Cardiac Effects of Infective and Obesity-Induced Inflammation

Nicol Janse van Rensburg

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, for the degree of Master of Science in Medicine.

Johannesburg, 2011

Abstract

The cause of cardiac pathology in a significant number of patients with heart failure is unclear. Although chronic inflammatory changes may contribute toward progressive heart failure, whether low-grade chronic systemic inflammation, produced by infective processes or obesity accounts in-part for the development of cardiac dysfunction and heart failure requires further study. In this regard, although lipopolysaccharide (LPS) administration, an inflammatory mediator derived from the walls of gram negative organisms has been shown to produce an increased cardiomyocyte apoptosis, the lowest dose of LPS previously employed is commensurate with doses that produce vascular shock. Hence this does not reflect the impact of inflammatory changes produced by low-grade systemic infections. Moreover, although inflammatory substances have been shown to be released from adipose tissue and obesity is a cause of cardiac dysfunction and heart failure, it is uncertain to what extent inflammatory changes mediate obesity-induced myocardial dysfunction. To clarify the role of LPS and obesity-induced inflammation as potential causes of cardiac damage and dysfunction, in the present dissertation I therefore evaluated the influence of pyrogenic, but non-septic doses of LPS on cardiomyocyte apoptosis and cardiac systolic function in rats and the contribution of inflammation as indexed by circulating high sensitivity C-reactive protein concentrations (hs-CRP) to the relationship between obesity and myocardial systolic function in humans.

In normal rats, core body temperature (surgically implanted [in the peritoneal cavity], temperature-sensitive radiotransmitters), cardiomyocyte apoptosis (Terminal Deoxynucleotidyl Transferase Mediated dUTP Nick End Labeling [TUNEL]) staining) and left ventricular (LV) systolic function (two-dimensional directed M-mode echocardiography) were evaluated following two doses of LPS (250 µg/kg), derived from *Eschericia coli,* delivered 24 hours apart. Cardiac assessments were performed 6 hours after the second LPS dose to ensure that cardiac measurements were obtained at the time of a febrile response, whilst the first LPS dose was employed to ensure that a sufficiently long period had occurred for apoptotic cell death to be

detected using a TUNEL system. In this study LPS was able to induce a febrile response ($p<0.05$, $n=26$, compared to normal circadian rhythm), yet failed to produce an increased cardiomyocyte apoptosis or decreased LV systolic chamber (LV endocardial fractional shortening-FSend) or myocardial (LV midwall fractional shortening-FSmid) function.

In 292 randomly selected participants from an urban, developing community not receiving antihypertensive therapy, I also assessed the independent relationship between indices of obesity or hs-CRP and LV FSend and FSmid. In this study indices of adiposity including waist circumference (partial $r=0.35$, $p<0.0001$) were independently related to log hs-CRP. Furthermore, waist circumference was independently and inversely associated with FSmid (standardized β-coefficient= -0.19 ± 0.07, $p<0.01$), but not with FSend. Although log hs-CRP was associated with FSmid on bivariate analysis, no independent relationship between these variables was noted ($p=0.21$). Furthermore, with the inclusion of both waist circumference and log hs-CRP in the same regression model, waist circumference remained independently associated with FSmid (standardized β-coefficient= -0.18 ± 0.08, $p<0.05$).

In conclusion, the results of the present dissertation do not support a role for pyrogenic, but non-septic doses of LPS in mediating cardiomyocyte apoptosis or dysfunction or a role for low grade inflammation, as indexed by hs-CRP concentrations in mediating obesity-induced myocardial systolic dysfunction.

Table of contents

Declaration

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

……

Nicol Janse van Rensburg

……day of …….. 2011

I certify that the studies contained in this dissertation have the approval of the Committee for Research in Human Studies of the University of the Witwatersrand, Johannesburg. The ethics approval numbers are M02-04-72 and renewed as M07-04-69 (human study) and AESC 2007/43/03 (animal study).

……

Nicol Janse van Rensburg

……day of ……2011

……. ……..

Gavin R. Norton (supervisor) Date

……. ……..

Angela J. Woodiwiss (supervisor) Date

Acknowledgements

I would like to thank my supervisors, Professors Gavin Norton and Angela Woodiwiss for their unending support, patience, enthusiasm and dedication that has made this degree possible. In addition, I would like to thank Patrick Dessein and Lois Harden for their academic support and guidance. I would also like to thank Linda Dessein, Margaret Badenhorst, Shan Singh, Leanda Vengethasamy and Olebogeng (Harold) Majane for their technical support and guidance.

Statement of my contributions to data collection and analysis

I designed the animal project in conjunction with my supervisors. The data from the animal project were largely collected and analysed by myself. I performed the echocardiography as well as the TUNEL staining technique and operated the computer network which recorded and catalogued the temperature readings. The haemotoxylin & eosin stains were performed by Margaret Badenhorst.

I designed the human project in conjunction with my supervisors. To ensure quality control the majority of the data collected for the human project was the result of work by several people. I catalogued the CRP samples and participated in anthropometric and echocardiographic measurements. The echocardiographic measurements were nevertheless conducted by a trained echocardiographer. The analysis of the data was conducted by me.

List of Abbreviations

µg	microgram
bax	bcl2-associated x protein
bcl2	B-cell lymphoma 2
BMI	body mass index
BP	blood pressure
BSA	body surface area
bts/min	beats per minute
cAMP	cyclic adenosine monophosphate
Cer	ceramide
cGMP	cyclic guanosine monophosphate
℃	degrees Celsius
℃.hr	degrees Celsius multiplied by hour
cm	centimetres
CRP	C-reactive protein
DBP	diastolic blood pressure
DM	diabetes mellitus
DNA	deoxyribonucleic acid
EC	extracellular
EF	ejection fraction
ERK1/2	extracellular regulated kinase 1/2
FSend	left ventricular endocardial fractional shortening
FSmid	left ventricular midwall fractional shortening
HbA_{1c}	glycated haemoglobin
hr	hour

HR	heart rate
IL-1	Interleukin-1
IL-6	Interleukin-6
IRAK-1	Interleukin-receptor associated kinase-1
JNK	c-Jun N-terminal Kinase
kg	kilogram
LPS	Lipopolysaccharide
LV	left ventricle
LVED	left ventricular end diastolic diameter
LVEDD	left ventricular end diastolic internal diameter
LVES	left ventricular end systolic diameter
LVESD	left ventricular end systolic internal diameter
LVH	left ventricular hypertrophy
LVM	left ventricular mass
LVMI	left ventricular mass index
m	metres
MAPK	mitogen-activated protein kinase
mmHg	millimeters of mercury
MMP	matrix metalloproteinase
NF-κB	nuclear factor kappa B
NO	nitric oxide
NSM	membrane associated neutral sphingomyelinase
p value	probability value
$PaCO_2$	partial pressure of arterial carbon dioxide
PP	pulse pressure
PWTed	end diastolic posterior wall thickness

PWTes	end systolic posterior wall thickness
RWT	left ventricular relative wall thickness
SBP	systolic blood pressure
SD	standard deviation
SEM	standard error of the mean
SeptalTed	end diastolic septal wall thickness
SeptalTes	end systolic septal wall thickness
Sph	Sphingomyelin
TIMP	Tissue Inhibitory Matrix Metalloproteinase
TNF-α	Tumor necrosis factor alpha
TRI	thermal response index
TUNEL	Terminal Deoxynucleotidyl Transferase Mediated dUTP Nick End Labeling
uPA	urokinase plasminogen activator
X^2- statistic	Chi-squared statistic

List of Tables

List of Figures

Preface

The impetus for the work described in the present dissertation was driven by the need to adequately address the potential role of low grade systemic inflammation in contributing toward the development as opposed to the progression of heart failure, particularly in a country such as South Africa. In this regard, there is no question that heart failure produces low-grade systemic inflammation, and that this could contribute toward progressive heart failure. However, there is little evidence to suggest that low-grade inflammation may cause the development of heart failure. In South Africa, a considerable proportion of patients with chronic heart failure develop cardiomyopathies of unknown origins (idiopathic dilated cardiomyopathy). Whether this has a genetic, environmental or combined basis as a cause of the disease is uncertain. South Africa is also a country where infectious diseases, often chronic infections, play a considerable role in morbidity and mortality. Furthermore, there is presently an epidemic of obesity in South Africa and obesity is a source of chronic inflammation. It is therefore not unreasonable to hypothesise that obesity or chronic low-grade systemic infections could contribute toward the development of cardiac dysfunction and ultimately heart failure. However, there are outstanding issues with respect to the role of low-grade inflammation produced by infectious diseases and obesity in the development of cardiac dysfunction and heart failure.

In the present dissertation I undertook two studies which were designed to answer basic questions. The first question was whether low-grade inflammation produced by components of infective agents could promote cardiomyocyte apoptosis, an important pathophysiological process that mediates heart failure. In this regard, previous studies had demonstrated that this effect could occur, but only used doses of the component of infective agents that is now recognized as producing septic (vascular) shock. In the present dissertation I therefore evaluated this hypothesis with a pyrogenic, but non-septic dose. Obviously in this study I assessed this hypothesis in animal models where cardiac tissue is readily available. I also evaluated whether inflammation, as indexed by circulating concentrations of an inflammatory

substance could account for the relationship that exists between obesity and cardiac systolic dysfunction. In this study I employed a randomly selected community sample with a high prevalence of obesity-associated increases in circulating concentrations of inflammatory markers. This study was conducted largely because no previous studies had assessed whether circulating markers of inflammation could in-part account for the relationship between indices of obesity and cardiac function.

The dissertation begins with a review chapter which highlights the current understanding and controversies in the field and leads the reader through a series of arguments in support of conducting the studies described in the present dissertation. The first chapter ends with a summary of the problem statements and the aims of the dissertation. Chapter 2 outlines the methodology employed, chapter 3 describes the results and chapter 4 summarizes the results and underscores the novelty of the findings by placing the studies in the context of our present understanding of the field, indicating how the work of the dissertation extends our knowledge within the field. Moreover, this chapter highlights the implications of the findings and the limitations of the studies performed, providing suggestions as to the way forward. The work presented in this dissertation will be prepared for publication, once sample sizes are sufficient to improve statistical power and once further long-term studies have been completed.

Chapter 1

Introduction

Inflammation as a potential pathophysiological mechanism responsible for chronic myocardial damage or dysfunction.

1.0 Introduction

Heart failure is a condition that contributes to a substantial proportion of morbidity and mortality. Indeed, from the time of diagnosis, survival rates are comparable to those of malignancies with the worst possible outcomes (Cowie et al 2000, Stewart et al 2001, Hobbs et al 2004, Lenfant et al 1994). Because of the appalling outcomes in patients with heart failure, and the marked burden to health care systems and patients alike produced by heart failure, over the past two-to-three decades there has been a considerable number of studies that have been performed to attempt to improve the current situation.

The past two-to-three decades have been highly successful with respect to research into an understanding of the pathophysiological mechanisms responsible for the progressive decline in cardiac function in heart failure, once heart failure is established. The identification of these mechanisms has culminated in a number of novel therapeutic approaches including the use of blockers of the renin-angiotensin-aldosterone system and β-blockers (Hunt et al 2001). However, in comparison to the success achieved with studies evaluating the mechanisms of the progression of heart failure once it is established, research into the mechanisms responsible for the development of heart failure has been comparatively less successful. In this regard there are still many aspects of heart failure and causes of heart failure where the pathophysiological mechanisms still require further elucidation.

Identifying the pathophysiological mechanisms responsible for the development of heart failure is particularly important in developing countries, such as South Africa. Indeed, a recent clinical audit conducted in a developing community in South Africa, indicates that the burden of cardiovascular disease in a hospital setting appears to be through hospitalizations or referrals for heart failure (Sliwa et al 2008, Stewart et al 2008). An important question that emerges from these data is whether this apparently high prevalence of heart failure in hospital settings in

emerging communities (Sliwa et al 2008, Stewart et al 2008) represents the outcomes of a failed health care system in general, or a high prevalence of disease processes the pathophysiology of which is uncertain.

Notwithstanding an important role for rheumatic heart disease (that may nevertheless be diminishing) and a potentially increasing role for ischaemic heart disease as causes of heart failure in emerging communities (Stewart et al 2008), of the causes of heart failure in these communities, the burden of heart failure is nevertheless mainly through hypertension and idiopathic dilated cardiomyopathy (dilated cardiomyopathy of unknown origin) with idiopathic dilated cardiomyopathy being the predominant cause (Stewart et al 2008). Although there are a number of hypotheses regarding the pathophysiology of idiopathic dilated cardiomyopathy, such as genetic causes (in familial dilated cardiomyopathy) (Hershberger et al 2005), the end stage of a myocarditis (Liu and Mason 2001), an immunological disorder (Luppi et al 1998, Brooksbank et al 2005a and b), or through excessive alcohol consumption and a concomitant thiamine deficiency (wet beriberi) (Olubodun et al 1996, Seftel 1972), to-date no clear cause for this condition has been identified. Thus, there is no question that hypotheses regarding the potential pathophysiological mechanisms of heart failure of unknown origin (idiopathic) in emerging communities need to be pursued. The consequences of these outcomes may be that novel approaches that prevent the development of heart failure will emerge.

In the present dissertation I tested the hypothesis that low grade inflammatory states mediated by chronic infections or obesity may contribute toward cardiac damage or dysfunction. These hypotheses were developed, as it is acknowledged that in emerging communities in Africa infective diseases play a significant role in contributing toward morbidity and mortality (Maher et al 2010, Nsubuga et al 2010), but there is currently little evidence to suggest that this morbidity and mortality related to infections is in-part through the development of heart failure. In addition, there is simultaneously an epidemic of obesity in South Africa (Puoane et al 2002) and

as shall be reviewed in the present chapter, obesity is considered to represent a chronic inflammatory state (Berg et al 2005). As shall also be reviewed in the present chapter, although obesity is associated with adverse effects on the heart, independent of other risk factors, whether these adverse effects are mediated by obesity-induced chronic inflammation is still uncertain.

In the present dissertation I therefore considered the possibility that if low grade inflammatory states mediated by either systemic infections (unrelated to the heart), or obesity can produce cardiac damage or dysfunction, these changes, if they persist for some time, may contribute toward progressive myocardial abnormalities and subsequently to cardiac dilatation and hence heart failure. These hypotheses can only be confirmed in longitudinal studies in large population samples. However, the studies performed in the present dissertation were conducted to test the hypotheses that low grade inflammation, produced by an infection or obesity, may indeed mediate myocardial damage or dysfunction. Data to show a relationship between obesity-induced inflammatory changes or low grade infection and cardiac damage or dysfunction would provide the “proof-of-principle” required to support a large scale clinical study.

In the present chapter I will therefore first describe the current evidence to suggest that infective states, where the origin of the infection is distant to the heart, could produce myocardial damage. I will also underscore the limitations of this evidence with respect to contributing to a hypothesis that low grade systemic infections may ultimately promote myocardial damage and hence heart failure. Second, I will describe the evidence to suggest that obesity may contribute toward myocardial damage, highlighting the data that supports or refutes a notion that these effects could occur through an impact of inflammatory mediators. Moreover, throughout the introduction to the present dissertation I will highlight the principle reasons for conducting the studies presented in the present dissertation.

Prior to developing the hypotheses that inflammation mediated by low-grade infective states or obesity may promote myocardial damage and ultimately contribute toward the development of heart failure, I will first highlight the evidence to indicate that inflammation, mediated by the presence of heart failure itself, may contribute toward progressive heart failure. This dissertation would be incomplete without this discussion, as historically, the hypothesis that inflammatory changes contribute to cardiac dysfunction has largely emerged from these studies.

2.0 Inflammation produced in heart failure may contribute toward progressive cardiac dysfunction.

The initial evidence that suggested that heart failure could be determined in-part, by a chronic inflammatory state, was provided by studies conducted in patients with heart failure who were free of infections at the time. In this regard early studies suggested an important role for increased circulating cytokine concentrations in heart failure. Indeed, concentrations of the pro-inflammatory cytokine, tumour necrosis factor-α (TNF-α), were noted to be increased in patients with heart failure despite the absence of clinical evidence for an infection (Levine et al 1990, Torre-Amione et al 1996b, Matsumori et al 1994). Moreover, increases in circulating cytokine concentrations in patients with heart failure were associated with more severe heart failure and pump dysfunction (Torre-Amione et al 1996b). Importantly, increased circulating concentrations of pro-inflammatory cytokines were observed to be decreased by heart failure therapy, including β-adrenergic blockers, and these decreases in circulating cytokine concentrations were also noted to be associated with an improved pump function (Mayer et al 2010, Cinquegrana et al 2005).

The origin of the increases in circulating pro-inflammatory cytokines in patients with heart failure, who have no clinical evidence of an infection, could be from a number of sources.

The inflammation may be intra-cardiac or systemic in origins (Torre-Amione et al 1996a, Torre-Amione et al 1999, Doyama et al 1996). Both sources of inflammation could contribute towards the progression of heart failure (Mann, 2002). What are the extra-cardiac sources of inflammation? Stimulation of cytokine production in heart failure could emanate from increased circulating concentrations of endotoxins (lipopolysaccharides) derived from the gastrointestinal tract (because of gastrointestinal oedema causing an increased permeability of the epithelial lining to endotoxins) (Niebauer et al 1999, Genth-Zotz et al 2002). Furthermore, a sustained TNF-α over-production has been reported to occur *ex vivo* in white cells obtained from patients with idiopathic dilated cardiomyopathy (Aukrust et al 1999, Brooksbank et al 2005a and 2005b). In one study, this effect persisted despite haemodynamic improvement subsequent to treatment with standard medical care (Brooksbank et al 2005b) and occurred independent of increases in circulating endotoxin concentrations, or an increased sensitivity of white cells to endotoxin stimulation (Brooksbank et al 2005a). The excessive white cell TNF-α production in patients with heart failure in that study (Brooksbank et al 2005a) was attributed to immune-mediated mechanisms, but the source of the immune response was not identified. What is the intra-cardiac source of inflammation? In this regard, as TNF-α is expressed in the myocardium, particularly the failing myocardium (Torre-Amione et al 1996a, 1996b, Doyama et al 1996), it is also possible that the heart itself is a source of increased circulating cytokines in patients with heart failure.

As previously reviewed (Mann 2002), pre-clinical studies have confirmed the clinical findings of increased circulating cytokine concentrations and an enhanced myocardial TNF-α expression in cardiac pathology, a change that may be mediated in part by increased angiotensin II (Kalra et al 2002). Furthermore, also as previously reviewed (Mann 2002, Kelly and Smith 1997), preclinical studies have suggested that pro-inflammatory cytokines, such as

TNF-α, could act on the myocardium to promote myocardial damage and dysfunction and the mechanisms thereof.

Figure 1.1 outlines the mechanisms by which TNF-α exerts deleterious effects on the myocardium. Briefly, these mechanisms include cardiomyocyte apoptosis, extracellular matrix degradation, and reduced contractility. Cardiomyocyte apoptosis occurs as a result of three different mechanisms, one of which is the TNF-α activation of macrophages (Tavener et al 2005), as well as the TNF-α inhibition of cAMP, both of which cause a reduction of cytochrome c activity, thereby activating caspases and resulting in apoptosis (Chagnon et al 2005, Prabhu 2004). A second mechanism is through the production of nitric oxide, which results in impaired mitochondrial respiration, resulting in apoptosis (Tavener et al 2005, Prabhu 2004, Suzuki et al 2007). A third mechanism is via a JNK signaling pathway which causes apoptosis (Dutta et al 2006).

Extracellular matrix degradation is the result of TNF-α binding to membrane associated neutral sphingomyelinase, which results in ERK1/2 signalling, and subsequent Urokinase Plasminogen Activator (uPA), as well as Matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) activation. These events result in matrix degradation (Cheng et al 2009).

Reduced contractility is the result of the activation of membrane associated neutral sphingomyelinase which causes an increased ceramide production. Ceramide produces sphingosine, which inhibits the release of calcium from the ryanodine receptor and ceramide inhibits the internal calcium channel (Prabhu et al 2004).

Together, all of the clinical and preclinical studies described in the aforementioned discussion have provided sufficient evidence to suggest that blockade of the pro-inflammatory cytokine, TNF-α, may have useful therapeutic benefits in heart failure. These data thus

prompted the initiation of a number of clinical studies to test this hypothesis. Unfortunately, the use of agents that block TNF-α effects in patients with heart failure, were discontinued at an early stage, as mortality appeared to increase rather than decrease in the group of patients receiving the TNF-α blockers (Chung et al 2003, Mann et al 2004). Although a number of reasons have been provided to explain these data, including a suggestion that inflammatory effects on the heart may have in fact increased, rather than decreased with these agents (Mann 2004, Clark et al 1998, Anker and Coats 2002), these studies have nevertheless generated some doubt as to the potential role of TNF-α and indeed pro-inflammatory cytokines in heart failure.

In contrast to the outcome of the clinical studies demonstrating a potential deleterious effect of blockade of pro-inflammatory cytokines (Anker and Coats 2002), there is nonetheless evidence to suggest that alternative agents, with general inhibitory effects on immune function, could produce benefits in heart failure. Indeed, the immunomodulatory agent pentoxifylline has been demonstrated to improve cardiac function in patients with heart failure and these effects are associated with reductions in circulating concentrations of TNF-α and other pro-inflammatory cytokines (Sliwa et al 1998, Martich et al 1992).

Nevertheless, the beneficial effects on cardiac function in patients with heart failure mediated by pentoxifylline (Sliwa et at al 1998, Martich et al 1992) may not have been produced as a consequence of the immunomodulatory properties of pentoxifylline as originally

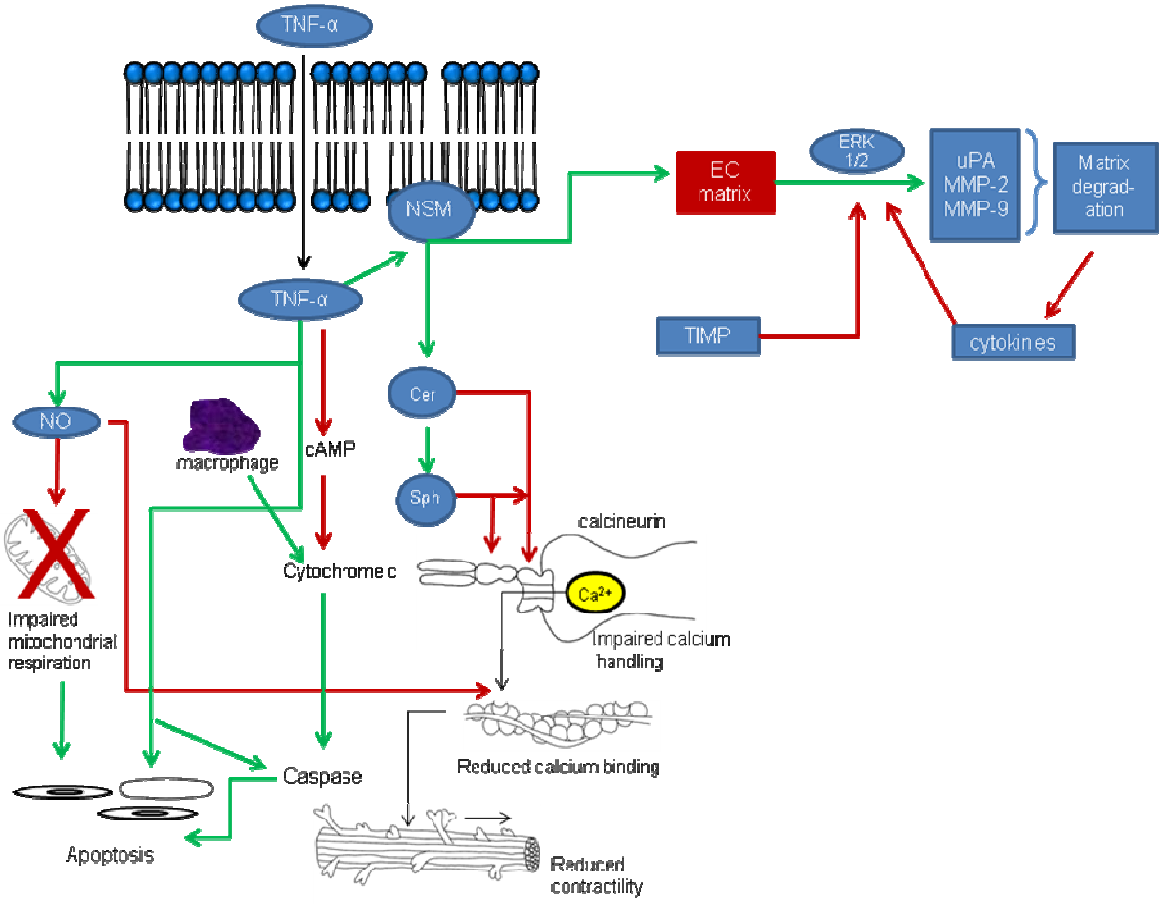


Figure 1.1. Cellular mechanisms of the deleterious effects of cytokines on the myocardium. Red arrows depict an inhibitory pathway; green arrows depict a stimulatory pathway. NF-kB, nuclear factor kappa B; JNK, c-Jun N-terminal Kinase, MAPK, membrane-associated protein kinase; IRAK-1,Interleukin-receptor associated kinase-1 ; NO, nitric oxide; TNF-α, tumour necrosis factor-α; NSM,membrane associated neutral sphingomyelinase; Cer, ceramide; Sph, Spingomyelin; EC, extracellular; TIMP, Tissue Inhibitory Matrix Metalloproteinase, MMP, matrix metalloproteinase; cAMP, cyclic adenosine monophosphate; uPA,urokinase plasminogen activator. The figure summarises pathways supported by the following references: Prabhu et al 2004; Liu et al 2008; Cheng et al 2009; Suzuki et al 2007;Chagnon et al 2005; Tavener et al 2005.

suggested by the authors. Indeed, pentoxifylline has been shown to produce potent inotropic effects through phosphodiesterase inhibitory properties (Osadchii et al 2005). In this regard, phosphodiesterase inhibitors, such as amrinone, milrinone and pentoxifylline, are well recognized as substances that increase intracellular cyclic adenosine monophosphate concentrations and hence mediate positive inotropic and lusitropic effects. Thus, the role of pro-inflammatory cytokines in the progression of heart failure still remains uncertain.

3.0 Systemic infections may produce myocardial damage and dysfunction

There is no question that infections of the myocardium (myocarditis) can produce marked myocardial damage and dysfunction with subsequent cardiac dilatation and pump dysfunction (Liu and Mason 2001,Doyama et al 1996, Hunt et al 2001). Indeed, myocarditis is a well-recognized cause of heart failure (Hunt et al 2001). Moreover, there is evidence to suggest that some patients with idiopathic dilated cardiomyopathy develop an auto-immune response in the myocardium (Luppi et al 1998) and that persistent activation of white cells to produce pro-inflammatory cytokines, even when assessed *ex vivo*, may occur in patients with idiopathic dilated cardiomyopathy (Aukrust et al 1999, Brooksbank et al 2005a and 2005b). However, whether common infections derived from sources other than the myocardium, if repeatedly occurring, can produce myocardial damage and dysfunction is a question that has not as yet been fully addressed.

