J Physiol. 1969; 202:44P-45P.

Opie LH, Mansford KR, Owen P. Effects of increased heart work on glycolysis and adenine nucleotides in the perfused heart of normal and diabetic rats. Biochem J. 1971; 124:475-490.

Owen P, Dennis S, Opie LH. Glucose flux rate regulates onset of ischemic contracture in globally underperfused rat hearts. Circ Res. 1990; 66:344-354.

Pagliassotti MJ, Gayles EC, Podolin DA, Wei Y, Morin CL. Developmental stage modifies diet-induced peripheral insulin resistance in rats. Am J Physiol Regul Integr Comp Physiol. 2000; 278:R66-R73.

Pagliassotti MJ, Prach PA, Koppenhafer TA, Pan DA. Changes in insulin action, triglycerides, and lipid composition during sucrose feeding in rats. Am J Physiol. 1996; 271:R1319-R1326.

Paradies G, Petrosillo G, Pistolese M, Di Venosa N, Serena D, Ruggiero FM. Lipid peroxidation and alterations to oxidative metabolism in mitochondria isolated from rat heart subjected to ischemia and reperfusion. Free Radic Biol Med. 1999; 27:42-50.

Parving HH. Microalbuminuria in essential hypertension and diabetes mellitus. J Hypertens Suppl. 1996; 14: S89-S93

Pascual M, Pascual DA, Soria F, Vicente T, Hernández AM, Tébar FJ, Valdés M. Effects of isolated obesity on systolic and diastolic ventricular function. Heart. 2003; 89:1152-1156.

Paulson DJ, Tahiliani AG. Cardiovascular abnormalities associated with human and rodent obesity. Life Sci. 1992; 51:1557-1569.

Perseghin G, Scifo P, De Cobelli F, Pagliato E, Battezzati A, Arcelloni C, Vanzulli A, Testolin G, Pozza G, Del Maschio A, Luzi L. Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H-13C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. Diabetes. 1999; 48:1600-1606.

Petersen KF, Shulman GI. Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. Am J Cardiol. 2002; 90:11G-18G.

Peterson LR, Herrero P, Schechtman KB, Racette SB, Waggoner AD, Kisrieva-Ware Z, Dence C, Klein S, Marsala J, Meyer T, Gropler RJ. Effect of obesity and insulin resistance on myocardial substrate metabolism and efficiency in young women. Circulation. 2004; 109:2191-2196.

Peterson LR, Waggoner AD, Schechtman KB, Meyer T, Gropler RJ, Barzilai B, Davila-Roman VG. Alterations in left ventricular structure and function in young healthy obese women: assessment by echocardiography and tissue Doppler imaging. J Am Coll Cardiol. 2004; 43:1399-1404.

Phillips DI, Caddy S, Ilic V, Fielding BA, Frayn KN, Borthwick AC, Taylor R. Intramuscular triglyceride and muscle insulin sensitivity: evidence for a relationship in nondiabetic subjects. Metabolism. 1996; 45:947-950.

Phillips RA, Krakoff LR, Dunaif A, Finegood DT, Gorlin R, Shimabukuro S. Relation among left ventricular mass, insulin resistance, and blood pressure in nonobese subjects. J Clin Endocrinol Metab. 1998; 83:4284-4288.

Piatti PM, Monti LD, Galli L, Fragasso G, Valsecchi G, Conti M, Gernone F, Pontiroli AE. Relationship between endothelin-1 concentration and metabolic alterations typical of the insulin resistance syndrome. Metabolism. 2000; 49:748-752.

Pickavance LC, Tadayyon M, Widdowson PS, Buckingham RE, Wilding JP. Therapeutic index for rosiglitazone in dietary obese rats: separation of efficacy and haemodilution. Br J Pharmacol. 1999; 128:1570-1576.

Pi-Sunyer FX. The obesity epidemic. Pathophysiology and consequences of obesity. Obes Res. 2002; 10:S97-S104.

