A Proposed Pathophysiological Role for TNFα in Obesity Induced Cardiac Hypertrophy

Maryam Rostami

Thesis presented in partial fulfillment of the requirements for the degree of Master of Physiology at the University of Stellenbosch

Project Leader: Dr EF Du Toit

Project co-Leader: Dr J van Rooyen

Declaration

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

SUMMARY

Background: Cardiac hypertrophy is an adaptive process occurring in response to mechanical overload or tissue injury. The stimuli for cardiac hypertrophy are diverse and vary from increased afterload on the heart to cardiac remodeling in response to cytokines. Amongst others, obesity is characterized by excessive body weight resulting in metabolic disorders. This excess body weight necessitates an increased blood and oxygen delivery to the peripheral tissues, which is achieved by an elevated cardiac output. Total blood volume is also increased in the obese due to the increased tissue volume and vascularity. With time, the obesity induced increase in cardiac preload results in left ventricular hypertrophy and dilatation. Obesity is also associated with complications such as hypertension, insulin resistance and impaired glucose metabolism.

In addition, adipose tissue has been implicated to contribute to elevated circulating TNF_{α} levels in obesity and may contribute to the pathophysiology of the heart in obese individuals. The heart is a major cytokine-producing organ that generates amongst others tumor necrosis factor $_{\alpha}$ (TNF_{α}). TNF_{α} is a proinflammatory cytokine, which acts to increase its own production, has cytotoxic and cytostatic effects on certain tumor cells and influences growth and differentiation in virtually all cell types including cardiomyocytes. Elevated levels of TNF_{α} are detected peripherally in almost all forms of cardiac injury and in hypertrophic cardiomyopathy. These elevations are proposed to be deleterious to the heart, although an adaptive role for low levels of TNF_{α} has been proposed.

Aim: The aim of the study was to determine whether there is a correlation between obesity and serum, myocardial, and adipose tissue TNF_{α} levels and cardiac hypertrophy. We also wished to determine whether the hearts from the obese animals functioned normally under normoxic conditions and whether they responded differently to ischaemia/reperfusion when compared with their concurrent controls.

Materials and Methods: Male Sprague-Dawley rats (n=100) were fed a high caloric diet (HCD) containing 33% rat chow, 33% condensed milk, 7% sucrose and 27% water, or standard laboratory rat chow for 6-12 weeks. Food consumption, body weight gain, heart weight and tibia length were measured. Serum glucose, insulin and lipid levels were also determined. Hearts were excised and perfused on the isolated Working Heart perfusion apparatus and cardiac function was monitored and documented. Hearts were then subjected to 15 minutes of total global ischaemia at 37^0C, and reperfused for 30 minutes. Cardiac function was again documented.

A separate series of hearts were freeze-clamped at different time points during the experimental protocol and stored in liquid nitrogen for the determination of myocardial TNF_{α} and cGMP levels. Serum TNF_{α} levels were determined after 12 weeks on the high caloric or normal/control diet. After 12 weeks on the diet myocardial TNF_{α} levels of the HCD fed animals and their concurrent controls were determined before and during ischaemia. Adipose tissue and myocardial tissue TNF_{α} levels were also determined after 6, 9 and 12 weeks on the respective diets. Myocardial cGMP levels were measured in the HCD fed rats and the control rats after 6, 9, and 12 weeks. These data were used as an indirect index to determine whether the myocardial NO-cGMP pathway was activated in the normoxic hearts on the respective diets.

Results: The body weight of the HCD fed animals was significantly higher compared with their respective controls after 12 weeks on the diet (459.9 $\pm$ 173.8 g and 271.5 $\pm$ 102.6 g respectively ($p<0.05$)). The HCD fed animals also had heart weight to body weight ratios that were significantly greater compared with the controls (4.2 $\pm$ 0.1 mg/g and 3.7 $\pm$ 0.1 mg/g respectively ($p<0.05$)).

The plasma glucose levels of the HCD fed animals were higher than their respective controls (9.2 $\pm$ 0.3 mmol/l and 7.8 $\pm$ 0.3 mmol/l respectively ($p<0.05$)), but their insulin levels were similar (12.87 $\pm$ 1.02 μIU/ml and 12.42 $\pm$ 5.06 μIU/ml). Plasma lipid profiles (plasma cholesterol, high density lipoprotein (HDL) cholesterol and plasma triacylglyceride (TAG)) were abnormal in the HCD fed animals compared with the control rats. Plasma TAG levels in the HCD fed animals were significantly higher compared with the control rats (0.664 $\pm$ 0.062 mmol/l and 0.503 $\pm$ 0.043 ($p<0.05$)), while plasma cholesterol levels (1.794 $\pm$ 0.058 mmol/l and 2.082 $\pm$ 0.062 mmol/l ($p<0.05$)) and HDL cholesterol levels were significantly lower (1.207 $\pm$ 0.031 mmol/l and 1.451 $\pm$ 0.050 mmol/l ($p<0.05$)).

Cardiac mechanical function was similar for both groups before ischaemia, but the percentage aortic output recovery was lower for the hearts from the HCD fed animals when compared with their controls (47.86 $\pm$ 7.87% and 66.67 $\pm$ 3.76 % respectively ($p<0.05$)).

Serum TNF_α levels of the HCD fed animals were higher compared with the control animals (51.04 $\pm$ 5.14 AU and 31.46 $\pm$ 3.72 AU respectively ($p<0.05$)), but myocardial TNF_α levels remained lower in these animals (312.0 $\pm$ 44.7 pg/gram ww and 571.4 $\pm$

132.9 pg/gram ww respectively ($p<0.05$)). During ischaemia these myocardial TNF_α levels increased above those of the controls (442.9 $\pm$ 12.4 pg/gram ww and 410.0 $\pm$ 12.5 pg/gram ww respectively ($p<0.05$)). The adipose tissue TNF_α levels were significantly increased after 12 weeks on the high caloric diet compared with the control animals (4.4 $\pm$ 0.4 pg/gram ww and 2.5 $\pm$ 0.3 pg/gram ww respectively ($p<0.05$)). There was no significant difference in the myocardial cGMP levels of the HCD rats compared with the conrol rats after 6, 9 and 12 weeks.

Conclusion: 1) The high caloric diet induced obesity, which lead to cardiac hypertrophy in this study. 2) There was a strong correlation between elevated adipose tissue and serum TNF_α levels, and cardiac hypertrophy. 3) Elevated serum TNF_α levels did not lead to activation of the myocardial NO-cGMP pathway in the normoxic hearts in this model. 4) The hypertrophied hearts from the HCD fed animals had poorer post-ischaemic myocardial functions than their concurrent controls.

OPSOMMING

Agtergrond: Miokardiale hipertrofie is 'n aanpassing wat gebeur as 'n gevolg van meganiese oorbelading of weefsel beskadiging. Verskillende stimuli kan tot miokardiale hipertrofie aanleiding gee soos byvoorbeeld 'n verhoging in nalading, of miokardiale hermodellering in respons op sitokiene. Verhoging van voorbelading in vetsug mag ook tot hipertrofie aanleiding gee. Vetsug word gekenmerk deur 'n oormatige liggaamsmassa wat tot metaboliese versteurings lei. Die oormatige liggaamsmassa vereis 'n verhoging in bloed- en suurstofverskaffing aan die perifere weefsel wat deur 'n verhoging in die kardiale uitset vermag kan word. Die bloed volume van 'n vetsugtige individu word ook verhoog as gevolg van 'n verhoging in weefselvolume en vaskulariteit en met verloop van tyd induseer die verhoogde kardiale voorbelading linker ventrikulêre hipertrofie en dilatasie. Vetsug word ook met verskeie ander siekte toestande soos hipertensie, insulien weerstandigheid en versteurde glukose metabolisme, geassosieer.

Vetweefsel dra ook by tot verhoging van tumor nekrose faktor alfa (TNF_{α}) vlakke in die bloed, wat op sy beurt tot miokardiale hipertrofie mag bydra. TNF_{α} is 'n pro-inflammatoriese sitokien wat sy eie produksie kan stimuleer. Dit het ook sitotoksiese en sitostatiese effekte op sekere tumor selle en kan groei en differensiasie in bykans alle seltipes, insluitende kardiomiosiete, stimuleer. Die hart kan ook TNF_{α} produseer en verhoogde TNF_{α} vlakke word feitlik in alle vorms van miokardiale besering en hipertrofiese kardiomiopatie waargeneem. Daar word voorgestel dat verhoogde TNF_{α} vlakke vir die hart nadelig is, ten spyte van die vermoeding dat die sitokien 'n potensiële aanpassings rol by laer vlakke het.

Doelstelling: Die doel van hierdie studie was om vas te stel of daar 'n verband tussen vetsug en serum, miokardiale en vetweefsel TNF_{α} vlakke en miokardiale hipertrofie, bestaan. Ons het ook gepoog om te bepaal of harte van vetsugtige diere normaal funksioneer en of die response van sulke harte op isgemie-herperfusie van die van ooreenstemmende kontroles verskil.

Materiaal en tegnieke: Manlike Sprague-Dawley rotte (n=100) is vir 6-12 weke op 'n hoë kalorie dieët (HKD) geplaas. Die HKD het uit 33% rotkos, 33% gekondenseerde melk, 7% sukrose en 27% water bestaan. Kontrole diere het standaard laboratorium rotkos ontvang. Voedselinname, liggaamsmassa toename, serum insulien, glukose en lipied vlakke is ook bepaal. Harte is geïsoleer en geperfuseer volgens die Werk Hart perfusie metode en hart funksie is gemonitor en gedokumenteer. Harte is vervolgens aan 15 minute globale isgemie by 37°C blootgestel en daarna weer vir 30 minute geherperfuseer waartydens hartfunksie weer gedokumenteer is. 'n Aparte groep harte is op spesifieke tydsintervalle gedurende die eksperimentele protokol gevriesklamp en in vloeibare stikstof gestoor vir die bepaling van miokardiale TNF_{α} en sGMP vlakke.

Serum TNF_{α} vlakke is bepaal na 12 weke op die dieët. Na die diere 12 weke op die HKD was, is hierdie diere en hul ooreenstemmende kontroles se miokardiale TNF_{α} vlakke voor en na isgemie bepaal. Vetweefsel en miokardiale TNF_{α} vlakke is ook onderskeidelik na 6, 9 en 12 weke bepaal. Miokardiale sGMP vlakke is in die HKD diere en in die kontrole diere na 6, 9 en 12 weke bepaal. sGMP vlakke is gebruik as 'n indirekte indeks van aktivering van die miokardiale NO-sGMP boodskapper pad.

Resultate: Na 12 weke op die dieët was die liggaamsmassa van die HKD diere beduidend hoër in vergeleke met hul ooreenstemmende kontroles (459.9 $\pm$ 173.8 g en 271.5 $\pm$ 102.6 g ($p<0.05$)). Die HKD diere se hart massa tot liggaam massa verhouding was ook beduidend hoër in vergelyking met die van kontroles (4.2 $\pm$ 0.1 mg/g en 3.7 $\pm$ 0.1 mg/g ($p<0.05$)).

Alhoewel insulien vlakke dieselfde was (12.42 $\pm$ 5.06 μIU/ml en 12.87 $\pm$ 1.02 μIU/ml), was serum glukose vlakke van die HKD diere hoër as die van die ooreenstemmende kontroles (9.2 $\pm$ 0.3 mmol/l en 7.8 $\pm$ 0.3 mmol/l ($p<0.05$)). Plasma lipied profiele (HDL cholesterol, plasma cholesterol en trigliseriede) was abnormaal in die HKD diere. Plasma TAG vlakke in die HKD diere was beduidend hoër as die van die kontroles (0.664 $\pm$ 0.062 mmol/l en 0.503 $\pm$ 0.043 ($p<0.05$)), terwyl plasma cholesterol vlakke (1.794 $\pm$ 0.058 mmol/l en 2.082 $\pm$ 0.062 mmol/l ($p<0.05$)) en HDL cholesterol vlakke beduidend laer was (1.207 $\pm$ 0.031 mmol/l en 1.451 $\pm$ 0.050 mmol/l ($p<0.05$)).

Miokardiale meganiese funksie was dieselfde vir beide groepe voor isgemie, maar die persentasie aorta omset herstel tydens herperfusie was laer in die HKD diere in vergelyking met die van kontrole diere (47.86 $\pm$. 7.87% en 66.67 $\pm$ 3.76% ($p<0.05$)).

Serum TNF_α vlakke van die HKD diere was beduidend hoër as die van kontrole diere (51.04 $\pm$ 5.14 AU en 31.46 $\pm$ 3.72 AU ($p<0.05$)), maar miokardiale TNF_α vlakke was laer (312.0 $\pm$ 44.7 pg/gram nat gewig en 571.4 $\pm$ 132.9 pg/gram nat gewig ($p<0.05$)). Die vetweefsel TNF_α vlakke was ook beduidend verhoog na 12 weke op 'n hoë kalorie dieët wanneer dit vergelyk word met die van kontrole diere (4.4 $\pm$ 0.4 pg/gram nat gewig en 2.5 $\pm$ 0.3 pg/gram nat gewig respektiewelik ($p<0.05$)). Daar was

geenbeduidende verskille in die miocardiale vlakke van sGMP in die HKD diere in vergelyking met die kontroles na 6, 9 en 12 weke.

Gevolgtrekkings: 1) 'n Hoë kalorie dieët het in dié studie vetsug geïnduseer en tot miokardiale hipertrofie gelei. 2) Daar was 'n positiewe korrelasie tussen verhoogde vetweefsel en serum TNF_{α} vlakke, en miokardiale hipertrofie. 3) Verhoogde serum TNF_{α} vlakke het nie tot die aktivering van die miokardiale NO-sGMP pad in hierdie model gelei nie. 4) Die hipertrofiese harte het tydens herperfusie ná isgemie swakker as hul ooreenstemmende kontroles gefunksioneer.

ACKNOWLEDGMENTS

I would like to express my gratitude to my supervisor, Dr Eugene F (Jos) du Toit for all his patience, guidance, assistance and motivation throughout this study.

I wish to thank Dr Barbara Huisamen and Prof Amanda Lochner for welcoming me into the department at Tygerberg Medical Campus.

For financial support I would like to thank the National Research Foundation.

I wish to thank Human for always being there for me when I needed him most, and without whose motivation this would not have been possible. And finally, in the memory of my parents, who are always with me.