As highlighted in the previous section, relatively low grade inflammation, where the inflammation originates as a consequence of heart failure, may be a key determinant of progressive heart failure. Thus, it is not unreasonable to hypothesize that low grade inflammatory changes derived from sources other than heart failure, may contribute toward progressive myocardial damage. Indeed, C-reactive protein, TNF-α, and interleukin-6 (Bahrami

et al 2008, Vasan et al 2003, Cesari et al 2003) as well as erythrocyte sedimentation rate (Ingelsson et al 2005b), a generalized marker of systemic inflammation, have all been shown to be independent predictors of heart failure. However, in these studies (Bahrami et al 2008, Vasan et al 2003, Cesari et al 2003, Ingelsson et al 2005b) the source of the inflammatory changes was not identified. Whether these inflammatory changes represented the presence of low-grade infections derived, for example, from poor dental hygiene causing periodontal disease (Moutsopoulos and Madianos 2006, Geerdts et al 2002) or excess adiposity (Ridker et al 2003) was not examined.

There are nevertheless a number of studies to suggest that systemic infections, rather than infections of the myocardium *per se*, promote myocardial damage and dysfunction. In this regard, patients with sepsis but without clinical evidence of myocarditis, may have depressed cardiac systolic function, in spite of adequate volume resuscitation to correct the reduced cardiac filling commonly found in septic shock (Calvin et al 1981, Parker et al 1984, Reilly et al 1989, Jafri et al 1990, Munt et al 1998, Poelaert et al 1997, Krishnagopalan et al 2002 [review]). With respect to the potential mechanisms thereof, there are a number of hypotheses that could explain this effect.

3.1 Endotoxin as a myocardial depressant factor

In cases of sepsis, the presence of a “myocardial depressant factor” was proposed more than 60 years ago (Wiggers 1947), but it is only recently that potential candidates for this factor have emerged. In this regard, infusion of lipopolysaccharide (LPS) (endotoxin), a component of the cell wall of gram negative bacteria, has been shown to partially mimic the myocardial and systemic cardiovascular effects of sepsis (Suffredini et al 1989, Hung 1993a and 1993b). However, because few patients with septic shock have detectable circulating concentrations of

LPS (Merx et al 2007) it is unlikely that LPS contributes to the myocardial effects of the majority of patients with sepsis. Nevertheless, the concept of LPS mediating decreases in myocardial function in septic shock has engendered a notion that LPS could also mediate myocardial abnormalities in any gram negative infection irrespective of whether septic shock is present or not.

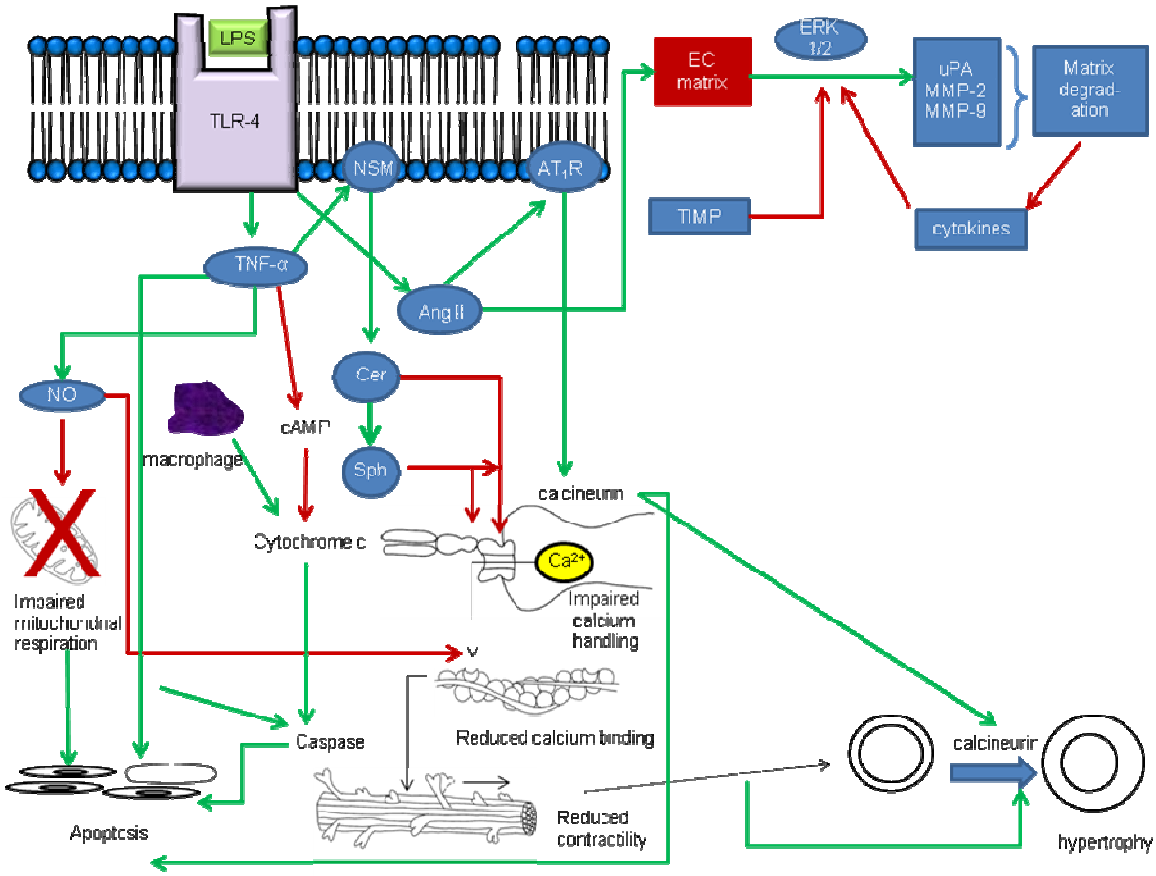
Over the past two decades, a number of studies have demonstrated that LPS depresses myocardial and cardiomyocyte function (Suffredini et al 1989, Brady et al 1992, Hung and Lew 1993a, 1993b, Tao and McKenna 1994, Nishikawa et al 1995, Nishikawa and Lew 1996, Lew et al 1996, 1997a, Stein et al 1996, Yasuda et al 1997a, 1997b, 1999, Tavener et al 2004) and induces cardiomyocyte apoptosis (Comstock et al 1998, Li et al 2002, Suzuki et al 2003, 2007, McDonald et al 2000, Chagnon et al 2005, Niu et al 2008). However, LPS failed to attenuate either baseline cardiomyocyte contractility or noradrenaline-induced increases in cardiomyocyte contractility in one study (Muller-Werdan et al 1998).

Although there is no doubt that a decreased myocardial function contributes toward heart failure, there has been some doubt expressed as to the clinical relevance of cardiomyocyte apoptosis (programmed cell death). However, there is now increasing evidence to indicate that cardiomyocyte apoptosis may be an important change that contributes toward cardiac dysfunction and heart failure. In this regard, an increased cardiomyocyte apoptosis occurs in transgenic models of heart failure (Sarkar et al 2004) and in human heart failure (Abbate et al 2003). Moreover, inhibitors of the caspase enzyme, an enzyme that is central to mediating the apoptotic process, have been demonstrated to reduce cardiomyocyte apoptosis and attenuate the reduction in left ventricular pump dysfunction in the transition to heart failure (Hayakawa et al 2003, Engel et al 2004). Furthermore, implantation of human bone-marrow−derived angioblasts prevents cardiomyocyte apoptosis and adverse cardiac remodeling (Kocher et al 2001). Thus, it appears that cardiomyocyte apoptosis may be a critical determinant

of a failing myocardium, and hence that LPS-induced cardiomyocyte apoptosis (Li et al 2002, Suzuki et al 2003, Suzuki et al 2007, Comstock et al 1998, McDonald et al 2000, Niu et al 2008, Chagnon et al 2005) may be a key determinant of heart failure as a consequence of severe or repeated systemic infections. Indeed, blockade of a critical enzyme involved in apoptosis prevents cardiomyocyte apoptosis and cardiac dysfunction in an animal model of sepsis (Neviere et al 2001).

The potential mechanisms of the LPS-induced decrease in contractile function and cardiomyocyte apoptosis have been summarized in Figure 1.2. Importantly, activation of pro-inflammatory cytokines such as TNF-α may not be a necessary pre-requisite to LPS-induced cardiomyocyte apoptosis or contractile dysfunction (Nishikawa et al 1996). The mechanisms of the effects are thought to be through LPS-induced effects on Toll-like receptors (Frantz et al 1999, Tavener et al 2004, Li et al 2002) found either on leukocytes or on cardiomyocytes. LPS-induced activation of Toll-like receptors on cardiomyocytes is thought to induce apoptosis in-part by stimulating cardiomyocyte angiotensinogen, and hence through angiotensin II, promoting calcineurin-induced cardiomyocyte programmed cell death (Suzuki et al 2007). TNF-α may however, play a role as LPS-induced apoptosis can be inhibited by administration of a TNF-α receptor fragment (Cowan et al 2001, Peng et al 2005). With respect to the mechanisms of the LPS-induced decrease in contractile function, this may occur through an impaired mitochondrial function (Tavener et al 2004), down-regulation of dihydropyridine receptors (the Ca^{2+} channels required for excitation-contraction coupling in the myocardium)(Lew et al 1996), or through the activation of inducible nitric oxide synthase which enhances nitric oxide synthesis and produces increases in cardiomyocyte cyclic guanosine monophosphate (cGMP) concentrations (Brady et al 1992, Stein et al 1996, Yasuda and Lew 1997a, 1997b, Lew et al 1997b, Yasuda and Lew 1999). With respect to the impact of cGMP, in contrast to cyclic adenosine monophosphate which promotes contractile function by stimulating

Figure 1.2. Potential cellular mechanisms of the deleterious effects of endotoxin on the myocardium. LPS, lipopolysaccharide; TLR-4, toll-like receptor-4; Ang II, angiotensin II; AT1 R, angiotensin type I receptor. See Figure 1.1 for additional abbreviations. The figure summarises pathways supported by the following references: Prabhu et al 2004; Liu et al 2008; Cheng et al 2009; Suzuki et al 2007;Chagnon et al 2005; Liu et al 2008; Tavener et al 2005.



intracellular Ca^{2+} release from the sarcoplasmic reticulum, cGMP reduces myocardial contraction by decreasing the myofilament response to Ca^{2+}. An additional mechanism by which LPS induces deleterious effects on the myocardium is through ventricular hypertrophy, which is mediated by calcineurin, the release of which was described in the previous paragraphs (Cheng et al 2009).

The now considerable data demonstrating that LPS promotes myocardial dysfunction and cardiomyocyte apoptosis (Suffredini et al 1989, Brady et al 1992, Hung and Lew 1993a, 1993b, Tao and McKenna 1994, Nishikawa et al 1995, Nishikawa and Lew 1996, Lew et al 1996, 1997a, Stein et al 1996, Comstock et al 1998, Yasuda and Lew 1997a, 1997b, 1999, Li et al 2002, Suzuki et al 2003), is of major clinical importance in sepsis and septic shock. Indeed, as previously indicated, sepsis and septic shock are frequently associated with a marked decrease in cardiac function (Calvin et al 1981, Parker et al 1984, Jafri et al 1990, Munt et al 1998, Poelaert et al 1997). However, can one extrapolate these data to a potential clinically relevant effect in chronic infections or infections which are not associated with the haemodynamic changes that accompany sepsis? Obviously it is unlikely that low-grade infections result in acute decreases in cardiac contractile or diastolic function and consequently cardiac decompensation unless co-existing cardiac pathology exists where febrile responses will increase haemodynamic loads on the heart. However, it is also possible that with repeated inflammatory responses, modest degrees of cardiomyocyte apoptosis, with no immediate effect on heart function, could nevertheless ultimately translate into a significant degree of myocardial damage, thus ultimately heralding the onset of cardiac dysfunction and heart failure.

The authors of the studies showing that cardiomyocyte apoptosis occurs in response to LPS (Li et al 2002, Suzuki et al 2003, 2007, Comstock et al 1998) have suggested that this effect occurs at sufficiently low enough concentrations of LPS as to be comparable with the LPS

concentrations that occur in chronic infections. However, as shall be discussed, there is both evidence to support and refute the notion that LPS-induced effects on the myocardium are indeed compatible with LPS concentrations that occur during the average chronic infection.

3.2 Is there sufficient evidence to suggest that low grade infections promote myocardial damage?

Table 1.1 summarizes all of the studies that to my knowledge have assessed the impact of LPS on cardiomyocyte apoptosis conducted *in vivo* or *ex vivo* (employing cell cultures). The Table also summarizes the doses of LPS employed in the *in vivo* studies. It should be apparent from this Table that the lowest dose of LPS employed by these investigators to produce cardiomyocyte apoptosis was I mg/kg (Suzuki et al 2003, Suzuki et al 2007, Li et al 2002). In this regard, this dose has recently been reported to produce a marked decrease in blood pressure and cerebral vasodilation, an increased circulating lactate concentration, and a metabolic acidosis with a compensatory decrease in $PaCO_2$ (respiratory drive) (Rosengarten et al 2008). It would thus appear that although increased circulating LPS concentrations occur in chronic infections (Moutsopoulos and Madianos 2006, Geerts et al 2002), the lowest dose of LPS administered in *vivo* to induce cardiomyocyte apoptosis (I mg/kg)(Suzuki et al 2003, Suzuki et al 2007, Li et al 2002) is more likely to induce all of the changes that one would normally consider to be congruent with septic shock (Rosengarten et al 2008). The question therefore remains as to whether low-grade infections without the changes associated with septic shock are indeed able to produce cardiomyocyte apoptosis. To address this issue, *as part of the present dissertation I therefore studied the effect of doses of LPS that have previously been shown to produce a febrile response consistent with low-grade infections but without producing septic shock (Harden et al 2006), on cardiomyocyte apoptosis*.

Table 1.1. Summary of studies assessing the effects of lipopolysaccharide (LPS) on myocardial apoptosis.

Author(s)	In vivo or in vitro	Dose of LPS in *in vivo* studies	Apoptosis		
			TUNEL	Caspase	Other
Suzuki et al 2007	In vivo†	1mg/kg	Increased between 4-24hr ($p<0.05$) (n=5)	Increased between 4 and 24hr ($p<0.05$) (n=7-19)	-
	In vitro†	10ng/ml	Increased after 24hr ($p<0.05$) (n=5-9)	-	-
Li et al 2002	In vitro†	10ng/ml	Increased at 24hr ($p<0.01$) (n=5).	Increased caspase-3 activity at 16hr $P<0.05$ (n=10)	Bcl2:bax decreased at 12hr ($p<0.05$) (n=6)
	In vivo†	1mg/kg iv	Increased at 1-3 days ($p<0.05$) (n=3)	-	-
Niu et al 2008	In vivo*	10 mg/kg ip	Increased ($p<0.01$) vs control after 12 hours	No increase in caspase-3/7 after 12 hours	-
Suzuki et al 2003	In vitro†	10ng/ml,24hr	Increased after 24hr ($p<0.05$) (n=13)	-	-
	In vivo†	1mg/kg iv	Increased after 24 hr ($p<0.05$) (n=5-7)	-	-
Comstock et al 1998	In vitro	100ng/ml			Comet assay. Increases in percentage apoptotic cells ($p<0.001$) (n=20)
Chagnon et al 2005	In vivo†	10 mg/kg		Increased caspase-3 ($p<0.05$) after 6hrs and 24hrs	Increased DNA fragmentation ($p<0.05$) at 6hrs. Increased cytochrome-c ($p<0.05$) at 6hrs
McDonald et al 2000	In vivo†	4mg/kg	Increase ($p<0.05$) at 24hr	Increased caspase-3 ($p<0.05$) at 24hrs	Increased bax ($p<0.05$) at 24hr. Increased bcl2 at ($p<0.05$) at 6hr
Chao et al 2005	In vitro†	500ng/ml	-	Reduced caspase-3 activation following serum deprivation.	LPS reduced histone-DNA fragmentation and DNA laddering after serum deprivation ($p<0.01$) (n=3)

† Sprague Dawley rats,*mice

4.0 Obesity-induced inflammatory changes and cardiac effects.

As shall be underscored in the present section of the dissertation, obesity is now a well-recognized source of systemic inflammation. Furthermore, as shall also be highlighted in subsequent sections, there is an emerging body of evidence to indicate that obesity produces myocardial damage and dysfunction independent of conventional cardiovascular risk factors. As there is now a substantial body of evidence to indicate that pro-inflammatory cytokines may produce myocardial dysfunction and damage (see section 2.0), it is not inconceivable that obesity-induced inflammatory changes may also mediate myocardial damage. Nevertheless, as shall be discussed, few studies have pursued the notion that obesity-induced inflammatory effects may mediate the adverse effects of obesity on the myocardium.

The question of the role of inflammation as a potential cause of obesity-induced myocardial damage and dysfunction is of importance in the context of the emerging world-wide epidemic of obesity. Indeed, the prevalence of obesity is increasing in both developed and in economically emerging countries (Ogden et al 2007, Flegal et al 2002, Puoane et al 2002). In the United States of America in 2004, ~31% of men and ~33% of women were obese and ~3% of men and ~7% of women were extremely obese (a body mass index [BMI] > 40 kg/m^2) (Ogden et al 2007). In South Africa, a country which is considered to be an emerging economy, at approximately the turn of the century, ~29% of men and ~57% of women were obese (Puoane et al 2002).

It may be argued that the solution to this problem is not to identify the mechanisms through which obesity produces morbidity and mortality, but rather to focus research efforts on weight loss programs. However, there is now increasing evidence to indicate that obesity, once established, is difficult to manage. Indeed, weight reduction programs seldom result in obese individuals reaching target body weights and frequently result in an inability to maintain

appropriate weight reduction (Anderson et al 2001, Klein et al 2004, Lang et al 2006). Thus, there is no question that the adverse effects of obesity on the heart and the potential mechanisms that explain these effects are of societal importance and of importance to the health care sector in South Africa.

4.1 Adipose tissue as a source of inflammation.

As indicated in the aforementioned discussion, one potential mechanism that may explain the deleterious effects of obesity on a number of organ systems is through the capacity of adipose tissue to increase circulating cytokine concentrations. Indeed, adipose tissue is a key determinant of an increased systemic inflammatory state and the evidence for relationships between an excess adiposity and the pro-inflammatory mediators C-reactive protein, TNF-α, interleukin-6, leptin, and serum amyloid A3 has been extensively reviewed (Berg et al 2005). Moreover, although derived from adipocytes, circulating concentrations of the anti-inflammatory adipokine, adiponectin, are decreased in obesity (Berg et al 2005). Importantly, reductions in circulating concentrations of inflammatory markers are associated with weight loss produced by liposuction (Giugliano et al 2004), gastric bypass (Kopp et al 2003) and diet (Heilbronn et al 2001) and in rats removal of visceral fat reduces TNF-α concentrations (Gabriely et al 2002). Is adipose tissue associated with the development of heart failure?

4.2 An excess adiposity is associated with heart failure independent of conventional cardiovascular risk factors.

Over the past decade, a nested case-control study and a number of prospective studies have demonstrated an independent relationship between the degree of adiposity and the

development of heart failure (Chen et al 1999, He et al 2001, Johansson et al 2001, Wilhelmsen et al 2001, Kenchaiah et al 2002, 2009, Ingelsson et al 2005a and 2005b, Nicklas et al 2006, Bahrami et al 2008, Spies et al 2009). The outcomes and characteristics of these studies have been summarized in Table 1.2. This relationship has been demonstrated in the general population (He et al 2001, Kenchaiah et al 2002, Ingelsson et al 2005b, Bahrami et al 2008), in the middle-aged (Ingelsson et al 2005a), in the elderly (Chen et al 1999, Nicklas et al 2006), in men (Wilhelmsen et al 2001, Ingelsson 2005b), in women (He et al 2001), in general practice (Johansson et al 2001), in physicians (Kenchaiah et al 2009) and in persons with established coronary artery disease (Spies et al 2009).

As compared to referent body mass indices between 20 and 25 kg/m^2, a body mass index of between 25 and 30 kg/m^2 may increase the risk of heart failure by 39-49% (Bahrami et al 2008, Kenchaiah et al 2009) and a body mass index greater than or equal to 30 kg/m^2 increases the risk of heart failure by 83-180% independent of conventional cardiovascular risk factors (Johansson et al 2001, Kenchaiah et al 2002, Bahrami et al 2008, Kenchaiah et al 2009). In one study however, no increased risk for heart failure was noted in persons with a body mass index of between 25 and 30 kg/m^2 (overweight) in a general practice setting (Johansson et al 2001). Moreover, in one study (Spies et al 2009), but not in other studies (Ingelsson et al 2005a, Nicklas et al 2006) the adipose tissue-heart failure relationship is better predicted by the use of indices of adiposity that reflect an accumulation of fat in central (abdominal) stores, such as waist circumference or waist-to-hip ratio, than indices such as body mass index. Depending on the study population and the study design, the independent risk for heart failure in people whom are overweight or obese is quantitatively comparable with the impact of for example the presence of hypertension, or diabetes mellitus in these same studies (Table 1.2).

Table 1.2. Characteristics and outcomes of studies showing a relationship between an excess adiposity and heart failure.

Author	Sample size	Age	Follow up	Number of new cases	Relationship between adiposity and heart failure After adjustments		
					BMI kgm^2	RR (95% CI)	p value
Chen et al 1999	1 749	65+ yrs	10 yrs	173	≥28kgm^2 compared with BMI <24kgm^2.	1.60 (1.0-2.4)	0.04
He et al 2001	13 643 5545 men 8098 women	25-74 yrs	19 yrs	1 382	≥ 27.8 kgm^2 (men) ≥27.3kgm^2 (women)	1.30 (1.12-1.52)	=0.001
Johansson et al 2001	689 467 cases; 5000 controls	40-84 yrs	-	938 (total) 489 men, 449 women	25-29.9kgm^2	1.0(0.7-1.2)	-
					≥30 kgm^2	2.1(1.5-2.8)	-
Kenchaiah et al 2002	5 881	55 yrs	14 yrs	496 (total) 238 men 258 women	25-29.9kgm^2	1.34(1.08-1.67)	=0.007
					≥30 kgm^2	2.04(1.59-2.63)	<0.001
Kenchiah et al 2009	21 094 men	53 yrs	20.5 yrs	1109	25-29.9kgm^2	1.49(1.32-1.69)	<0.0001
					≥ 30kgm^2	2.80(2.34-3.50)	<0.0001
Ingelsson et al 2005a	1 187 men	≥70 yrs	7-12 yrs	104		1.35 (1.11-1.65)	Not shown
Ingelsson et al 2005b	2 314 men		29.6 yrs	282		1.48(1.33-1.65)	<0.001
Nicklas et al 2006	2 435. 1081 men 1354 women	70-79 yrs	6.1 yrs	166	versus <25 kgm^2	1.25(1.02-1.53)	p=0.003
Bahrami et al 2008	6 814	55-84 yrs	4 yrs	79	versus <25 kgm^2	-	-
Spies et al 2009	979	65 yrs	4.9 yrs	128 hospitalised. 152 cardiovascular events	versus <25 kgm^2	1.6 (1.2-2.1)	
Wilhelmson et al 2001	7495 men	47-55 yrs	27 yrs	937	≥30 kgm^2	1.06 (1.03-1.09)	=0.0001

BMI, body mass index; CHD, coronary heart disease; RR, relative risk.

Although the relationship between an excess adiposity and the development of heart failure is modified by adjustments for conventional cardiovascular risk factors, including hypertension, diabetes mellitus and cholesterol concentrations as well as with adjustments for coronary events, the impact of these adjustments is surprisingly modest. Indeed, considering overweight and obese persons overall, the percentage risk for heart failure is diminished by only 1-13% with these adjustments (He et al 2001, Ingelsson et al 2005a, 2005b, Nicklas et al 2006, Spies et al 2009) and by only 55% in obese individuals in a study in which obesity increased the risk for heart failure by 180% (Kenchaiah et al 2009). Thus, although there is no question that to prevent overweight/obesity-induced heart failure, targeting modifiable cardiovascular risk factors with lifestyle interventions and medication is an essential approach, the large residual risk for heart failure after adjustments for conventional cardiovascular risk factors indicates that this may not be the most appropriate solution.

4.3 Adiposity and cardiac dysfunction

It is important to acknowledge that heart failure is a clinical syndrome with many causes that are frequently, but not necessarily directly related to adverse myocardial effects. As such, relationships between an excess adiposity and heart failure do not provide direct evidence for adverse effects on the heart. Is there evidence to indicate that an excess adiposity is associated with adverse cardiac functional changes that could represent an intermediate process in the development of heart failure? If so, are there intervention studies with weight reduction to provide a proof of principle that an excess adiposity promotes myocardial damage?

In the Multiethnic Study of Atherosclerosis, the independent relationship between obesity and congestive heart failure was reduced from an 83% to a 58% risk of heart failure with the inclusion of left ventricular ejection fraction in the regression model (Bahrami et al 2008). These

data indicate that decreases in pump function, which in otherwise well individuals is a well established risk factor for the development of heart failure (Bahrami et al 2008), is a potentially important mechanism through which obesity promotes heart failure. However, not all studies support the view that the relationship between an excess adiposity and heart failure can be explained by the development of cardiac dysfunction. Indeed, the risk of heart failure that is explained by waist-to-hip ratio may persist even with adjustments for measures of cardiac function, including ejection fraction and measures of diastolic dysfunction (Spies et al 2009). Is there additional evidence to support a view that an excess adiposity may mediate heart failure through direct effects on the heart?

A number of pre-clinical studies have provided evidence to suggest that cardiomyocyte dysfunction or damage to the heart may occur in obese states (Caroll et al 1997, Relling et al 2006, Dong et al 2006, Ren et al 2000, Barouch et al. 2006, Zhou et al 2000). However, as many of the animal models employed in these studies (Caroll et al 1997, Relling et al 2006, Dong et al 2006, Ren et al 2000, Barouch et al. 2006, Zhou et al 2000) also have associated conventional cardiovascular risk factors, whether the outcomes of these studies are indeed beyond all conventional risk factors is uncertain. Nevertheless, in human studies, load-independent tissue Doppler indices of both systolic and diastolic myocardial function have been shown to be reduced in overweight and obese people without conventional cardiovascular risk factors (Peterson et al 2004, Wong et al 2004). Further, at a population level, indices of excess adiposity are independently and inversely related to diastolic chamber function beyond a number of conventional cardiovascular risk factors (Redfield et al 2003, Ammar et al 2008, Fischer et al 2003, Tsioufis et al 2009, Libhaber et al 2009) including blood glucose control as determined from glycated haemoglobin measurements, ambulatory blood pressure and arterial stiffness (Libhaber et al 2009). In contrast, the independent relationship between an excess adiposity and systolic chamber function is controversial, with some studies showing a

relationship between an excess adiposity and systolic function (Ammar et al 2008, Chinali et al 2006, Peterson et al 2004, Wong et al 2004, Scaglione et al 1992, Karason et al 1998, Alpert et al 1995, Alpert et al 1993), whilst others have failed to do so (Pascual et al 2003, de Devitiis et al 1981, Zarich et al 1991, Stoddard et al 1992, De Simone et al 1996, Mureddu et al 1996, Iacobellis et al 2002).