Pogwizd SM, Corr PB. Electrophysiologic mechanisms underlying arrhythmias due to reperfusion of ischemic myocardium. Circulation. 1987; 76:404-426.

Porte D, Kahn SE. β cell dysfunction and failure in type 2 diabetes: potential mechanisms. Diabetes. 2001; 50:S160-S163.

Porth C. Pathophysiology: Concepts of altered health states. Philadelphia, Toronto. JB Lippincott Company. 1982; p148-p151.

Pratipanawatr W, Pratipanawatr T, Cusi K, Berria R, Adams JM, Jenkinson CP, Maezono K, DeFronzo RA, Mandarino LJ. Skeletal muscle insulin resistance in normoglycemic subjects with a strong family history of type 2 diabetes is associated with decreased insulin-stimulated insulin receptor substrate-1 tyrosine phosphorylation. Diabetes. 2001; 50:2572-2578.

Puoane T, Steyn K, Bradshaw D, Laubscher R, Fourie J, Lambert V, Mbananga N. Obesity in South Africa: the South African demographic and health survey. Obes Res. 2002; 10:1038-1048.

Puri PS, Varley KG, Kim SW, Barwinsky J, Cohen M, Dhalla NS. Alterations in energy metabolism and ultrastructure upon reperfusion of the ischemic myocardium after coronary occlusion. J Cardiol. 1975; 36:234-243.

Rajala MW, Scherer PE. Mini review: The adipocyte-At the crossroads of energy homeostasis, inflammation, and atherosclerosis. Endocrinology. 2003; 144: 3765-3773.

Rana JS, Mukamal KJ, Morgan JP, Muller JE, Mittleman MA. Obesity and the risk of death after acute myocardial infarction. Am Heart J. 2004; 147:841-846.

Rashid S, Uffelman KD, Lewis GF. The mechanism of HDL lowering in hypertriglyceridemic, insulin resistant states. J Diabetes Complications. 2002; 16:24-28.

Rea TD, Heckbert SR, Kaplan RC, Psaty BM, Smith NL, Lemaitre RN, Lin D. Body mass index and the risk of recurrent coronary events following acute myocardial infarction. Am J Cardiol. 2001; 88:467-472.

Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes. 1988; 37:1595-607.

Reaven GM. Insulin resistance/compensatory hyperinsulinemia, essential hypertension and cardiovascular disease. J Clin Endocrinol Metab. 2003; 88:2399-2403.

Reaven GM. The metabolic syndrome: Requiscat in pace. Clin Chem. 2005; 51:931-938.

Reaven GM, Risser TR, Chen YD, Reaven EP. Characterization of a model of dietary induced hypertriglyceridemia in young non-obese rats. J Lipid Res. 1979; 20:371-378.

Regitz-Zagrosek V, Friedel N, Heymann A, Bauer P, Neuss M, Rolfs A, Steffen C, Hildebrandt A, Hetzer R, Fleck E. Regulation, chamber localization, and subtype distribution of angiotensin II receptors in human hearts. Circulation. 1995; 91:1461-1471.

Reinecke K, Lucius R, Reinecke A, Rickert U, Herdegen T, Unger T. Angiotensin II accelerates functional recovery in the rat sciatic nerve in vivo: role of the AT_2 receptor and the transcription factor NF-kappaB. FASEB J. 2003; 17:2094-2096.

Reiser S, Bohn E, Hallfrisch J, Michaelis OE 4th, Keeney M, Prather ES. Serum insulin and glucose in hyperinsulinemic subjects fed three different levels of sucrose. Am J Clin Nutr. 1981; 34:2348-2358.

Reynisdottir S, Ellerfeldt K, Wahrenberg H, Lithell H, Arner P. Multiple lipolysis defects in the insulin resistance (metabolic) syndrome. J Clin Invest. 1994; 93:2590-2599.

Rimm EB, Stampfer MJ, Giovannucci E, Ascherio A, Spiegelman D, Colditz GA, Willett WC. Body size and fat distribution as predictors of coronary heart disease among middle-aged and older US men. Am J Epidemiol. 1995; 141:1117-1127.