ABBREVIATIONS

Units of measurement

%	percentage
µl	microliter
µIU	micro International Units
^{0}C	degrees Celsius
cm	centimeters
g	gram
gram ww	gram wet weight
M	molar
mg	milligram
min	minutes
mm	millimeter
mmol	millimole
mol	mole
mW	milliwatts
pg	picogram
pmol	picomol
v	volume
w	weight

Chemical Compounds

Ca^{2+}	calcium
$CaCl_2.2H_20$	calciumchloride 2-hydrate
CO_2	carbondioxide

H_2O	water
K^+	potassium
KCl	potassium chloride
KH_2PO_4	potassium dihydrogenphosphate
$MgSO_4.7H_2O$	magnesiumsulphate 7-hydrate
NaCl	sodium chloride
$NaHCO_3$	sodium hydrogen carbonate
O_2	Oxygen
TRIS	tris(hydroxymethyl) aminomethane hydrochloride

Other abbreviations

Ab	antibody
AC	adenylyl cyclase
ACE	angiotensin converting enzyme
ADP	aorta diastolic pressure
ANG II	angiotensin II
ANP	atrial natriuretic peptide
AO	aortic output
AP-1	activating protein-1
ASK1	apoptotic signal-regulated kinase 1
ASP	aorta systolic pressure
ATP	adenosine triphosphate
AT-TNF	adipose tissue-derived TNF
AU	arbitrary units
BP	blood pressure
cAMP	cyclic 3',5'-adenosine monophosphate

CAM	calmodulin
CF	coronary flow
cGMP	cyclic 3',5'-guanosine monophosphate
CHF	congestive heart failure
CN	calcineurin
CoA	coenzyme-A
CT-1	cardiotrophin-1
DAG	diacylglycerol
DIO	diet induced obesity
DNA	deoxyribonucleic acid
ECM	extracellular matrix
ELISA	enzyme-linked immunosorbent assay
eNOS	endothelial NOS
ERK	extracellular-regulated kinases
ET-1	endothelin-1
FAD	flavine adenine nucleotide
FADD	Fas-associated death domain
FAK	focal adhesion kinase
FFA	free fatty acids
FMN	flavin mononucleotide
FRAP	FKBP-12-rapamycin associated protein
GDP	guanosine diphosphate
gp130	glycoprotein 130
G-protein	guanine nucleotide-binding proteins
GTP	guanosine triphosphate
HCD	high caloric diet

HDL	high density lipoprotein
HR	heart rate
I-κB	inhibitory-κB
IE genes	immediate early genes
IL-β	interleukin-beta
IL-6	interleukin-6
INF$_\gamma$	interferon-gamma
iNOS	inducible nitric oxide synthase
IP3	inositol-triphosphate
JAK	janus-associated kinases
JNK	c-Jun N-terminal protein kinases
LDL	low density lipoprotein
L-NAME	N^G-nitro-L-arginine methyl ester
LPL	lipoprotein lipase
MADD	mitogen-activated protein kinase-activating death domain
MAPK	mitogen-activated protein kinases
MAPKAP	MAPK-associated protein kinase
MEK	MAPK/ERK
MEK	MEK kinases
MHC	myosin heavy chain
MLC	myosin light chain
Mort 1	mediator of receptor induced toxicity (alternative for FADD)
mRNA	messenger ribonucleic acid
NADPH	nicotinamide adenine dinucleotide phosphate-reduced form
N	Normal
NEFA	non-esterified fatty acids

NF/AT	nuclear factor of activated T-lymphocytes
NF-$_K$B	nuclear factor-kappa B
NIK	NF-$_K$B inducing kinase
nNOS	neural NOS
NO	nitric oxide
NOS	nitric oxide synthase
PDE	phosphodiesterase
PIP2	phosphatidylinositol-bisphosphate
PKA	cAMP dependent protein kinase activity
PKC	protein kinase C
PKG	cGMP dependent protein kinase
PLA	phospholipase A_2
PLB	phospholamban
PLC	phospholipase C
PLD	phospholipase D
RIP	receptor interacting protein
RNA	ribonucleic acid
ROI	reactive oxygen intermediates
ROS	reactive oxygen species
SAC	stretch activated ion channels
SAPK	stress activated protein kinases
SEM	standard error of the mean
SERCA	sarcoplasmic reticulum Ca^{2+} ATPase
sGC	soluble guanylyl cyclase
SHR	spontaneously hypertensive rats
SMase	sphingomyelinase

STAT	signal transducers and activators of transcription
sTNF-R	soluble TNF-receptors
TAG	triacylglyceride
TGF-β	transforming growth factor-beta
THB4	tetrahydrobiopterin
TNF-α	tumor necrosis factor-alpha
TNF-R	TNF-receptors
TRADD	TNF receptor-associated death domain
TRAF-1	TNF receptor-associated factor 1
TRAF-2	TNF receptor-associated factor 2
VEGF	vascular endothelial growth factor
Wt	total work

LIST OF FIGURES

Chapter 2

Chapter 4

LIST OF TABLES

Chapter 1

INTRODUCTION

Cardiac hypertrophy is an adaptive process that occurs in response to mechanical overload or tissue injury. It is characterized by increased myocyte size, proliferation of cardiac fibroblasts and progression of interstitial and perivascular fibrosis. The stimuli for cardiac hypertrophy are diverse and vary from increased afterload on the heart to cardiac remodeling in response to cytokines (Chiariello and Perrone-Filardi, 1999).

Pressure overload induces pathological hypertrophy with impairment in systolic function, myocardial relaxation and an increase in diastolic stiffness (Lecarpentier *et al.*, 1987; Doering *et al.*, 1988). The diastolic stiffness is accounted for by an increase in interstitial collagen (Doering *et al.*, 1988; Brilla *et al.*, 1991). These changes also represent an initial step in the pathogenesis of many cardiac diseases that ultimately progress to ventricular failure. The mechanisms by which this condition of cardiac hypertrophy eventually progresses to heart failure are not fully understood.

Hypertrophied hearts are more vulnerable to brief periods of ischaemia because of an impaired reactive hyperaemic response, which results in a delayed metabolic recovery (McAinsh *et al.*, 1998). These abnormalities may contribute to the increased morbidity that is associated with cardiac hypertrophy (McAinsh *et al.*, 1998).

Hypertrophy has been conceptualized as possibly having two components, mediated by cardiac myocytes (MC) and nonmyocytes (NMC) (Kuwahara *et al.*, 1999). Interaction between MC's and surrounding NMC's is likely to be an important

component of the hypertrophic process (Weber and Brilla, 1991). Many growth factors and cytokines acting as autocrine/paracrine factors are probably involved in the response and cross talk between MC and NMC. These routes appear to play an important role in the pathophysiology of cardiac hypertrophy.

Obesity has been linked to left ventricular hypertrophy and is a metabolic disorder in which multiple clinical and biochemical alterations coexist. Abnormal carbohydrate and lipid profiles are features that are usually present in the obese individual. They usually have elevated triglycerides, total cholesterol, and low-density lipoprotein cholesterol levels and reduced high-density lipoprotein cholesterol concentrations (Kannel *et al.*, 1971; LaRosa *et al.*, 1990). Due to the excessive weight associated with obesity, the overall metabolic demands are elevated, and in order to deliver more oxygen to the peripheral tissue, the cardiac output is increased. In this case increased stroke volume accounts for the increased cardiac output. Blood volume is also increased, due to increased tissue volume and vascularity. With time, the obesity induced increase in preload on the heart results in left ventricular hypertrophy and dilatation (Paulson and Tahiliani, 1992).

Hypertrophic obesity (maturity-onset obesity) is the predominant type of obesity present in man, and is associated with complications such as hypertension, insulin resistance and impaired glucose metabolism (Paulson and Tahiliani, 1992). It is difficult to implicate obesity directly to cardiovascular disease, because obesity usually coexists with a number of secondary cardiovascular risk factors such as the diabetes, hypercholesterolemia and hypertension just mentioned.

Recent research findings have shown that serum TNF_{α} levels are elevated in obese

individuals and it has been proposed that these cytokines may play a role in cardiac remodeling. The heart is a major cytokine-producing organ that generates TNF_{α}. Both myocardial macrophages and cardiac myocytes are known to synthesize this proinflammatory cytokine, which acts to increase its own production and the synthesis of small inflammatory mediators such as platelet activating factor, eicosanoides and oxidative radicals. TNF_{α} has cytostatic and cytotoxic effects on certain tumor cells, influencing growth and differentiation in virtually every cell type, including cardiomyocytes. TNF_{α} is also an endogenous pyrogen that stimulates the production of other endogenous pyrogens, such as interleukin-β (IL-β) (Meldrum, 1998). Adipose tissue is another strong source of TNF_{α} and has been implicated in the elevation of circulating TNF_{α} levels and endothelial dysfunction (Yudkin *et al.*, 1999).

Elevated levels of TNF_{α} are detected peripherally in almost all forms of cardiac injury as well as hypertrophic cardiomyopathy (Matsumori *et al.*, 1994). Elevated myocardial TNF_{α} levels are deleterious to the heart, although a beneficial and adaptive role has also been proposed (Nakano *et al.*, 1998). The short-term expression of myocardial TNF_{α}, and possibly other cytokines such as IL-β and cardiotrophin-1 (CT-1) may provide the heart with additional homeostatic responses to environmental stress. These may include hypertrophic growth, increased regional myocardial blood flow and increased resistance to ischaemia-induced arrythmias through the generation of nitric oxide (Yokoyama *et al.*, 1997).

It may be possible that elevations in serum TNF_{α} levels induces iNOS and leads to decreased mechanical function of the heart of obese individuals. iNOS is the cytokine inducible isoform of nitric oxide synthase (NOS), which is expressed by a number of

paranchymal cells within the myocardium, including the endothelium of the coronary microvasculature and the myocardium. Infiltrating inflammatory cells are all able to express iNOS in response to soluble inflammatory mediators including specific cytokines and bacterial cell wall components (Balligand *et al.*, 1993; Balligand *et al.*, 1995). iNOS induction has been demonstrated to diminish both basal and catecholamine enhanced chronotropic and inotropic function in isolated myocytes and in the intact heart (Ungureanu-Longrois *et al.*, 1995). The mechanisms by which NO mediates these effects include increased activation of soluble guanylyl cyclase (sGC) causing elevated cGMP levels (Kinugawa *et al.*, 1997), inhibition of electron transport by mitochondrial respiratory chain, and the production of oxidants, such as peroxynitrite (Beckman and Koppenol 1996). At low physiological concentrations NO may protect the myocytes from harmful stimuli such as mechanical stress or norepinephrine. At higher physiological levels it may not only decrease function, but also cause the loss of myocytes due to apotosis (Sawyer and Colucci, 1998).

It remains controversial whether cardiac hypertrophy results solely from the blood volume overload associated with obesity. Since cytokines are thought to be involved in cardiac remodeling and play a role in certain forms of cardiac hypertrophy, we wished to determine whether these paracrine/autocrine factors, and in particular TNF_{α}, may be involved in stimulating cardiac hypertrophy in obesity. This is particularly important, as obesity has been associated with chronic elevations in serum TNF_{α} levels (Yudkin *et al.*, 1999). This study was therefore designed to investigate whether there is a possible link between myocardial TNF_{α} levels, adipose tissue TNF_{α} levels, serum TNF_{α} levels, and myocardial hypertrophy. We also wished to investigate whether the elevated TNF_{α} levels in the obese animals had direct effects on cardiac function and the severity of ischaemia/reperfusion injury.

Chapter 2

LITERATURE REVIEW

2.1 Cardiac Hypertrophy

Cardiac hypertrophy is a fundamental process of adaptation to an increased workload due to hemodynamic overload, such as increased blood pressure and/or blood volume (Cooper, 1987; Mondry and Swynghedauw, 1995). Myocardial hypertrophy is defined as an increased myocardial mass beyond the normal range. The upper heart weight limit for men is 450 grams while that for women is 400 grams. The development of cardiac hypertrophy is initially compensatory and therefore beneficial. This period occurs when the work-induced growth of the heart compensates for the increased workload per heart mass ratio. Gross mechanical function is normal, but sensitive tests shows a decreased rate of shortening velocity, delayed relaxation, and a diminished coronary vascular reserve (Meerson, 1983). Hypertrophy augments the number of contractile units and reduces ventricular wall stress to normal levels, following the law of Laplace, whereby an increased wall thickness decreases wall stress. Many of the key proteins involved in cardiac function undergo changes of qualitative and quantitative nature in response to hemodynamic overload (Lompre *et al.*, 1991). Therefore the adult heart adapts to a superimposed environmental stress by three interrelated and integrated mechanisms, namely cardiac hypertrophy, cardiac remodeling and cardiac repair (Mann, 1996). Compensated hypertrophy often progresses to decompensated cardiac hypertrophy. This condition occurs when the work output per unit of cardiac mass decreases. This is usually due to the progressively decreasing ability of the heart to fill normally and to generate force (Meerson, 1983).

The hypertrophy of cardiomyocytes is initiated by endocrine, paracrine, and autocrine factors that stimulate a wide array of membrane bound receptors. Their activation results in the triggering of multiple cytoplasmic signal transduction cascades, which affects nuclear factors and the regulation of gene expression.

The functional changes during the development of cardiac hypertrophy include impairment in systolic function, myocardial relaxation and an increase in diastolic stiffness (Lecarpentier *et al.*, 1987; Doering *et al.*, 1988). Abnormalities in diastolic relaxation and systolic function are thought to be due to the down-regulation of intracellular calcium handling proteins such as the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) and phospholamban (PLB). SERCA fascilitates the transport of Ca^{2+} into the sarcoplasmic reticulum, while the phosphorylation of PLB increases the affinity of SERCA for Ca^{2+}. Besides changes in the status of functional proteins an increase in interstitial collagen and other structural proteins has also been shown to account for the increase in diastolic stiffness in cardiac hypertrophy (Doering *et al.*, 1988; Brilla *et al.*, 1991).

During the development of cardiac hypertrophy specific changes have been observed in cardiomyocytes, namely rapid induction of proto-oncogenes and heat shock protein genes, quantitative and qualitative changes in gene expression, and increased rate of protein synthesis. The first response to hemodynamic overload is the induction of proto-oncogenes such as c-fos, c-jun and c-myc, and heat shock protein gene hsp70, collectively called immediate-early (IE) genes (Mulvagh *et al.*, 1987; Komuro *et al.*, 1988; Izumo *et al.*, 1988).

With hypertrophy the expression of several genes that encode sarcomeric proteins are switched to expression of fetal isoforms, such as the transition from cardiac α-actin to skeletal α-actin and from the α-form of myosin heavy chain (MHC) to the β-MHC form in rodents (Schwartz *et al.*, 1986). In addition several shifts in isogene expression of proteins involved in energy metabolism have been described (Revis *et al.*, 1977; Meerson and Javich, 1982). Atrial natriuretic peptide (ANP), which is restricted to the atria until shortly after birth, is also re-expressed in the ventricle upon hemodynamic overload (Izumo *et al.*, 1988).

2.1.1 Stimuli causing cardiac hypertrophy

Mechanical stress and neural or humoral factors

The primary stimuli for cardiac hypertrophy are mechanical stress and/or an accompanying increase in neural or humoral factors. Yet cardiac hypertrophy can be induced even after adrenoreceptor blockade (humoral) or sympathectomy (neural) (Cooper *et al.*, 1985). Thus mechanical stress by itself induces cardiac hypertrophy in response to hemodynamic overload. This view is supported by several studies done *in vivo* and *in vitro*. In the isolated heart, increased cardiac load stimulated protein synthesis (Kira *et al.*, 1984) and stretching cultured cardiac myocytes stimulated protein synthesis and induced alterations in gene expression without involvement of humoral factors (Mann *et al.*, 1989; Komuro *et al.*, 1990; Sadoshima *et al.*, 1992; Kira *et al.*, 1994; Vandenburgh *et al.*, 1995).