More direct evidence to support a role for an excess adiposity in producing cardiac dysfunction is that obtained from studies of weight reduction. Weight loss induced either by gastric bypass surgery (Willens et al 2005, Rider et al 2009) or by lifestyle intervention (Wong et al 2006) results in improvements in myocardial diastolic function independent of conventional cardiovascular risk factors. However, even with the use of load-independent tissue Doppler measures of myocardial as opposed to chamber function, or with chamber function assessments, weight loss produced by either lifestyle modification or gastric bypass does not influence left ventricular systolic function (Willens et al 2005, Rider et al 2009, Wong et al 2006, Skilton et al 2007). The ability of gastric bypass surgery to improve myocardial diastolic function (Willens et al 2005, Rider et al 2009) independent of conventional cardiovascular risk factors and in association with weight loss, nevertheless provides more convincing evidence for a role of obesity in promoting heart failure through direct effects on the heart. Furthermore, as weight loss following gastric bypass surgery avoids the use of dietary interventions, this evidence (Willens et al 2005, Rider et al 2009) suggests that obesity rather than dietary constituents contribute toward this beneficial effect of weight loss on the heart.

4.3 Is there a relationship between an excess adiposity and cardiac dysfunction beyond all potential haemodynamic mediators?

There are a number of potential hypotheses to explain the relationship between obesity and myocardial damage or dysfunction independent of conventional cardiovascular risk factors. Before I discuss the potential role of inflammatory substances derived from adipose tissue as determinants of obesity-cardiac dysfunction relations, it is important that I first exclude a role for haemodynamic factors that are not normally measured as part of risk assessment. In this regard, a number of haemodynamic factors may mediate obesity-induced decreases in cardiac systolic or diastolic function. These haemodynamic factors include the following:

A decrease in total peripheral resistance with a subsequent increase in venous return may occur in obese people, an alteration that may increase volume preloads on the heart (Messerli et al 1983, Carabello and Gittens 1987). Furthermore, an increase in large artery stiffness is associated with an excess adiposity (Sutton-Tyrrell et al 2001, Majane et al 2008), a change that may increase central blood pressure by promoting earlier wave reflections. Hence, there is a possibility that an enhanced cardiac afterload may occur in people with an excess adiposity, mediated by changes in central as opposed to peripheral arteries. Measurements of peripheral blood pressures may not reveal these increases in central pressures because of the effects of pressure amplification that occurs from central to peripheral arteries. Last, the relationship between an excess adiposity and 24 hour blood pressures may not be the same as that between an excess adiposity and office blood pressures (Majane et al 2007). Thus, measurements of office blood pressure may not reveal the full extent of obesity-induced increases in blood pressure on the heart.

Is there evidence to indicate that an excess adiposity promotes cardiac dysfunction beyond all of the aforementioned haemodynamic changes? Indeed, at a community level, the

independent relationship between central obesity and a reduction in left ventricular chamber diastolic function is independent of left ventricular filling volumes, large artery stiffness and 24-hour ambulatory blood pressure (Libhaber et al 2009). Consequently, a potential role of non-haemodynamic factors mediating obesity-induced decreases in cardiac dysfunction requires consideration.

4.4 Obesity-induced inflammation as a potential determinant of cardiac dysfunction.

There are a number of hypotheses that could potentially explain the independent relationship between obesity and either heart failure or decreases in cardiac systolic or diastolic function. However, with respect to the topic of the present dissertation, I will restrict the discussion to the hypothesis that inflammatory mediators derived from adipose tissue may explain this relationship. Before discussing the evidence to suggest that obesity-derived inflammatory changes mediate heart failure, it is nevertheless important to highlight the fact that C-reactive protein, TNF-α, and interleukin-6 (Bahrami et al 2008, Vasan et al 2003, Cesari et al 2003) as well as erythrocyte sedimentation rate (Ingelsson et al 2005b), a generalized marker of systemic inflammation, have all been shown to be independent predictors of heart failure.

In support of a role for obesity-derived inflammatory substances as mediators of heart failure, after adjustments for either interleukin-6 or C-reactive protein in the regression analysis, the independent relationship between obesity and heart failure failed to achieve statistical significance (Bahrami et al 2008). However, in that study (Bahrami et al 2008) the increased risk for heart failure in obese patients decreased from an 83% risk (hazards ratio=1.83) to a 50-58% risk (hazards ratio=1.50-1.58) after adjustments for inflammatory markers. Thus, a potential residual risk for heart failure remained even after adjustments for pro-inflammatory markers (Bahrami et al 2008). In support of this notion, in another study (Spies et al 2009), the

independent relationship between waist-to-hip ratio and the subsequent development of heart failure in patients with established coronary artery disease was only partially modified with adjustments for C-reactive protein (CRP), TNF-α, and interleukin-6. In that study, the independent relationship between an excess adiposity and the development of heart failure remained even after adjustments for these inflammatory markers (Spies et al 2009). Thus, there is still considerable controversy as to the role of adipose tissue-derived pro-inflammatory substances in the development of obesity-induced heart failure. Is there evidence for a role of anti-inflammatory substances derived from adipose tissue as possibly mediating protective effects on the myocardium?

Pre-clinical studies suggest that the adipose tissue-derived anti-inflammatory substance adiponectin protects the heart against adverse myocardial effects. Indeed, adiponectin knock-out promotes (Shibata et al 2004, Sam et al 2010) and delivery of adiponectin attenuates (Shibata et al 2004) adverse cardiac remodelling in response to a pressure overload. Furthermore, adiponectin knock-out augments diastolic dysfunction and promotes the development of diastolic heart failure in pressure overload states (Sam et al 2010). However, prospective studies have failed to show adiponectin as an independent risk factor for the development of heart failure (Ingelsson et al 2006, Frankel et al 2009).

4.4 Further work is required to explore the role of inflammation as a determinant for the impact of obesity on the myocardium.

Clearly further evidence is still required to identify whether increased concentrations of pro-inflammatory substances and decreased concentrations of anti-inflammatory substances mediate the independent relationship between obesity and cardiac failure and dysfunction. As there are presently only two studies to-date that have explored whether an excess cytokine

production contributes toward obesity-induced heart failure (Kenchaiah et al 2002, Spies et al 2009), there is an obvious need to pursue this question further. In this regard, both animal and human studies are required to either support or refute this notion.

Human studies are required to assess whether the relationship between obesity and cardiac dysfunction or the beneficial impact of weight reduction on cardiac function can be accounted for by the presence of circulating inflammatory markers. Animal studies are required to inhibit cytokine production in models of obesity and determine whether this improves cardiac function. In our laboratory, although we have access to an animal model of dietary-induced obesity, members of our group have recently demonstrated that this model is not associated with left ventricular systolic dysfunction (du Toit et al 2008). Therefore, this is not a model which can be employed to determine the mechanisms of obesity-induced systolic dysfunction.

In contrast, our laboratory is studying left ventricular structure and function in a community-based sample with a high prevalence of obesity (Norton et al 2008, 2009, Woodiwiss et al 2008, 2009, Majane et al 2007, 2008). *Consequently, as part of the present dissertation, I explored the possibility that a marker of inflammation, CRP, may in-part explain the association between an excess adiposity and left ventricular systolic dysfunction in a relatively large population sample obtained from an ongoing study being conducted in an urban, developing community of African ancestry with a high prevalence of obesity* (Norton et al 2008, 2009, Woodiwiss et al 2008, 2009, Majane et al 2007, 2008).

To evaluate the impact of CRP on systolic cardiac function, I selected to assess the relationship between CRP and cardiac function in a community sample, as compared to a sample of patients in heart failure. I employed this approach to dissect out the effect of “obesity-mediated” as compared to “heart failure-mediated” inflammatory-induced changes (see previous sections). However, there is some controversy as to the method of assessing cardiac systolic function in studies where heart failure is not present. Therefore, before describing the studies

performed as part of the present dissertation, it is important that I first outline the controversies regarding the detection of preclinical abnormalities of systolic cardiac function in human studies.

5.0 The detection of an abnormal systolic cardiac function in human studies of obesity

A number of studies have been conducted to assess the relationship between obesity and cardiac function in human studies. In this regard, the majority of studies have reported on a deleterious effect of obesity on diastolic function of the heart, but studies assessing the impact of obesity on systolic chamber function (pump function) independent of conventional cardiovascular risk factors have produced conflicting outcomes with the majority showing a lack of effect of obesity on cardiac pump function as indexed by left ventricular ejection fraction or other measures of chamber function (Peterson et al 2004, Wong et al 2004, Pascual et al 2003, Scaglione et al 1992, de Devitiis et al 1981, Chakko 1991, Zarich et al 1991, Stoddard et al 1992, De Simone et al 1996, Mureddu et al 1996, Iacobellis et al 2002, Karason et al 1998). Nevertheless, subsequent studies performed using tissue Doppler imaging techniques, which assess myocardial function, have demonstrated a reduction in myocardial systolic function in obesity, despite a normal systolic chamber function (Peterson et al 2004, Wong et al 2004).

Although I did not have access to tissue Doppler imaging techniques for the assessment of myocardial function, one measure of myocardial function that has not previously been explored with respect to a potential impact of obesity independent of hypertension, is left ventricular midwall fractional shortening. In contrast to left ventricular endocardial fractional shortening, which is an index of systolic chamber function, left ventricular midwall fractional shortening is an assessment of myocardial function (Norton et al 2002). A number of studies have now demonstrated that under circumstances of a normal systolic chamber function, as

indexed by endocardial fractional shortening, when the left ventricle is concentrically remodelled, midwall fractional shortening may be reduced (Mayet et al 2002,De Simone et al 1994, Li et al 2001,Muiesan et al 2000). Although a lack of relationship between body size and left ventricular midwall fractional shortening has been reported on in hypertensives (De Simone et al 1996), this relationship has not been studied in the absence of the confounding effects of hypertension (i.e, in the general population). Moreover, this relationship has not been assessed in a population sample with a high prevalence of obesity or in a population sample with a high prevalence of concentric cardiac remodelling produced by an excess adiposity. As we have recently demonstrated that obesity produces marked concentric remodelling of the left ventricle (Woodiwiss et al 2008), the use of midwall fractional shortening to assess the relationship between obesity and cardiac systolic function is entirely appropriate.

Therefore, in the present dissertation I evaluated the relationship between *CRP and left ventricular midwall fractional shortening, in a relatively large population sample obtained from an ongoing study being conducted in an urban, developing community of African ancestry with a high prevalence of obesity.*

6.0 Summary of problem statements

Problem statement 1: Although there is evidence to indicate that LPS, derived from gram negative organisms, can promote cardiomyocyte apoptosis, there is a question as to whether the doses of LPS employed in these studies produce effects that represent septic shock or low-grade infections.

Problem statement 2: Although there is evidence to indicate that obesity can promote cardiac dysfunction independent of conventional cardiovascular risk factors, only two studies have explored whether a relationship with heart failure could be mediated by inflammatory

changes associated with obesity and no studies have assessed whether an independent relationship with cardiac dysfunction could be mediated by inflammatory changes associated with obesity.

7.0 Aims of the dissertation

The major aim of the present dissertation was to provide further proof of principle that low grade inflammation may contribute toward myocardial damage and dysfunction. In this regard the specific aims of the present dissertation were:

1) To assess whether pyrogenic, but not septic doses of LPS promote cardiomyocyte apoptosis in rats.
2) To determine whether indices of adiposity are independently associated with left ventricular midwall as opposed to endocardial fractional shortening in a randomly selected community sample with a high prevalence of excess adiposity and subsequently to evaluate whether this relationship could be accounted for by circulating concentrations of the inflammatory marker, CRP.

Chapter 2

Methods

As indicated in chapter 1, in the present dissertation, two separate studies were conducted, one in rats and one in a cross-sectional population sample. The justification for using rats in one study and humans in another has already been provided. For ease of reading, the methodology for these two studies will be described in separate sections.

2.1 Methodology for the study to assess whether pyrogenic doses of LPS promote cardiomyocyte apoptosis in rats.

2.1.1 Study design and animals

Male Sprague-Dawley rats weighing between 400g and 500g were studied. Rats were housed individually in cages where the room temperature was maintained at approximately 22ºC and the lighting set to a 12:12 hour light: dark cycle. Food and water were provided *ad libitum.* Rats were handled and injected with saline daily at the volume employed to administer LPS (1ml/kg) for a period of two weeks prior to the study to habituate them to the experimental procedures. The habituation took place to prevent stress-induced hyperthermia, which commonly results from novel handling procedures (Kluger et al 1987,Cabanac et al 1992).

To assess the effects of a pyrogenic dose of *lipopolysaccharide* (LPS) on the heart, rats were randomly assigned to receive a subcutaneous injection of either saline (1 ml/kg) or Lipopolysaccharide (LPS) (250 µg/kg). The LPS was derived from Escherichia coli endotoxin (serotype 0111:B4, Sigma, St. Louis, MO, USA) and reconstituted with saline (sterile, pyrogen-free 0.9% saline, Sabax, Johannesburg, South Africa). A stock solution of 5000µg/ml was prepared and diluted to a concentration of 250µg/ml. As LPS is present in the environment, sterility was maintained in the procedures involving preparation of the stock solution. It has previously been documented that LPS adheres to plastic whilst in storage, and therefore the

stock solution was prepared specifically with the aim of standardizing the concentration of the solution contained therein. Tubes containing LPS were placed in an ultrasonic bath which removed any LPS adherent to the tube. LPS or saline were injected subcutaneously (Harden et al 2006) on two separate, consecutive days and the second LPS injection was exactly 24hour after the first injection for each rat. The rats were killed 6 hours after the second LPS injection. Two LPS injections were given to ensure that cardiomyocyte apoptosis was assessed at the time of a febrile response (second injection), but also following a sufficiently long period to ensure that apoptotic cell death could be detected using a TUNEL system. The LPS was administered at approximately the same time of the day in each rat (between 7 and 9am), to eliminate the possible effect of circadian alterations in temperature, which could possibly affect the amplitude of the fever response.

Rats were anaesthetized 6 hours after the second injection of LPS or the vehicle in order to perform echocardiography. Echocardiography was performed to exclude the possibility that a sufficiently large dose of LPS was administered to produce myocardial dysfunction and could therefore be considered to produce haemodynamic changes consistent with sepsis. The hearts were then rapidly removed under anaesthesia and placed in ice cold water, dissected and stored in 10% phosphate buffered formaldehyde for histological assessments (for the evaluation of cardiomoycyte apoptosis).

To evaluate the pyrogenic effect of the LPS, febrile responses to LPS or the saline vehicle were determined in a separate group of 10 rats using temperature-sensitive radiotransmitters.The dose of LPS administered to rats was considered physiologically effective if a fever developed. A fever was defined as an increase in core body temperature above 1℃.

2.1.2 Core body temperature measurements and assessment of fever responses

Core body temperature was measured continuously and in real-time using surgically implanted, temperature-sensitive radiotransmitters (resolution: 2ºC, Data Sciences) (Figure 2.1) implanted into the peritoneal cavity of rats. One radiotransmitter was surgically implanted into each rat, following the induction of anaesthesia with a combination of xylazine (4mg/kg intramuscularly) and ketamine hydrochloride (80mg/kg intramuscularly). The radiotransmitters were coated in non-toxic plastic which reduced the risk of infection and the extent of tissue adhesion, both of which could influence the accuracy of the results. Core body temperature was measured by the temperature-sensitive radiotransmitters which employ remote biotelemetry. The output frequency (Hz) of the transmitter was monitored at 5 minute intervals by a receiver plate (RTA 500, Mini-Mitter, Sunriver, OR, USA) below the cage of each rat (figure 2.1, bottom panel). The frequency transmitted by each plate was fed into a peripheral processor (DP-24 Data-Port, VitalView, Minimitter, Sunriver, OR, USA) connected to a personal computer and the output was measured in degrees centigrade. Rats were allowed to recover for two weeks following the surgery before the effect of LPS was determined.

To obtain average daily temperatures, the temperature over time was calculated as the area under the graph of a time-temperature graph (Figure 2.2). To ensure that the increase in core body temperature following LPS administration was a true reflection of a fever, and not a result of daily fluctuations in core body temperature during the circadian rhythm, the average area under the curve (thermal response index) was calculated for each rat over a period of three days prior to LPS or vehicle administration. The difference between the thermal response index following LPS or vehicle administration and the average daily temperature obtained from the average area under the graph over 3 days prior to either LPS or saline administration was assessed.

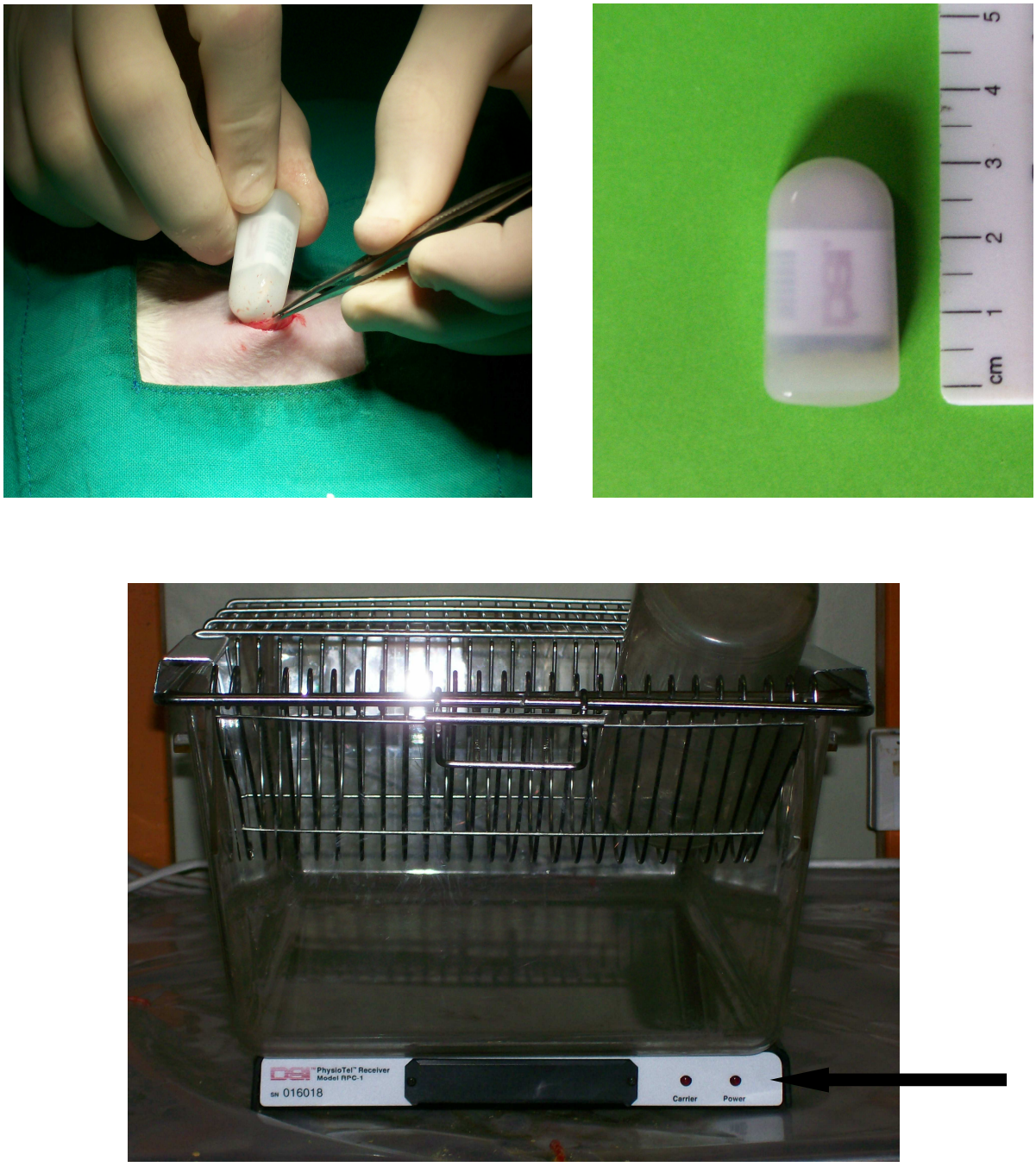


Figure 2.1. Photograph of the surgically implanted, temperature-sensitive radiotransmitter used to determine core body temperature (top). The bottom panel shows the recording plate placed underneath the cage that an individual rat was housed in, as illustrated by the arrow.

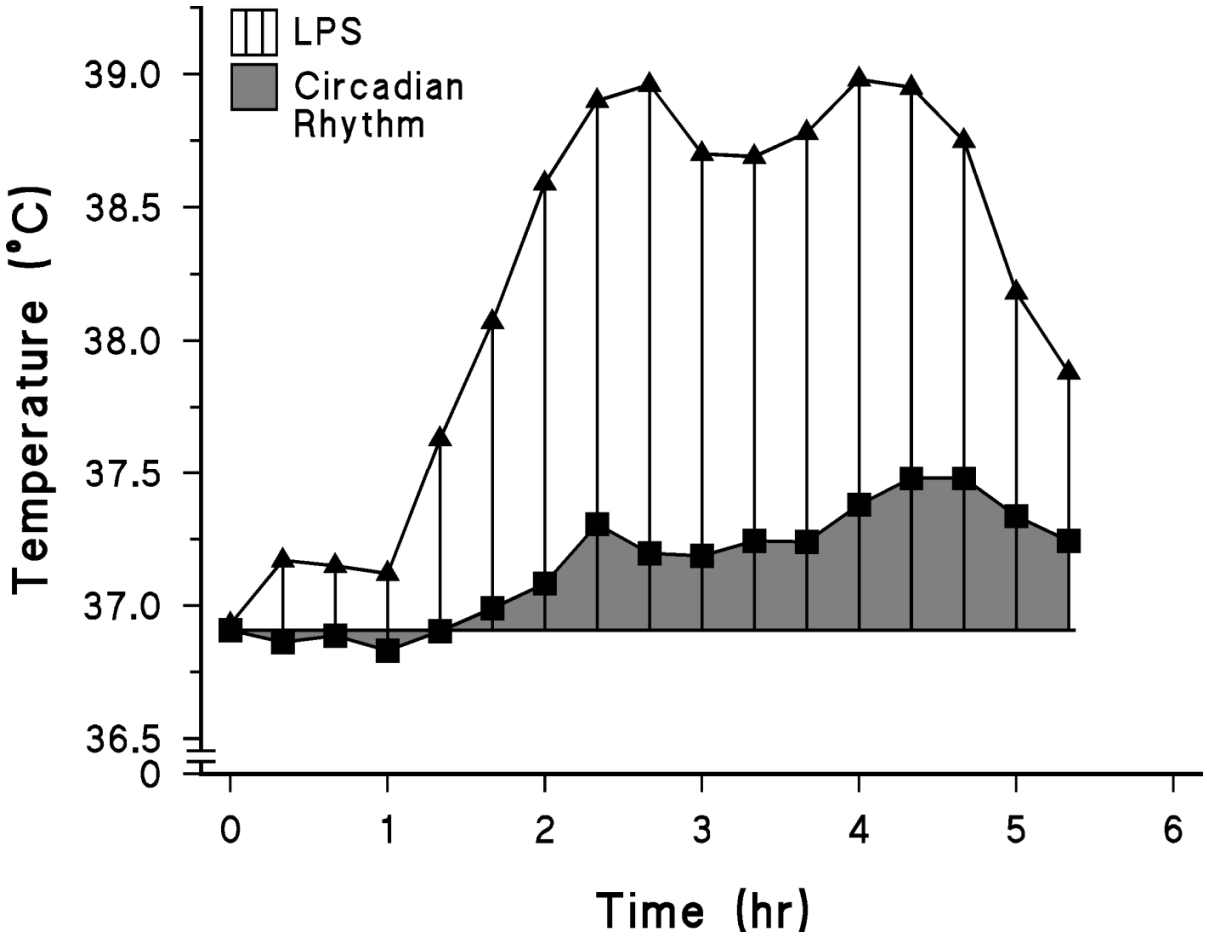


Figure 2.2. Method of assessing the magnitude of a fever. The area under curve for the typical core body circadian rhythm fluctuation in temperature (grey) is subtracted from the area under the curve for the core body temperature response to lipopolysaccharide (LPS) (vertical lines) administration. Data shown are obtained from a single animal.

2.1.3 Cardiac structure and function in rats

To determine the impact of LPS on left ventricular dimensions and systolic chamber or myocardial function in anaesthetized rats, echocardiography was performed using previously described methods (Norton et al 2002, Woodiwiss et al 2001). To perform echocardiography rats were anaesthetized with 50mg/kg of ketamine and 3mg/kg of xylazine to ensure immobility during the procedure. A high resolution ultrasonic probe (7 MHz paediatric probe) was used to obtain two-dimensional guided M-mode images of the rat left ventricle using an approach previously described (Chung et al 1998, Norton et al 2002) (Figure 2.3) on an Accuson echocardiograph. Care was taken to ensure that an M-mode image was obtained at the level of the papillary muscles. As the right ventricular cavity was difficult to visualize, septal wall thickness values were not determined. However, left ventricular end diastolic and end systolic internal diameter and posterior wall thickness values were determined (Figure 2.3). Left ventricular internal dimensions during systole and diastole were measured using the American Society for Echocardiography's (Sahn et al 1978) leading edge method (Norton et al 2002). Measurements were made from 3 consecutive beats. Left ventricular chamber and myocardial systolic function were determined from endocardial (FS_{end}) and midwall (FS_{mid}) fractional shortening respectively, as previously described (Norton et al 2002) using the following equations:

$$FS_{end} = (EDD-ESD/EDD) \times 100$$

$$FS_{mid} = \frac{[(EDD+ED\ PWT)/2-(ESD+ES\ PWT)/2]}{(EDD+ED\ PWT)/2} \times 100$$

where EDD = end diastolic diameter, ESD = end systolic diameter, ED PWT = end diastolic posterior wall thickness and ES PWT = end systolic posterior wall thickness.