Roach PD, Balasubramaniam S, Hirata F, Abbey M, Szanto A, Simons LA, Nestel PJ. The low-density lipoprotein receptor and cholesterol synthesis are affected differently by dietary cholesterol in the rat. 1993; 1170:165-172.

Roden DM, Balser JR, George AL Jr, Anderson ME. Cardiac ion channels. Annu Rev Physiol. 2002; 64:431-475.

Rosenthal M, Haskell WL, Solomon R, Widstrom A, Reaven GM. Demonstration of a relationship between level of physical training and insulin-stimulated glucose utilization in normal humans. Diabetes. 1983; 32:408-411.

Rossi GP, Sacchetto A, Rizzoni D, Bova S, Porteri E, Mazzocchi G, Belloni AS, Bahcelioglu M, Nussdorfer GG, Pessina AC. Blockade of angiotensin II type 1 receptor and not of endothelin receptor prevents hypertension and cardiovascular disease in transgenic (mREN2)27 rats via adrenocortical

steroid-independent mechanisms. Arterioscler Thromb Vasc Biol. 2000; 20:949-956.

Russell FD, Davenport AV. Secretory pathways in endothelin synthesis. Br J Pharmacol. 1999; 126:391-398.

Ryu MH, Cha YS. The effects of a high-fat or high-sucrose diet on serum lipid profiles, hepatic acyl-CoA synthetase, carnitine palmitoyltransferase-I, and the acetyl-CoA carboxylase mRNA levels in rats. J Biochem Mol Biol. 2003; 36:312-318.

Saad MJ, Velloso LA, Carvalho CR. Angiotensin II induces tyrosine phosphorylation of insulin receptor and its association with phosphatidylinositol 3-kinase in rat heart. Biochem J. 1995; 310:741-744.

Sadoshima J, Izumo S. Molecular characterization of angiotensin II--induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. Critical role of the AT_1 receptor subtype. Circ Res. 1993; 73:413-423.

Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation. 1978; 58:1072–1083.

Sainani GS, Maru VG. Role of endothelial cell dysfunction in essential hypertension. J Assoc Physicians India. 2004; 52:966-969.

Sakata K, Yoshida H, Ono N, Matsunaga Y, Hoshino T, Kaburagi T, Mochizuki M, Yoshimura M. A clinical feature of myocardial stunning associated with acute myocardial infarction. Ann Nucl Med. 1994; 8:153-157.

Sale EM, Atkinson PG, Sale GJ. Requirement of MAP kinase for differentiation of fibroblasts to adipocytes, for insulin activation of p90 S6 kinase and for insulin or serum stimulation of DNA synthesis. EMBO J. 1995; 14:674–684.

Santos AD, Miller RP, Mathew PK, Wallace WA, Cave WT Jr, Hinojosa L. Echocardiographic characterization of the reversible cardiomyopathy of hypothyroidism. Am J Med. 1980; 68:675-682.

Sasson Z, Rasooly Y, bhesania T, Rasooly I. Insulin resistance is an important determinant of left ventricular mass in the obese. Circulation. 1993; 88:1431-1436.

Saupe KW, Sobol SC, Koh SG, Apstein CS. Effects of AT_1 receptor block begun late in life on normal cardiac aging in rats. J Cardiovasc Pharmacol. 2003; 42:573-580.

Schaper J, Mulch J, Winkler B, Schaper W. Ultrastructural, functional, and biochemical criteria for estimation of reversibility of ischemic injury: a study on

the effects of global ischemia on the isolated dog heart. J Mol Cell Cardiol. 1979; 11:521-541.

Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P. Nitric oxide release accounts for insulin's vascular effects in humans. J Clin Invest. 1994; 94:2511-2515.

Schmieder RE, Langenfeld MR, Friedrich A, Schobel HP, Gatzka CD, Weihprecht H. Angiotensin II related to sodium excretion modulates left ventricular structure in human essential hypertension. Circulation. 1996; 94:1304-1309.