Other stimuli such as growth factors and hormones have been implicated in the induction of cardiac hypertrophy. The expression or release of these factors have been reported in hearts that are hypertrophied due to hemodynamic overload and in cardiomyocytes that are hypertrophied due to stretch (Ruwhof and Van der Laarse,

2000). These factors include endothelin-1 (ET-1) (Arai *et al.*, 1995) angiotensin II (ANG II) (Sadoshima and Izumo, 1993; Miyata *et al.*, 1996; Tamura *et al.*, 1998), transforming growth factor-β (TGF-β) (Villareal and Dillman, 1992; Calderone *et al.*, 1995), insulin-like growth factor-1 (IGF-1) (Calderone *et al.*, 1995), myotrophin (Sen *et al.*, 1990) and vascular endothelial growth factor (VEGF) (Li *et al.*, 1997a; Seko *et al.*, 1999). Cardiac myocytes, cardiac fibroblasts, endothelial cells and vascular smooth muscle cells, all possibly secrete growth promoting factors after a mechanical stress stimulus, which induce hypertrophy of cardiomyocytes in an autocrine/paracrine fashion (Ruwhof and Van der Laarse 2000).

There are mechanosensors such as integrins and the cytoskeleton, and sarcolemmal proteins that are also implicated in cardiac hypertrophy. Integrins are a family of cell-surface receptors that link the extracellular matrix (ECM) to the cellular cytoskeleton at sites called focal adhesion sites (Hynes, 1992; Juliano and Haskill, 1993; Schwartz *et al.*, 1995).

Integrins are composed of α and β subunit heterodimers that consist of a large extracellular domain, a transmembrane region, and usually a short cytoplasmic domain. The extracellular domain binds to proteins of the ECM or to counter-receptors on other cells, whereas the cytoplasmic domain forms links with cytoskeletal proteins and intracellular signaling molecules such as α-actinin and focal adhesion kinase (FAK) (Hynes, 1992; Lewis and Schwartz, 1995). Integrins can act as mechanotransducers in cardiac cells (MacKenna *et al.*, 1998), since cardiac fibroblasts caused activation of two signal transduction pathways, the ERK and JNK pathway once the fibroblasts were stretched in an integrin (β1)-dependent manner.

Studies have confirmed the influence of integrins in the hypertrophic response in cardiomyocytes (Ross *et al.*, 1998).

Mechanical stress may also cause deformation of the sarcolemma, which may directly or indirectly induce conformational changes in proteins that are anchored to the inner surface of the cell membranes, or in transmembrane proteins. Sarcolemmal proteins include several effector enzymes such as phospholipases and protein kinase C (PKC) isoenzymes, ion channels such as stretch-activated channel (SAC), or ion exchangers such as the Na^+/H^+ exchanger (Ruwhof and Van der Laarse, 2000).

Protein kinase C and cardiac hypertrophy

Phospholipases are enzymes that catalyze the breakdown of plasma membrane phospholipids, thereby generating second messenger molecules. Activated PLC can hydrolyze phosphatidylinositol-bisphophate (PIP2) into inositol-triphosphate (IP3) and diacylglycerol (DAG). DAG is a second messenger that causes the translocation of PKC isoenzymes from the cytosol to a membrane fraction, thereby activating them (Newton, 1995). Activation of PKC may then reduce the action of PLC and stimulate that of PLD (Nishizuka, 1992). Experimental evidence suggests that the activation of PLC and/or PLD may play a role in mechanical stress-induced cardiac hypertrophy (Von Harsdorf *et al.*, 1989; Sadoshima and Izumo, 1993; Dassouli *et al.*, 1993).

Protein kinase C (PKC) is a serine/ threonine protein kinase. The PKC family consists of several isoenzymes that differ in distribution, regulation, and enzyme activity. The isoenzymes are classified into three subclasses, namely the classical PKCs which are regulated by DAG, phosphatidylserine, and Ca^{2+}; novel PKCs which are regulated by DAG and phosphatidylserine, but not Ca^{2+}, and finally the atypical PKCs

whose regulation has to be defined although DAG and Ca^{2+} appear not to be involved (Puceat and Vassort, 1996; Newton, 1997). Activation of PKC in cardiomyocytes has been found to stimulate the expression of c-fos and skeletal α-actin genes (Komuro *et al.*, 1991) and to activate transcription of β-MHC, MLC-IIa, and ANP (Kariya *et al.*, 1991; Shubeita *et al.*, 1992) indicating that activation of PKC can induce hypertrophy.

Mechanosensitive ion channels and cardiac hypertrophy

Activation of mechanosensitive ion channels has been observed to be involved in the transduction mechanism between mechanical stress and cardiac hypertrophy (Hu and Sachs, 1997). The passage of Na^+, K^+ and Ca^{2+} is allowed through these stretch activated ion channels (SACs) (Ruknudin *et al.*, 1993). Several mechanisms for Ca^{2+} involvement in the development of cardiac hypertrophy have been proposed (Ruknudin *et al.*, 1993). Increased concentrations of Ca^{2+} may enhance the PKC activity followed by direct or indirect alterations in gene expression. Ca^{2+} can also regulate IE gene expression, such as c-fos (Rosen *et al.*, 1995).

Activation of Na^+/H^+ exchanger has also been implicated in the process of mechanotransduction through the SACs, since its activation increases intracellular pH, which is known to stimulate the expression of hypertrophic marker genes and protein synthesis (Fuller, 1997).

G-proteins and cardiac hypertrophy

Another candidate mechanism for mechanotransduction involves guanine nucleotide-binding proteins (G-proteins) that couple cell surface receptors to the appropriate effectors. There are two forms of signal transducing G-proteins, the small G-proteins

and the heterotrimeric G-proteins. These G-proteins share common characteristics: they exist in two interconvertible conformational states, i.e. an inactive guanosine diphosphate (GDP)-bound state and an inactive guanosine triphosphate (GTP)-bound state (Hall, 1990). The heterotrimeric G-proteins are associated with signal transduction originating from cell surface receptors. Heterotrimeric G-protein subunits are localized at sites of focal adhesions that provide contact via integrins with ECM thereby functioning as a sensor of mechanical stress (Hansen *et al.*, 1994).

Activation of PKC may be crucial in this G-protein induced hypertrophy. Therefore it has been hypothesized that integrins, heterotrimeric G-proteins, PLC and PKC have an integrated action in the mechanotransduction of cardiac hypertrophy (Molkentin *et al.*, 1998; Mende *et al.*, 1999).

2.1.2 Signal transduction pathways in cardiac hypertrophy

The signal transduction pathways possibly involved in the mechanical stress-induced hypertrophy include two major pathways, the mitogen-activated protein kinases (MAPK) pathway and the janus-associated kinases /signal transducers and activators of transcription (JAK/STAT)- pathway.

The MAPKs are serine/threonine kinases that become activated upon tyrosine/threonine phosphorylation and additional modifications, and they in turn phosphorylate and activate nuclear substrates such as c-myc, c-jun, ATF-2, and $p62^{TCF}$ and other kinases such as $p90^{RSK}$ and MAPKAP kinase 2 (Sturgill and Wu, 1991; Dalby *et al.*, 1998). This pathway consists of several subfamilies of which the extracellular-regulated kinases (ERK) pathway, the c-Jun N-terminal protein kinases (JNK) pathway and the p38 MAPK pathway are perhaps the most important. They

are activated by heterotrimeric G proteins, such as Ras, cdc42, Rac, by protein kinases such as Src and FAK and by PKC via activation of PLC and/or PLD or by JAKs via gp130. Their downstream targets are cytosolic kinases such as $p90^{RSK}$ and MAPKAPK2/3 and nuclear transcription factors such as c-jun, c-myc and Elk1 (Ruwhof and Van der Laarse, 2000). The JAK/STAT pathway is directly activated probably via gp130. Upon activation of STATs by JAKs, STATs translocate to the nucleus and induce gene transcription (Ruwhof and Van der Laarse, 2000).

Cardiac hypertrophy can also be induced by the Ca^{2+}/calmodulin-dependent phosphatase calcineurin (Molkentin *et al.*, 1998). Studies on transgenic mice expressing activated forms of calcineurin developed cardiac hypertrophy, and upon cyclosporine (an inhibitor of calcineurin) administration, cardiac hypertrophy was prevented in these mice (Molkentin *et al.*, 1998). Cyclosporine not only inhibited calcineurin, but also suppressed the re-expression of fetal genes in cardiomyocytes when stimulated *in vitro* with Ang II and phenylephrine. Activation of calcineurin dephosphorylates the cytoplasmic transcription factor NF-AT3, which migrates into the nucleus and interacts with the GATA4 transcription factor to synergistically up-regulate gene expression (Molkentin *et al.*, 1998).

However, other studies failed to show that cyclosporine suppresses the development of cardiac hypertrophy in rodents with hemodynamic overload *in vivo* (Zhang *et al.*, 1999a; Ding *et al.*, 1999). The involvement of calcineurin in the development of hypertrophy is therefore still controversial (Molkentin *et al.*, 1998; Ruwhof and Van der Laarse, 2000) (Figure 2.1.1).

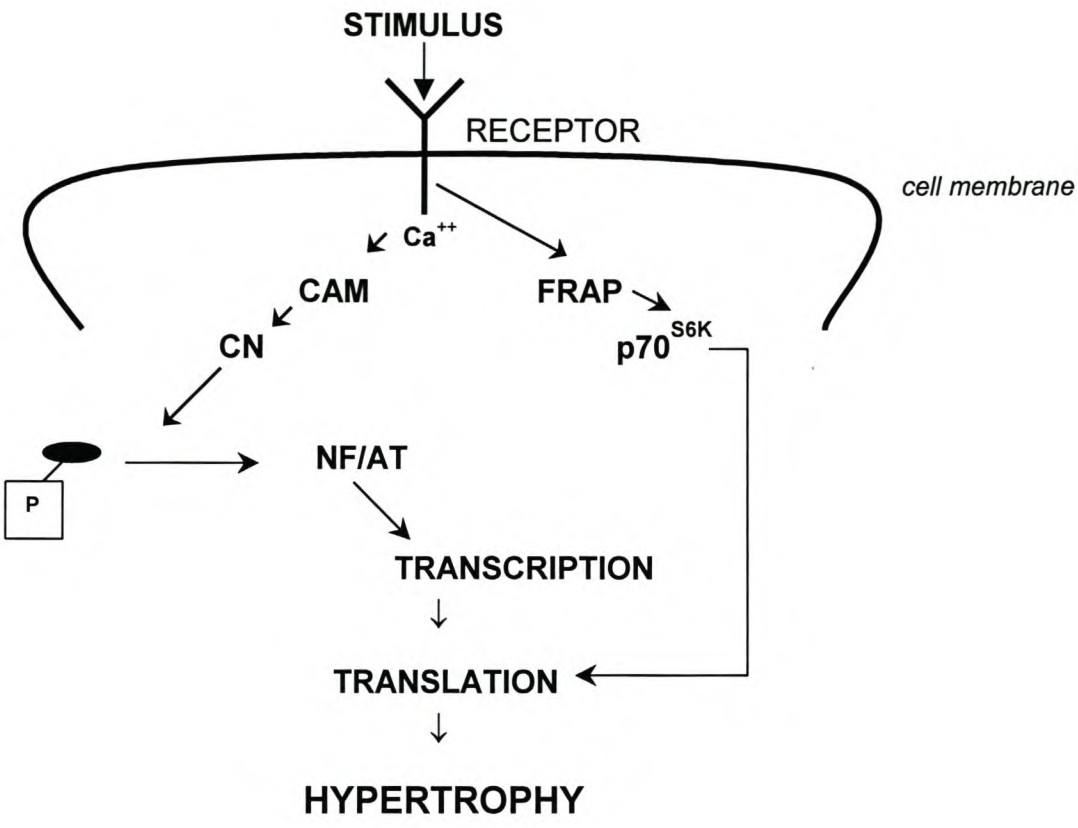


Figure 2.1.1: Signal transduction via the calcineurin and p70 S6 kinase pathway. Activation of each of these pathways has been linked to cardiac hypertrophy. CN, calcineurin; FRAP, FKBP-12-rapamycin associated protein; CAM, calmodulin; NF/AT, nuclear factor of activated T-lymphocytes. (Adapted from Boluyt and Bing, 2000)

2.1.3 Transition from cardiac hypertrophy to heart failure

Impaired functional performance despite hypertrophic enlargement characterizes the decompensated failing heart. Features of heart failure include depressed myocardial function, extensive interstitial fibrosis, and apoptotic myocyte loss (Bing *et al.*, 1995; Conrad *et al.*, 1995; Li *et al.*, 1997b). In fact, interstitial fibrosis is a hallmark of cardiac hypertrophy in heart failure (Anderson *et al.*, 1979; Pearlman *et al.*, 1982; Huysman *et al.*, 1989) and other pathological conditions such as dilated cardiomyopathy (Beltrami *et al.*, 1995) as well as in aging (Besse *et al.*, 1994; Boluyt *et al.*, 1994). Manifestations of decompensated function include ventricular dilatation,

reduced fractional shortening, diminished ejection fraction, and decreased myocardial force production.

Several factors have been suggested to play roles in the transition from compensated hypertrophy to the decompensated state. These include abnormalities in: (i) calcium handling (Feldman *et al.*, 1993), (ii) contractile proteins (Boluyt *et al.*, 1994), and (iii) extracellular matrix proteins (Boluyt *et al.*, 1994). As previously mentioned, myocyte hypertrophy is not only modulated by loading conditions, but also by systemic or neurohormonal processes (Dzau, 1993). The cardiac renin-angiotensin system is activated in the pressure-overloaded heart (Zhang *et al.*, 1995), and has been shown to play a deleterious role in the transition toward congestive heart failure (CHF) (Litwin *et al.*, 1995). The expression of chemokines and pro-inflammatory cytokines also play a role in the development of hypertrophy and eventually cardiac failure (Feron *et al.*, 1995). Oxidative stress, which is induced by pro-inflammatory cytokines, is also involved in the process (Schreck *et al.*, 1991; Dhalla *et al.*, 1996).

Age-associated changes in the myocardium also contribute to the progression from stable cardiac hypertrophy to dysfunction and heart failure (Kannel and Belanger, 1991). Fibronectin is an integral protein of the extracellular matrix that acts to bind together various components of the heart. The levels of fibronectin mRNA decreases between development and adulthood (Mamuya *et al.*, 1992). Yet the levels of this protein are increased again in the aging left ventricle (LV) and atria (Boluyt and Lakatta, 1998).

Fibrosis increases the stiffness of the myocardium leading to diastolic dysfunction and exacerbation of heart failure (Weber *et al.*, 1993). Fibrosis results, in part, from

the increased expression of genes encoding extracellular matrix proteins that make up the scaffolding that provides the framework in which myocytes function (Boluyt and Bing, 2000).