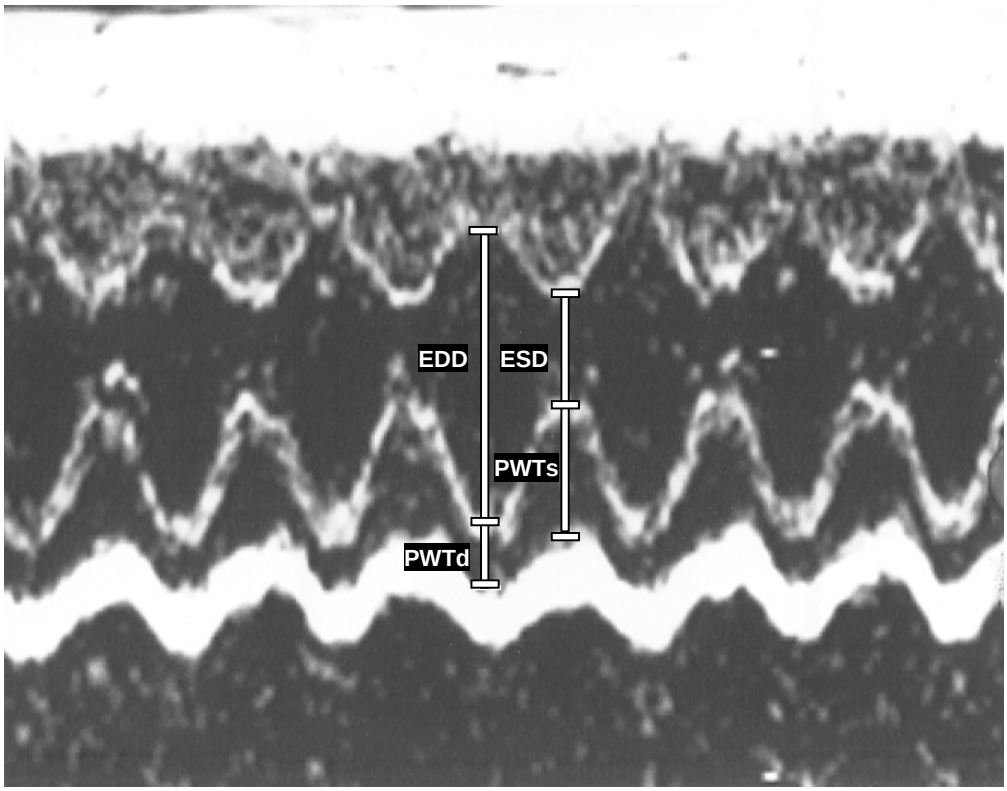


Figure 2.3. A representative M-mode image used to determine left ventricular structure and function in rats. EDD, end diastolic diameter; ESD, end systolic diameter; PWTd, posterior wall thickness at the end of diastole; PWTs, posterior wall thickness at the end of systole.

The calculation of midwall diameter at either end diastole or end systole is based on an assumption that PWT = septal wall thickness, and hence that half of PWT = half of septal wall thickness.

2.1.4 Assessment of cardiomyocyte apoptosis.

After removal of the heart from the thoracic cavity under anaesthesia, the heart was placed in ice cold water, weighed, dissected and stored in 10% phosphate buffered formaldehyde for a maximum period of 24-hours. Myocardial tissue was subsequently processed and embedded in paraffin wax. The degree of apoptosis was quantified on 5μm thick tissue sections. Nuclear deoxyribonucleic acid (DNA) fragments in the tissue sections were detected using a non-radioactive *in situ* apoptotic cell death detection kit (DeadEnd™ Colorimetric TUNEL system, Promega, Madison, WI, USA), where terminal deoxynucleotidyl transferase (TdT) was used to incorporate biotinylated nucleotide at the 3'-OH DNA ends. Horseradish-peroxidase-labeled streptavidin binds to biotinylated nucleotides, which subsequently stain dark brown in response to hydrogen peroxide and diaminobenzidine (Agarwala and Kalil, 1998). Both positive (DNase treated) and negative (no addition of TdT) control tissue sections were incorporated into each assay.

Paraffin embedded sections were first immersed in xylene for 5 minutes to de-paraffinize the tissue sections. The tissue sections were then washed by immersing the slides in 100% ethanol for 5 minutes and again for 3 minutes. The sections were rehydrated in graded ethanol washes (95%, 85%, 70% and 50%) for 3 minutes each. The slides were washed in a 0.85% NaCl solution for 5 minutes and in phosphate buffered saline for 5 minutes. The tissue sections were fixed by immersing the slides in a 4% paraformaldehyde solution for 15 minutes and the

slides were then immersed in phosphate buffered saline for 5 minutes, dried, and placed on a flat surface.

To increase the permeability of the cell membrane, 100µl of a 20µg/ml proteinase K solution was added to the slides to cover each tissue section and incubated for 30 minutes at room temperature. The tissue sections were washed by immersing the slides in phosphate buffered saline for 5 minutes and re-fixed by immersing in a 4% paraformaldehyde solution and washed again in phosphate buffered saline for 5 minutes. At this point the positive control slide was treated with DNase I to cause DNA fragmentation whilst the experimental slides remained in a phosphate buffered saline solution. 100µl of DNase I buffer was added to the positive control slide to cover the tissue sections and incubated at room temperature for 5 minutes. DNase I buffer containing DNase was added to cover the tissue sections and the slides were incubated for 10 minutes at room temperature. The positive control slide was washed 4 times in distilled water and in phosphate buffered saline for 5 minutes.

The tissue sections were covered with equilibration buffer for 8 minutes. 10µl of biotinylated nucleotide mix and 10µl of rTDT Enzyme were added to 980µl of equilibration buffer for the reaction mix. A control incubation buffer was prepared for the negative control slide by adding 1µl of biotinylated nucleotide mix and 1µl of distilled water to 98µl of equilibration buffer. After equilibration the slides were blotted with tissue paper to remove excess liquid and 100µl of the rTDT reaction mix was then added to each tissue section. The sections were covered with plastic cover slips and incubated at 37˚C for 60 minutes inside a humidified chamber to allow the end-labeling reaction to occur. After 60 minutes the slides were removed from the incubator and the plastic cover slips removed. 20X SSC was diluted with distilled water and the rTDT reaction terminated by immersing the slides in 20X SSC solution for 15 minutes. The tissue sections were subsequently washed in phosphate buffered saline twice for 5 minutes each to remove unincorporated biotinylated nucleotides. The slides were immersed in 0.3% hydrogen

peroxide for 5 minutes to block the endogenous peroxides and washed with phosphate buffered saline for 5 minutes.

Streptavidin horseradish peroxidase was diluted in phosphate buffered saline and 100µl was added to each slide to cover the tissue sections. The slides were incubated at room temperature for 30 minutes and washed with phosphate buffered saline for 5 minutes. 50µl of DAB Substrate 20X Buffer, 50µl of DAB 20X Chromogen and 50µl of Hydrogen Peroxide 20X were added to 950µl of distilled water. 100µl of the DAB solution was then added to each slide to cover the tissue sections for 8 minutes at room temperature. The slides were rinsed 4 times with distilled water, dehydrated by immersing the slides in graded ethanol washes (50%, 70%, 85% and 95%) and immersed in xylene. The slides were subsequently mounted using permanent mounting medium.

The number of apoptotic cardiomyocyte nuclei and the total number of cardiomyocyte nuclei (haematoxylin and eosin stain) in each slide were counted on ten evenly spaced fields from the apex to the base using a computer-based image acquisition and analysis system at 400 times magnification (Axiovision 3, Carl Zeiss, Gottingen, Germany). Apoptotic cardiomyocyte nuclei were expressed as a percentage of the total number of cardiomyocyte nuclei. Representative examples of stained sections for the samples assessed and from positive and negative controls are illustrated in Figure 2.4. All sections were coded and a single observer “blinded” to the identity of the rat from which the section was obtained recorded the number of apoptotic nuclei, and counted the total number of cardiac myocyte nuclei from the haemotoxylin and eosin slides.

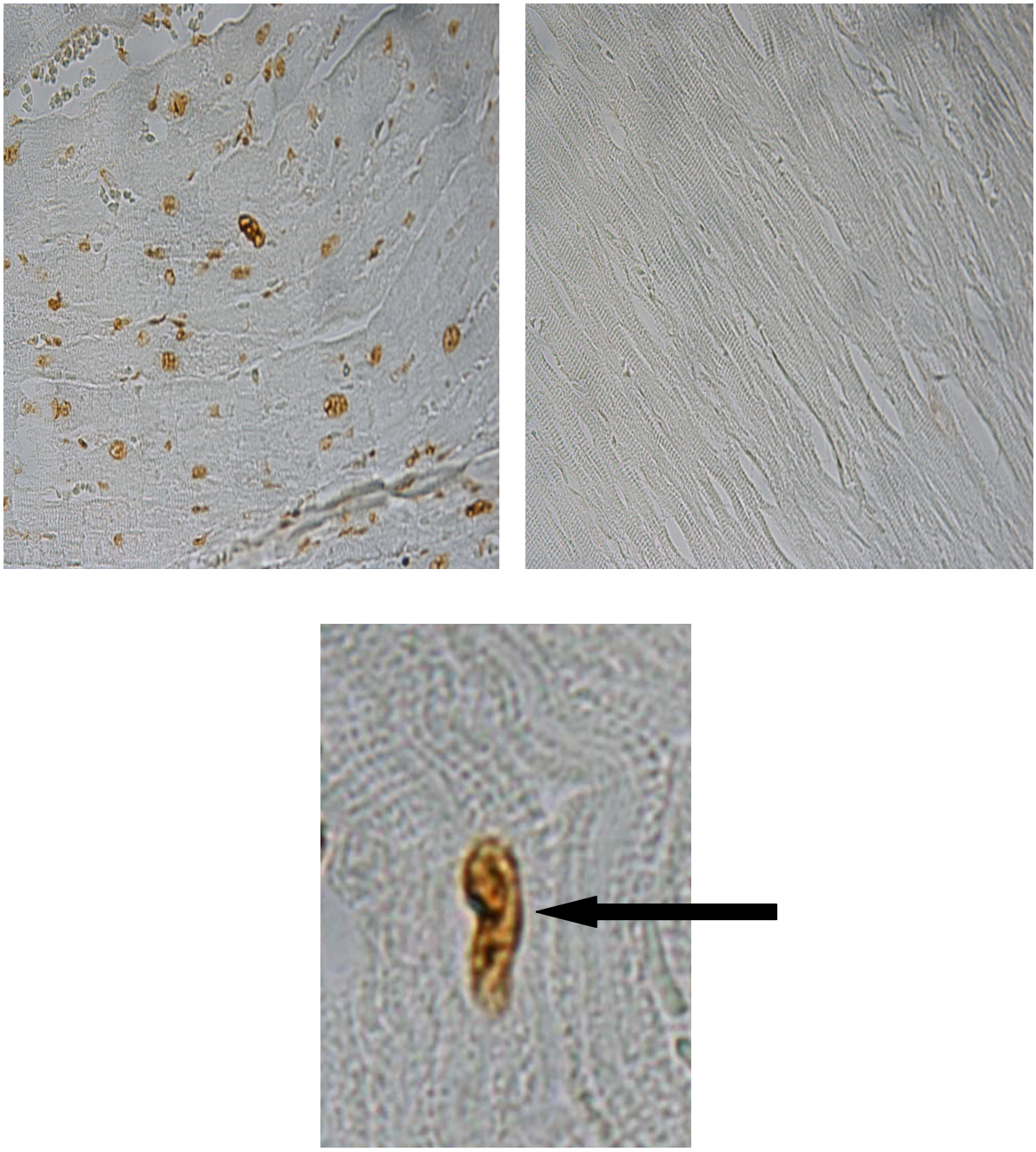


Figure 2.4. Histological sections of the myocardium stained for apoptotic nuclei. The upper panels illustrate sections obtained from a positive (left) and a negative (right) control and the lower panel a section from the myocardium of a heart showing one apoptotic cardiomyocyte nucleus (arrow). Note the numerous apoptotic nuclei in the positive control section.

2.2 Methodology for the study to assess whether inflammation in-part explains the relationship between obesity and myocardial dysfunction.

2.2.1 Study participants

The study protocol was approved by the University of the Witwatersrand Committee for Research in Human Subjects (approval number: M02-04-72 and renewed as M07-04-69). Participants of African ancestry, with a minimum age for participation being 16 years, but without an upper age limit, were recruited from nuclear families of the South-Western township (Soweto) of Johannesburg. A lower age limit was included as BP rapidly increases with age below this value. Random recruitment of spouses and siblings living in households from formal dwellings represented in the last census conducted (2001) was performed. Street names and addresses of households with at least one parent and two siblings or two parents and one sibling were obtained from the Department of Home Affairs**.** These households were allocated numbers and numbers were selected from a random number generator. People residing in informal dwellings or institutions/homes were not recruited.

Recruitment for the present study was initiated in October 2003 and data obtained up until November 2006 were used for the present dissertation. Up until November 2006, of the 1082 participants that were invited to be part of the study, 671 (62%) agreed to participate. Of the 671 participants enrolled, 418 (62%) agreed to echocardiograph assessments. Of these, data from 19 were discarded because of poor quality echocardiograph assessments and 16 participants had insufficient serum to assess CRP concentrations. As cardiac function is influenced by antihypertensive therapy, analysis was restricted in participants who were not receiving treatment for hypertension. Thus, in total, data from 292 participants were assessed in this study. 199 (68%) of the participants recruited were of the Nguni chiefdom (Zulu, Xhosa,

Ndebele, Swati) and 93 (32%) were of the Sotho chiefdom (South Sotho, North Sotho and Tswana). The lack of representation from the Venda chiefdom reflects a lack of individuals of this chiefdom residing in these areas of Johannesburg. No participants of mixed, Asian, or European ancestry were recruited and no Khoi-San subjects were recruited. All participants gave written, informed consent to participate in the study.

2.2.2 Questionnaire

Participants completed a standard questionnaire, where in order to avoid translational errors, the questionnaire was not translated into an African language, but study assistants familiar with all languages spoken in these townships and who either previously lived in Soweto or currently reside in Soweto assisted with the completion of each questionnaire. Only same sex assistants were used to assist each family member with the completion of the questionnaire. Assistance was only provided when requested. The majority of participants were reasonably proficient in English. Study assistants first visited homes of participants in order to develop a trusting relationship. The questionnaire was only completed at a subsequent clinic visit and then ambiguities checked by performing a follow-up home visit. If family members were absent at follow-up home visits, data was checked with them personally via telephonic conversations whenever possible. Ambiguities in answers to the questionnaire were detected by an independent observer prior to the second home visit. A pilot study was conducted in 20 subjects to ensure that data obtained in the questionnaires were reproducible when obtained with the assistance of two separate study assistants.

The questionnaire requested specific answers to date of birth, gender, previous medical history, the presence of hypertension, diabetes mellitus and kidney disease, prior and current drug therapy (analgesic use included), prior and current occupation, level of education, smoking

status (including the number of cigarettes smoked in the past and at the present time), daily alcohol consumption (beer, traditional beer or other forms of alcohol and the daily quantity), caffeine consumption (number of cups of tea or coffee and whether they are decaffeinated and the number of cola's a day), exercise frequency and family history of hypertension and cardiovascular events. For females, menstrual history, history of pregnancies and oral contraceptive use was evaluated. Most of the questions simply required a "Yes"-"no" answer, but understanding was assessed by requesting some short answers. If subjects were unable to provide the name of medication taken these were obtained on the second home visit. Although information on education, present occupation and annual incomes were obtained, these data have not been analysed at this point as an appropriate score has not been derived.

2.2.3 Blood pressure, pulse rate and anthropometric measurements and blood tests

Trained nurse-technicians measured blood pressure using a standard mercury sphygmomanometer during a clinic visit. A standard cuff with a 12 $\times$ 24 cm inflatable bladder was used, but if upper arm circumference exceeded 31 cm, larger cuffs with a 15 $\times$ 35 cm inflatable bladder were used. After 10 minutes of rest in the seated position, five consecutive blood pressure readings were taken 30 to 60 seconds apart, followed by a pulse rate count. The cuff was deflated at approximately 2 mm Hg per second and phase I (systolic) and phase V (diastolic) blood pressure recorded to the nearest 2 mm Hg according to the recommendations of the European Society of Hypertension (O'Brien et al 2003). The average of the five readings was taken as the clinic blood pressure. In the present study quality control of blood pressure assessments was assessed as previously described (Majane et al 2007). Only 0.68% of visits had fewer than the planned blood pressure recordings. The frequency of identical consecutive recordings was 0% for systolic blood pressure and 0% for diastolic blood pressure. The

occurrence of blood pressure values recorded as an odd number was 0%. Of the 2912 systolic and diastolic blood pressure readings, 28.8% ended on a zero (expected =20%). A diagnosis of hypertension was made if subjects were receiving antihypertensive therapy and/or if the average of the mean values for the clinic readings was ≥140/90 mm Hg.

Body height, weight, waist and hip circumference and triceps and subscapular skin-fold thickness (Harpenden Skinfold Calliper, Bedfordshire, UK) were measured during the clinic visit by a trained observer. Height and weight were measured with the participants standing and wearing indoor clothes with no shoes. Waist circumference (WC) and hip circumference were measured according to conventional techniques (World Health Organisation, 2000). Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters and waist-to-hip ratio calculated as an index of central obesity. Mean skin-fold thickness was calculated as the mean of sub-scapular and triceps skin-fold thickness values. Participants were identified as being overweight if their BMI was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2. Central obesity was defined as an enlarged WC (≥88 cm in women and ≥102 cm in men).

Blood samples were obtained on the day of the clinic visit and sent to the South African National Health Systems Laboratories to perform a full blood count and differential count, to measure urea, creatinine and electrolyte concentrations, to assess liver function (from alanine transaminase, aspartate transaminase, gamma gluteryl transaminase, alkaline phosphatase, albumin, total protein and plasma albumin, total bilirubin, and conjugated and unconjugated bilirubin concentrations) and plasma urate concentrations, to obtain a lipid profile (total cholesterol, low density lipoprotein cholesterol concentrations, high density lipoprotein cholesterol concentrations and triglyceride concentrations), a blood glucose measurement, percentage glycated haemoglobin (HbA1c) (Roche Diagnostics, Mannheim, Germany) and a follicle stimulating hormone concentration in females (to confirm menopausal status in females).

Diabetes mellitus or abnormal blood glucose control was defined as the use of insulin or oral hypoglycaemic agents or a glycated haemoglobin value greater than 6.1% (Bennett et al 2007).

Serum C-reactive protein (CRP) concentrations were measured using a quantitative immunoturbidimetric assay (Kit 6K2602, Architect, Sentinel Diagnostics, USA) which employs an method of detection with the lowest detection limit being 0.01 mg/dl. The analysis was performed on an Olympus OSR 6185 (Olympus Diagnostics, Lismeehan, Ireland) with inter- and intra-assay coefficients of variation of 1.3% and 0.4%, respectively. In this assay serum containing CRP agglutinates when it comes into contact with polyclonal anti-CRP antigens bound to latex particles in a 0.2% suspension. Once CRP and anti-CRP have agglutinated, the concentration of CRP is determined by spectrophotometry, as assessed by an absorbance change from an initial 572 nm. The lowest detection limit of the CRP assay of 0.01 mg/dl is lower than that for the high-sensitivity CRP assay of 0.02 mg/dl. Samples with concentrations that exceeded the upper limit were diluted at a ratio of 1:10 with normal saline and re-analyzed. The presence of a generalized systemic infection was diagnosed if CRP concentrations were ≥10 mg/dl.

2.2.4 Echocardiography in human participants

Echocardiographic measurements were performed by an experienced observer using a pulse colour Doppler Hewlett Packard model 4500-5500 recorder coupled to a 2.5 MHz transducer. Left ventricular dimensions were determined using two-dimensional directed M-mode echocardiography in the short axis view and these recordings analyzed according to the American Society of Echocardiography convention (Sahn et al 1978). During recordings, the transducer was placed perpendicular to the chest wall or pointed slightly inferiorly and laterally at the end of the long axis. All measurements were recorded on videotape and analyzed off-line

by the same observer who was unaware of the clinical details of the participants. The interventricular septal wall thickness (IVS) at end diastole and end systole, the posterior wall thickness (PWT) at end diastole and end systole and the end diastolic and end systolic internal dimensions of the left ventricle were measured only when appropriate visualization of both the right and the left septal surfaces occurred. Figure 2.5 shows a representative M-mode image employed to assess left ventricular mass and systolic function.

Left ventricular mass was derived according to an anatomically validated formula (Devereux et al 1986) ($LVM = 0.8 \times [1.04 (LVEDD + IVS + PWT)^3 - (LVEDD)^3] + 0.6g$) and indexed to height$^{2.7}$ (LVM index, LVMI). Left ventricular relative wall thickness (RWT) was calculated as (LV diastolic posterior wall thickness x2)/LV end diastolic diameter (Ganau et al1992). Left ventricular mean wall thickness was determined from the mean of LV septal and posterior wall thickness. Left ventricular ejection fraction, and endocardial and midwall fractional shortening will be calculated from dimension measurements using standard formulae, where:

$$\text{LV ejection fraction (EF)} = \frac{(\text{LVED volume} - \text{LVES volume})}{\text{LVED volume}} \times 100$$

$$\text{LV endocardial fractional shortening (FSend)} = \frac{(\text{LVEDD} - \text{LVESD})}{\text{LVEDD}} \times 100$$

$$\text{LV midwall fractional shortening (FSmid)} = \frac{[([\text{PWTed} + \text{SeptalTed}]/2 + \text{LVEDD}) - ([\text{PWTes} + \text{SeptalTes}]/2 + \text{LVESD})]}{([\text{PWTed} + \text{SeptalTed}]/2 + \text{LVEDD})} \times 100$$

where LVED = left ventricular end diastolic, LVES = left ventricular end systolic, LVEDD = LVED internal diameter, LVESD= LVES internal diameter, PWTed = end diastolic posterior wall thickness, PWTes = end systolic posterior wall thickness, SeptalTed = end diastolic septal wall

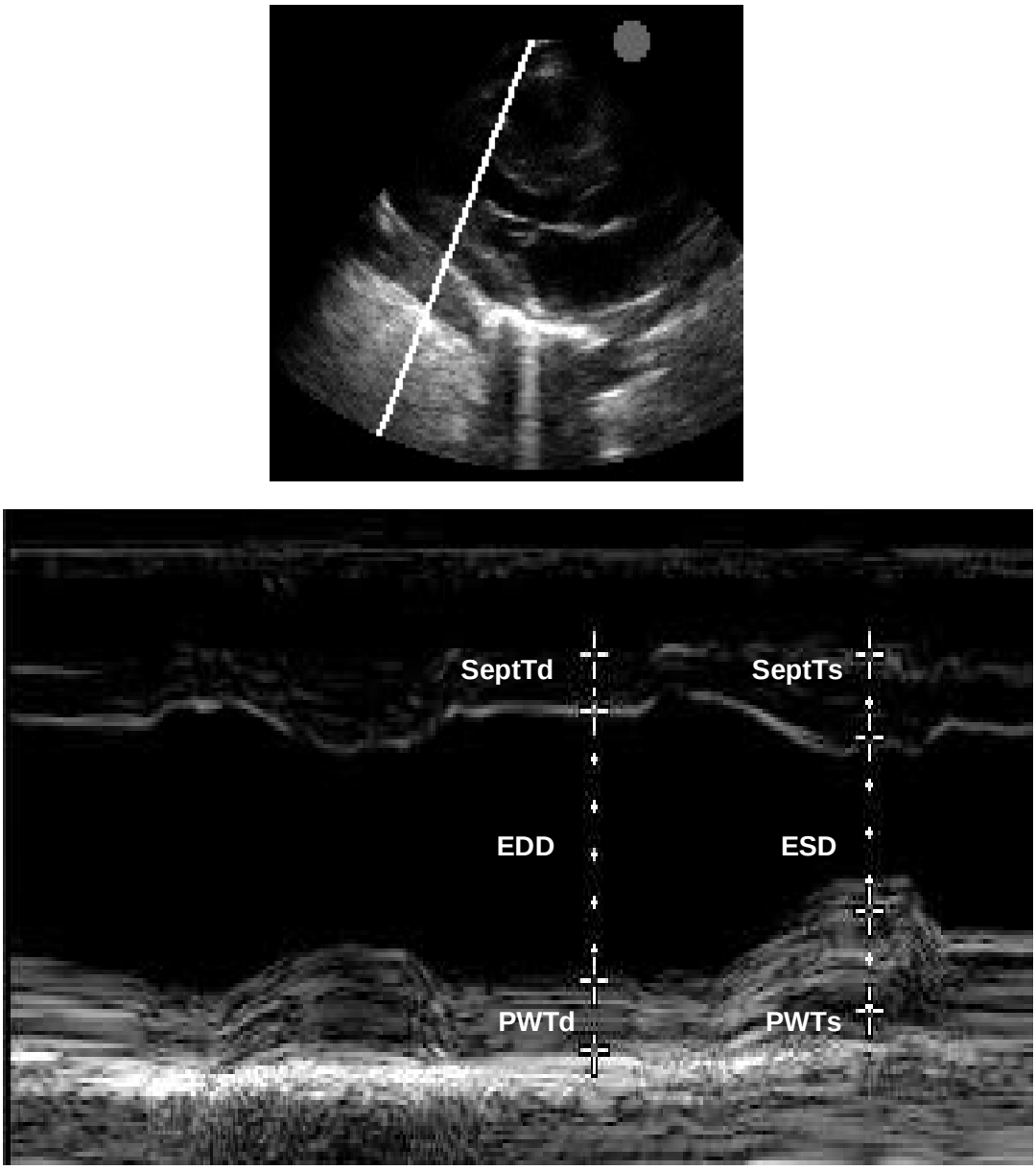


Figure 2.5. A two-dimensional guided (upper panel) M-mode echocardiographic image (lower panel) derived from a Hewlett Packard model 5500 utilised to assess left ventricular dimensions and function.EDD, end diastolic diameter, ESD, end systolic diameter, PWTd, posterior wall thickness at the end of diastole, PWTs, posterior wall thickness at the end of systole, SeptTd, end diastolic septal wall thickness, SeptTs, end systolic septal wall thickness.

thickness, and SeptalTes = end systolic septal wall thickness. Left ventricular volumes were calculated from left ventricular diameters using the Teichholz formula (Teichholz et al 1976).

Left ventricular hypertrophy (LVH) was defined as a LVMI >51 $g/m^{2.7}$ for both women and men (Nunez et al 2005). A concentric LV was defined as a RWT>0.45. A concentric LVH was defined as the presence of LVH and a RWT>0.45. Eccentric LVH was defined as the presence of LVH and a RWT≤0.45. Concentric remodelling was defined as the presence of a RWT>0.45, but without LVH.

Intra-observer variability studies were conducted on 29 subjects on whom repeat echocardiographic measurements have been performed within a two week period of the initial measurements. The Pearson's correlation coefficients for LV end diastolic diameter, septal wall thickness and posterior wall thickness were 0.76, 0.94 and 0.89 (all $p<0.0001$) respectively, and the variances (mean % difference ± SD) were 0.12±5.95%, -0.77±4.47% and 0.67±5.57% respectively. In addition, no significant differences between repeat measurements were evident on paired t-test analysis (p=0.99, p=0.42 and p=0.48 respectively).

2.3 Data analysis.

In the animal study, data are represented as either mean±SEM or mean±SD. For comparisons of groups in the animal study, an unpaired Student's t test was performed. For a comparison of the thermal response index after versus before LPS injection, a paired Student's t test was performed.

Database management and statistical analyses for the study conducted on the community sample was performed with SAS software, version 9.1 (The SAS Institute Inc., Cary, North Carolina, USA). Data from individual subjects were averaged and expressed as mean±SD. The X^2-statistic was used to compare means and proportions. To assess the relationship between

indices of adiposity or serum hs-CRP concentrations and indices of left ventricular function, multivariate linear regression analyses was performed with age, blood pressure, gender, regular alcohol abuse, regular smoking and the presence or absence of diabetes mellitus or abnormal blood glucose control in the regression models. Probability values were obtained with further adjustments for non-independence of family members (mixed model as outlined in the SAS package). As hs-CRP concentrations were not normally distributed they were log transformed to achieve a more uniform distribution.