Schmieder RE, Messerli FH. Does obesity influence early target organ damage in hypertensive patients. Circulation. 1993; 87:1482-1488.

Schonekess BO, Allard MF, Lopaschuk GD. Recovery of glycolysis and oxidative metabolism during postischaemic reperfusion of hypertrophied rat hearts. Am J Physiol. 1996; 271:H798-H805.

Schulz R, Dodge KL, Lopaschuk GD, Clanachan AS. Peroxynitrite impairs cardiac contractile function by decreasing cardiac efficiency. Am J Physiol. 1997; 272:1212-1219.

Schwarz ER, Pollick C, Meehan WP, Kloner RA. Evaluation of cardiac structures and function in small experimental animals: transthoracic, transesophageal, and intraventricular echocardiography to assess contractile function in rat heart. Basic Res Cardiol. 1998; 93:477-486.

Schwemmer M, Sommer O, Bassenge E. Blockade of angiotensin signaling improves myocardial function in hypercholesterolemia independent of changes in eicosanoid release. Cardiovasc Drugs Ther. 2000; 14:317-327.

Sengul AM, Altuntas Y, Kurklu A, Aydin L. Beneficial effect of lisinopril plus telmisartan in patients with type 2 diabetes, microalbuminuria and hypertension. Diabetes Res Clin Pract. 2005; [Epub ahead of print]

Seria C. A critical appraisal of the intrinsic pancreatic angiotensin-generating system. J Pancreas. 2001. 2:50-55.

Shen AC, Jennings RB. Kinetics of calcium accumulation in acute myocardial ischemic injury. Am J Pathol. 1972; 67:441-452.

Shepherd PR, Navé BT, Siddle K. Insulin stimulation of glycogen synthesis and glycogen synthase activity is blocked by wortmannin and rapamycin in 3T3-L1 adipocytes: evidence for the involvement of phosphoinositide 3-kinase and p70 ribosomal protein-S6 kinase. Biochem J. 1995; 305:25–28.

Shimada Y, Avkiran M, Susceptibility to ischaemia and reperfusion arrhythmias in myocardial hypertrophy: Due to flow of injury current? Jpn Heart J. 2003; 44:989-1004.

Silverthorn DU. Human physiology: An integrated approach. San Fransico, Boston, New York, Cape Town, Hong Kong, London, Madrid, Mexico City, Montreal, Munich, Paris, Singapore, Sydney, Tokyo, Toronto. Pearsons Inc. 2004; p464, p480, p514, p637.

Skolnik EY, Batzer A, Li N, Lee CH, Lowenstein E, Mohammadi M, Margolis B, Schlessinger J. The function of GRB2 in linking the insulin receptor to Ras signaling pathways. Science. 1993; 260:1953-1955.

Skoumal R, Seres L, Soos P, Balogh E, Kovats T, Rysa J, Ruskoaho H, Toth M, Horkay F. Endothelin Levels in Experimental Diabetes Combined with Cardiac Hypertrophy. J Cardiovasc Pharmacol. 2004; 44:S195-S197.

Soria A, D'Alessandro ME, Lombardo YB. Duration of feeding on a sucrose-rich diet determines metabolic and morphological changes in rat adipocytes. J Appl Physiol. 2001; 91:2109-2116.

Sorrentino F, Busa A, Averna M, Nigro P. Ventricular arrhythmias in hypertensive patients with mild metabolic alterations. Minerva Cardioangiol. 2003; 51:63-67.

Speth R.C, Daubert D.L, Grove K.L. Angiotensin II: a reproductive hormone too? Regul Pept.1999; 79:25-40.

Spinale FG, Holzgrefe HH, Mukherjee R, Webb ML, Hird RB, Cavallo MJ, Powell JR, Koster WH. Angiotensin converting enzyme inhibition and angiotensin II subtype-1 receptor blockade during the progression of left ventricular dysfunction: differential effects on myocyte contractile processes. J Pharmacol Exp Ther. 1997; 283:1082-1094.