In a study on spontaneously hypertensive rats (SHR), the transition to heart failure is accompanied by marked changes in the expression of an array of genes in the heart. Amid the most prominant changes are increased levels of mRNAs encoding fibronectin, collagen Type I, collagen Type III and osteopontin, which are all components of the extracellular matrix (Boluyt *et al.*, 1994; Singh *et al.*, 1999). Interstitial and perivascular localization of collagen mRNA has been implicated in failing hearts (Bing *et al.*, 1997), and since increases in fibrillar collagen in the interstitium contributes to tissue stiffness, increases in fibronectin and collagen gene expressions are likely to contribute to the impaired function. The strong relationship between fibrosis and myocardial stiffness is very well demonstrated in studies where an angiotensin converting enzyme (ACE) inhibitor was used to treat young adult SHR in dosages allowing the dissociation of hypertrophy and fibrosis. The experiments demonstrated that the ACE inhibitor could regress fibrosis and normalize LV stiffness in the aged SHR, thereby preventing LV dysfunction (Brilla *et al.*, 1996).

Other studies have demonstrated that the transforming growth factor β_1 ($TGF\beta_1$) family of cytokines plays a key role in regulating many aspects of the remodeling process including the up-regulation of extracellular matrix (ECM) genes (Boluyt *et al.*, 1994). The up-regulation of $TGF\beta_1$ gene expression observed in failing hearts may be involved in controlling accumulation of extracellular matrix, as seen during wound repair (Zhang *et al.*, 1999b). Fibrosis and collagen formation were reduced in a study

done where $TGF\beta_1$ was inhibited (Smith *et al.*, 1999). $TNF\alpha$ and its role in the decompensated heart will be discussed later.

2.2 Obesity

Obesity essentially results from an imbalance between food intake and energy utilization. The condition is usually characterized by a large increase in adipose tissue and its triglyceride content. This increase in adipose tissue may be partly due to an increase in the number of adipose cells (termed hyperplastic obesity) or an increase in size of the adipose tissue (termed hypertrophic obesity). Obesity is normally associated with a number of secondary conditions, including hypertension, abnormal lipid metabolism and insulin resistance leading to impaired glucose tolerance or type II diabetes mellitus. These conditions are all cardiovascular risk factors known to have an adverse effect on the cardiovascular system (Paulson and Tahiliani, 1992).

Increasing adiposity as a function of increasing age also contributes to the increase in the incidence of diabetes and hypertension, which are both cardiovascular risk factors (Holbrook *et al.*, 1989; Caro, 1991; Busby *et al.*, 1992; Coldritz *et al.*, 1995). Although the specific cause for obesity is unknown, abnormalities in leptin homeostasis have been proposed to increase the propensity of obesity (Caro *et al.*, 1996). Leptin is released into the plasma (Frederich *et al.*, 1995; Leroy *et al.*, 1996) and the concentrations are related to body fat mass (Frederich *et al.*, 1995). Development of obesity in some rodent models is related to mutations in leptin or the leptin receptor. Administration of leptin to obese rats lacking leptin result in immediate normalization of body weight, metabolism and the regulation of the hypothalamic-

pituary axis (Caro *et al.*, 1996). The role of leptin in humans however remains unclear (Widdowson *et al.*, 1997).

2.2.1 Obesity and cardiac remodeling

Due to the excessive weight associated with obesity the overall metabolic demands, including oxygen consumption, are increased. In order to deliver more oxygen to peripheral tissues, cardiac output is increased. An increased stroke volume rather than heart rate accounts for the increase in cardiac output (Garavaglia *et al.*, 1988). The increased stroke volume is achieved by an augmented end-diastolic volume and increased pre-load. Both these parameters increase due to the increase in blood volume associated with obesity. Blood volume is also increased because of the increased peripheral vascularity (Messerli, 1982). Over an extended period this elevated pre-load on the heart results in left ventricular hypertrophy and eventually dilatation. Other factors may also be involved in cardiac remodeling such as TNF_{α}, which is released by adipose tissue and/or produced *de novo* in the heart (Hotamisligil *et al.*, 1994; Kapadia *et al.*, 1997).

2.2.2 Obesity and insulin resistance

Insulin resistance occurs when there is a decreased tissue responsiveness to insulin in the body. Insulin resistance has been associated with pathologic conditions such as diabetes (both type I and type II), hypertension and coronary heart disease (Ferrannini and Natali, 1991). Visceral adiposity appears to be a strong risk factor for hypertension, dyslipidemia, coronary heart disease and insulin resistance of type II diabetes in various species (Pouliot *et al.*, 1992; Spiegelman *et al.*, 1992; Tschernof *et al.*, 1996; Banerji *et al.*, 1997). Furthermore, in clinical studies, detectable cardiac dysfunction has been reported to occur as early as the glucose intolerance phase

that follows insulin resistance (Celentano *et al.*, 1995). Diabetic-like cardiomyopathy has also been demonstrated at very early stages of type II diabetes and is therefore associated with insulin resistance (Dutta *et al.*, 2001).

Diabetes is a principle cause of morbidity and mortality in human populations. Only a small population of patients suffer from type I diabetes, which is caused by pancreatic β-cell failure and leads to an absolute loss of insulin (Nathan, 1993). Type II diabetes mellitus is characterized by target-tissue resistance to insulin and cannot be overcome by β-cell hypersecretion (Taylor, 1999).

Although type II diabetes mellitus is associated with insulin resistance, in both rodent and human models, the connection between increased adiposity and insulin resistance remains unclear (Kahn *et al.*, 1996; Kopelman, 2000).

The mechanisms for the decreased insulin responsiveness in obese individuals is unknown. Adipocytes however secrete a variety of other polypeptides, such as tumor necrosis factor-α (TNF_α), adipsin, Acrp 30/adipoQ and resistin, that may affect insulin action in other tissues (Spiegelman and Flier, 1996; Mohamed-Ali *et* al. 1998; Friedman and Halaas, 1998; Hotamisligil, 1999; Shimomura *et al.*, 1999; Moller, 2000; Steppan *et al.*, 2001). Insulin resistance in persons with visceral obesity may relate in part to the metabolic characteristics of visceral fat cells, which when compared with peripheral fat cells, are more resistant to the metabolic effects of insulin and more sensitive to lipolytic hormones (Tandia *et al.*, 1989; Jensen *et al.*, 1989). The preferential channeling of different fuels to fat and changes in the transcription profile of adipose tissue remain poorly understood in the pathogenesis

of obesity and insulin resistance. Carbohydrate and lipid metabolism play an important role in this context (Fabris *et al.*, 2001).

Carbohydrates and lipids share common pathways, which may be important in the regulation of energy metabolism and fuel partitioning. Insulin stimulates glucose oxidation in skeletal muscle and adipose tissue, but other macronutrients, which increase may interfere with glucose oxidation (Ferrannini *et al.*, 1983). Free fatty acid (non-esterified fatty acid [NEFA])-induced insulin resistance saves scarce glucose for central nervous system requirements, and this becomes counterproductive in obesity because it inhibits glucose utilization when there is no need to save it (Fabris *et al.*, 2001). Glucose and NEFA might thus be channeled toward adipose tissue in which insulin sensitivity is maintained or improved, and contribute to triglyceride synthesis, although the cellular and molecular mechanisms are still under debate.

2.3 TNFα

Tumor necrosis factor α is the term originally used to describe a humoral factor that was released by activated macrophages and induced hemorrhagic necrosis of transplanted tumors in mice (Carswell *et al.*, 1975). TNFα is now recognized as a cytokine (general term for protein intercellular messengers that influence cells of the immune system) with pleiotropic biological capabilities. Besides its cytostatic and cytotoxic effects on certain tumor cells, TNFα influences growth, differentiation and function of virtually every cell including cardiac myocytes (Gulick *et al.*, 1989; Yokoyama *et al.*, 1993). Thus, TNFα is part of an integral network of interactive signal pathways that orchestrate inflammatory and immunological events.

TNFα is expressed as a 26 kDa cell surface transmembrane protein; cleavage by TNFα converting enzyme generates a 17 kDa soluble form of TNFα, which is thought

to mediate most of its biological effects. Two distinct membrane receptors, a 55 kDa isoform (p55) and a 75 kDa isoform (p75) mediate all actions of TNF_{α} (Moller, 2000). TNF_{α} binds to these two specific cell surface receptors, namely type 1 (p55) and type 2 (p75) or termed TNF-R55 and TNF-R75. The extracellular portions of the TNF receptors have been identified in the serum in soluble forms (sTNF-Rs). Endogenous formation of TNF_{α} and release causes shedding of the extracellular portion (sTNF-Rs). $sTNF_{\alpha}$-Rs will now compete with the membrane bound receptors for the released TNF_{α}, interfering with the binding of TNF_{α} to cell-surface bound TNF-Rs. Therefore the sTNF-R concentration reflects the activation state of the TNF_{α}/TNF receptor system (Zahorska-Markiewicz *et al.*, 2000), (Figure 2.3.1).

2.3.2 TNF_{α} and the heart

It has been observed that elevated levels of TNF_{α} are detected peripherally in almost all forms of cardiac injury, including cardiac allograft rejection (Arbustini *et al.*, 1991; Sakobar *et al.*, 1993), myocardial infarction (Maury and Teppo, 1989; Basaran *et al.*, 1993), myocardial reperfusion injury (Lefer *et al.*, 1990; Herskowitz *et al.*, 1995) hypertrophic cardiomyopathy (Matsumori *et al.*, 1994) and end stage congestive heart failure (Levine *et al.*, 1990; Dutka *et al.*, 1993).

The observation that TNF_{α} gene expression is also linked to almost all forms of injury suggests that it may serve as a 'stress response' gene in the heart (Mann, 1996). TNF α is expressed *de novo* in the heart within 30 minutes after a stressful stimulus (Kapadia *et al.*, 1995). TNF_{α} mRNA levels rapidly return towards baseline levels once the stressful stimulus is removed, hence suggesting that TNF_{α} gene expression is tightly regulated in the heart (Kapadia *et al.*, 1995).

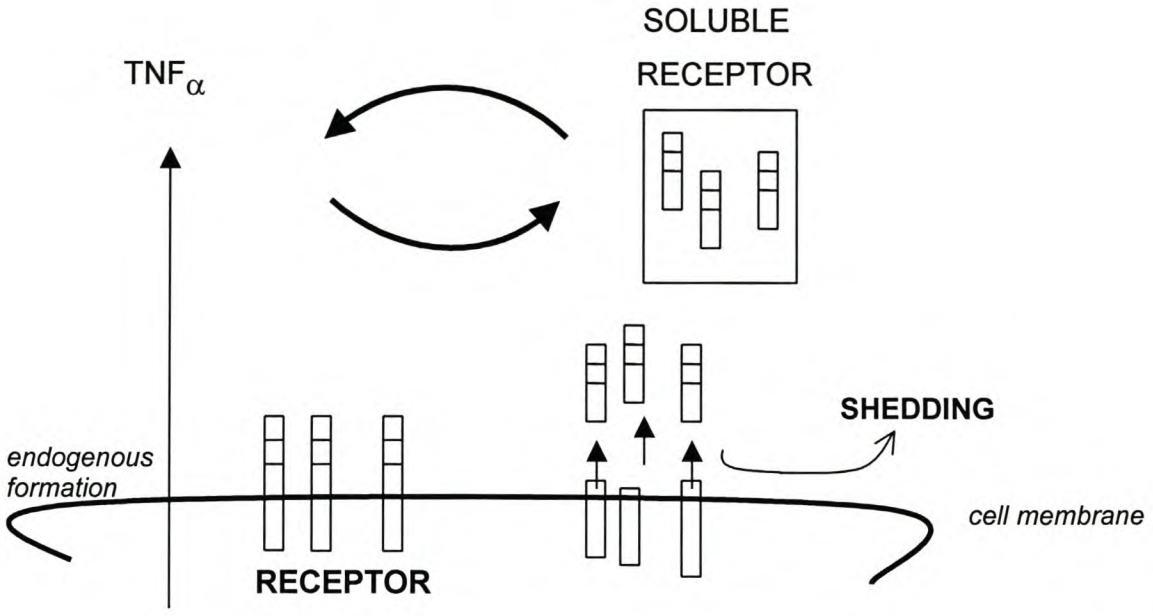


Figure 2.3.1: Cytokine bioactivity. When soluble TNF_α receptors are bound to TNF_α, they are capable of preventing TNF_α from binding to the TNF_α receptors on the cell surface membranes. (Modified from Bozkurt *et al.*, 1998).

Since TNF_α is a mediator of inflammation and septic shock, its effects are considered as harmful or beneficial to the host. It triggers the release of practically all known mediators of inflammation such as other cytokines and all metabolites of arachidonic acid, the final results ranging from haemorrhagic necrosis to extensive local fibrosis (Aggarwal and Natarajan, 1996).

TNF_α can also initiate its own transcription and induce self-amplification (Ferrari, 1998). It damages the host by inducing and enhancing, often in association with IFN-$_\gamma$, the production of reactive oxygen derivatives, such as nitric oxide. However under these pathological conditions, TNF_α may have dual effects, allowing the host self-protection against this type of injury by the induction of oxygen free radical-scavenging enzymes, such as mitochondrial superoxide dismutase and of protective substances such as heat shock proteins (Aggarwal and Natarajan, 1996).

The bi-functional effects TNF_{α} are thought to be dependent on concentrations of its expression in the myocardium. This cytokine is harmful to the myocardium when expressed in high concentrations, causing left ventricular dysfunction and apoptosis (Kubota *et al.*, 1997).

However, acute exposure of the heart to low levels of TNF_{α} may be cardioprotective. Sack and colleagues (2000) have proposed that post-ischaemic recovery was improved by acute/subacute low concentrations of TNF_{α} in the isolated rat heart. On the other hand inhibition of the synthesis of TNF_{α} by an inhibitor of p38 MAP kinase decreased the production of this cytokine and improved post-ischaemic function (Cain *et al.*, 1999) (Figure 2.3.2.1). The different results observed in these studies may be due to differences in the method of induction of ischaemia, the duration of ischaemia and the species used in the studies (Sack *et al.*, 2000).

2.3.3 TNF_{α} and cardiac hypertrophy

Clinical studies show that circulating levels of TNF_{α} are elevated in patients with chronic heart failure due to ischaemic heart disease and dilated cardiomyopathy (Levine *et al.*, 1990; Swedberg *et al.*, 1990; Matsumori *et al.*, 1994; Torre-Amione *et al.*, 1996). Cardiac myocyte hypertrophy is a principle feature of such cardiac diseases (Gilbert and Bristow, 1994; Beltrami *et al.*, 1994), and TNF_{α} is an important factor that may induce hypertrophy.