Chapter 3

Results

As indicated in chapters 1 and 2, in the present dissertation two separate studies were conducted, one in rats and one in a cross-sectional population sample. For ease of reading, the results for these two studies will also be described in separate sections.

3.1 Results for the study to assess whether pyrogenic doses of LPS promote cardiomyocyte apoptosis in rats.

3.1.1 Diurnal temperatures and thermal responses to lipopolysaccharide

Figure 3.1 shows the mean and SD of core body temperatures as determined in rats prior to LPS administration over 3 days. A typical circadian rhythm of core body temperature was observed, with night body temperatures being higher than day body temperatures. Core body temperature was lowest first thing in the morning and highest in the evening, whilst a gradual increase in body temperature was noted through the day. Figure 3.2 shows the thermal response index (upper panel) and the temperatures measured over 6 hours (lower panel) in response to a single LPS or vehicle injection. Data are compared to that obtained at the same time of day over 3 days of temperature measurement in the same rats. Rats receiving LPS exhibited a marked thermal response as compared to rats receiving the saline vehicle. In this regard, rats developed a fever, as indicated by a temperature increase of greater than 1℃. The fever response peaked at 3 hours post LPS administration. Figure 3.3 shows the thermal response index after a single (upper panel) and after a second (lower panel) injection of LPS or the vehicle injection in rats. The thermal response index after LPS administration was similar on the second as compared to the first day.

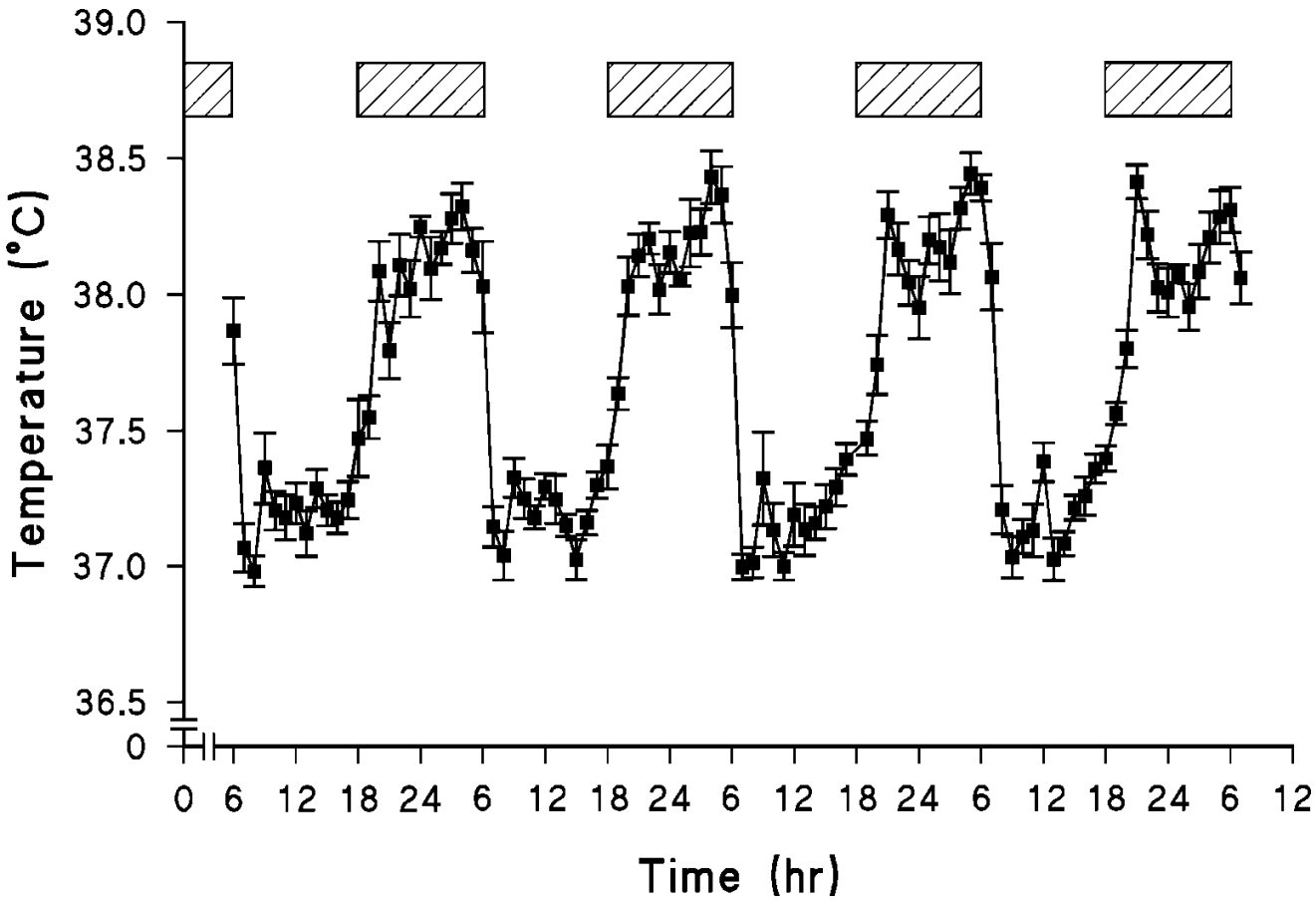


Figure 3.1. Body temperature variations for 13 rats over a period of three days prior to lipopolysaccharide (LPS) administration. Hatched bars indicate periods of darkness. Data are means and SD.

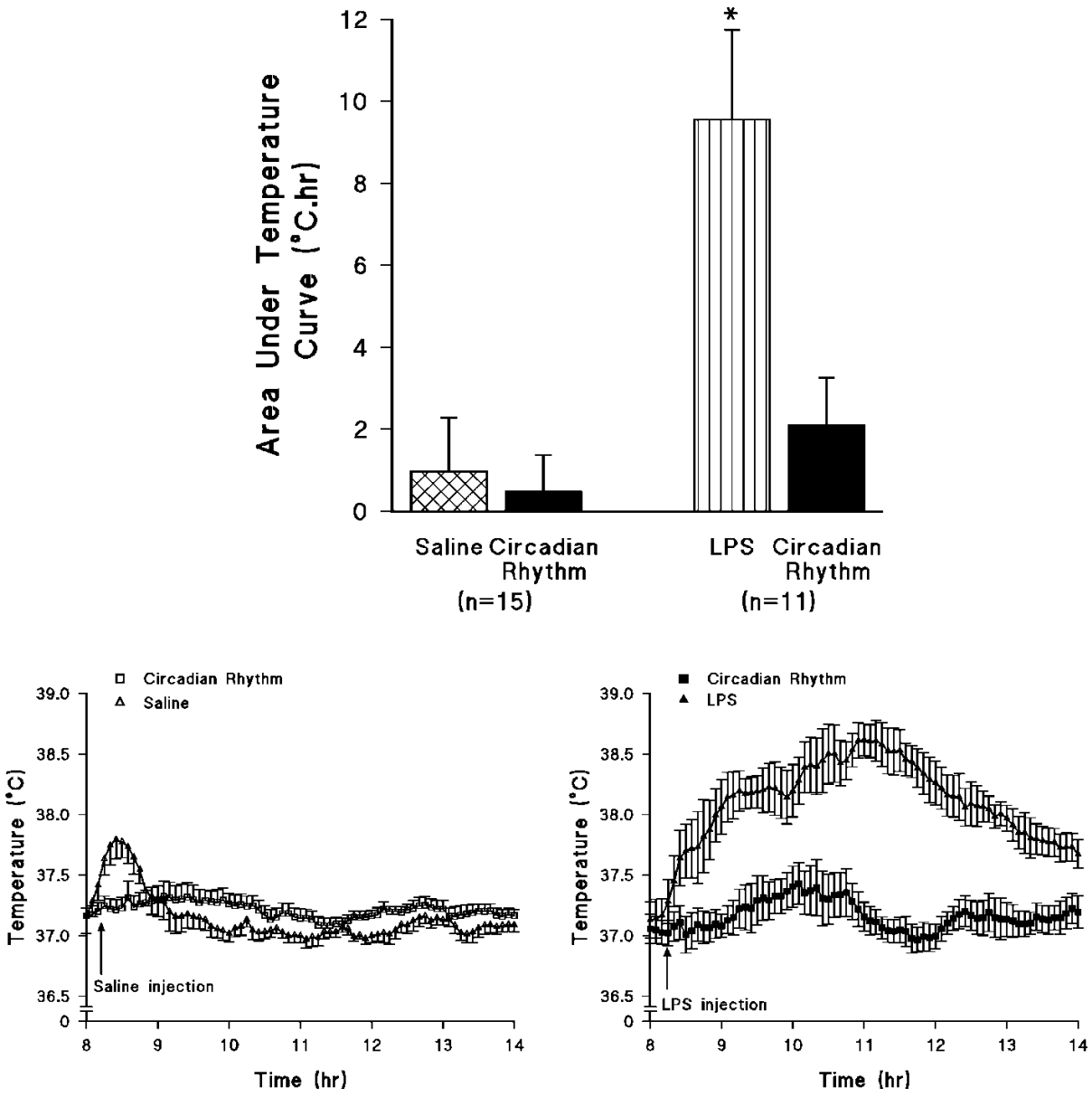


Figure 3.2. Thermal response index (upper panel) and the temperatures measured over 6 hours (lower panel) in response to lipopolysaccharide (LPS) at 250 µg/kg or the vehicle injection in rats. Also shown are data obtained at the same time of day over 3 days of temperature measurement in the same rats (circadian rhythm).*$p<0.05$, vs LPS and saline circadian rhythm.

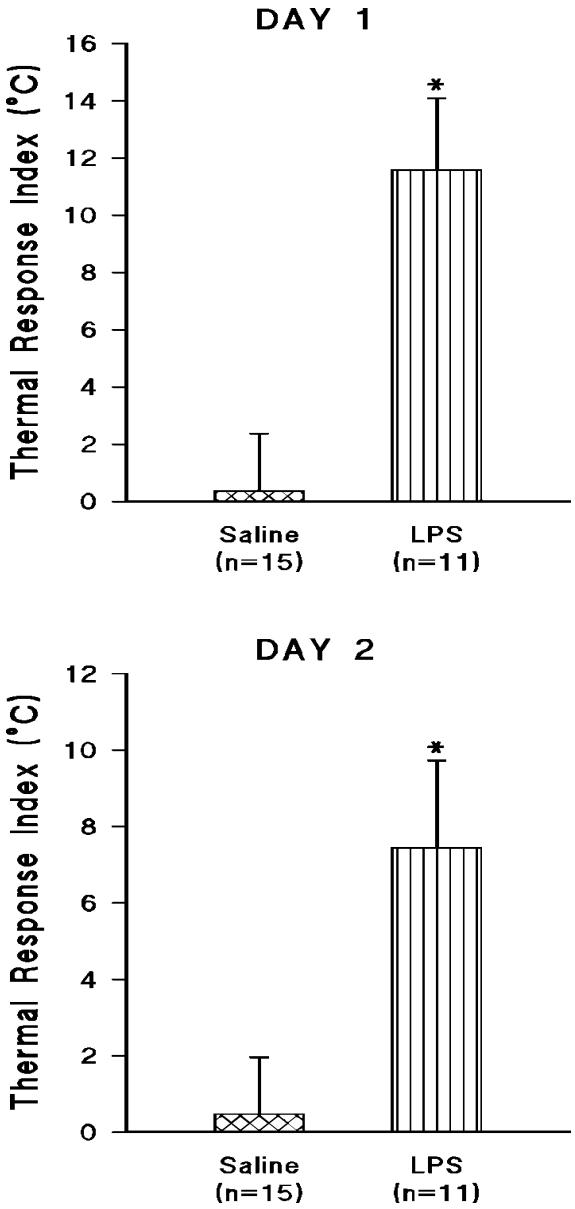


Figure 3.3. Thermal response index after a single (upper panel) and after a second (lower panel) injection of lipopolysaccharide (LPS) at 250 µg/kg or the vehicle injection in rats. Data shown are obtained over 6 hours at the same time of day.* $p<0.05$, vs saline and LPS circadian rhythm.

3.1.2 Echocardiographic data in rats

Figure 3.4 shows left ventricular endocardial and midwall fractional shortening values, heart rates, and end diastolic diameters obtained in rats after having received either LPS or the vehicle. No differences in either left ventricular systolic chamber function (endocardial fractional shortening), myocardial function (midwall fractional shortening), heart rate, or left ventricular end diastolic diameters were noted between rats receiving LPS or the saline vehicle.

3.1.3 Percentage cardiomyocyte apoptosis

Figure 3.5 shows the percentage cardiomyocytes that were TUNEL positive stained in the left ventricle of rats 6 hours after receiving a second dose of either LPS or the saline vehicle. Lipopolysaccharide administration, despite producing a marked febrile response (see section 3.1.1) failed to induce a significant increase in TUNEL positive stained cardiomyocytes.

3.2 Results of the study to assess whether inflammation in-part explains the relationship between obesity and myocardial dysfunction.

3.2.1 Characteristics of the participants.

Table 3.1 gives the characteristics of the study participants not receiving antihypertensive therapy in whom echocardiography was performed as well as the characteristics of the participants not receiving antihypertensive therapy in whom did not consent to echocardiography, had poor quality echocardiograms, or had insufficient serum for

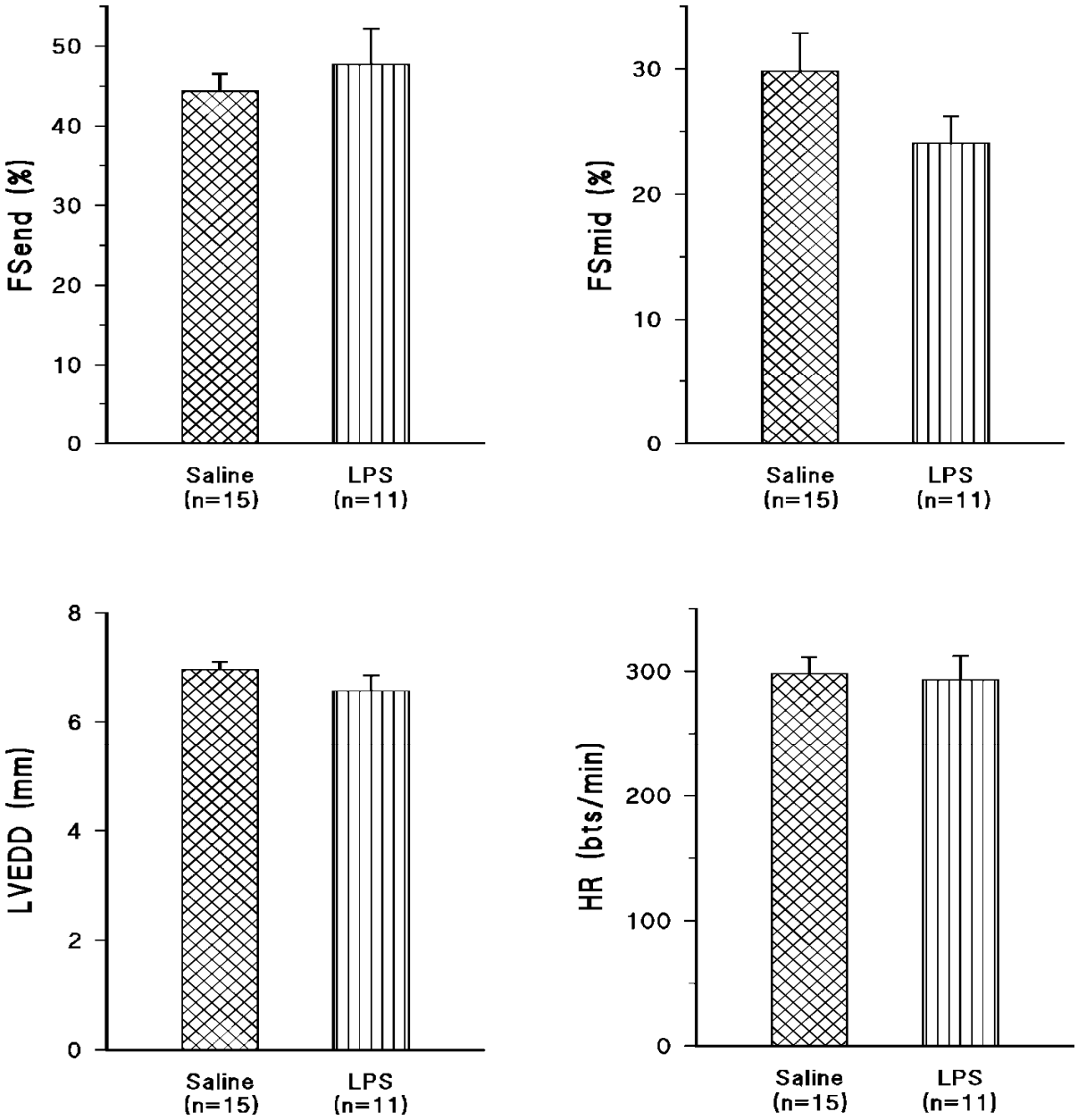


Figure 3.4. Left ventricular function, diameters and heart rate in rats 6 hours after having received a second dose of either lipopolysaccharide (LPS) at 250 µg/kg or the saline vehicle. FSend, endocardial fractional shortening; FSmid, midwall fractional shortening; EDD, end diastolic diameter; HR, heart rate.

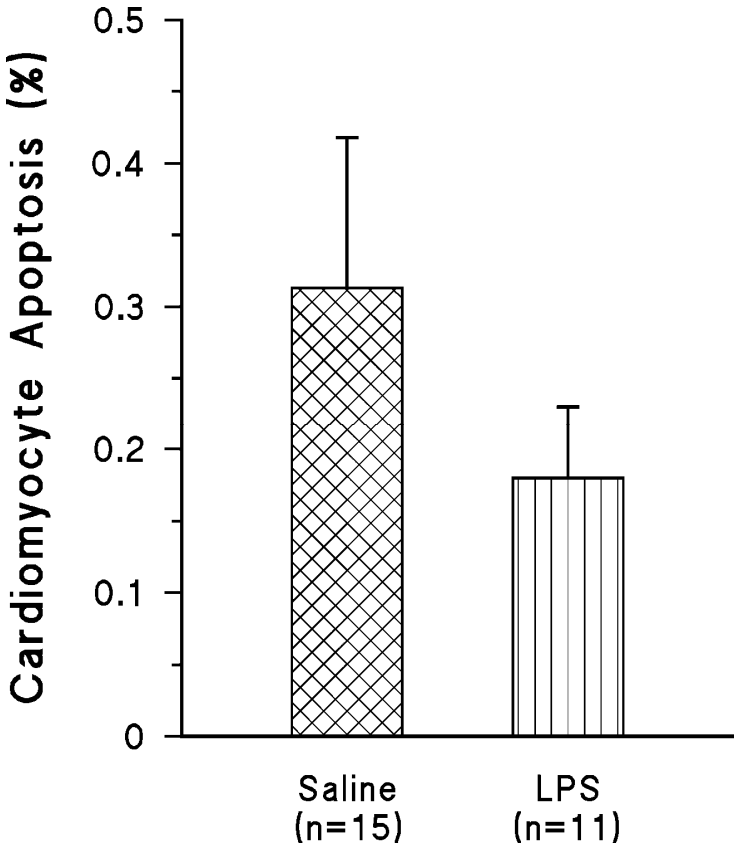


Figure 3.5. Percentage of rat left ventricular cardiomyocytes showing TUNEL positive staining, 6 hours after rats have received a second dose of either lipopolysaccharide (LPS) at 250 µg/kg or the saline vehicle.

Table 3.1. Characteristics of study participants and participants excluded from analysis.

	Study group	Excluded participants
Number (% female)	292 (62.0)	248 (60.0)
Age (years)	39±17*	36±16
Height (m)	162.5±8.6	161.1±8.9
Weight (kg)	74.7±18.8	72.4±18.2
Body mass index (kg/m^2)	28.4±7.3	28.0±7.2
Waist circumference (cm)	87.9±15.3	86.7±15.7
Mean skin-fold thickness (cm)	2.08±1.13	2.05±1.16
% overweight/obese	25.0/37.7	22.1/36.4
% with central obesity	37.0	36.9
% with hypertension	24.9	21.1
% with DM or HbA_{1c}>6.1%	17.5	12.1
Regular smoking (%)	14.0	12.5
Regular alcohol (%)	24.0*	15.3
Conventional SBP/DBP (mm Hg)	128±21/83±12	125±19/83±11
Conventional PP (mm Hg)	44.5±13.8	42.3±12.2
LVED diameter (cm)	4.74±0.50	-
LVED septal thickness (cm)	1.01±0.15	-
LVED posterior wall thickness (cm)	0.97±0.13	-
LVED mean wall thickness (cm)	0.99±0.14	-
LVM (g)	167±47	-
LVM indexed for BSA (g/m^2)	93.6±22.8	-
LVM indexed for height$^{2.7}$ (g/m$^{2.7}$)	45.2±12.6	-

DM, diabetes mellitus; HbA_{1C}, glycated haemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; LV, left ventricle; LVED, LV end diastolic; LVM, LV mass; BSA, body surface area. *p<0.05 vs excluded participants.

CRP measurements. More women than men participated. In general the group had a high BMI, with ~63% of participants being either overweight (~25%) or obese (~38%) and ~37% having central obesity. More women than men were obese with 50% of women and 18% of men being obese and 53% of women and 12% of men having central obesity. ~25% of participants were hypertensive. ~18% had diabetes mellitus or an impaired blood glucose control (HbA1c>6.1%). A relatively low proportion of participants reported smoking or a regular intake of alcoholic beverages. ~29% of men and ~20% of women with echocardiographic data had LVH. ~6.5% of participants with echocardiographic data had concentric LVH, ~17% eccentric LVH and ~15.4% concentric LV remodelling. Except for being slightly younger and consuming less alcohol no differences were noted in the characteristics of participants excluded from the analysis because of a lack of echocardiographic data or available hs-CRP concentrations (Table 3.1). Mean (±SD) CRP concentrations in mg/l was 6.0± 8.8, with the mean CRP concentrations for women being 7.2±9.4 and for men being 4.1±7.4 ($p<0.0001$). Fifteen % of participants had elevated CRP concentrations.

3.2.2 Factors associated with log C-reactive protein.

On univariate analysis, all adiposity indexes (Figure 3.6), age ($r=0.34$, $p<0.0001$), female gender ($r=0.23$, $p<0.0001$), physical activity ($r=0.26$, $p<0.0001$), menopausal status ($r=-0.14$, $p<0.05$), use of medication in the last two weeks ($r=-0.15$, $p=0.01$), and diabetes mellitus or inappropriate glucose control ($r=0.33$, $p<0.0001$) were all associated with log CRP. In multivariate models, both BMI (partial $r=0.36$, $p<0.0001$), WC (partial $r=0.35$, $p<0.0001$) and skin-fold thickness (partial $r=0.27$, $p<0.001$) were strong independent predictors of log CRP. Similarly, in gender-specific analysis, BMI was the strongest independent predictor of log CRP in both men ($r=0.28$, $p<0.005$) and women ($r=0.39$, $p<0.0001$).

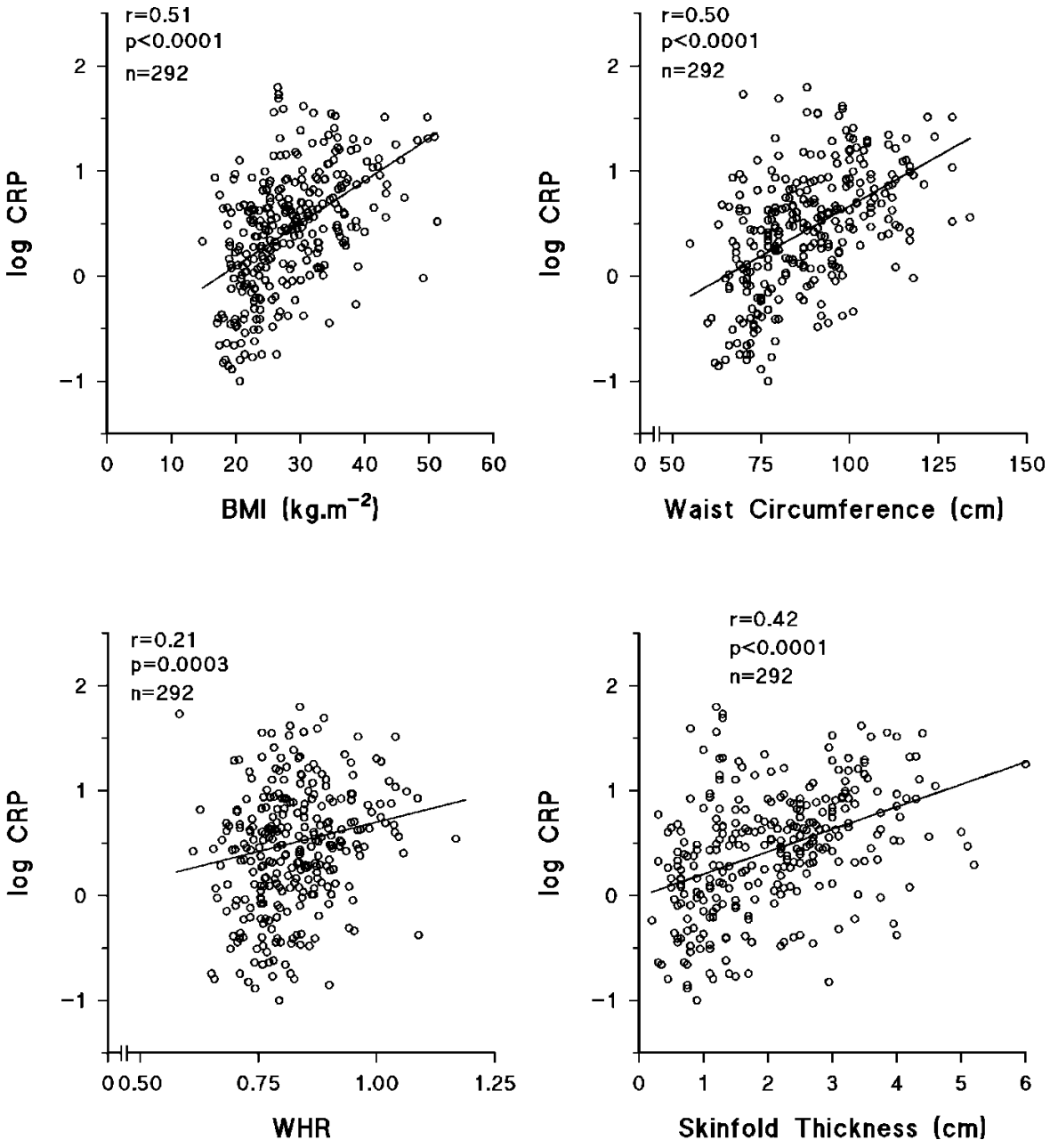


Figure 3.6. Relationship between indices of adiposity and C-reactive protein. BMI, body mass index; WHR, waist-to-hip ratio.