Stegbauer J, Vonend O, Oberhauser V, Rump LC. Effects of angiotensin-(1-7) and other bioactive components of the renin–angiotensin system on vascular resistance and noradrenaline release in rat kidney. J Hypertens 2003; 21:1391-1399.

Stein O, Stein Y. Atheroprotective mechanisms of HDL. Atherosclerosis. 1999; 144:285-301.

Stoll M, Steckelings UM, Paul M, Bottari SP, Metzger R, Unger T. The angiotensin AT_2-receptor mediates inhibition of cell proliferation in coronary endothelial cells. J Clin Invest. 1995; 95:651-657.

Stowe DF, Bosnjak ZJ, Kampine JP. Cardiac cell action potential duration is dependent upon induced changes in free Ca^{2+} activity during pH changes in vitro. J Electrocardiol. 1986; 19:143-154.

Sun Y, Weber KT. Angiotensin II receptor binding following myocardial infarction in the rat. Cardiovasc Res. 1994; 28:1623-1628.

Sullivan JM, Vander Zwaag RV, el-Zeky F, Ramanathan KB, Mirvis DM. Left ventricular hypertrophy: effect on survival. J Am Coll Cardiol. 1993; 22:508-513.

Surawicz B, Knilans TK. Chou's electrocardiology in clinical practice. Adult and pediatric. Fifth edition. W.B Saunders Company. Philidelphia. 2001; p389.

Switzer JL, Osterman F. The coincidence in autopsy material of nephro-sclerosis and cardiac hypertrophy with a clinical history of hypertension. Am J Med Sci. 1950; 219:523-527.

Tagle R, Acevedo M, Vidt DG. Microalbuminuria: is it a valid predictor of cardiovascular risk? Cleve Clin J Med. 2003; 70:255-261.

Takahashi N, Atsumi H, Nakada S, Takeishi Y, Tomoike H. Alterations in the inotropic responses to forskolin and Ca^{2+} and reduced gene expressions of Ca^{2+}-signaling proteins induced by chronic volume overload in rabbits. Jpn Circ J. 2000; 64:861-867.

Takeo S, Tanonaka K, Miyake K, Fukumoto T. Role of ATP metabolites in induction of incomplete recovery of cardiac contractile force after hypoxia. Can J Cardiol. 1988; 4:193-200.

Tani M, Neely JR. Role of intracellular Na^{+} and Ca^{2+} overload and depressed recovery of ventricular function of perfused ischaemic rat hearts. Possible involvement of Na^{+}-H^{+} and Na^{+}-Ca^{2+} exchange. Cardiovasc Res. 1989; 65:1045-1056.

Tanko LB, Bagger YZ, Qin G, Alexandersen P, Larsen PJ, Christiansen C. Enlarged waist combined with elevated triglycerides is a strong predictor of accelerated atherogenesis and related cardiovascular mortality in postmenopausal women. Circulation. 2005; 111:1883-1890.

Tatarkova Z, Aplan P, Matejovicova M, Lehotsky J, Dobrota D, Flameng W. Effect of ischemia and reperfusion on protein oxidation in isolated rabbit hearts. Physiol Res. 2005; 54:185-191

Tenenbaum A, Motro M, Schwammenthal E, Fisman EZ. Macrovascular complications of the metabolic syndrome: an early intervention is imperative. Int J Cardiol. 2004; 97:167-172.

Tennant R. Factors concerned in the arrest of contraction in an ischaemic myocardial area. Am J Physiol. 1935; 113:677-682.

Thibault G, Arguin C, Garcia R. Cardiac endothelin-1 content and receptor subtype in spontaneously hypertensive rats. J Mol Cell Cardiol. 1995; 27:2327-2336.

Tilton RG, Cole PA, Zions JD, Daugherty A, Larson KB, Sutera SP, Kilo C, Williamson JR. Increased ischemia-reperfusion injury to the heart associated

with short-term, diet-induced hypercholesterolemia in rabbits. Circ Res. 1987; 60:551-559.

Tkáč I. Metabolic syndrome in relationship to type 2 diabetes and atherosclerosis. Diabetes Res Clin Prac. 2005; 68:S2-S9.