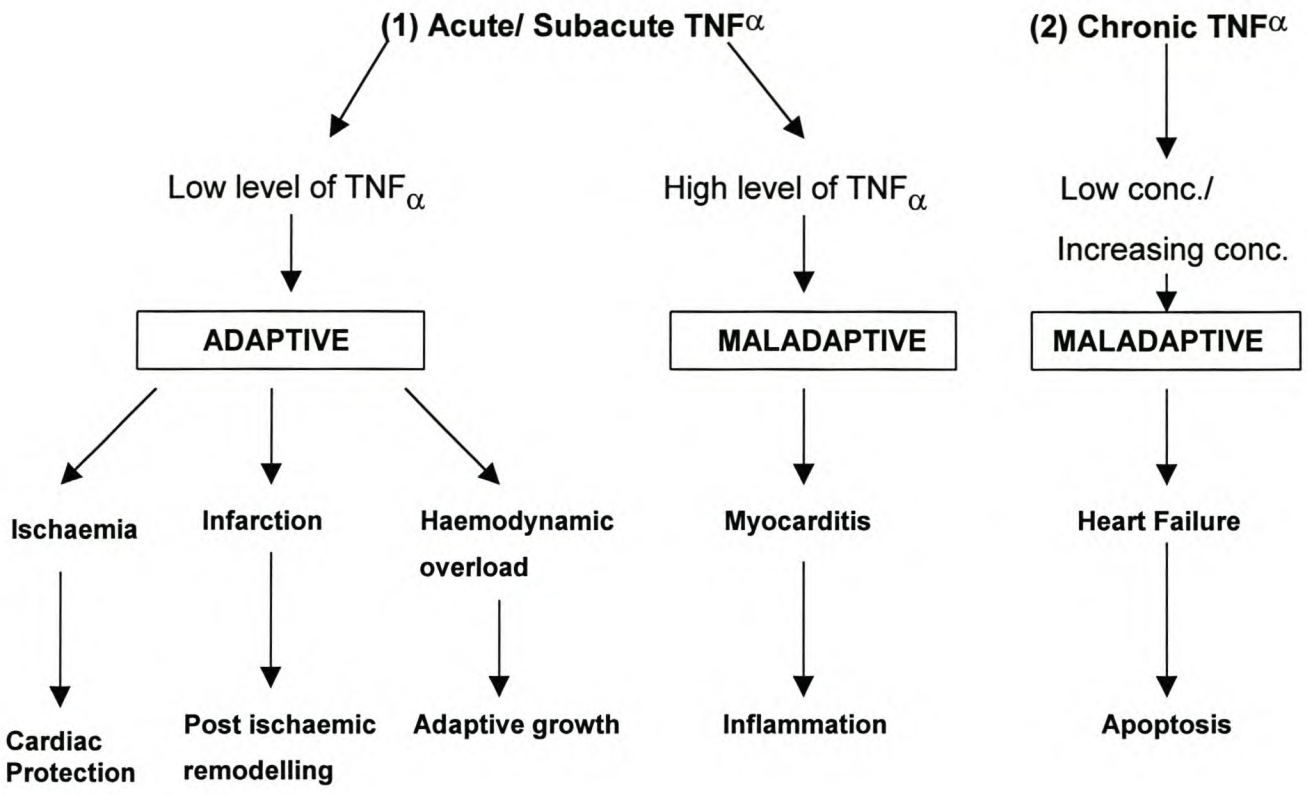


Figure 2.3.2.1: Model for an adaptive and maladaptive roles of TNF_{α}. (Modified from Sack *et al.*, 2000)

The direct effect of TNF_{α} on cardiac cells has been demonstrated in cultured myocytes (Long *et al.*, 1991; Yokoyama *et al.*, 1997). The phenotypic alterations are thought to be an adaptive response in order to maintain normal contractility and homeostasis in response to an increased workload. A simple passive stretch of cat papillary muscle induced the expression of TNF mRNA and it has been suggested that physiologically relevant concentrations of TNF_{α} provoke hypertrophic response by increasing the synthesis of both structural and contractile protein in adult feline cardiocytes (Kapadia *et al.*, 1997; Yokoyama *et al.*, 1997).

TNF_{α} exerts cytotoxic activity on some types of tumor cells, in part via the generation of reactive oxygen intermediates (ROIs) (Matthews *et al.*, 1987; Wong *et al.*, 1989; Goossens *et al.*, 1995) and JNK activation in cardiac myocytes. ROIs are involved in

many biological processes as they are involved in the defense against micro-organisms, and they can cause host cell injury directly. ROIs also take part in regulating the expression of various genes and cell growth (Rao and Berk, 1992; Marui *et al*., 1993). ROIs specifically stimulate DNA synthesis and the expression of proto-oncogenes such as c-myc and c-fos in vascular smooth muscle (Rao and Berk, 1992). Nakamura and colleagues (1998) demonstrated that antioxidants can inhibit TNF_{α} induced cardiac hypertrophy in cultured neonatal rat myocytes. However, the exact signaling pathways involved in ROI induced cardiac hypertrophy need further investigation (Nakamura *et al*., 1998).

TNF_{α} binds to its cognate homotrimeric receptor, forming multimolecular signal transduction complexes, which can rapidly activate several divergent downstream signaling pathways (Sack *et al*., 2000). TNF-R1 and TNF-R2 are co-expressed on most cell types, but signaling from TNF-R1 is thought to be more important of the two receptor types in the heart, since transfection experiments, receptor specific antibodies, and experiments with gene-knock-out mice have shown that TNF-R1 is the dominant signaling receptor (Tartaglia and Goeddel, 1992; Vandenabeele *et al*., 1995). TNF-R1 can signal apoptosis and activate the transcription factor nuclear factor-kappa B ($NF\text{-}_{K}B$), while TNF-R2 can directly signal certain activities in lymphocytes such as $NF\text{-}_{K}B$ activation and help deliver TNF_{α} to TNF-R1 (Tartaglia *et al*., 1993b). The inflammatory response, but not the hypertrophic response, can be attenuated by genetic attenuation of the TNF-R1 signaling pathway (Bounoutas *et al*., 1998).

The TNF-R2 signaling complex involves proteins of the intracellular domain required for signaling, and these are called TRAF-1 and TRAF-2 (Rothe *et al*., 1994, Rothe *et*

al., 1995). TRAF-1 and TRAF-2 can form both homodimers and heterodimers through their TRAF domains, but only TRAF-2 interacts directly with TNF-R2 (Rothe *et al.* 1994). TNF-R1 contains a death domain in its intracellular domains that signals apoptosis and NF-$_K$B activation (Tartaglia *et al.*, 1993a; Hsu *et al.*, 1995; Hsu *et al.*, 1996b), and these are termed as TNF receptor-associated death domain (TRADD). Upon TNF$_\alpha$ binding to its receptors, TNF-R2 forms a complex with TRAF2, activating both NF$_K$B-dependent and independent transcriptional events implicated in the cytoprotective genes involved in cellular growth, survival and proliferation (Natoli *et al.*, 1998). The cytoprotective pathway includes the hypertrophic growth programme downstream of PKC (Baines et al., 1999), NF$_K$B, stress activated protein kinases (SAPK) and JNK (Baines et al., 1999).

Receptor interacting protein (RIP) is a cytoplasmic serine/threonine protein kinase that contains a C-terminal death domain. It was originally described as a Fas-interacting protein (Stanger *et al.*, 1995), however it has been suggested that it does not interact with Fas in mammalian cells, but rather TRADD interacts strongly with RIP and recruits RIP to the TNF-R1 complex (Hsu *et al.*, 1996a) (Figure 2.3.3.1).

2.3.4 TNF$_\alpha$ and the sphingomyelin pathway

Sphingomyelin is a phospholipid found in the plasma membrane of mammalian cells. Signaling through the sphingomyelin pathway is associated with generation of ceramide, which acts as a second messenger in activating a variety of cellular functions. Ceramide belongs to the group of sphingosine based lipid second messenger molecules that are critically involved in the regulation of signal transduction of diverse cell surface membrane receptors (Kronke, 1999). Following membrane receptor triggering by TNF$_\alpha$, neutral and acid isoforms of

sphingomyelinases are rapidly activated, generating ceramide through sphingomyelin hydrolysis (Kronke, 1999). Increased intracellular ceramide concentrations have been implicated in the induction of apoptosis in a number of cell types (Cifone *et al.*, 1994; Haimovitz-Friedman *et al.*, 1994; Jayadev *et al.*, 1995).

Ceramide is produced primarily by the hydrolysis of sphingomyelin through sphingomyelinase (SMase), or from palmitoyl CoA and serine through *de novo* synthesis. Ceramide, once generated, can be metabolized or converted to other molecules by various enzymes such as ceramidase, glucosylceramidase synthase, ceramide kinase, and sphingomyelin synthase (Luberto and Hannun, 1999; Hannun and Luberto, 2000).

With respect to the alteration of intracellular ceramide levels in response to cytokines, it has been demonstrated that this alteration mainly resulted from the two modulations of two isoforms of SMase, the neutral (N-) and acidic (A-) SMase, and ceramidase in a variety of mammalian tissues and cells.

A- and N- SMase were found to be stimulated by TNF_{α}, IL-1_{β}, and interferon-$_{\gamma}$, and ceramidase can be activated or inhibited by these cytokines (Kim *et al.*, 1991; Mathias *et al.*, 1993; Kolesnick and Golde, 1994; Hannun, 1994; Coroneos *et al.*, 1995; Nikolova-Karakashian *et al.*, 1997). Zhang and colleagues (2001) demonstrated that N-SMase, A-SMase and ceramidase are present in the myocardium of rats. They concluded that increases in tissue ceramide levels during ischaemia/reperfusion are not associated with enhanced SMase activity, but rather with inhibition of ceramidase, which is contrary to the findings of other studies (Hernandez *et al.*, 2000), where hypoxia and reoxygenation in cultured rat myocytes

was reported to induce N-SMase and then the accumulation of ceramide. Reasons for these discrepancies are unknown (Zhang *et al.*, 2001) (Figure 2.3.3.1).

2.3.5 Other Cytokine signal transduction pathways in cardiac hypertrophy

Janus-associated kinases (JAKs) were first identified as protein tyrosine kinases associated with cytokine receptors that regulate signal transduction of these receptors (Ihle, 1995). The JAK family consists of Jak1, Jak2, Jak3 and Tyk2. They have a molecular mass ranging from 120 to 130 kDa (Ihle, 1995). Signal transducers and activators of transcription (STATs) are latent transcription factors located in the cytoplasm, which become activated by phosphorylation of a tyrosine residue.

They are both named after their dual functions in signal transduction in the cytoplasm and activation of transcription in the nucleus (Schindler and Darnell, 1995; Horvath and Darnell, 1997). Binding of ligand to their cytokine receptors leads to phosphorylation and activation of the receptor-JAK complex with subsequent recruitment and activation of STATs by phosphorylation. The phosphorylated STATs dimerize, migrate into the nucleus and bind response elements in the promoters of target genes to stimulate gene transcription (Ihle, 1995; Schindler and Darnell, 1995).

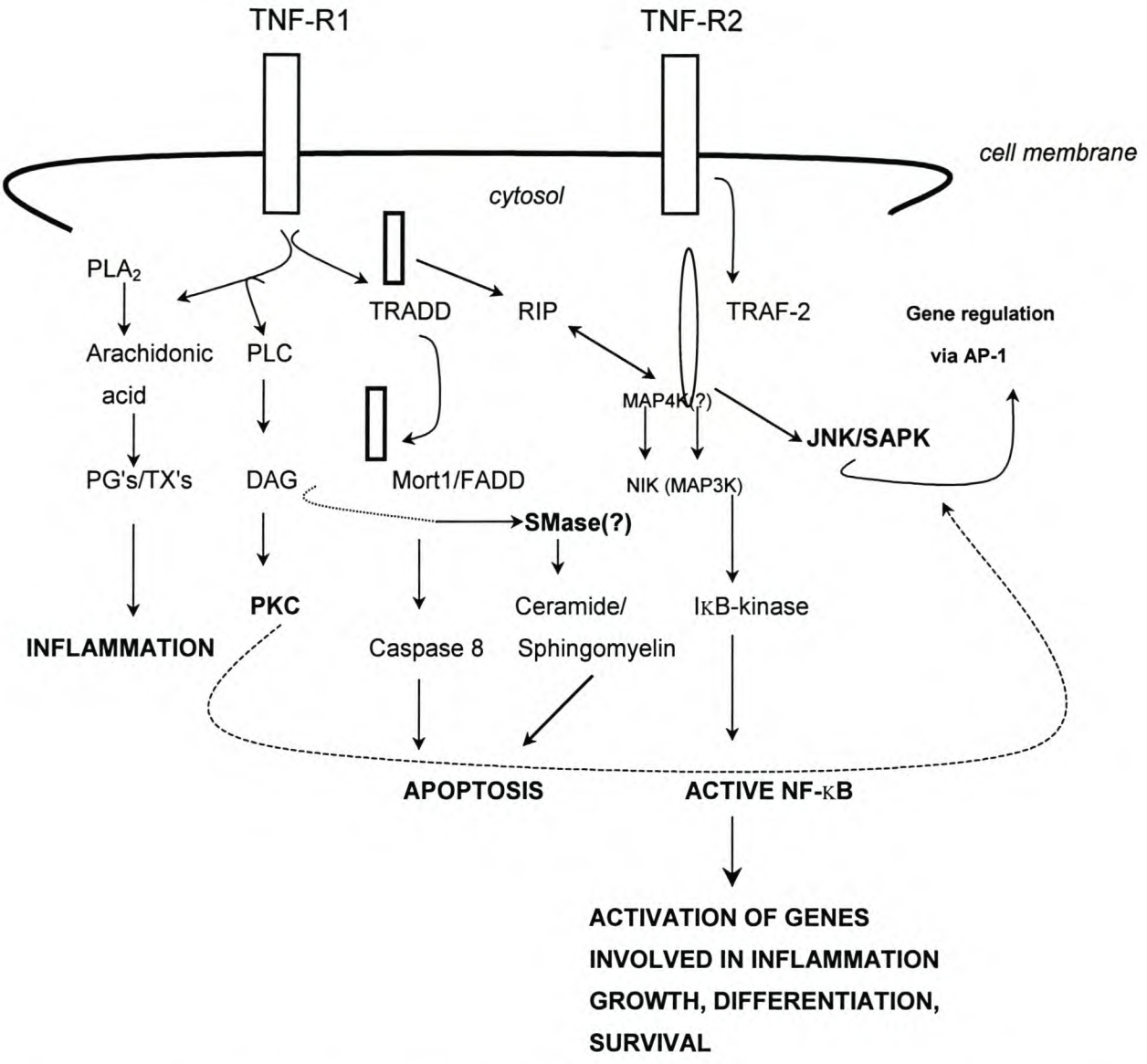


Figure 2.3.3.1 Signalling pathways of TNFRs after activation by TNF_{α}. The figure shows the recruitment of various docking proteins to TNF-R1 and TNF-R2. These include: tumor receptor associated death domain (TRADD), receptor interacting protein (RIP), TNF receptor associated factor 2 (TRAF2), and Fas-associated death domain protein (FADD), alternate name, MORT1, (mediator of receptor induced toxicity). Recruitment of these docking proteins is coupled to the activation of numerous signalling pathways leading to inflammation, apoptosis or multiple gene activation. The phospholipase A_2 (PLA_2) include: cyclooxygenase (COX); prostaglandines (PGs), and thromboxanes (TXs). The phospholipase C (PLC) pathway includes diacylglycerole (DAG), sphingomyelinase (SMase) and protein kinase C (PKC). The mitogen activated kinase (MAPK) include NF_KB inducing kinase (NF_KB), the NF_KB transcription factor subtypes-p-50 and p65 and the NF_KB cytosolic anchoring protein complex or inhibitory protein I_KB. The TRAF2 or PKC activated pathway is represented by Janus N-terminal kinase/stress activated protein kinase (JNK/SAPK) with activation of genes via activation protein-1 (AP-1). (Adapted from Sack *et al.*, 2000).