3.2.3 Individual factors correlated with left ventricular systolic function.

On bivariate analysis, waist circumference and skin-fold thickness were inversely correlated with left ventricular midwall fractional shortening (Figure 3.7) and BMI (r=-0.14, p=0.02) and waist-to-hip ratio (r=-0.12, p<0.05) were marginally and inversely correlated with left ventricular midwall fractional shortening. Importantly, log CRP was also inversely correlated with left ventricular midwall fractional shortening (Figure 3.7). In addition, age (r=-0.16, p<0.01), diabetes mellitus or an HbA1c>6.1% (r=-0.15, p=0.01), systolic BP (r=-0.13, p<0.05), pulse pressure (r=-.013, p<0.05), and regular alcohol use (r=-0.12, p<0.05) were inversely associated with left ventricular midwall fractional shortening. Neither diastolic blood pressure (r=-0.08, p=0.17), regular tobacco intake (r=0.03, p=0.67), gender (r=-0.007, p=0.91) nor left ventricular mass index (r=0.02, p=0.76) were correlated with midwall fractional shortening.

On bivariate analysis neither waist circumference (Figure 3.7), skin-fold thickness (Figure 3.7), BMI (r=0.09, p=0.13), waist-to-hip ratio (r=0.03, p=0.56), sex (r=0.006, p=0.92), age (r=0.04, p=0.50), systolic blood pressure (r=0.02, p=0.69), diastolic blood pressure (r=0.07, p=0.22), pulse pressure (r=-0.03, p=0.63), diabetes mellitus or an HbA1c>6.1% (r=0.03, p=0.67), regular tobacco intake (r=0.09, p=0.13), regular alcohol intake (r=-0.05, p=0.44), or left ventricular mass index (r=-0.008, p=0.89) were correlated with left ventricular endocardial fractional shortening. Moreover, log CRP was not correlated with left ventricular endocardial fractional shortening (Figure 3.8).

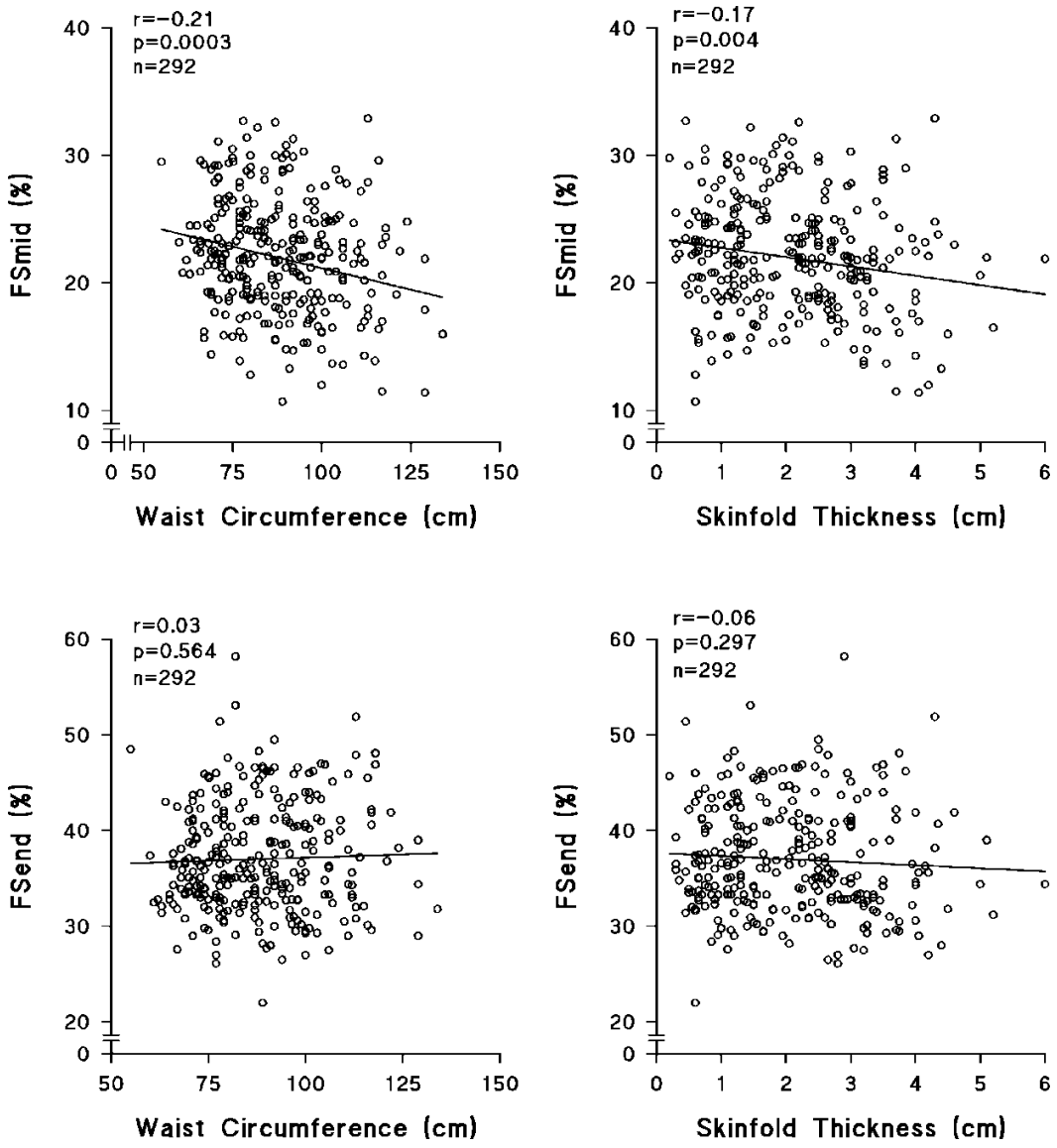


Figure 3.7. Relationship between indices of adiposity and left ventricular midwall (FSmid) and endocardial (FSend) fractional shortening.

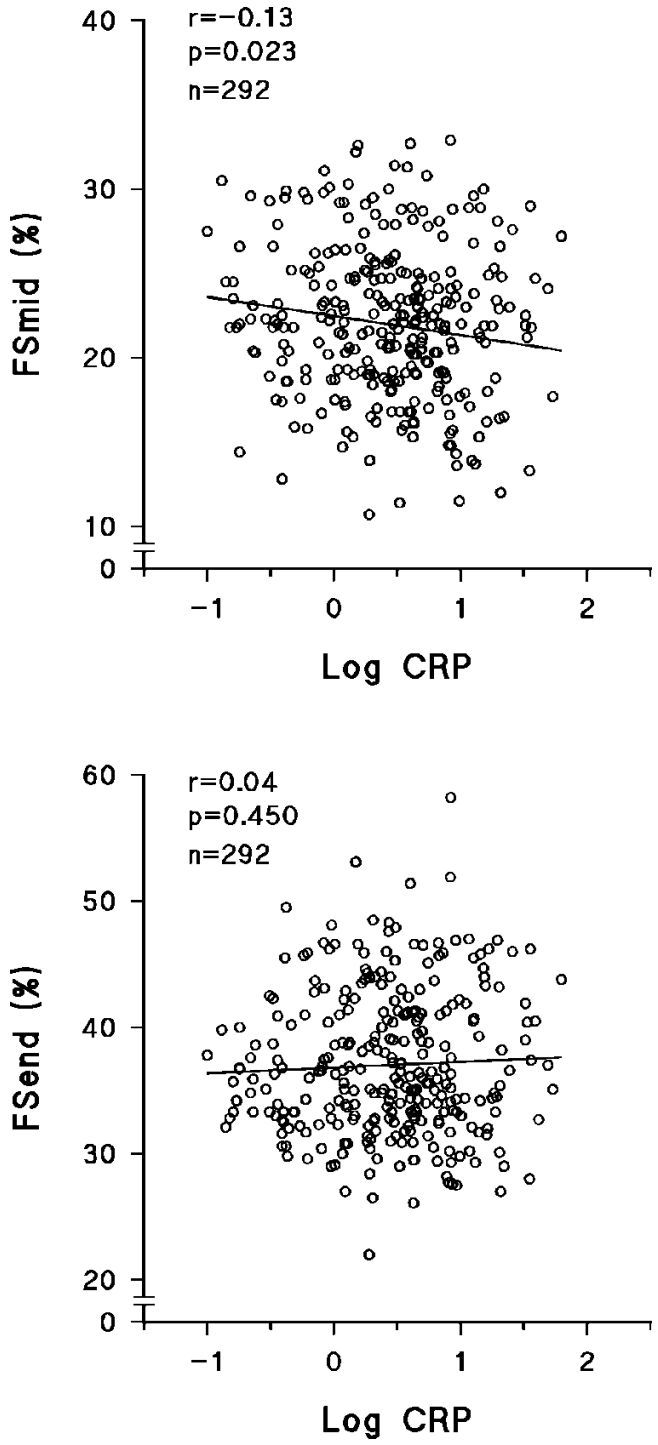


Figure 3.8. Relationship between log C-reactive protein (CRP) and left ventricular midwall (FSmid) or endocardial (FSend) fractional shortening.

3.2.4 Independent associations with left ventricular systolic function.

Table 3.2 shows the factors independently associated with left ventricular midwall fractional shortening. On multivariate regression analysis with waist circumference included as an index of adiposity. In a multivariate model without log CRP in the model, only waist circumference was independently and inversely associated with left ventricular midwall fractional shortening. In a multivariate model with log CRP but without waist circumference included in the model, log CRP was not independently associated with left ventricular midwall fractional shortening.

3.2.5 Independent associations with left ventricular systolic function with both waist circumference and C-reactive protein in the same model.

With both waist circumference and log hs-CRP in the same regression model, waist circumference still showed an independent association with fractional midwall shortening (standardized β-coefficient=-0.18±0.08, p=0.021). The slope of the relationship (standardized β-coefficient) between waist circumference was similar before (see Table 3.2) and after the inclusion of log hs-CRP in the regression model.

Table 3.2. Independent associations with left ventricular midwall fractional shortening in multivariate regression models in the study group (n=292).

Left ventricular midwall fractional shortening vs	Standardized β-coefficient±SEM	p value for relationship
	With waist circumference but not log CRP in the model	
Age	-0.05±0.08	0.52
Waist circumference	-0.19±0.07	**<0.01**
Systolic blood pressure	-0.07±0.08	0.35
Female sex	0.05±0.06	0.28
DM or HbA1c>6.1%	0.07±0.06	0.03
Left ventricular mass index	0.16±0.07	0.02
Regular alcohol	0.13±0.06	0.02
	With log CRP but not waist circumference in the model	
Age	-0.11±0.08	0.16
Log CRP	-0.08±0.07	0.21
Systolic blood pressure	-0.09±0.08	0.26
Female sex	0.03±0.06	0.59
DM or HbA1c>6.1%	0.08±0.06	0.23
Left ventricular mass index	0.14±0.07	<0.05
Regular alcohol	0.13±0.06	<0.05

Probability values were further adjusted for non-independence of family members. Significant probability values indicating inverse associations with left ventricular midwall fractional shortening are indicated in bold.

Chapter 4

Discussion

In the present chapter, consistent with the sequence followed in chapters 2 and 3 of the present dissertation, I will first discuss how the studies performed in my dissertation have furthered our understanding of the potential role of low-grade infections in promoting adverse cardiac changes. Second, I will discuss how the studies performed in my dissertation have enhanced our understanding of the role of obesity-induced inflammatory changes in promoting cardiac dysfunction.

4.1. Lipopolysaccharide effects on cardiomyocyte apoptosis

As reviewed in chapter 1 of the present dissertation, a number of studies indicate that LPS mediates cardiomyocyte apoptosis (Li et al 2002, Suzuki et al 2003, Suzuki et al 2007, Comstock et al 1998, McDonald et al 2000). The potential mechanism of the LPS-induced cardiomyocyte apoptotic effect is through Toll-like receptor activation, which stimulates cardiomyocyte angiotensinogen expression, and hence through angiotensin II, promotes calcineurin-induced cardiomyocyte programmed cell death (Suzuki et al 2007). In addition, the LPS-induced cardiomyocyte apoptotic effect may also be mediated through TNF-α effects (Cowan et al 2001, Peng et al 2005). These findings of an effect of LPS on cardiomyocyte apoptosis (Li et al 2002, Suzuki et al 2003, Suzuki et al 2007, Comstock et al 1998, McDonald et al 2000) are of major clinical importance in sepsis and septic shock which are frequently associated with a profound decrease in cardiac function (Calvin et al 1981, Parker et al 1984, Jafri et al 1990, Munt et al 1998, Poelaert et al 1997). Indeed, inhibition of caspase, the key enzyme involved in promoting apoptotic pathways, prevents cardiac dysfunction and cardiomyocyte apoptosis in an animal model of sepsis (Neviere et al 2001). However, can one extrapolate these data to a potential clinically relevant effect in chronic infections or infections which are <u>not</u> associated with the haemodynamic changes that accompany sepsis?

It is possible that with repeated inflammatory responses, modest degrees of cardiomyocyte apoptosis, with no immediate effect on heart function, could ultimately translate into a significant degree of myocardial damage, thus heralding the onset of heart failure after a prolonged time-period. This hypothesis is based on the findings that increased circulating LPS concentrations do indeed occur in chronic sub-clinical infections associated for example with a poor dental hygiene (Moutsopoulos and Madianos 2006, Geerts et al 2002). The authors of some of the studies showing that cardiomyocyte apoptosis occurs in response to LPS (Li et al 2002, Suzuki et al 2003, 2007) have suggested that the cardiomyocyte apoptotic effect occurs at sufficiently low enough concentrations of LPS as to be comparable with the LPS concentrations that occur in chronic infections. However, the LPS dose employed in these studies (I mg/kg) (Suzuki et al 2003, Suzuki et al 2007, Li et al 2002) has recently been reported to produce a marked decrease in blood pressure and cerebral vasodilation, an increased circulating lactate concentration, and a metabolic acidosis with a compensatory decrease in $PaCO_2$ (respiratory drive) (Rosengarten et al 2008).

It would thus appear that the dose of LPS administered to induce cardiomyocyte apoptosis is more likely to induce all of the changes that one would normally consider to be congruent with septic shock. Thus, an outstanding question is whether low-grade infections, without the changes associated with septic shock, are indeed able to produce cardiomyocyte apoptosis.

4.1.1 Lipopolysaccharide-induced effects on cardiomyocyte apoptosis: Outcomes of the present study and comparison with previous studies

In the present study although LPS given at significantly lower doses (250 µg/kg) than the doses given in those studies that have shown an LPS-induced cardiomyocyte apoptotic effect (I

mg/kg) (Suzuki et al 2003, Suzuki et al 2007, Li et al 2002) induced a marked febrile response, cardiomyocyte apoptosis was not observed. Although the apparent inconsistencies in LPS-induced cardiomyocyte apoptosis between the results in the present dissertation and those previously reported on (Comstock et al 1998, Li et al 2002, Suzuki et al, 2003, 2007, McDonald et al 2000, Chagnon et al 2005, Niu et al 2008) are likely to be explained by differences in the LPS doses employed, other explanations need to be considered.

Importantly, in previous studies, cardiomyocyte apoptosis as assessed from caspase 3 activity was observed within 4 hours of LPS administration and did not dissipate over 24 hours (Suzuki et al 2007). Consequently, it is unlikely that differences in the time taken to assess apoptosis (6 hours after a second dose of LPS in the present study) contributed to the differences in the outcomes of the present and previous (Suzuki et al 2003, Suzuki et al 2007, Li et al 2002) studies. Importantly, the time period of 6 hours post LPS administration was selected to assess cardiomyocyte apoptosis in the present study to ensure that hearts were obtained in a period where there was still evidence of a febrile response. Indeed, the time of termination of rats coincided with a body temperature that was still higher than rats receiving the vehicle control and higher than the body temperature of the same rats measured at the same time of day on a preceding day in the absence of LPS. Moreover, cardiomyocyte apoptosis was assessed not only 6 hours after a dose of LPS administered on the same day, but also 24-hours after a dose of LPS administered on the preceding day. Consequently in the present study I was able to assess the impact of LPS on cardiomyocyte apoptosis over a 6-24 hour period, which encompasses the time periods within which LPS-induced cardiomyocyte apoptosis would be expected to occur (Suzuki et al 2007). A study with further time periods included would obviously strengthen the conclusions of the present study. However, based on previous evidence of the optimal time period of 4-24 hours (Suzuki et al 2007), this is unlikely to occur. Moreover, I could have considered assessing the effect of daily doses of LPS for prolonged

periods thus evaluating whether a cumulative effect on the heart could have occurred. However, consideration would have to be given to repeated injections of LPS and the possibility of LPS tolerance developing (Roth et al 1994). Indeed, in the present study, although there was no statistically significant tolerance, the thermal response index did tend to decrease on day 2. Further injections of LPS are therefore likely to have resulted in significant tolerance.

Although not measured in the present study, but as would be expected, a febrile response induced by LPS administration, such as noted in the present study, is associated with increased circulating concentrations of inflammatory mediators, such as TNF-α (Givalois et al 1994, Roth et al 1991). In this regard, although TNF-α at high concentrations has been shown to reduce cardiomyocyte function and promote myocardial damage (reviewed by Mann 2002, Kelly and Smith 1997) TNF-α at sufficiently low concentrations has cardio-protective effects on the heart (Deuchar et al 2007, Lecour et al 2002, Tanno et al 2003, Kurrelmeyer et al 2000). It is therefore possible that in response to LPS, increased circulating TNF-α concentrations, or an increased myocardial TNF-α expression in the present study may have only been sufficient to produce protective, rather than destructive effects on the heart. In contrast, at higher LPS doses such as those employed by previous authors (Suzuki et al 2003, Suzuki et al 2007, Li et al 2002), the extent to which TNF-α concentrations, or an increased myocardial TNF-α expression may have increased could have been sufficient to promote myocardial damage. Neither in our study, nor in previous studies have circulating TNF-α concentrations, or an increased myocardial TNF-α expression been measured in response to LPS and correlated with cardiomyocyte apoptosis or the lack thereof. Further studies are required to evaluate these possibilities.

4.1.2 Lipopolysaccharide-induced effects on cardiac function: Outcomes of the present study and comparison with previous studies.

As reviewed in chapter 1, LPS depresses myocardial and cardiomyocyte function (Suffredini et al 1989, Brady et al 1992, Hung and Lew 1993a, 1993b, Tao and McKenna 1994, Nishikawa et al 1995, Nishikawa and Lew 1996, Lew et al 1996, 1997a, Stein et al 1996, Yasuda and Lew 1997a, 1997b, 1999, Tavener et al 2004). In contrast, in the present study I failed to show an effect of LPS on cardiac systolic function and this outcome is consistent with a lack of adverse effects of LPS on noradrenaline-mediated increases in contractile function in isolated cardiomyocytes previously reported on in one study (Muller-Werdan et al 1998). The explanation for the lack of effect of LPS on cardiac systolic function in the present study is most likely because of the low dose of LPS employed in the present as compared to previous studies (Suffredini et al 1989, Brady et al 1992, Hung and Lew 1993a, 1993b, Tao and McKenna 1994, Nishikawa et al 1995, Nishikawa and Lew 1996, Lew et al 1996, 1997a, Stein et al 1996, Yasuda and Lew 1997a, 1997b, 1999, Tavener et al 2004). In the present study the purpose of LPS administration was to mimic the inflammatory effects of a low-grade systemic infection, rather than septic shock. In previous studies (Suffredini et al 1989, Brady et al 1992, Hung and Lew 1993a, 1993b, Tao and McKenna 1994, Nishikawa et al 1995, Nishikawa and Lew 1996, Lew et al 1996, 1997a, Stein et al 1996, Yasuda and Lew 1997a, 1997b, 1999, Tavener et al 2004) however, the purpose of these studies was to attempt to mimic septic shock.

In the present study the evidence to support a view that the dose of LPS does not induce septic shock was the presence of heart rates and left ventricular end diastolic diameters in rats receiving LPS that were similar to the control rat values. In septic shock, left ventricular end diastolic diameter is likely to decrease as a consequence of a reduced venous return (through the effects of peripheral pooling). Furthermore, if blood pressures had decreased acutely as a

consequence of a septic shock state, a baroreceptor response is likely to have resulted in an increased heart rate. As this was not apparent, it is unlikely that septic shock occurred in the present study.

4.1.3 Lipopolysaccharide-induced effects on the heart: Study strengths and limitations

The strengths of the present study are as follows. First, I used surgically implanted radiotelemeters to establish the physiological response to LPS and to ensure that cardiomyocyte apoptosis was assessed as guided by changes in core body temperature as well as time after injection. Second, to ensure that LPS did not produce changes consistent with septic shock, echocardiography was performed to assess filling diameters, heart rates and systolic cardiac function.

The weaknesses of the present study are as follows: The only method that I employed to assess cardiomyocyte apoptosis was a TUNEL technique. Importantly, in our groups hands this has previously been successful in showing hypertensive associated (Veliotes et al 2005) and adrenergic-induced (Osadchii et al 2007) cardiomyocyte apoptosis. However, TUNEL may overestimate the number of apoptotic nuclei, as it labels both DNA fragmentation and cells undergoing DNA repair. This is obviously not a concern in the present study as this would have biased against a result of a lack of effect of LPS on cardiomyocyte apoptosis. Alternative techniques such as the assessment of myocardial caspase-3 activity or other methods including DNA laddering could have been employed to support the present outcomes.

A second limitation of the present study is that ideally, the present study would have benefitted from a group of rats receiving a high dose of LPS, a dose previously shown to induce cardiomyocyte apoptosis (I mg/kg) (Suzuki et al 2003, Suzuki et al 2007, Li et al 2002) to act as a positive control group. However, this was not possible for ethical reasons due to the possibility

that this dose is capable of producing haemodynamic and other changes consistent with septic shock (Rosengarten et al 2008). Indeed, in discussion with the chairperson of the Animal Ethics Screening Committee, it is unlikely that this committee would ever approve an application of a model of septic shock in unanaesthetised animals.

Additional limitations of the present study include the following: I assessed the effect of a relatively short-term period of LPS exposure on cardiomyocyte apoptosis. In this regard I cannot exclude the possibility that further exposure to LPS in long-term studies may ultimately result in increases in cardiomyocyte apoptosis. Moreover, although I assessed the impact of LPS on cardiomyocyte apoptosis, I failed to evaluate the effect on cardiomyocyte autophagy. Further work is therefore required to address these questions.

4.1.4 Lipopolysaccharide-induced effects on the heart: Clinical implications.

The outcomes of the present study support the view that LPS at concentrations that induce a low-grade systemic infection do not result in increases in cardiomyocyte apoptosis. Hence, the present study suggests that LPS-induced cardiomyocyte apoptosis demonstrated in previous studies (Li et al 2002, Suzuki et al 2003, Suzuki et al 2007, Comstock et al 1998, McDonald et al 2000) is probably important with respect to the impact of LPS in sepsis but not in low-grade systemic infections. Further long-term studies are nevertheless still required to substantiate these outcomes. Moreover, the outcomes of the present study need to be confirmed using alternative assessments of cardiomyocyte apoptosis or assessments of alternative cell death mechanisms including cardiomyocyte autophagy.

4.2 Outstanding question regarding the role of inflammation in obesity-induced cardiac dysfunction

As described in chapter 1 of the present dissertation, few studies have assessed whether circulating inflammatory markers may explain the association that exists between obesity and heart failure or cardiac dysfunction. In support of a role for obesity-derived inflammatory substances as mediators of heart failure, after adjustments for either interleukin-6 or C-reactive protein in the regression analysis, the independent relationship between obesity and heart failure failed to achieve statistical significance (Bahrami et al 2008). However, in that study (Bahrami et al 2008) the increased risk for heart failure in obese patients decreased from an 83% (hazards ratio=1.83) to a 50-58% (hazards ratio=1.50-1.58) risk after adjustments for inflammatory markers. Thus, a potential residual risk for heart failure remained even after adjustments for pro-inflammatory markers (Bahrami et al 2008). In support of this notion, in another study (Spies et al 2009), the independent relationship between waist-to-hip ratio and the subsequent development of heart failure in patients with established coronary artery disease was only partially modified with adjustments for CRP, TNF-α, or interleukin-6. In that study (Spies et al 2009), the independent relationship between an excess adiposity and the development of heart failure remained even after adjustments for these inflammatory markers. Thus, there is still considerable controversy as to the role of adipose tissue-derived pro-inflammatory substances in the development of obesity-induced heart failure. Before describing the main outcomes of the present study assessing whether obesity-induced cardiac systolic dysfunction can be accounted for by serum CRP concentrations, it is important to first place the study group in context. In this regard, the first important question is whether in the present study cohort there was evidence that obesity contributes toward circulating concentrations of serum CRP?

4.2.1 Relationship between obesity and CRP: Outcomes of the present study and comparison with previous studies.

In the present study I noted relatively strong relationships between serum CRP concentrations and indices of an excess adiposity, which persisted even when correcting for conventional risk factors in multivariate regression models. These findings are in agreement with previous studies demonstrating independent relationships between indices of adiposity and circulating concentrations of inflammatory markers, including CRP (Bahrami et al 2008, Berg and Scherer 2005, Despres et al 2006, Ridker et al 2003 and Sattar et al 2007, Yudkin et al 1999, Cartier et al 2009a, Piche et al 2005). Although associations do not indicate cause and effect, other studies have provided sufficient evidence to suggest that these relations are indeed cause and effect, as weight loss with liposuction (Giugliano et al 2004), gastric bypass (Kopp et al 2003) and diet (Heilbronn et al 2001) have all been shown to decrease circulating concentrations of inflammatory markers.

In keeping with previous studies demonstrating correlation coefficients of.0.21-0.61 for relationships between adiposity indices and circulating CRP concentrations (Yudkin et al 1999, Cartier et al 2009a, 2009b, Lemieux et al 2001, Piche et al 2005), in the present study I could similarly show correlation coefficients for the relationships between indices of an excess adiposity and circulating CRP concentrations ranging from 0.21-0.51, with BMI and waist circumference showing the strongest correlations. Although a greater number of women participated in the present study than men, and consistent with previous studies (Khera et al 2009) female gender was independently associated with circulating CRP concentrations, the relationship between indices of adiposity and serum CRP was similar in both gender groups.

Importantly, in the present study BMI was equally as strongly associated with serum CRP concentrations as was waist circumference. This is in contrast to the stronger relationships

noted between indices of central obesity and circulating CRP concentrations as compared to alternative adiposity indices and circulating CRP concentrations previously suggested (Piche et al 2005, Yudkin et al 1999, Lemieux et al 2001, Cartier et al 2009a, 2009b). However, in previous studies, despite the claim that central adiposity is the primary determinant of CRP (Yudkin et al 1999, Cartier et al 2009a, 2009b, Hak et al 1999), only one study (Hak et al 1999) showed that the relationship between central adiposity and inflammation was independent of the effect of BMI.