Thresher JS, Podolin DA, Wei Y, Mazzeo RS, Pagliasotti MJ. Comparison of the effects of sucrose and fructose on insulin action and glucose tolerance. Am J Physiol. 2000; 279:R1334-R1340.

Timmermans P.B, Wong P.C, Chiu A.T, Herblin W.F, Benfield P, Carini D.J, Lee R.J, Wexler R.R, Saye J.A, Smith R.D. Angiotensin II receptors and angiotensin II receptor antagonists. Pharmacol Rev. 1993; 45:205-251.

Tisne Versailles J, Verscheure Y, Pourrias B. Comparison of the effects of regional ischaemia and of reperfusion on the hypertrophied heart of spontaneously hypertensive rats and the heart of Wistar-Kyoto rats with isolated perfused heart via the left atrium. Effects of lidocaine, propranonol and verapamil. J Pharmacol. 1983; 14:19-34.

True LD. Morphometric applications in anatomic pathology. Hum Pathol. 1996; 27:450-467.

Tsotetsi OJ, Woodiwiss AJ, Netjhardt M, Qubu D, Brooksbank R, Norton GR. Attenuation of cardiac failure, dilatation, damage, and detrimental interstitial remodeling without regression of hypertrophy in hypertensive rats. Hypertension. 2001; 38(4):846-851.

Tsutsumi Y, Matsubara H, Masaki H, Kurihara H, Murasawa S, Takai S, Miyazaki M, Nozawa Y, Ozono R, Nakagawa K, Miwa T, Kawada N, Mori Y, Shibasaki Y, Tanaka Y, Fujiyama S, Koyama Y, Fujiyama A, Takahashi H, Iwasaka T. Angiotensin II type 2 receptor overexpression activates the vascular kinin system and causes vasodilation. J Clin Invest. 1999; 104:925-935.

Van Itallie TB. Health implications of overweight and obesity in the United States. Ann Intern Med. 1985; 103:983-988.

Veliotes DG, Woodiwiss AJ, Deftereos DA, Gray D, Osadchii O, Norton GR. Aldosterone receptor blockade prevents the transition to cardiac pump dysfunction induced by beta-adrenoreceptor activation. Hypertension. 2005; 45:914-920.

Velliquette RA, Friedman JE, Shao J, Zhang BB, Ernsberger P. Therapeutic actions of an insulin receptor activator and a novel peroxisome proliferator-activated receptor gamma agonist in the spontaneously hypertensive obese rat model of metabolic syndrome X. J Pharmacol Exp Ther. 2005; 314:422-430.

Velloso LA, Folli F, Sun XJ, White MF, Saad MJA, Kahn CR. Cross-talk between the insulin and angiotensin signaling systems. Proc Natl Acad Sci.1996; 93:12490-12495.

Vitarius JA. The metabolic syndrome and cardiovascular disease. Mt Sinai J Med. 2005; 72:257-262.

Vetter U, Kupferschmid C, Lang D, Pentz S. Insulin-like growth factors and insulin increase the contractility of neonatal rat cardiocytes in vitro. Basic Res Cardiol. 1988; 83:647-654.

Victor E, Zipes DP. Cardiac electrophysiologic alterations during myocardial ischaemia. Am J Physiol. 233:H329-H345

Wambolt RB, Henning SL, English DR, Bondy GP, Allard MF. Regression of cardiac hypertrophy normalizes glucose metabolism and left ventricular function during reperfusion. J Mol Cell Cardiol. 1997; 29:939-948.

Wang P, Chatham JC: Onset of diabetes in Zucker diabetic fatty (ZDF) rats leads to improved recovery of function after ischemia in the isolated perfused heart. Am J Physiol Endocrinol Metab. 2004; 286:E725-E736.