In case of receptor complexes sharing the glycoprotein 130 (gp130), such as members of the interleukin (IL)-6 family (cardiotrophin-1) (Latchman, 2000), signal transduction is triggered by the formation of dimers of gp130 (Kishimoto *et al.*, 1994). Activation of STATs also occurs through the receptor families other than the cytokine receptor family such as tyrosine kinase receptors (Takahashi *et al.*, 1999) and G-protein-coupled receptors (Bhat *et al.*, 1994; McWhinney *et al.*, 1997; Mascareno *et al.*, 1998). The JAK/STAT pathway was activated in rat hearts with pressure overload-induced cardiac hypertrophy and this is mediated by gp130 (Pan *et al.*, 1998).

Ligand binding to the gp130 and low affinity leukemia inhibitor factor receptor (LIFR) complex results in phosphorylation of Janus kinase (Jak) signaling factors, which in turn phosporylates gp130, generating a docking site for SH2 domain-containing proteins (Narazaki *et al.*, 1994). The MAPK pathway is activated through Ras by activated gp130 (Nakafuku *et al.*, 1992; Kumar *et al.*, 1994; Kunisada *et al.*, 1996) (Figure 2.3.4.1).

2.4 TNFα and Obesity

TNF$_\alpha$ is over-expressed in adipose tissue of obese insulin-resistant rodents and humans, and TNF$_\alpha$ antibody administration has been shown to counteract insulin resistance in the fat and muscle of obese Zucker rats (Hotamisligil *et al.*, 1994). These data would suggest a role for TNF$_\alpha$ in the pathophysiology of insulin resistance.

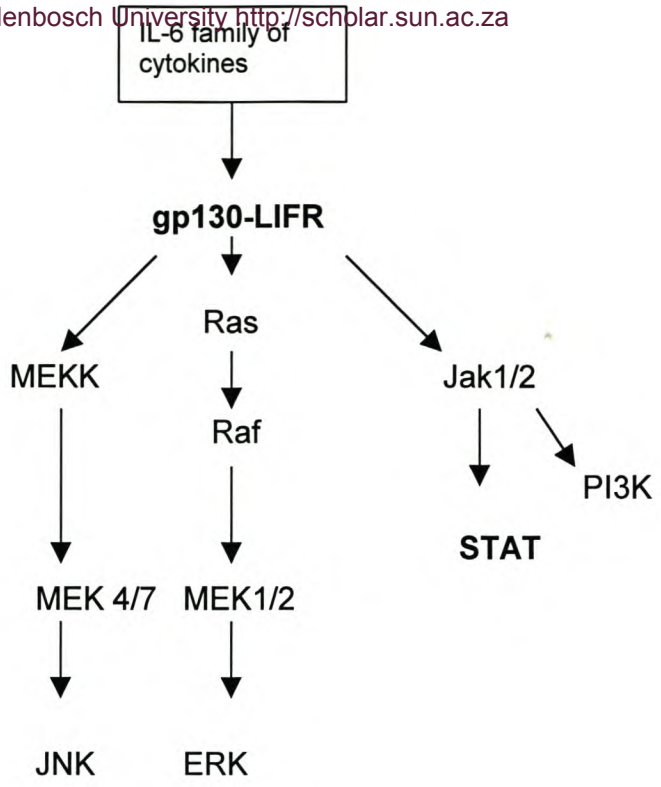


Figure 2.3.4.1: gp130 and Jak/STAT signalling pathway. Once gp130 is activated by LIF, cardiotrophin or other cytokines of the IL-6 cytokine family, this receptor interacts with Janus kinase 1 causing its activation, which in turn leads to STAT phosphorylation promoting dimerization and nuclear entry. gp130 also promotes the activation of phosphatidylinositol 3-kinase (PI3K, Ras and mitogen-activated protein kinase kinase kinases (MEKK). (Adapted from Molkentin and Dorn, 2001)

Although several studies have demonstrated an over-expression of TNF_{α} in adipose tissue and elevated plasma concentrations of the two soluble TNF receptors in obese animals (Hotamisligil *et al.*, 1995; Kern *et al.*, 1995; Hube *et al.*, 1997; Hauner *et al.*, 1998), it still remains unclear whether an increased adipose tissue production of TNF_{α} is reflected by elevated circulating plasma TNF_{α} levels. TNF_{α} activity represents the summation of TNF_{α} synthesis, secretion and the amount of soluble inhibitors present and several studies have only measured membrane TNF_{α} receptor density or mRNA levels (Hauner *et al.*, 1998).

In the past it was believed that the effects of TNF_{α} *in vivo* was confined to activated macrophages and lymphocytes (Beutler and Cerami, 1988). However further research provided strong evidence that TNF_{α} is found in adipose tissue and that levels increase in obese and insulin-resistant rodents (Hotamisligil *et al.*, 1993). Administration of exogenous TNF_{α} induced increased circulating TNF_{α} that has marked effects on lipid metabolism (Hotamisligil *et al.*, 1995). Increases in triglycerides, attributed to the induction of lipolysis and stimulation of hepatic lipogenesis have been observed (Beutler and Cerami, 1988; Grunfeld and Feingold, 1991; Spiegelman and Hotamisligil, 1993).

TNF_{α} has multiple actions in adipose tissue, which include increased insulin resistance associated with the defects in insulin-stimulated glucose disposal, decreased activity of lipoprotein lipase and increased hormone-sensitive lipase, preventing lipid accumulation (Kern *et al.*, 1995). TNF_{α} may be a local regulator of fat cell size, hence the overproduction of TNF_{α} in adipocytes of obese animals could represent a form of adipostat designed to limit the adipocyte size enlargement (Hotamisligil *et al.*, 1995).

When a soluble TNF_{α} binding protein was infused into *fa/fa* rats, which have high levels of adipose tissue TNF_{α}, there was a 2-3 fold increase in insulin stimulated glucose uptake, along with improved insulin receptor autophosphorylation in both adipose tissue and muscle (Hotamisligil *et al.*, 1993; Hotamisligil *et al.*, 1994). TNF_{α} neutralization however did not affect the insulin-mediated suppression of hepatic glucose output, and it is not clear whether the peripheral insulin resistance of the *fa/fa* rats was partially or completely normalized, since no parallel lean rats were investigated (Hotamisligil *et al.*, 1993). These findings were supported by studies on

Zucker *fa/fa* rats *in vivo*, where reduced insulin levels, free fatty acid levels and partial improvement in peripheral insulin sensitivity were observed (Cheung *et al.*, 1998). In contrast, neutralization of circulating TNF_{α} with anti-TNF_{α} antibodies did not affect hyperlipidemia in another study involving Zucker fa/fa rats (Lopez-Soriano *et al.*, 1997). Hence the down-regulation of any component that regulates the metabolic effects of insulin represents a plausible mechanism for how TNF_{α} may mediate insulin resistance. However controversy surrounds the role of adipose tissue-derived TNF (AT-TNF) in insulin resistance.

In *in vitro* systems such as isolated adipocytes or cultured 3T3-Li adipocytes, TNF_{α} can potentially suppress the expression of genes encoding proteins that normally regulate fatty acid uptake or lipogenesis. These include acetyl CoA carboxylase, LPL and glycerophosphate dehydrogenase (Zhang *et al.*, 1996). The cytokine has also been shown to inhibit preadipocyte differentiation and it might also induce *in vitro* differentiation of 3T3-Li adipocytes (Zhang *et al.*, 1996). These negative effects on adipocyte differentiation correlate with the suppression of other adipocyte genes, such as those encoding adipsin and the insulin-responsive glucose transporter (GLUT 4) (Stephens and Pekala, 1991; Szalkowski *et al.*, 1995; Zhang *et al.*, 1996).

There also seems to be a discrepancy between the role of AT-TNF on the insulin action when studied *in vitro* or *in vivo* (Morin *et al.*, 1998). AT-TNF activity was increased in 14 month- in comparison with 3-month-old Fisher 344 rats and in the retroperitoneal fat pad only the activity of TNF_{α} was weakly related to fasting plasma insulin (Morin *et al.*, 1997). However, the TNF_{α} activity was strongly correlated with cell size in both the epididymal and the retroperitoneal pads, and this cell size increases with age (Morin *et al.*, 1998).

Studies on the role of TNF_α originating from adipose tissue are therefore controversial and do not confirm or refute its importance in the development of insulin resistance or other pathological conditions. To our knowledge nothing is known about the effect of AT-TNF_α on the cardiovascular system.

2.5 TNFα and iNOS

The wide range of TNF_α activities and effects are explained by the presence of TNF-receptors on almost all nucleated cell types. The idea that the heart itself is a target for TNF is supported by the evidence, that circulating levels of TNF_α are high in CHF patients and myocardial TNF-Rs are low (Torre-Amione *et al.*, 1996). It has also been reported that TNF_α is responsible for the expression of the cytokine-induced high output isoform of nitric oxide synthase (NOS), iNOS or NOS2 (Goldhaber *et al.*, 1996). The expression of cationic amino acid transporters necessary for the uptake of the NO-precursor L-arginine and enzymes required for the production of tetrahydrobiopterin, a cofactor essential for iNOS activation, are also induced by TNF_α (Latini *et al.*, 1994; Packer, 1995; Torre-Amione *et al.*, 1996).

The three isoforms of NOS originally identified in the brain (nNOS), macrophages (iNOS) and endothelial cells (eNOS) share 50% to 60% homology of their amino acid sequence and are encoded by three different genes (NOS 1, NOS 2, NOS 3) (Bredt *et al.*, 1991; Lyons *et al.*, 1992; Lamas *et al.*, 1992; Nishida *et al.*, 1992). All three isoforms combine two functionally complementary portions, a carboxyl-terminal reductase domain homologous to cytochrome P450 reductase and an amino-terminal oxygenase domain containing binding sites for heme, L-arginine, and tetrahydrobiopterin (THB4), the two portions being connected by a calmodulin-

binding domain in the middle. On activation, the three isoforms presumably function as homodimers. Within each monomer, electrons provided by NADPH are transferred from the flavins (FAD or FMN) in the carboxyl-terminal portion of the molecule to heme iron, which is activated to bind O_2 and in the presence of the substrate L-arginine, to catalyze the synthesis of NO and L-citrulline.

iNOS generates a prolonged release of large amounts of NO which may be cytotoxic and/or inhibit myocyte contractility (Vejstrup *et al.*, 1998). The resulting increase in NO production from cardiac myocytes and other cell types further impairs ventricular function by increasing intracellular cGMP or by altering specific sarcolemmal ion channels (Ungureanu-Longrois *et al.*, 1995; Kelly *et al.*, 1996; Campbell *et al.*, 1996; Gross *et al.*, 1996; Kelly and Smith, 1997).

TNF_α induced desensitization of myofilaments to intracellular calcium is possibly mediated by NO. It has been demonstrated that TNF-induced, sphingosine-mediated disruption of calcium induced calcium release occurs early and NO mediates TNF_α induced desensitization of myofilaments to calcium (Gurevitch *et al.*, 1996; Vila-Petroff *et al.*, 1999) (Figure 2.5.1). Depressed myocardial contractile responses to Ca^{2+} have also been reported in pathological settings such as myocardial infarction and allograft rejection, where there is abundant expression of iNOS in macrophages infiltrating the myocardium (Yang *et al.*, 1994; Worrall *et al.*, 1995; Suzuki *et al.*, 1996).

2.5.1 cGMP-dependent pathway

Studies have suggested that NO is capable of inducing either a positive or negative inotropic response in cardiac myocytes. The cGMP-activated pathway is involved in the molecular mechanisms by which NO influences myocardial performance (Ignarro, 1990). NO and cGMP can decrease the cardiac myocyte L-type calcium current and contraction through activation of cGMP-stimulated cAMP phosphodiesterase (Balligand and Cannon, 1997). There is therefore a decrease in intracellular cAMP levels, initiating a lowered cAMP dependent protein kinase activity (PKA), which in turn alters the phosphorylated state of several target proteins. These include the α-subunit of the L-type calcium channel (Mery *et al.*, 1993; Han *et al.*, 1994 Han *et al.*, 1995; Han *et al.*, 1996). The down-regulation of calcium current is induced by the phosphorylation of the channel or poshosphorylation of an intermediate protein, namely cAMP phosphodiesterase (PDE), opposing the effect of PKA (Mery *et al.*, 1993). In addition cGMP dependent protein kinase (PKG) also decreases myofilament sensitivity to calcium, thereby promoting relaxation (Balligand and Cannon, 1997). On the other hand it has been suggested that this inhibitory effect of PKG may be due to the direct phosphorylation of the Ca^{2+} channel or an associated regulatory protein. There may be a balance between activities of PKA and PKG in regulating the Ca^{2+} channels at two separate sites (Sumii and Sperelakis, 1995).

A recent study has also suggested that NO is capable of inducing either a positive or negative inotropic response in cardiac myocytes depending on the concentration of NO donor used, in the presence or absence of specific inhibitors of soluble guanylyl cyclase (sGC), PKG and PKA. Vila-Petroff and colleagues (1999) demonstrated that although the decrease in contractile response observed at higher levels of NO can be attributed mainly to a cGMP-dependent reduction in myofilament responsiveness to

Ca^{2+}, the enhanced contractile response at lower NO levels is due to increased intracellular cAMP levels. These levels are possibly mediated in part through a novel NO-dependent, cGMP independent activation of adenylyl cyclase (AC) (Vila-Petroff *et al.*, 1999). It therefore seems that the NO levels being generated may be critical, and we know that iNOS generates high levels of NO. We therefore expect that these high levels would reduce the contractile function of the heart.

2.5.2 cGMP-independent pathway

Some or all of the cGMP-dependent pathways coexist in some cells (Wang and Lipsius, 1995; Han *et al.*, 1996; Kojda *et al.*, 1996), and the predominance of any one to produce the observed effect may vary according to the species used or the region of the heart, as well as the stimulus and the experimental conditions used (Balligand and Cannon, 1997). However, NO or its redox-related derivatives may also regulate Ca^{2+} channel function and cardiac contraction through mechanisms independent from cGMP.