It is possible that in the present study the inability to show a stronger relationship between indices of central adiposity as compared to alternative indices of adiposity and circulating CRP concentrations may be explained by the strength of the relationship between BMI and waist circumference in the population sampled (Majane et al 2008). It may also be argued that because more women than men participated in the present study, and that circulating CRP concentrations are related to subcutaneous rather than visceral fat in women and visceral rather than subcutaneous fat in men (Cartier et al 2009b), that this may explain the similarity in the strength of the associations between indices of central or general adiposity and circulating CRP concentrations. However, alternative studies indicate that there is a stronger association between indices of central obesity and circulating CRP concentrations in both genders (Khera et al 2009). Moreover, in the present study, gender-specific analysis revealed that BMI was the strongest independent predictor of circulating CRP concentrations in both men and women. Further studies conducted in a much larger cohort of participants are required to explain the equivalence of the strength of the relationship between BMI and serum CRP concentrations as compared to waist circumference and serum CRP concentrations in the present community.

4.2.2 Relationship between obesity and cardiac systolic function: Outcomes of the present study and comparison with previous studies.

In the present study I noted an inverse relationship between two indices of adiposity (waist circumference and skin-fold thickness) and an index of myocardial systolic function (left ventricular midwall fractional shortening), and the relationship between waist circumference and left ventricular midwall fractional shortening persisted with adjustments for potential confounders. However, I was unable to show an unadjusted or multivariate adjusted relationship between indices of adiposity and left ventricular chamber systolic function.

The independent relationship between an excess adiposity and left ventricular myocardial systolic function noted in the present study is consistent with decreases in tissue Doppler indices of systolic myocardial function noted to occur in overweight and obese people without conventional cardiovascular risk factors previously reported on (Peterson et al 2004, Wong et al 2004). Whether the relationships between an excess adiposity and myocardial systolic function are cause-effect relationships is nevertheless unclear. Indeed, even with the use of load-independent tissue Doppler measures of myocardial as opposed to chamber function, or with chamber function assessments, weight loss produced by either lifestyle modification or gastric bypass does not influence left ventricular systolic function (Willens et al 2005, Rider et al 2009, Wong et al 2006, Skilton et al 2007).

In contrast to the present study one previous study has failed to demonstrate a relationship between body size and left ventricular midwall fractional shortening (De Simone et al 1996). Nevertheless, in contrast to the present study where the participants studied comprised largely of normotensives (~75% of the sample) not receiving antihypertensive therapy, in the study by De Simone et al (1996), the relationship between obesity and left ventricular midwall fractional shortening was studied in hypertensives only. As hypertension is a

well-recognized cause of myocardial systolic dysfunction, this may have obscured the impact of obesity on myocardial function. Differences in the prevalence of obesity could also account for differences in the relationship between adiposity indices and left ventricular midwall fractional shortening in the present study as compared to the study by De Simone et al (1996) where no independent relationship was noted. Indeed, the prevalence of obesity in the present study (~37%) was greater than that reported on by De Simone et al (1996)(26.1%). In addition, in the study by De Simone et al (1996), the relationship between indices of an excess adiposity and left ventricular midwall fractional shortening was unlikely to have been assessed in a cohort in which the prevalence of concentric cardiac remodelling produced by an excess adiposity was as high as that described in the present study. Indeed, in the present study 25% of the total sample had either concentric left ventricular hypertrophy or remodelling and we have previously demonstrated that a substantial proportion on this change is accounted for by obesity (Woodiwiss et al 2008). As concentric left ventricular remodelling is thought to be a compensatory change that maintains chamber function in the presence of decreases in myocardial systolic function as indexed by left ventricular midwall fractional shortening (Norton et al 2002), the present study sample is ideally suited to assess whether obesity is associated with left ventricular midwall fractional shortening.

The lack of relationship between indices of excess adiposity and either unadjusted or multivariate adjusted values for left ventricular chamber systolic dysfunction (endocardial fractional shortening) is not surprising as the independent relationship between an excess adiposity and systolic chamber function is controversial, with some studies showing a relationship between an excess adiposity and systolic chamber function (Ammar et al 2008, Chinali et al 2006, Peterson et al 2004, Wong et al 2004, Scaglione et al 1992, Karason et al 1998, Alpert et al 1995, Alpert et al 1993), whilst others have failed to do so (Pascual et al 2003, Devitiis et al 1981, Zarich et al 1991, Stoddard et al 1992, De Simone et al 1996, Mureddu et al

1996, Iacobellis et al 2002). A lack of relationship between indices of an excess adiposity and chamber systolic function despite the relationship between indices of an excess adiposity and myocardial systolic function is also not surprising considering the high prevalence of concentric left ventricular remodelling reported on in the present study, a change attributed to a large extent to obesity (Woodiwiss et al 2008). In this regard, as previously indicated, concentric remodelling may indeed be a compensatory response to myocardial dysfunction.

4.2.3 Relationship between CRP and cardiac systolic function: Outcomes of the present study and comparison with previous studies.

In the present study, an inverse univariate correlation, albeit weak, was noted between CRP concentrations and left ventricular midwall fractional shortening, a relationship which nevertheless did not persist with adjustments for potential confounders. In addition, the inverse relationship between adiposity indices and left ventricular myocardial function remained when waist circumference was included in the same regression model with log CRP, whilst log CRP was not independently associated with left ventricular systolic myocardial function in this same model. These data therefore suggest that inflammatory changes as indexed by log CRP, are unlikely to account for the impact of an excess adiposity on left ventricular systolic myocardial function.

The lack of independent relationship between CRP concentrations and left ventricular midwall fractional shortening in the present study is in apparent contrast to the evidence to suggest that inflammatory markers, including CRP, independently predict the development of heart failure (Bahrami et al 2008, Vasan et al 2003, Cesari et al 2003, Ingelsson et al 2005b). However, in the present study I assessed left ventricular systolic myocardial function rather than heart failure. In this regard, chronic inflammation in the group studied may not have been

present for sufficiently long to have produced early changes in myocardial function. In addition, this observation may be attributed to the impact of co-linearity in the regression models, an effect produced by the close relationship between inflammation and obesity or other factors. Only prospective follow-up of participants will provide the necessary evidence to evaluate whether in the community studied, CRP concentrations independently predict a reduced myocardial systolic function or the development of heart failure.

The lack of ability of adjustments for CRP concentrations to nullify the inverse relationship between adiposity indices and left ventricular myocardial function is in apparent contrast to data demonstrating a potential role for obesity-derived inflammation as a mediator of heart failure. Indeed, previous studies have demonstrated that after adjustments for either interleukin-6 or CRP in the regression analysis, the independent relationship between obesity and heart failure failed to achieve statistical significance (Bahrami et al 2008). Moreover, Spies et al (2009), have demonstrated that the independent relationship between waist-to-hip ratio and the subsequent development of heart failure in patients with established coronary artery disease was partially modified with adjustments for CRP, TNF-α, and interleukin-6. However, the present data are also in-part in support of that study (Spies et al 2009), as these authors (Spies et al 2009), demonstrated that a residual independent relationship between an excess adiposity and the development of heart failure remained even after adjustments for these inflammatory markers. It is also possible that the ability of adjustments of circulating markers of inflammatory changes in the regression analysis to reduce the independent relationship between obesity and heart failure (Bahrami et al 2008, Spies et al 2009) could be attributed to effects of inflammatory changes on diastolic rather than systolic myocardial function, a possibility not explored in the present dissertation.

4.2.4 Inflammation and obesity-induced cardiac dysfunction: Strengths and limitations.

The outcomes of the present study need to be interpreted in the context of the study strengths and limitations. The strengths of the present study include the random selection techniques of the study sample, and the fact that the question was assessed in a community well recognized as having a high prevalence of obesity and where indices of obesity were strongly related to circulating CRP concentrations.

The weaknesses of the present study include the following: First, the sample size prevented me, in sensitivity analysis, from assessing relationships between obesity or circulating CRP concentrations and myocardial systolic function in subgroups of the study. Important subgroups could have been sex-specific groups as gender was associated with log CRP. A second limitation of the study was the cross-sectional design. Cross-sectional studies prevent the ability to draw conclusions regarding cause and effect. Only longitudinal studies assessing the ability of CRP to predict the development of systolic dysfunction or intervention studies specifically targeting inflammation in obese individuals will allow for cause-effect relationships to be established. Third, myocardial tissue doppler measurements of myocardial systolic function were not available in the present study and these measurements could be more sensitive than calculations of midwall fractional shortening for detecting myocardial systolic dysfunction. Fourth, the study is limited by the lack of measurements of circulating concentrations of alternative pro-inflammatory and anti-inflammatory adipokine measurements. However, no study has yet demonstrated that these measurements offer a greater sensitivity to detect adverse effects on the heart than circulating high or CRP concentrations.

4.2.5 Inflammation and obesity-induced cardiac dysfunction: Clinical implications.

The outcomes of the present study suggest that obesity mediates deleterious effects on myocardial systolic function through actions that are independent of inflammatory changes. If these data are replicated in longitudinal or intervention studies targeting obesity or obesity induced inflammatory changes, these data may suggest that targeting obesity-induced inflammation may not be an important therapeutic intervention in preventing heart failure associated with systolic myocardial dysfunction. Further large community or population-based studies and intervention studies are therefore required to evaluate this hypothesis.

4.3 Overall conclusions

In conclusion, the present studies indicate the following: First, in contrast to the cardiomyocyte apoptosis associated with high doses of LPS, the inflammatory response produced by doses of LPS associated with a marked pyretic response, but not with sepsis, do not result in cardiomyocyte apoptosis as assessed using a TUNEL technique. Second, circulating concentrations of the inflammatory marker, CRP, do not account for obesity-induced decreases in systolic myocardial function. Although these studies require further work to substantiate these outcomes, these outcomes lend insights into the potential role of chronic inflammation produced either by infections or obesity on the heart. In this regard, the data obtained in the present dissertation suggest that low grade inflammation associated with infections or with obesity are unlikely to contribute toward the development of myocardial damage or myocardial systolic dysfunction.

References

Abbate A, Biondi-Zoccai GGL, Bussani R, Dobrina A, Camilot D, Feroce F, Rossiello R, Baldi F, Silvestri F, Biasucci LM, Baldi A. Increased myocardial apoptosis in patients with unfavorable left ventricular remodeling and early symptomatic post-infarction heart failure. J Am Coll Cardiol 2003;41:753-760.

Alpert MA, Lambert CR, Panayiotou H, Terry BE, Cohen MV, Massey CV, Hashimi MW, Mukerji V. Relation of duration of morbid obesity to left ventricular mass, systolic function, and diastolic filling, and effect of weight loss. Am J Cardiol 1995;76:1194-1197.

Alpert MA, Terry BE, Lambert CR, Kelly DL, Panayiotou H, Mukerji V, Massey CV, Cohen MV. Factors influencing left ventricular systolic function in non hypertensive morbidly obese patients and effect or weight loss. Am J Cardiol 1993;15:733-737.

Ammar KA, Redfield MM, Mahoney DW, Johnson M, Jacobsen SJ, Rodeheffer RJ. Central obesity: association with left ventricular dysfunction and mortality in the community. Am Heart J 2008;156:975-981.

Anderson JC, Konz, EC, Frederich RC, Wood CL. Long-term weight-loss maintenance: a meta-analysis of US studies. Am J Clin Nutr 2001;74**:**579–584.

Anker SD, Coats AJ. How to RECOVER from RENAISSANCE? The significance of the results of RECOVER, RENAISSANCE, RENEWAL and ATTACH. Int J Cardiol 2002;86:123-130.

Agarwala S, Kalil RE. Axotomy-induced neuronal death and reactive astrogliosis in the lateral geniculate nucleus following a lesion of the visual cortex in the rat. J Comp Neurol 1998;392:252-263.

Aukrust P, Ueland T, Lien E, Bendtzen K, Müller F, Andreassen AK, Nordøy I, Aass H, Espevik T, Simonsen S, Frøland SS, Gullestad L. Cytokine network in congestive heart failure

secondary to ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol 1999;83:376-382.

Bahrami H, Bluemke DA, Kronmal R, Bertoni AG, Lloyd-Jones DM, Shahar E, Szklo M, Lima JA. Novel metabolic risk factors for incident heart failure and their relationship with obesity: the MESA (Multi-Ethnic Study of Athersosclerosis) study. J Am Coll Cardiol 2008;51:1775-1783.

Barouch LA, Gao D, Miller KL, Xu W, Phan AC, Kittleson MM, Minhas KM, Berkowitz DE, Wei C, Hare JM. Cardiac myocyte apoptosis is associated with increased DNA damage and decreased survival in murine models of obesity. Circ Res 2006;98:119-124.

Bennett CM, Guo M, Dharmage SC. HbA1c as a screening tool for the detection of type 2 diabetes: a systematic review. Diabet Med 2007;24:333-343.

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

Brady AJP, Poole-Wilson PA, Harding SE, Warren JB. Nitric oxide production within cardiac myocytes reduces their contractility in endotoxaemia. Am J Physiol 1992;263:H1963-H1966.

Brooksbank[a] R, Woodiwiss AJ, Sliwa K, Badenhorst D, Defterios D, Wadee AA, Essop MR, Sareli P, Norton GR. Endotoxin-independent white cell cytokine production in heamodynamically stable patients with idiopathic dilated cardiomyopathy. Cardiovasc J South Afr 2005;16:260-265.

Brooksbank[b] R, Woodiwiss AJ, Sliwa K, Badenhorst D, Defterios D, Essop MR, Sareli P, Norton GR. Sustained white cell cytokine activation in idiopathic dilated cardiomyopathy despite haemodynamic improvement with medical therapy. Cardiovasc J South Afr 2005;16:200-204.

Cabanac A, Briese E. Handling elevates the colonic temperature of mice. Physiol Behav 1992;51:95-98.

Calvin JE, Driedger AA, Sibbald WJ. An assessment of myocardial function in human sepsis utilizing ECG gated cardiac scintigraphy. Chest 1981;80:579-586.

Carabello BA, Gittens L. Cardiac mechanisms and function in obese normotensive persons with normal coronary arteries. Am J Cardiol 1987;15:469-473.

Cartier[a] A, Cote M, Lemieux I, Perusse L, Tremblay A, Bouchard C, Despres JP. Age-related differences in inflammatory markers in men: contribution of visceral adiposity. Metabolism 2009;58**:**1452-1458.

Cartier[b] A, Cote M, Lemieux I, Perusse L, Tremblay A, Bouchard C, Despres JP. Sex differences in inflammatory markers: what is the contribution of visceral adiposity? Am J Clin Nutr 2009;89:1307-1314.

Carroll JF, Jones AE, Hester RL, Reihart GA, Cockrell K, Mizelle HE. Reduced cardiac contractile responsiveness to isoproterenol in obese rabbits. Hypertension 1997;30:1376-1381.

Cesari M, Penninx BWJH, Newman AB, Kritchevsky SB, Nicklas BJ, Sutton-Tyrrell K, Rubin SM, Ding J, Simonsick EM, Harris TB, Pahor M. Inflammatory markers and onset of cardiovascular events: Results from the Health ABC Study. Circulation 2003;108:2317-2322.

Chagnon F, Metz CN, Bucala R, Lesur O. Endotoxin-induced myocardial dysfunction: effects of macrophage migration inhibitory factor neutralization. Circ Res 2005;96**:**1095-1102.

Chakko S, Mayor M, Allison MD, Kessler KM, Materson BJ, Myerburg RJ. Abnormal left ventricular filling in eccentric left ventricular hypertrophy of obesity. Am J Cardiol 1991;68:95-98.

Chao W, Shen Y, Zhu X, Zhao H, Novikov M, Schmidt U, Rosenzweig A. Lipopolysaccharide improves cardiomyocyte survival and function after serum deprivation. J Biol Chem 2005;280: 1997-2005.

Chen YT, Vaccarino V, Williams CS, Butler J, Berkman LF, Krumholz HM. Risk factors for heart failure in the elderly: a prospective community based study. Am J Med 1999;106:605-612.

Cheng YC, Chen LM, Chang MH, Chen WK, Tsai FJ, Tsai CH, Lai TY, Kuo WW, Huang CY, Liu CJ. Lipopolysaccharide upregulates uPA, MMP-2 and MMP-9 via ERK1/2 signaling in H9c2 cardiomyoblast cells. Mol Cell Biochem 2009;325:15-23.

Chinali M, DeSimone G, Roman MJ, Lee ET, Best LG, Howard BV, Devereux RB. Impact of obesity on cardiac geometry and function in a population of adolescents: the Strong Heart Study. J Am Coll Cardiol 2006;47**:**2267-2273.

Chung ES, Perlini S, Aurigemma GP, Fenton RA, Dobson JG, Meyer TE. Effects of chronic adenosine uptake blockade on adrenergic responsiveness and left ventricular chamber function in pressure overload hypertrophy in the rat. J Hypertens 1998;16:1813-1822.

Chung ES, Packer M, Lo KH, Fasanmade AA, Willerson JT. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumour necrosis factor-alpha, in patients with moderate-to-severe heart failure: results of the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial. Circulation 2003;107:3133-3140.

Cinquegrana G, D'Aniello L, Landi M, Spinelli L, Grande G, De Prisco F, Petretta M. Effects of different degrees of sympathetic antagonism on cytokine network in patients with ischemic dilated cardiomyopathy. J Card Fail 2005;11:213-219.

Clark MA, Plank LD, Connolly AB, Streat SJ, Hill AA, Gupta R, Monk DN, Shenkin A, Hill GL. Effect of a chimeric antibody to tumor necrosis factor-alpha on cytokine and physiologic

responses in patients with severe sepsis--a randomized, clinical trial. Crit Care Med 1998;26:1650-1659.

Comstock KL, Krown KA, Page MT, Martin DM Ho P, Pedraza M, Castro EN, Nakajima N, Glembotski CC, Quintana PJE, Ra S. LPS-Induced TNF–α release from and apoptosis in rat cardiomyocytes: obligatory role for CD14 in mediating the LPS response. J Mol Cell Biol 1998;30:2761-2775.

Cowan DB, Noria S, Stamm C, Garcia LM, Poutias DN, del Nido PJ, McGowan FX. Lipopolysaccharide internalization activates endotoxin-dependent signal transduction in cardiomyocytes. Circ Res 2001;88:491-498.

Cowie MR, Wood DA, Coats AJS, Thompson SG, Suresh V, Poole-Wilson PA, Sutton GC. Survival of patients with a new diagnosis of heart failure: a population based study. Heart 2000;83:505-510.

De Simone G, Deveroux RB, Mureddu GF, Roman MJ, Ganau A, Saba PS, Alderman MH, Laragh JH. Assessment of left ventricular function by the midwall fractional shortening/end-systolic stress relation in human hypertension. J Am Coll Cardiol 1994;23:1444-1451.

De Simone G, Deveroux RB, Mureddu GF, Roman MJ, Ganau A, Alderman MH, Contaldo F, Laragh JH. Influence of obesity on left ventricular midwall mechanics in arterial hypertension. Hypertension 1996;28:276-283.

Deuchar GA, Opie LH, Lecour S. TNFα is required to confer protection in an in vivo model of classical ischaemic preconditioning. Life Sci 2007;80:1686-1691.

Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N. Echocardiograph assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol 1986;57:450-458.

de Devitiis O, Fazio S, Petitto M, Maddalena G, Contaldo F, Mancini M. Obesity and cardiac function. Circulation 1981;64:477-482.

Dong F, Zhang X, Yang X, Esberg LB, Yang H, Zhang Z, Culver B, Ren J. Impaired cardiac contractile function in ventricular myocytes from leptin deficient ob/ob obese mice. J Endocrinol 2006;188:25-36.

Dutta J, Fan Y, Gupta N, Fan G, Gėlinas C. Current insights into the regulation of programmed cell death by NF-κβ. Oncogene 2006;25:6800-6816.

du Toit EF, Smith W, Muller C, Strijdom H, Stouthammer B, Woodiwiss AJ, Norton GR, Lochner A. Myocardial susceptibility to ischemic-reperfusion injury in a pre-diabetic model of dietary-induced obesity. Am J Physiol Heart Circ Physiol 2008;294:H2336-H2343.

Doyama K, Fujiwara H, Fukimoto M, Tanaka M, Fujiwara Y, Oda T, Inada T, Ohtani S, Hasegawa K, Fujiwara T, Sasayama S. Tumour necrosis factor is expressed in the cardiac tissue of patients with heart failure. Int J Cardiol 1996;31:391-398.

Engel D, Peshock R, Armstrong RC, Sivasubramanian N, Mann DL. Cardiac myocyte apoptosis provokes adverse cardiac remodeling in transgenic mice with targeted TNF overexpression. Am J Physiol Heart Circ Physiol 2004;287**:**H1303-H1311.

Fischer M, Baessler A, Hense HW, Hengstenberg C, Muscholl M, Holmer S, Doring A, Broeckel U, Riegger G, Schunkert H. Prevalence of left ventricular diastolic dysfunction in the community: Results from a Doppler echocardiographic-based survey of a population sample. Eur Heart J 2003;24:320-328.

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

Frankel DS, Vasan RS, D'Agostino RB, Benjamin EJ, Levy D, Wang TJ, Meigs JB. Resistin, adiponectin and risk of heart failure. J Am Coll Cardiol 2009;53:754-762.

Frantz S, Kobzik L, Kim YD, Fukazawa R, Medzhitov R, Lee RT, Kelly RA. Toll4 (TLR4) expression in cardiac myocytes in normal and failing myocardium. J Clin Invest 1999;104:271-280.

Gabriely I, Ma XH, Yang XM, Atzmon G, Rajala MW, Berg AH, Scherer P, Rosetti L, Barzilai N. Removal of visceral fat prevents insulin resistance and glucose intolerance of aging: an adipokine-mediated process? Diabetes 2002;51**:**2951-2958.

Ganau A, Devereux RB, Roman MJ, deSimone G, Pickering TG, Saba PS, Vargiu P, Simongini I, Laragh JH. Patterns of left ventricular remodeling in essential hypertension. J Am Coll Cardiol 1992;19:1550-1558.

Geerdts SO, Nys M, De MP, Charpentier J, Albert A, Legrand V, Rompen EH. Systemic release of endotoxins induced by gentle mastication: association with periodontitis severity. J Periodontol 2002;73:73-78.

Genth-Zotz S, von Haehling S, Bolger AP, Kalra PR, Wensel R, Coats AJ, Anker SD. Pathophysiologic quantities of endotoxin-induced tumour necrosis factor-alpha release in whole blood from patients with chronic heart failure. Am J Cardiol 2002;90:1226-1230.

Giugliano G, Nicoletti G, Grella E, Giugliano F,Esposito K,Scuderi N, D'Andrea F. Effect of liposuction on insulin resistance and vascular inflammatory markers in obese women. Br J Plast Surg 2004;57:190-194.

Givalois L, Dornand J, Mekaouche M, Solier MD, Bristow AF, Ixart G, Siaud P, Assenmacher I, Barbanel G. Temporal cascade of plasma level surges in ACTH, corticosterone, and cytokines in endotoxin-challenged rats. Am J Physiol 1994;267:R164-R170.

Harden LM, du Plessis I, Poole S, Laburn HP. Interleukin-6 and leptin mediate lipopolysaccharide-induced fever and sickness behaviour. Physiol Behav 2006;89:146-155.

Hayakawa Y, Chandra M, Miao W, Shirani J, Brown JH, Dorn GW 2nd, Armstrong RC, Kitsis RN. Inhibition of cardiac myocyte apoptosis improves cardiac function and abolishes mortality in the peripartum cardiomyopathy of Galpha(q) transgenic mice. Circulation 2003;108:3036-3041.

He J, Ogden LG, Bazzano LA, Vuppturi S, Loria C, Whelton PK. Risk factors for congestive heart failure in US men and women:NHANES I epidemiologic follow-up study. Arch Intern Med 2001;161:996-1002.

Heilbronn LK, Noakes M, Clifton PM. Energy restriction and weight loss on very-low-fat diets reduce C-reactive protein concentrations in obese, healthy women. Arterioscler Thromb Vasc Biol ;2001:21:968-970.

Hershberger RE. Familial dilated cardiomyopathy. Prog Ped Cardiol 2005;20:161-168.

Hobbs RE. Guidelines for the diagnosis and management of heart failure. Am J Ther 2004;11:467-472.

Hung[a] J, Lew WY. Cellular mechanisms of endotoxin-induced myocardial depression in rabbits. Circ Res 1993;73:125-34.

Hung[b] J, Lew WY. Temporal sequence of endotoxin-induced systolic and diastolic myocardial depression in rabbits. Am J Physiol 1993;265:H810-819.

Hunt SA, Baker DW, Chin MH, Cinquegrani MP, Feldman AM, Francis GS, Ganiats TG, Goldstein S, Gregoratos G, Jessup ML, Noble RJ, Packer M, Silver MA, Stevenson LW, Gibbons RJ, Antman EM, Alpert JS, Faxon DP, Fuster V, Jacobs AK, Hiratzka LF, Russell RO, Smith SC, Jr. ACC/AHA guidelines for the evaluation and management of chronic heart failure in the adult: Executive summary, a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to revise the 1995 Guidelines for the Evaluation and Management of Heart Failure): Developed in Collaboration with the International Society for Heart and Lung

Transplantations; endorsed by the Heart Failure Society of America. Circulation 2001;104:2996-3007.

Iacobellis G, Ribaudo MC, Leto G, Zappaterreno A, Vecci E, DiMario U, Leonetti F. Influence of excess fat on cardiac morphology and function. Study in uncomplicated obesity. Obes Res 2002;10:767-773.

Ingelsson[a] E, Sundstrom J, Arnlov J, Zethelius B, Lind L. Insulin resistance and risk of congestive heart failure. JAMA 2005;294:334-341.

Ingelsson[b] E, Arnlov J, Sundstrom J, Lind L. Inflammation, as measured by the erythrocyte sedimentation rate, is an independent predictor for the development of heart failure. J Am Coll Cardiol 2005;45:1802-1806.

Ingelsson E, Riserus U, Berne C, Frystyk J, Flyvbjerg A, Axelsson T, Lundmark P, Zethelius B. Adiponectin and risk of congestive heart failure JAMA 2006;295:1772-1774.

Jafri SM, Levine S, Field BE, Bahorozian MT, Carlson RW. Left ventricular diastolic function in sepsis. Crit Care Med 1990;18:709-714.

Johansson S, Wallander MA, Ruigomez A, Garcia Rodriguez LA. Incidence of newly diagnosed heart failure in UK general practice. Eur J Heart Fail 2001;3:225-231.

Kalra D, Svasubramnian N, Mann D. Angiotensin II induces tumour necrosis factor alpha biosynthesis in the adult mammalian heart through protein kinase-C dependent pathway. Circulation 2002;105:2198-2205.

Karason K, Wallentin I, Larsson B, Sjostrom L. Effects of obesity and weight loss on cardiac function and valvular performance. Obes Res 1998;6:422-429.

Kelly R, Smith T. Cytokines and cardiac contractile function. Circulation 1997;95:778-781.

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

Kenchaiah S, Sesso HD, Gaziano MJ. Body mass index and vigorous physical activity and the risk of heart failure among men. Circulation 2009;119:44-52.

Khera A, Vega GL, Das SR, Ayers C, McGuire DK, Grundy SM, De Lemos JA. Sex differences in the relationship between C-reactive protein and body fat. J Clin Endocrinol Metab 2009;94**:**3251-3258.

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

Kluger MJ, O'Reilly B, Shope TR, Vander AJ. Further evidence that stress hyperthermia is a fever. Physiol Behav 1987;39:763-766.

Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, Homma S, Edwards NM, Itescu S. Neovascularization of ischemic myocardium by human bone-marrow−derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med 2001;7:430-436.

Kopp HP, Kopp CW, Festa A, Krzyzanowska K, Kriwanek S, Minar E, Roka R, Schernthaner G. Impact of weight loss on inflammatory proteins and their association with the insulin resistance syndrome in morbidly obese patients. Arterioscler Thromb Vasc Biol 2003;23:1042-1047.

Krishnagopalan S, Kumar A, Parrillo JE, Kumar A. Myocardial dysfunction in the patient with sepsis. Curr Opin Crit Care 2002;8:376-388.

Kurrelmeyer KM, Michael LH, Baumgarten G, Taffet GE, Peschon JJ, Sivasubramanian N, Entman ML, Mann DL. Endogenous tumor necrosis factor protects the adult cardiac

myocyte against ischemic-induced apoptosis in a murine model of acute myocardial infarction. Proc Natl Acad Sci USA 2000;97:5456-5461.

Lang A, Froelicher ES. Management of overweight and obesity in adults: behavioral intervention for long-term weight loss and maintenance. Eur J Cardiovasc Nurs 2006;5:102-114.

Lecour S, Smith RM, Woodward B, Opie LH, Rochette L, Sack MN. Identification of a novel role for Sphingolipid signaling in TNFα and ischemic preconditioning mediated cardioprotection.J Mol Cell Cardiol 2002;34:509-518.

Lemieux I, Pascot A, Prud'homme D, Almeras N, Bogaty P, Nadeau A, Bergeron J, Despres JP. Elevated C-reactive protein: another component of the atherothrombotic profile of abdominal obesity. Arterioscler Thromb Vasc Biol 2001;21**:**961-967.

Lenfant C. Report of the task force on research in heart failure. Circulation 1994;90:1118-11123.

Levine B, Kalman J, Mayer L, Fiuit HM, Packer M. Elevated circulating levels of tumour necrosis factor alpha in severe chronic heart failure. N Engl J Med 1990;323:236-241.

Lew WY, Yasuda S, Yuan T, Hammond HK. Endotoxin-induced cardiac depression is associated with decreased cardiac dihydropyridine receptors in rabbits. J Mol Cell Cardiol 1996;28:1367-1371.

Lew[a] WY, Lee M, Yasuda S, Bayna E. Depyrogenation of digestive enzymes reduces lipopolysaccharide tolerance in isolated cardiac myocytes. J Mol Cell Cardiol 1997;29:1985-1990.

Lew[b] WY, Ryan J, Yasuda S. Lipopolysaccharide induces cell shrinkage in rabbit ventricular cardiac myocytes. Am J Physiol 1997;272:H2989-H2993.

Li HL, Suzuki J, Bayna E, Zhang FM, Dalle Molle E, Clark A, Engler RL, Lew WY. Lipopolysaccharide induces apoptosis in adult rat ventricular myocytes via cardiac AT_1 receptors. Am J Physiol 2002;283:H461-H467.

Li L, Shigematsu Y, Hamada M, Hiwada K. Relative wall thickness is an independent predictor of left ventricular systolic and diastolic dysfunctions in essential hypertension. Hypertens Res 2001;24:493-499.

Libhaber CD, Norton GR, Majane OHI, Libhaber E, Essop, MR, Brooksbank R, Maseko M, Woodiwiss AJ. Contribution of central and general adiposity to an abnormal left ventricular diastolic function in a community sample with a high prevalence of obesity. Am J Cardiol 2009;104:1527-1533.

Liu CJ, Cheng YC, Lee KW, Hsu HH, Chu CH, Tsai FJ, Tsai CH, Chu CY, Liu JY, Kuo WW, Huang CY. Lipopolysaccharide induces cellular hypertrophy through calcineurin/NFAT-3 signaling pathway in H9c2 myocardiac cells. Mol Cell Biochem 2008;313:167-178.

Liu PP, Mason JW. Advances in understanding of myocarditis. Circulation 2001;104:1076-1082.

Luppi P, Rudert WA, Zanone MM, Stassi G, Trucco G, Finegold D, Boyle GJ, Del Nido P, McGowan FX Jr, Trucco M. Idiopathic dilated cardiomyopathy: A superantigen-driven autoimmune disease. Circulation 1998;98:777-785.

Maher D, Smeeth L, Sekajugo J. Health transition in Africa: practical policy proposals for primary care. Bull World Health Organ 2010;88:943-948.

Majane OHI, Norton GR, Maseko MJ, Makaula S, Crowther N, Paiker J, Thijs L, Brooksbank R, Sareli P, Staessen JA, Woodiwiss AJ. The association of waist circumference with ambulatory blood pressure is independent of alternative adiposity indices. J Hypertens 2007;25:1798-1806.

Majane OHI, Woodiwiss AJ, Maseko MJ, Crowther N, Dessein P, Norton GR. Impact of age on the independent association of adiposity with pulse wave velocity in a population sample of African ancestry. Am J Hypertens 2008;21:936-942.

Mann DL. Inflammatory mediators and the failing heart: past, present and the foreseeable future Circ Res 2002;91:988-998.

Mann DL, McMurray JJ, Packer M, Swedberg K, Borer JS, Colucci WS, Djian J,Drexler H, Feldman A, Kober L, Krum H, Liu P, Nieminen M, Tavazzi L, van Veldhuisen DJ, Waldenstrom A, Warren M, Westheim A, Zannad F, Fleming T. Targeted anticytokine therapy in patients with chronic heart failure: results of the Randomized Etanercept Worldwide Evaluation (RENEWAL). Circulation 2004;109:1594-1602.

Martich GD, Parker MM, Cunnion RE, Suffredinin AF. Effects of ibuprofen and pentoxifylline on the cardiovascular response of normal humans to endotoxin. J Appl Physiol 1992;73:925-931.

Matsumori A, Yamada T, Suzuki H, Matonba Y, Sasayama S. Increased circulating cytokines in patients with myocarditis and cardiomyopathy. Br Heart J 1994;72:561-566.

Mayer B, Holmer SR, Hengstenberg C, Lieb W, Pfeifer M, Schunkert, H. Functional improvement in heart failure patients treated with beta-blockers is associated with a decline of cytokine levels. Int J Cardiol 2010;103:182-186.

Mayet J, Ariff B, Wasan B, Chapman N, Shahi M, Senior R, Foale RA, Thom SA. Midwall myocardial shortening in athletic left ventricular hypertrophy. Int J Cardiol 2002;86:233-238.

McDonald TE, Grinman Mn, Carthy CM, Walley KR. Endotoxin infusion in rats induces apoptotic and survival pathways in hearts. Am J Physiol 2000;279:H2053-H2056.

Merx MW, Weber C. Sepsis and the heart. Circulation 2007;116:793-802.

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

Mosterd A, Cost B, Hoes AW, de Bruijne MC, Deckers JW, Hofman A, Grobbee DE. The prognosis of heart failure in the general population. The Rotterdam Study. Eur Heart J 2001;22:1318-1327.

Moutsopoulos NM, Madianos PN. Low-grade inflammation in chronic infectious diseases: paradigm of periodontal infections. Ann N Y Acad Sci 2006;1088:251-264.

Muiesan ML, Salvetti M, Monteduro C, Rizzoni D, Corbellini C, Castellano M, Porteri E, Agabiti-Rosei E. Changes in midwall systolic performance and cardiac hypertrophy reduction in hypertensive patients. J Hypertens 2000;18:1651-1656.

Muller-Werdan U, Schumann H, Loppnow H, Fuchs R, Darmer D, Stadler J, Holtz J, Werdan K. Endotoxin and tumour necrosis factor alpha exert a similar proinflammatory effect in neonatal rat cardiomyocytes, but have different cardiodepressant profiles. J Mol Cell Cardiol 1998;30:1027-1036.

Munt B, Jue J, Gin K, Fenwick J, Tweeddale M. Diastolic filling in human severe sepsis: an echocardiographic study. Crit Care Med 1998;26:1829-1833.

Mureddu GF, De Simone G, Greco R, Rosato GF, Contaldo F. Left ventricular filling pattern in uncomplicated obesity. Am J Cardiol 1996;77:509-514.

Neviere R, Fauvel H, Chopin C, Formstecher P, Marchetti P. Caspase inhibition prevents cardiac dysfunction and heart apoptosis in a rat model of sepsis. Am J Resp Crit Care Med 2001;163:218-225.

Nicklas BJ, Cesari M, Penninx BWJH, Kritchevsky SB, Ding J, Newman A, Kitzman DW, Kanaya AM, Pahor M, Harris TB. Abdominal obesity is an independent risk factor for chronic heart failure in older people. J AM Geriatr Soc 2006;54:413-420.

Niebauer J, Volk HD, Kemp M, Dominguez M, Schumann RR, Rauchhaus M, Poole-Wilson PA, Coats AJ, Anker SD. Endotoxin and immune activation in chronic heart failure: a prospective cohort study. Lancet 1999;353:1838-1842.

Niu J, Azfer A, Kolattukudy PE. Protection against Lipopolysaccharide-induced myocardial dysfunction in mice by cardiac-specific expression of soluble Fas. J Mol Cell Cardiol 2008;44:160-169.

Nishikawa Y, Lew WY. Endotoxin-induced left ventricular depression is blocked by nitrogen mustard or dimethylthiourea in rabbits. Am J Physiol 1995;269:H1098-H1105.

Nishikawa K, Mathison J, Lew WY. Serum tumour necrosis factor-α does not mediate endotoxin-induced myocardial depression in rabbits. Am J Physiol 1996;39:H485-H491.

Norton GR, Woodiwiss AJ, Gaasch WH, Mela T, Chung ES, Aurigemma GP, Meyer TE. Heart failure in pressure overload hypertrophy: The relative roles of ventricular remodeling and myocardial dysfunction. J Am Coll Cardiol 2002;39:664-671.

Norton GR, Majane OHI, Libhaber E, Libhaber C, Maseko MJ, Makaula S, Libhaber C, Woodiwiss AJ. The relationship between blood pressure and left ventricular mass index is dependent on an excess adiposity. J Hypertens 2009;27:1873-1883.

Norton GR, Maseko M, Libhaber E, Libhaber CD, Majane OHI, Dessein P, Sareli P, Woodiwiss AJ. Is pre-hypertension an independent predictor of target organ changes in young-to-middle aged persons of African descent? J Hypertens 2008;26:2279-2287.

Nsubuga P, Nwanyanwu O, Nkengasong JN, Mukanga D, Trostle M. Strengthening public health surveillance and response using the health systems strengthening agenda in developing countries. BMC Public Health 2010;10:S5.

Nunez E, Arnett DK, Benjamin EJ, Liebson PR, Skelton TN, Taylor H, Andrew MS. Optimal threshold value for left ventricular hypertrophy in blacks from the Atherosclerosis Risk in Communities study. Hypertension 2005;45:58-63.

O'Brien E, Asmar R, Beilin L, Imai Y, Mallion JM, Mancia G, Mengden T, Myers M, Padfield P, Palatini P, Parati G, Pickering T, Redon J, Staessen J, Stergiou G, Verdecchia P. European Society of Hypertension Working Group on Blood Pressure Monitoring. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. J Hypertens 2003;21:821-848.

Ogden CL, Yanovski SZ, Carroll MD, Flegal KM. The epidemiology of obesity. Gastroenterology 2007;132:2087-2102.

Olubodun JO Lawal SO. Alcohol consumption and heart failure in hypertensives. Int J Cardiol 1996;53**:**81-85.

Osadchii O, Norton G, Woodiwiss A. Inotropic responses to phosphodiesterase inhibitors in cardiac hypertrophy in rats. Eur J Pharmacol 2005;514:201-208.

Osadchii OE, Norton GR, McKechnie R, Defterios D, Woodiwiss AJ. Cardiac dilatation and pump dysfunction without intrinsic myocardial systolic failure following chronic β-adrenoreceptor activation. Am J Physiol 2007;292:246-253.

Parker MM, Shelhamer JH, Bacharach SL, Green MV, Natanson C, Frederick TM, Damske BA, Parrillo JE. Profound but reversible myocardial depression in patients with septic shock. Ann Intern Med 1984;100:483-490.

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

Peng T, Lu X, Feng Q. NADH oxidase signalling induces cyclooxygenase-2 expression during lipopolysacchardie stimulation in cardiomyocytes. FASEB J 2005; 19:293-295.

Peterson LR, Waggoner AD, Schechtmann KB, Meyer T, Gropler RJ, Barzilia B, Davila-Romain VG. Alterations in left ventricular structure and function in young healthy obese women. J Am Coll Cardiol 2004;43:1399-1404.

Piche ME, Lemieux S, Weisnagel SJ, Corneua L, Nadeau A, Bergeron J. Relation of high-sensitivity C-reactive protein, interleukin-6, tumor necrosis factor-alpha, and fibrinogen to abdominal adipose tissue, blood pressure, and cholesterol and triglyceride levels in healthy postmenopausal women. Am J Cardiol 2005;96**:**92-97.

Poelaert J, Declerek C, Vogelaers D, Colardyn F, Visser CA. Left ventricular systolic and diastolic function in septic shock. Intensive care Med 1997;23:553-560.

Prabhu SD. Cytokine-induced modulation of cardiac function. Circ Res 2004;95:1140-1153.

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.

Redfield MM, Jacobsen SJ, Burnett JC, Mahoney DW, Bailey KR, Rodeheffer RJ. Burden of systolic and diastolic ventricular dysfunction in the community: Appreciating the scope of the heart failure epidemic. JAMA 2003;289:194-202.

Reilly JM, Cunnion RE, Burch-Whitman C, Parker MM, Shelhamer JH, Parrillo JE. A circulating myocardial depressant substance is associated with cardiac dysfunction and peripheral hypoperfusion (lactic acidemia) in patients with septic shock. Chest 1989;95:1072-1080.

Relling DP, Esberg LB, Fang CX, Johnson WT, Murphy EJ, Carlson EC, Saari JT and Ren J. High-fat diet induced juvenile obesity leads to cardiomyocyte dysfunction and upregulation of Foxo3a transcription factor independent of lipotoxicity and apoptosis. J Hypertens 2006;24:549-561.

Ren J, Sowers JR, Walsh MF, Brown RA. Reduced contractile response to insulin and IGF-I in ventricular myocytes from genetically obese Zucker rats. Am J Physiol 2000;279:H1708-H1714.

Rider OJ, Francis JM, Ali MK, Peterson SE, Robinson M, Robson MD, Byrne JP, Clarke K, Neubauer S. Beneficial cardiovascular effects of bariatric surgical and dietary weight loss in obesity. J Am Coll Cardiol 2009;54:718-726.

Ridker PM, Buring JE. C-Reactive Protein, the metabolic syndrome and risk of cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation 2003;107:391-397.

Rosengarten B, Hecht M, Wolff S, Kaps M. Autoregulative function in the brain in an endotoxic rat shock model. Inflamm Res 2008;57:542-546.

Roth J, Conn C, Kluger MJ, Zeisberger E. Release of interleukin-6 and tumor necrosis factor during lipopolysaccharide induced fever in guinea pigs. 8th International Symposium Pharmacology of Thermoregulation, Kananaskis, Alberta, Canada. 1991.

Roth J, Hubschle T, Pehl U, Ross G, Gerstberger R. Influence of systemic treatment with cyclooxygenase inhibitors on lipopolysaccharide-induced fever and circulating levels of cytokines and cortisol in guinea pigs. Pflugers Arch 2002;443:411-417.

Roth J, McClellan JL, McClellan JL, Kluger MJ, Zeisberger E. Attenuation of fever and release of cytokines after repeated injections of lipopolysaccharide in guinea-pigs. J Physiol 1994;477:177-185.

Sahn DJ, de Maria A, Kisslo J, Weyman A. The committee on M-mode standardization of the American Society of Echocardiography: Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 1978;58:1072-1083.

Sam F, Duhaney TA, Sato K, Wilson RM, Ohashi K, Sono-Romanelli S, Higuchi A, De Silva DS, Walsh K, Ouchi N. Adiponectin defeiciency, diastolic dysfunction, and diastolic heart failure. Endocrinology 2010;151:322-331.

Sarkar S, Chawla-Sarkar M, Young D, Nishiyama K, Rayborn ME, Hollyfield JG, Sen S. Myocardial cell death and regeneration during progression of cardiac hypertrophy to heart failure. J Biol Chem 2004;279:52630-52642.

Seftel HC. Cardiomyopathies in Johannesburg Bantu. Part I: Aetiology and characteristics of Beriberi heart disease. S Afr Med J 1972;46:1707-1713.

Scaglione R, Dichiara MA, Indovina A, Lipari R, Ganguzza A, Parrinello G, Capuana G, Merlino G, Licata G. Left ventricular diastolic and systolic function in normotensive obese subjects: influence of degree and duration of obesity. Eur Heart J 1992;13:738-742.

Shibata R, Ouchi N, Ito M, Kihara S, Shiojima I, Pimentel DR, Kumada M, Sato K, Schiekofer S, Ohashi K, Funahashi T, Colucci WS, Walsh K.. Adiponectin-mediated modulation of hypertrophic signals in the heart. Nat Med 2004;10:1384-1389.

Skilton MR, Sieveking DP, Harmer JA, Franklin J, Loughnan G, Nakhla S, Sullivan DR, Caterson JD, Celermajer DS. The effects of obesity and non-pharmacological weight loss on vascular and ventricular function and structure. Diabet Obes Metab 2007;10:874-884.

Sliwa K, Skudicky D, Candy G, Wisenbaugh T, Sareli P. Randomised investigation of effects of pentoxifylline on left ventricular performance in idiopathic dilated cardiomyopathy. Lancet 1998;351:1091-1093.

Sliwa K, Wilkinson D, Hansen C, Ntyintyane L, Tibazarwa K, Becker A, Stewart S. Spectrum of heart disease and risk factors in a black urban population in South Africa (the Heart of Soweto Study): a cohort study. Lancet 2008;371:915-922.

Spies C, Farzaneh-Far R, Na B, Kanaya A, Schiller N, Whooley MA. Relations of obesity to heart failure hospitalization and cardiovascular events in persons with stable coronary heart disease (from the Heart and Soul Study). Am J Cardiol 2009;104:883-889.

Stein B, Frank P, Schmitz W, Scholz H, Thoenes M. Endotoxin and cytokines induce direct cardiodepressive effects in mammalian cardiomyocytes via induction of nitric oxide synthase. J Cell Mol Cardiol 1996;28:1631-1639.

Stewart S, MacIntyre K, Hole DJ, Capewell S, McMurray JJ. More 'malignant' than cancer? Five-year survival following a first admission for heart failure. Eur J Heart Fail 2001;3:315-322.

Stewart S, Wilkinson D, Hansen C, Vaghela V, Mvungi R, McMurray J, Sliwa K. Predominance of heart failure in the heart of Soweto study cohort: Emerging challenges for urban African communities. Circulation 2008;118:2360-2367.

Stoddard MF, Tseuda K, Thomas M, Dillon S, Kupersmith J. The influence of obesity on left ventricular filling and systolic function. Am Heart J 1992;124:694-699.

Suffredini, AF, Fromm RE, Parker MM, Brenner M, Kovacs JA, Wesley RA, Parrillo JE. The cardiovascular response of normal humans to the administration of endotoxin. N Engl J Med 1989;321:280-287.

Sutton-Tyrrell K, Newman A, Simonsick EM, Havlik R, Pathor M, Lakatta E, Spurgeon H, Vaitkevicius P. Aortic stiffness is associated with visceral adiposity in older adults enrolled in the study of health, aging, and body composition. Hypertension 2001:38:429-433.

Suzuki J, Bayna E, Molle ED, Lew WY. Nicotine inhibits cardiac apoptosis induced by lipopolysaccharide in rats. J Am Coll Cardiol 2003;41:482-488.

Suzuki J, Bayna E, Li HL, Molle ED, Lew WY. Lipopolysaccharide activates calcineurin in ventricular myocytes. J Am Coll Cardiol 2007;49:491-499.

Tanno M, Gorog DA, Bellahcene M, Cao X, Quinlan RA, Marber MS. Tumor necrosis factor-induced protection of the murine heart is independent of p38-MAPK activation. J Mol Cell Cardiol 2003;35:1523-1527.

Tao S, McKenna TM. In vitro endotoxin exposure induces contractile dysfunction in adult rat cardiac myocytes. Am J Physiol 1994;267:H1745-H1752.

Tavener SA, Long EM, Robbins SM, McRae KM, Van Remmen HV, Kubes P. Immune cell Toll-Like Receptor 4 is required for cardiac myocyte impairment during endotoxemia. Circ Res 2004;95:700-707.

Teichholz LE, Krelen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence of absence of synergy. Am J Cardiol 1976;37:7-11.

Torre-Amione[a] G, Kapadia S, Lee J, Durand JB, Bies RD, Young JB, Mann DL. Tumour necrosis factor and tumour necrosis factor receptors in the failing human heart. Circulation 1996;93:704-711.

Torre-Amione[b] G, Capaslera S, Benedict C, Oral Y, Young JB, Mann DL. Pro-inflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the Studies of Left Ventricular Dysfunction (SOLVD). J Am Coll Cardiol 1996;27:1201-1206.

Torre-Amione G, Stetson SJ, Youker KA, Durand JB, Radovancevic B, Delgado RM, Frazier OH, Entman ML, Noon GP. Decreased expression of TNFα in failing human myocardium after mechanical circulatory support: A potential mechanism of cardiac recovery. Circulation 1999;100:1189-1193.

Tsioufis CP, Tsiachris DL, Selima MN, Diminitriadis KS, Thomopoulos CG, Tsiliggiris DC, Gennadi AS, Syrseloudis DC, Stefanadi ES, Toutouzas KP, Kallikazaros IE, Stefanadis CI. Impact of waist circumference on cardiac phenotypes in hypertensives according to gender. Obesity 2009;17:177-182.

Vasan R, Sullivan LM, Roubenoff R, Dinarello CA, Harris T, Benjamin EJ, Sawyer DB, Levy D, Wilson PWF, D'Agostino RB. Imflammatory markers and risk of heart failure in elderly subjects without prior myocardial infarction: The Framingham Heart Study. Circulation 2003;107:1486-1491.

Veliotes DGA, Woodiwiss AJ, Deftereos D, Gray D, Osadchii O, Norton GR. Aldosterone receptor blockade prevents the transition to cardiac pump dysfunction induced by β-adrenoreceptor activation. Hypertension 2005;45:914-920.

Wiggers CJ. Myocardial depression in shock: a survey of cardiodynamic studies. Am Heart J 1947;33:633-650.

Wilhelmsen L, Rosengren A, Eriksson H, Lappas G. Heart failure in the general population of men: morbidity, risk factors and prognosis. J Intern Med 2001;249:253-261.

Willens HJ, Chakko SC, Byers P, Chirinos JA, Labrador E, Castrillon JC, Lowery MH. Effects of weight loss after gastric bypass on right and left ventricular function assessed by tissue Doppler imaging. Am J Cardiol 2005;95:1521-1524.

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.

Wong CY, Byrne NM, O'Moore-Sullivan T, Hills AP, Prins JB, Marwick TH. Effect of weight loss due to lifestyle intervention on subclinical cardiovascular dysfunction in obesity (body mass index >30 kg/m^2). Am J Cardiol 2006;98:1593-1598.

Woodiwiss AJ, Tsotetsi OJ, Sprott S, Lancaster EJ, Mela T, Chung ES, Meyer TE, Norton GR. A reduction in myocardial collagen cross-linking parallels left ventricular dilatation in rat models of systolic chamber dysfunction. Circulation 2001;103:155-160.

Woodiwiss AJ, Libhaber CD, Majane OHI, Libhaber E, Maseko M, Norton GR. Obesity promotes left ventricular concentric rather than eccentric geometric remodeling and hypertrophy independent of blood pressure. Am J Hypertens 2008;21:1144-1151.

Woodiwiss AJ, Molebatsi N, Maseko MJ, Libhaber E, Libhaber C, Majane OHI, Paicker J, Dessein P, Brooksbank R, Sareli P, Norton GR. Nurse-recorded auscultatory blood pressure at a single visit predicts target organ changes as well as ambulatory blood pressure. J Hypertens 2009;27:287-297.

Yasuda[a] S, Lew WY. Lipopolysaccharide depresses cardiac contractility and β-adrenergic contractile response by decreasing myofilament response to Ca^{2+} in cardiac myocytes. Circ Res 1997;81:1011-1020.

Yasuda[b] S, Lew WY. Endothelin-1 ameliorates contractile depression by lipopolysaccharide in cardiac myocytes. Am J Physiol 1997;273:H1403-H1407.

Yasuda S, Lew WY. Angiotensin II exacerbates lipopolysaccharide-induced contractile depression in rabbit cardiac myocytes. Am J Physiol 1999;276:H1442-H1449.

Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 1999;19**:**972-978.

Zarich SW, Kowalchuk GJ, McGuire MP, Benotti PN, Mascioli EA, Nesto RW. Left ventricular filling abnormalities in asymptomatic morbid obesity. Am J Cardiol 1991;68:377-381.

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. Proc Nat Acad Sci 2000;97:1784-1789.

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Woodiwiss/Norton

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER MO70469**
PROJECT	Gene Candidates As Determinants of Blood Pressure and Intermediary Phenotypes in Pathogenesis of Hypertension in Black S Africans
INVESTIGATORS	Profs A/G Woodiwiss/Norton
DEPARTMENT	School of Physiology
DATE CONSIDERED	07.05.09
DECISION OF THE COMMITTEE*	Approved unconditionally (refer M020472)

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 07.05.09 **CHAIRPERSON** .....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Woodiwiss A Prof

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Woodiwiss/Norton et al

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M02-04-72

PROJECT Gene Candidates As Determinants of Blood Pressure And Intermediary Phenotypes In Pathogenesis of Hypertension In Black South Africans

INVESTIGATORS Prof's AJ/G et al Woodiwiss/Norton et al

DEPARTMENT School of Physiology, Wits Medical School

DATE CONSIDERED 02-04-26

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON-JONES
HREC (MEDICAL)
2007 -05- 09
JOHANNESBURG

This clearance is valid and within the 5-year validity

DECISION OF THE COMMITTEE *

Approved unconditionally

DATE 02-05-14 **CHAIRMAN** .....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Prof AJ Woodiwiss
Dept of School of Physiology, Wits Medical School

Works2\lain0015\HumEth97.wdb\M 02-04-72

==========

DECLARATION OF INVESTIGATOR(S).

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

AESC 3

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2007/43/03

APPLICANT: Ms N Janse Van Rensburg

SCHOOL: Physiology
DEPARTMENT:
LOCATION: Medical School

PROJECT TITLE: The impact of tumour necrosis alpha on adrenergenic-induced cardiomyocyte apopotosis in rats.

Number and Species

Approval was given for to the use of animals for the project described above at an AESC meeting held on 20070529. This approval remains valid until 20070529.

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and to the following additional conditions:

Pilot only approved

Signed: _____ Date: 26:6:06.
(Chairperson, AESC)

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ Date: 26/06/07
(Registered Veterinarian)

cc: Supervisor: #
Director: CAS

Works 2000\lain0015\AESCCert.wps