Wang TD, Chen WJ, Su SS, Lo SC, Lin WW, Lee YT. Increased cardiomyocyte apoptosis following ischemia and reperfusion in diet-induced hypercholesterolemia: relation to Bcl-2 and Bax proteins and caspase-3 activity. Lipids. 2002; 37:385-394. (a)

Wang YX, Fitch R, Li W, Werner M, Halks-Miller M, Lillis B, Vergona R, Post J, Sullivan ME, Verhallen PF. Reduction of cardiac functional reserve and elevation of aortic stiffness in hyperlipidemic Yucatan minipigs with systemic and coronary atherosclerosis. Vascul Pharmacol. 2002; 39:69-76. (b)

Wang X, Qi Y, Yang J, Tong L, Pang Y, Tang C. Intracellular redistribution of cardiac endothelin-1 receptor in rat during myocardial hypertrophy. Chin Med Sci J. 2001; 16:86-92. (c)

Warner TD, de Nucci G, Vane JR. Rat endothelin is a vasodilator in the isolated perfused mesentery of the rat. Eur J Pharmacol. 1989; 159:325-326.

Warnholtz A, Nickenig G, Schulz E, Macharzina R, Brasen JH, Skatchkov M, Heitzer T, Stasch JP, Griendling KK, Harrison DG, Bohm M, Meinertz T, Munzel T. Increased NADH-oxidase-mediated superoxide production in the early stages of atherosclerosis: evidence for involvement of the renin-angiotensin system. Circulation. 1999; 99:2027-2033.

Weibel ER, Palade GE. New cytoplasmic components in arterial endothelia. J Cell Biol. 1964; 23:101-112.

Welborn TA, Breckenridge A, Rubinstein AH, Dollery CT, Fraser TR. Serum-insulin in essential hypertension and in peripheral vascular disease. Lancet. 1966; 1:1136-1137.

Werrmann JG, Cohen SM. Comparison of effects of angiotensin-converting enzyme inhibition with those of angiotensin II receptor antagonism on functional and metabolic recovery in postischemic working rat heart as studied by [31P] nuclear magnetic resonance. J Cardiovasc Pharmacol. 1994; 24:573-586.

West JB. Best and Taylor's physiology basis of medical practice. Twelfth edition. Baltimore. Williams and Wilkins. 1990; p190.

Wharton J, Morgan K, Rutherford RA, Catravas JD, Chester A, Whitehead BF, De Leval MR, Yacoub MH, Polak JM. Differential distribution of angiotensin AT_2 receptors in the normal and failing human heart. J Pharmacol Exp Ther. 1998; 284:323-336.

Whitebread S, Mele M, Kamber B, de Gasparo M. Preliminary biochemical characterization of two angiotensin II receptor subtypes. Biochem Biophys Res Commun. 1989; 163:284-291.

Wit AL, Cranefield PF. Triggered and automatic activity in the canine coronary sinus. Circ Res. 1977; 41:434-445.

Wolk R. Arrhythmogenic mechanisms in left ventricular hypertrophy. Europace. 2000; 2:216-223.

Wong CY, O'Moore-Sullivan T, Leano R, Byrne N, Beller E, Marwick TH. Alterations of left ventricular myocardial characteristics associated with obesity. Circulation. 2004; 110:3081-3087.

Wu L, Iwai M, Nakagami H, Chen R, Suzuki J, Akishita M, De Gasparo M, Horiuchi M. Effect of angiotensin II type 1 receptor blockade on cardiac remodeling in angiotensin II type 2 receptor null mice. Arterioscler Thromb Vasc Biol. 2002; 22:49-54.

Xia Z, Kuo KH, McNeill JH, Ansley DM. Endothelin A and B receptor antagonist bosentan reduces postischemic myocardial injury in the rat: critical timing of administration. Can J Physiol Pharmacol. 2005; 83:259-266.

Xie Z, Koyama T, Abe K, Fuji Y, Sawa H, Nagtashima K. Upregulation of P53 protein in rat heart subjected to a transient occlusion of the coronary artery followed by reperfusion. Jpn J Physiol. 2000; 50:159-162.