NO produces oxidants such as peroxynitrite and it has been demonstrated that peroxynitrite inhibits enzymes involved in the citric acid cycle, such as the interaction between cis-aconitase and iron-sulfur clusters (Beckman and Koppenol, 1996; Hausladen and Fridovich, 1994). Reduced oxygen consumption in muscle slices has been reported where NO has presumably inhibited mitochondrial electron transfer, an effect that was reproduced in neonatal rat ventricular myocytes treated with IL-1β (MacDonald and Moss, 1993; Shen *et al.*, 1994; Shen *et al.*, 1995; Oddis and Finkel, 1995). It is suggested that this effect could involve the inactivation of the heme moiety of cytochrome *c* oxidase by NO (Torres *et al.*, 1995) (Figure 2.5.1).

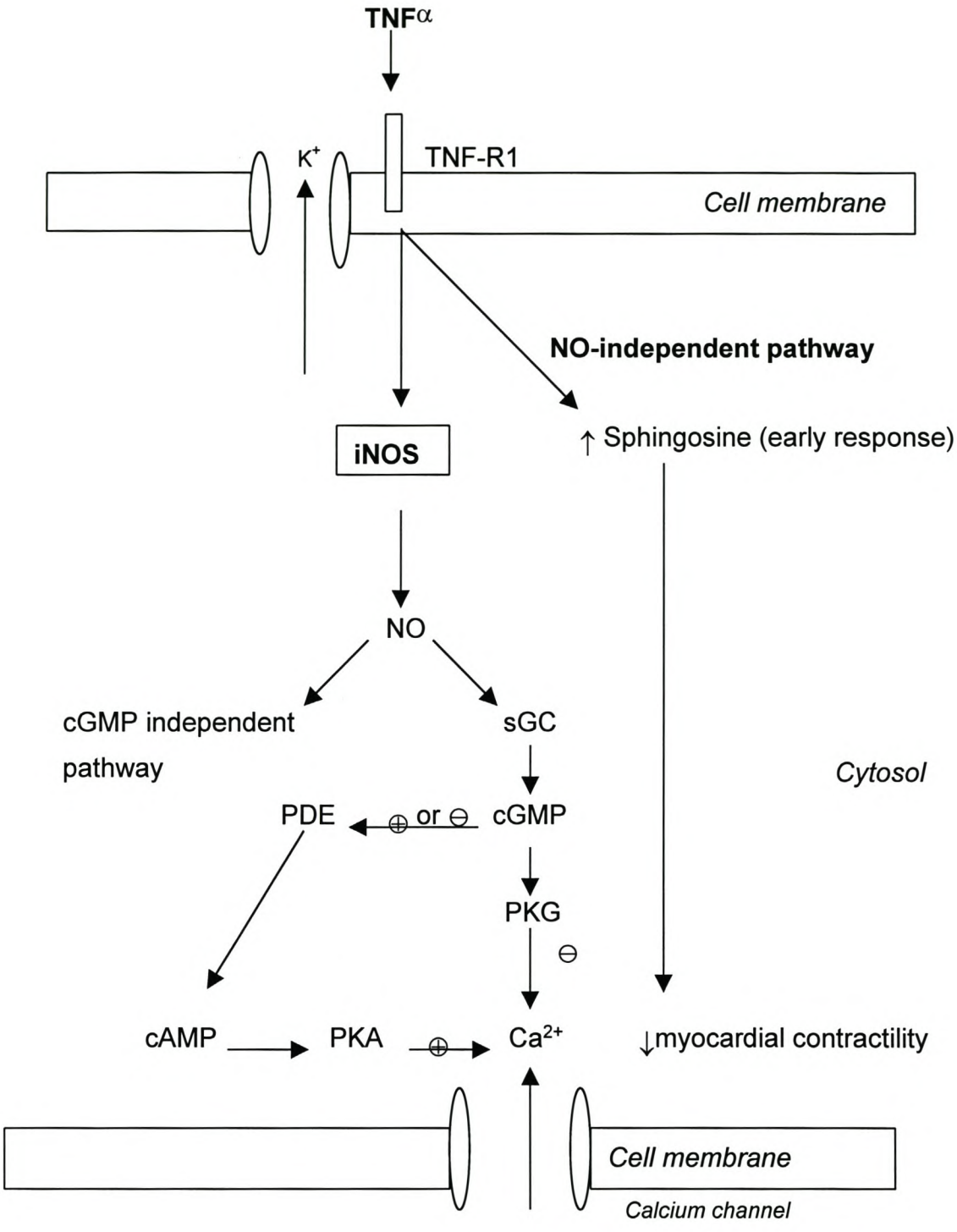


Figure 2.5.1: Simplified signalling pathway for the action of NO in cardiac myocytes. These include: Tumor necrosis factor alpha (TNF_{α}), inducible nitric oxide synthase (iNOS), soluble guanylyl cyclase (sGC), phosphodiesterase (PDE), and cAMP dependent protein kinase (PKA). (Modified from Balligand and Cannon, 1997; Meldrum, 1998).

2.5.3 TNF_{α}, iNOS and apoptosis

Programmed cell death (apoptosis) is a process by which cells undergo inducible non-necrotic cellular suicide. In contrast with necrotic cell death, programmed cell death is dependent on the de novo synthesis of proteins that initiate a cellular suicide program in response to specific stimuli (Steller, 1995; Thompson, 1995; Vaux and Strausser, 1996). For most cells of hematopoeitic lineage, apoptosis is a constitutive process but can also be induced by noxious stimuli (Ayala *et al.*, 1996). It has become apparent that apoptosis contributes to the pathophysiology of neurodegenerative diseases, autoimumune diseases, acquired immunodeficiency syndrome, cancer and a number of myocardial diseases (Steller, 1995; Thompson, 1995; Vaux and Strausser, 1996).

Cardiac myocyte apoptosis occurs in chronic heart failure, ischaemia, arrhythmogenic right ventricular dysplasia, myocarditis and sudden cardiac death (Gottlieb *et al.*, 1994; Packer, 1995; Krown *et al.*, 1996; Olivetti *et al.*, 1997). Apoptosis is characterized by cell death, but the cell membrane integrity is maintained. Therefore apoptotic cardiac myocytes do not release creatine kinase and retain their ability to exclude dyes such as trypan blue (Kajstura *et al.*, 1996). It has been proposed that cardiac myocytes also retain their ability to contract in response to calcium inophores (Krown *et al.*, 1996). By contrast, necrosis is associated with a high influx of calcium where myocytes are maximally contracted and unable to contract further in response to a calcium inophore (Meldrum *et al.*, 1996).

Studies have shown that chronic TNF_{α} production in the heart may lead to permanent functional impairment and apoptosis via NO-dependent and independent pathways (Szaboles *et al.*, 1996; Haywood *et al.*, 1996; Natoli *et al.*, 1997). When

TNF_{α} binds to its receptor, a number of TNF receptor-associated proteins assemble and associate to the cytoplasmic end of TNF receptors. These include TRADD, which recruits FADD (also known as MORT1) protein and TRAF2. TRAF2 interacts with TRADD and an N-terminal ring finger required for signaling the activation of $NF\text{-}_{K}B$ and JNK/SAPK (Rothe *et al.*, 1995; Liu *et al.*, 1996; Natoli *et al.*, 1997). Activation of TRADD is also associated with the recruitment and activation of apoptotic proteases with subsequent progression to programmed cell death (Sack *et al.*, 2000) (Figure 2.3.3.1).

TNF_{α} also induces iNOS release and NO induced cardiac myocytes apoptosis may possibly be mediated by a cGMP independent mechanism, namely via the generation of oxygen-derived free radicals such as peroxynitrite (Ing *et al.*, 1999). However, it remains unclear as to how and to what extent NO itself, or peroxynitrite, contributes to iNOS-dependent cell death in cardiac myocytes (Arstall *et al.*, 1999).

Several studies have shown that NO donors can trigger apoptosis in macrophages, vascular smooth muscle cells and cardiac myocytes (Albina *et al.*, 1993; Cui *et al.*, 1994; Shimaoka *et al.*, 1995; Pinsky *et al.*, 1995; Nishio *et al.*, 1996). *In vivo* studies have also demonstrated that apoptosis of macrophages and cardiac myocytes occurs in parallel with iNOS induction in experimental models of cardiac allograft rejection and myocardial infarction (Szaboles *et al.*, 1996; Suzuki *et al.*, 1996). NO produced by iNOS may diminish cardiac function as a result of cell death, which is possibly triggered by apoptosis (Beckman and Koppenol, 1996; Szaboles *et al.*, 1996).

There is evidence that programmed cell death of myocytes occur in the decompensated human heart, although there is an enhanced expression of Bcl-2, the proto-oncogene, which protects cells from apoptosis (Gravanis *et al.*, 1994; Szaboles

et al., 1996; Olivetti *et al.*, 1997). It has also been demonstrated that some cytokines (IL-1β and IFNγ) induce apoptosis associated with an increased Bax relative to Bcl-2 expression in isolated neonatal rat ventricular myocytes (Ing *et al.*, 1999; Arstall *et al.*, 1999).

2.6 Ischaemia/reperfusion injury

Ischaemia by definition exists whenever the flow of arterial blood through coronary blood vessels is reduced to a volume below that required by the myocardium for normal function. The myocardium is therefore deprived of oxygen, and a shift from aerobic to anaerobic metabolism occurs (Jennings 1970, Jennings and Yellon 1992). The severe and sustained reduction in blood flow to the myocardium reduces the oxidative phosphorylation leading to failure to resynthesize energy-rich phosphates such as ATP and creatine phosphate. With this profound reduction in energy stores active tension generation is decreased. As a result ion homeostasis is lost, causing a leakage of K^+ into the extracellular environment and Ca^{2+} into the cytoplasm. These changes contribute to the electrical instability in the cells and a failure of relaxation (Maxwell and Lip, 1996).

Ischaemia induced alterations in the myocardium strictly depend on the duration and severity of ischaemia. Prolonged myocardial ischaemia inevitably results in myocardial death (Buerke *et al.*, 1999). Acute myocardial ischaemia results in an intense inflammatory reaction (Engler *et al.*, 1986; Jones *et al.*, 1999).

Reperfusion is defined as the full restoration of blood flow to the ischaemic area. However experimental models have demonstrated that reperfusion can inflict microvascular and myocyte damage, which compromises return of normal coronary

perfusion and cardiac function (Ryan *et al.*, 1990; Knabb *et al.*, 1987). The resultant reperfusion injury was originally defined as the metabolic, functional and structural consequence of restoring coronary artery flow that can be avoided or even reversed by the modification of the conditions of reperfusion (Rosenkranz and Buckberg, 1983). Yet despite the risk of exacerbating the ischaemic injury, it is an absolute prerequisite for the survival of ischaemic tissue (Hearse, 1977; Braunwald and Kloner, 1985; Becker and Ambrosio, 1987; Opie, 1989).

Myocardial ischaemia/reperfusion injury is exacerbated by cytosolic Ca^{2+} overload (Du Toit and Opie, 1992). Ischaemia/reperfusion also generates high levels of free radicals composed of both reactive oxygen intermediates and NO (Kilgore and Lucchesi, 1993). When these are generated in sufficient concentrations, free radicals directly injure the myocardium and may even cause cell death. Free radicals also activate redox-sensitive transcription factors, including NF-$_K$B, and trigger the expression of IL-1β, TNFα and other proinflammatory mediators (Mercurio *et al.*, 1997; Mercurio and Manning, 1999; Bowie and O'Neill, 2000; Bonizzi *et al.*, 2000).

Although high levels of NO may contribute to certain cardiovascular disorders, there is experimental evidence to suggest that low levels of NO may be essential for normal cardiac homeostasis. It plays a key role in normal myocardial physiology, and it is suggested that at low physiological concentrations NO may protect myocytes from deleterious stimuli such as mechanical stress, ischaemia and norepinephrine (Sharma *et al.*, 2000). The mechanisms involved in NO protection during mechanical stress and norepinephrine administration remain unclear (Sharma *et al.*, 2000). Du Toit and colleagues (1998) demonstrated that stimulation of the NO-cGMP pathway during severe ischaemia with NO-donors protected the heart against ischaemic and

reperfusion injury, possibly by decreasing cytosolic Ca^{2+} overload during ischaemia and reperfusion.

On the other hand studies have also shown that a NO-inhibitor L-NAME reduces infarct size (Patel *et al.*, 1993; Woolfson *et al.*, 1995). The mechanisms by which NO results in these contrasting effects may involve decreases and increases in oxidative stress, respectively (Sawyer and Colucci, 1998). Curtis and Pabla (1997) suggest that the protective effects of some NOS inhibitors may occur by NO-independent mechanisms.

2.6.1 TNF_{α} and ischaemia/reperfusion injury

There are three main theories as to why there are high levels of TNF_{α} in CHF. One hypothesis is that the heart is the main source of this cytokine, since it has been shown that the failing myocardium is capable of producing TNF_{α} (Torre-Amione *et al.*, 1996). *In vivo* studies have also reported that haemodynamic pressure overload is capable of stimulating TNF_{α} mRNA synthesis (Kapadia *et al.*, 1997). The second hypothesis is that the bowel wall oedema, which occurs in CHF is responsible for bacterial translocation with subsequent endotoxin release and immune activation (Anker *et al.*, 1997). The third hypothesis is that hypoxia may be a stimulus for increased TNF_{α} production in CHF patients (Hasper *et al.*, 1998).

Although little is known about the mechanisms of ischaemia and reperfusion induced myocardial TNF_{α} production (Gurevitch *et al.*, 1996), it has been established, that reperfusion of the ischaemic myocardium imposes an oxidant burden on the myocardium in which the reduction product of molecular oxygen and hydrogen peroxide contribute to myocardial injury (Brown *et al.*, 1988). Hydrogen peroxide

induces activation of p38 MAP kinase, and this may also contribute to the ischaemia-reperfusion induced TNF_{α}-production (Guyton *et al.*, 1996; Huot *et al.*, 1997). Oxidant stress may also activate $NF_{K}B$, involved in the sequence of ischaemia/reperfusion induced TNF_{α} production.

In most cells, $NF_{K}B$ exists in a latent state, unable to induce TNF_{α} production (Mallinin *et al.*, 1997). While in this latent state, $NF_{K}B$ is bound to its inhibitory proteins, called Inhibitory $_{K}B$ ($I_{K}B$). After activation by ischaemia-reperfusion, phosphorylation of $I_{K}B$ results in the disruption of the $NF_{K}B$-$I_{K}B$ complex and the degradation of $I_{K}B$ (Li and Sedivy, 1993; Trede *et al.*, 1995; Sweet and Hume, 1996). Liberated $NF_{K}B$ then translocates from the cytoplasm to the nucleus, where it docks to DNA at one of four $NF_{K}B$ sites (TNF promoter region) (Shakov *et al.*, 1990). Evidence shows that $NF_{K}B$ is activated directly either by phosphorylation of $I_{K}B$ or by oxidant stress, after which it translocates to the nucleus to activate TNF gene transcription (Meldrum, 1998) (Figure 2.6.1.1).