Xu D, Emoto N, Giaid A, Slaughter C, Kaw S, deWit D, Yanagisawa M. ECE-1: A membrane-bound metalloprotease that catalyzes the proteolytic activation of big endothelin-1. Cell. 1994; 78:473-485.

Yahiro E, Ideishi M, Wang LX, Urata H, Kumagai K, Arakawa K, Saku K. Reperfusion-induced arrhythmias are suppressed by inhibition of the angiotensin II type 1 receptor. Cardiology. 2003; 99:61-67.

Yang BC, Phillips MI, Ambuehl PE, Shen LP, Mehta P, Mehta JL. Increase in angiotensin II type 1 receptor expression immediately after ischemia-reperfusion in isolated rat hearts. Circulation. 1997; 96:922-926.

Yin FC, Spurgeon HA, Rakusan K, Weisfeldt ML, Lakatta EG. Use of tibial length to quantify cardiac hypertrophy: application in the aging rat. Am J Physiol. 1982; 243:H941-H947.

Yoshiyama M, Kim S, Yamagishi H, Omura T, Tani T, Yanagi S, Toda I, Teragaki M, Akioka K, Takeuchi K. Cardioprotective effect of the angiotensin II type 1 receptor antagonist TCV-116 on ischemia-reperfusion injury. Am Heart J. 1994; 128:1-6.

Youn TJ, Kim HS, Oh BH. Ventricular remodeling and transforming growth factor-beta 1 mRNA expression after nontransmural myocardial infarction in rats: effects of angiotensin converting enzyme inhibition and angiotensin II type 1 receptor blockade. Basic Res Cardiol. 1999; 94:246-253.

Yue TL, Bao W, Gu JL, Cui J, Tao L, Ma XL, Ohlstein EH, Jucker BM. Rosiglitazone treatment in Zucker diabetic Fatty rats is associated with ameliorated cardiac insulin resistance and protection from ischemia/reperfusion-induced myocardial injury. Diabetes. 2005; 54:554-562.

Yusuf S, Hawken S, Ounpuu S, Bautista L, Franzosi MG, Commerford P, Lang CC, Rumboldt Z, Onen CL, Lisheng L, Tanomsup S, Wangai P Jr, Razak F, Sharma AM, Anand SS; INTERHEART Study Investigators. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. Lancet. 2005; 366:1640-1649.

Zeller M, Steg PG, Ravisy J, Laurent Y, Janin-Manificant L, L'Huillier I, Beer JC, Oudot A, Rioufol G, Makki H, Farnier M, Rochette L, Verges B, Cottin Y. Prevalence and impact of metabolic syndrome on hospital outcomes in acute myocardial infarction. Arch Intern Med. 2005; 165:1192-1198.

Zeng G, Quon MJ. Insulin-stimulated Production of Nitric Oxide Is Inhibited by Wortmannin. J. Clin. Invest. 1996; 98:894-898.

Zerkowski HR, Broede A, Kunde K, Hillemann S, Schafer E, Vogelsang M, Michel MC, Brodde OE. Comparison of the positive inotropic effects of serotonin, histamine, angiotensin II, endothelin and isoprenaline in the isolated human right atrium. Naunyn Schmiedebergs Arch Pharmacol. 1993; 347:347-352.

Zhao ZQ, Nakamura M, Wang NP, Wilcox JN, Shearer S, Ronson RS, Guyton RA, Vinten-Johansen J. Reperfusion induces myocardial apoptotic cell death. Cardiovasc Res. 2000; 45:651-660.

Zhou Y-T, Grayburn P, Karim A, Shimabukuro M, Higa M, Baetens D, Orci L, Unger RH. Lipotoxic heart disease in obese rats: Implications for human obesity. PNAS. 2000; 97:1784-1789.

Zhuo J, Song K, Harris PJ, Mendelsohn FA. In vitro autoradiography reveals predominantly AT_1 angiotensin II receptors in rat kidney. Ren Physiol Biochem. 1992; 15:231-239.

Zipes DP, Wellens HJJ. Sudden cardiac death. Circulation. 1998; 98:2334-2351.