The association of elevated inflammatory cytokine levels with a poor prognosis in heart failure, together with their known adverse effects on myocardial structure and function, suggests that cytokines are likely to be detrimental in this condition (Suffredini *et al.*, 1989; Torre-Amione *et al.*, 1995; Torre-Amione *et al.*, 1996). Studies have suggested that the increased levels of TNF_{α} following ischaemia/reperfusion contribute to infarct development, and the inflammatory response causes a degree of myocyte loss (Hansen, 1995; Gurevitch *et al.*, 1997). However it has been established that the primary function of the proinflammatory response is to protect the body from infections and other stresses to which it is exposed. It is only when the

inflammatory response becomes overactive or is inappropriately stimulated, that it begins to have harmful effects.

It has been proposed that cytokines expressed within the myocardium in response to environmental injury, may be important initiating and integrating homeostatic responses within the heart (Mann, 1996; Sharma *et al*., 2000). Therefore it is suggested that the short-term expression of inflammatory cytokines may provide the myocardium with an adaptive response to stress, whereas the long-term expression of these molecules may be maladaptive by producing cardiac decompensation (Sack *et al*., 2000).

Nelson and colleagues (1995) demonstrated that pretreatment of rabbits with intravenous TNF_{α} 24 hours before ischaemia and reperfusion resulted in improved cardiac contractile functional recovery. Sack and colleagues (2000) confirmed these results, but induced milder ischaemia with less production of TNF_{α}, allowing a positive response to the added low concentrations of TNF_{α} in the myocardium. It was therefore suggested that the known production of TNF_{α} by the myocardium in response to an ischaemic/reperfusion insult, may in fact be an endogenous pathway activated by the heart to induce short-term intrinsic cardioprotection against subsequent ischaemia/reperfusion injury. Conversely, Nutt and colleagues (1998) demonstrated that cardiac contractile function was markedly attenuated at high concentrations of TNF_{α} infusion. It remains controversial whether TNF_{α}'s role in inducing an acute reduction of contractility is adaptive and confers cardioprotection or whether it is maladaptive leading to long-term myocardial dysfunction during ischaemia and reperfusion. However, to our kowledge, the effects of TNF_{α} from

other sources such as AT-TNF$_\alpha$ during ischaemia and reperfusion have not been documented.

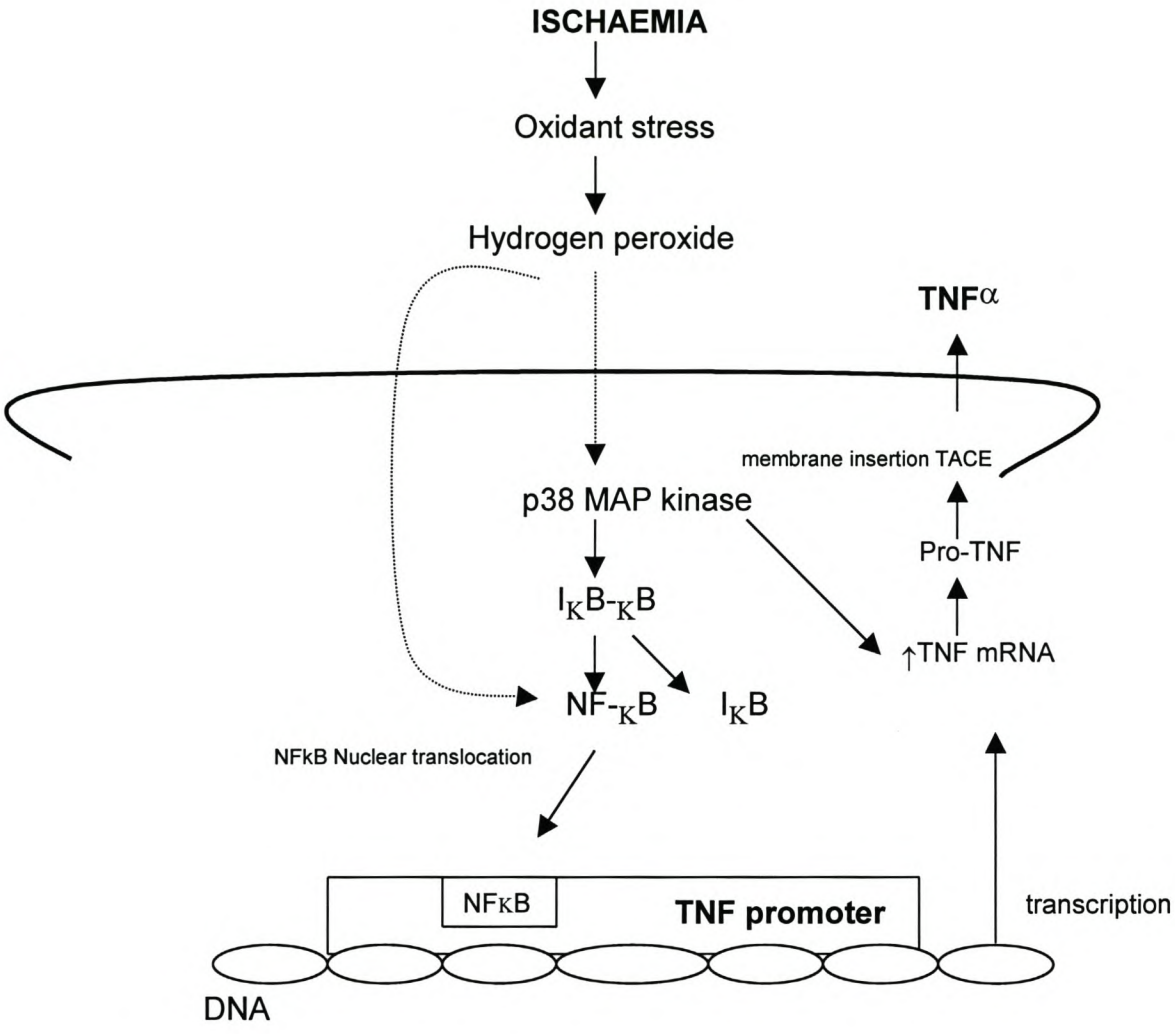


Figure 2.6.1.1: TNF$_\alpha$ synthesis in myocytes. Ischaemia/reperfusion, oxidant stress, and hydrogen peroxide directly activate p38 MAPK and NF$_K$B, to induce TNF$_\alpha$ production. Once transcription to pro-TNF$_\alpha$ occurs in the cytosol, myristoylation permits membrane insertion, where pro-TNF remains until it is cleaved to its mature form by TNF$_\alpha$ -converting enzyme (TACE) (modified from Meldrum, 1998).

Chapter 3

MATERIALS AND METHODS

3.1 Experimental Model

3.1.1 Rat Diet

Human obesity is often associated with a Western lifestyle, which includes the availability of highly palatable foods. A model of diet induced obesity (DIO) in the rat (Pickavance *et al.*, 1999) was therefore chosen for this study. The DIO rat model is characterized by hyperphagia, increased thermogenesis, hyperleptinaemia and mild insulin resistance (MCormack *et al.*, 1989; Wilding *et al.*, 1992; Widdowson *et al.*, 1997).

The diet used contained a slightly elevated carbohydrate content, and for the purpose of this study was referred to as a high caloric diet. The diet provided 65% of the energy in the form of carbohydrates, 19% as protein and 16% as fat. This diet was designed to induce hyperphagia, without employing major changes in macro-nutrient composition, when compared with normal rat chow. This model was found reliable for inducing weight gain and mild insulin resistance (Wilding *et al.*, 1992; Widdowson *et al.*, 1997; Pickavance *et al.*, 1999). The control rats were fed a rodent pellet chow (standard rat chow), which provided 60% of energy in the form of carbohydrates, 30% as protein and 10% as fat.

3.2 Indices for Identifying Cardiac Hypertrophy

3.2.1 Heart weight to body weight ratio

The use of the heart weight to body weight ratio to identify cardiac hypertrophy was established by Walter and Addis in 1939, and has subsequently been used as an index of cardiac hypertrophy in other studies (Villa *et al.*, 1998; Wambolt *et al.*, 2000).

3.2.2 Heart weight to tibia length ratio

This parameter offers another method of identifying cardiac hypertrophy and is one that is physiologically related to the metabolic demands placed on the heart by the body and reflects relative lean body size throughout life. The tibia length in rats change minimally beyond maturity even though the epiphysis does not close. The tibia growth curve therefore appears to be independent of the presence or absence of gross pathological lesions appearing during the lifetime of the rat and seems to be independent of changes in the body weight (Berg and Harmison, 1958). Yin and colleagues (1982) only managed to apply the tibia length to heart weight ratio as a parameter for cardiac hypertrophy in older (fully grown) rats. This method may therefore be used as an alternative parameter for quantifying hypertrophy in fully grown rats.

3.3 Animals

Male Sprague-Dawley rats were weaned at 3 weeks and put on a high caloric diet for 12 weeks. The control animals had free access to standard rat chow and water, while those on the high caloric diet had unlimited access to food containing 33% chow, 33% condensed milk, 7% sucrose and 27% water, and drinking water. They were maintained in animal quarters at a constant temperature (22^0C) and humidity (40%) with a 12-hour day/night cycle. The South African Medical Research Council's guide

for the humane use of laboratory animals was followed throughout.

3.4 Measurements made during the study

3.4.1 Food consumption

Food intake was measured daily for both the groups and expressed in grams of food consumed per week (grams/week).

3.4.2 Body weight gain

Animals were weighed weekly and their weights documented. Their respective body weight gain is expressed in both weight gain/week and total weight gain over 12 weeks.

3.4.3 Heart weight

At the end of the perfusion protocol, the hearts were carefully removed from the perfusion apparatus, dried off and incisions were made through the atria to the ventricles. Hearts where then blotted on paper towel to remove excess water and perfusion buffer. Heart weights are expressed in milligrams. The heart weight to body weight ratio was determined and used as an index of cardiac hypertrophy. The tibia length was measured in centimeters using a vernier. These lengths were also expressed relative to heart weight and used as an index of cardiac hypertrophy.

3.4.4 Glucose levels

Blood glucose levels were measured in non-fasted animals with an Accutrend ® blood glucose analyser and Accutrend® glucose test strips (Boehringer Mannheim, Germany).

3.4.5 Insulin

An Insulin solid phase ^{125}I radioimmunoassay (COAT-A-COUNT®, Diagnostic Products Corporation, Los Angeles, CA) was used to measure insulin in the serum

3.4.6 Lipid profiles

Lipid profiles were determined by spectrophotometric analysis (Technicon RA 1000 Auto analyser) using Technicon assay reagents, measuring in mmol/l plasma Cholesterol, HDL cholesterol and plasma TAG. LDL cholesterol quantification requires a more specialized assay procedure (beta-quantification). We therefore chose not to measure LDL cholesterol in this study.

3.5 Heart perfusions

3.5.1 The isolated Rat Heart Model

The system consists of two parts; the Langendorff perfusion side, used to perfuse the heart in a retrograde fashion, and the working heart side used to perfuse the heart in the working heart mode. The retrograde Langendorff perfusion apparatus was used to allow the hearts to stabilize at the beginning of the experiment and during reperfusion. The apparatus was first described by Langendorff in 1895, and later modified by Neely and co-workers (1967). In order to measure mechanical function of the heart, the working heart model was used.

3.5.2 Perfusion Solution

The perfusion solution was a crystalloid Krebs- Henseleit buffer, containing in mM: NaCl 118,46; $NaHCO_3$ 24,995; KCl 4,748; KH_2PO_4 1,185; $MgSO_4.7H_2O$ 1,19 and $CaCl_2.2H_2O$ (Merck) 1,25 and glucose 10. The solution was gassed with 95% O_2, 5%

CO_2 for 20 minutes before and throughout the experiment. The buffer was prepared on the day of use and filtered through a 0,45 μm Millipore filter.

3.5.3 Organ Isolation

The rats were anaesthetised by an intraperitoneal injection with Intramed Thiopentone sodium (sodium pentobarbital) 0,1mg/g. Once under anaesthesia, their hearts were rapidly excised and placed in ice-cold Krebs-Henseleit solution to minimize ischaemic injury while being transferred to the perfusion apparatus. During this time blood and adipose tissue was collected from the animal. The blood was centrifuged and immediately frozen, while the peri-renal adipose tissue was frozen in liquid nitrogen.

The isolated heart was then mounted on the aortic cannula and perfused with an oxygenated normothermic Krebs-Henseleit buffer in the Langendorff mode within 60 seconds of excision from the animal. All excess non-cardiac tissue was removed from the heart. The left atrium was cannulated. The initial Langendorff perfusion period lasted 10 minutes during which the heart was allowed to stabilize and blood and other enzymes were washed out. A Sarns Model 5500 pump kept the buffer in the resevoir at a constant level so as to maintain the perfusion pressure at 100cm H_2O.

After this initial period the heart perfusion system was switched to the working heart mode. The perfusate temperature was maintained at 37.0 ^{0}C by means of a water-bath (Thermomix 1460, B. Braun Melsungen, Germany). A thermistor probe was inserted into the right coronary sinus to monitor myocardial temperature before, during and after ischaemia. The atrial perfusion pressure (preload) was maintained at

a perfusion pressure of 20 cmH_2O while the afterload was 100 cmH_2O. Heart rate, coronary flow, aorta systolic and diastolic pressures and aortic output were measured every 5 minutes during the experiment. The left ventricular pressure was monitored, using a pressure transducer connected to a BP Amp, GSR Amp and stimulus isolator (Powerlab, AD Instruments, Australia). After each experiment the hearts were freeze clamped with Wollenberger metal tongs pre-cooled in liquid nitrogen and stored in a -64 ^0C freezer for biochemical analysis.

3.6 Experimental Protocol

3.6.1 Myocardial functional data collection

Myocardial functional data was collected after animals were on the diet for 12 weeks:

Pre-ischaemic perfusion (0-30 minutes): During the pre-ischaemic phase, hearts were perfused in the Langendorff mode for 10 minutes for stabilization. They were then switched to the working heart mode for 20 minutes. During the working heart mode, functional data was documented at 5 minute intervals. (n=10)

Ischaemia (30-45 minutes): Hearts were subjected to 15 minutes total global ischaemia and myocardial temperature was maintained at 36^0C.

Reperfusion (45-75 minutes): The hearts were reperfused for 10 minutes in the Langendorff mode before being switched over to the working heart mode for a further 20 minutes. Functional data was documented at 5 minute intervals.

3.6.2 Myocardial tissue sample collection

Tissue samples were collected from a separate series of animals after 6 weeks, 9 weeks and 12 weeks for biochemical analysis.

Sample collection times

After 12 weeks on the diet:

a) Hearts were perfused under standard conditions, 10 minutes in Langendorff mode and 20 minutes in working heart mode and then freeze clamped for storage at -64^{0}C. (n=7)

b) Hearts were again perfused under standard conditions, 10 minutes in Langendorff mode and 20 minutes in working heart mode. These hearts were also subjected to total global ischaemia (at 36^{0}C). At the end of ischaemia the hearts were freeze clamped and stored at -64^{0}C.

After 6 weeks and 9 weeks on the diet:

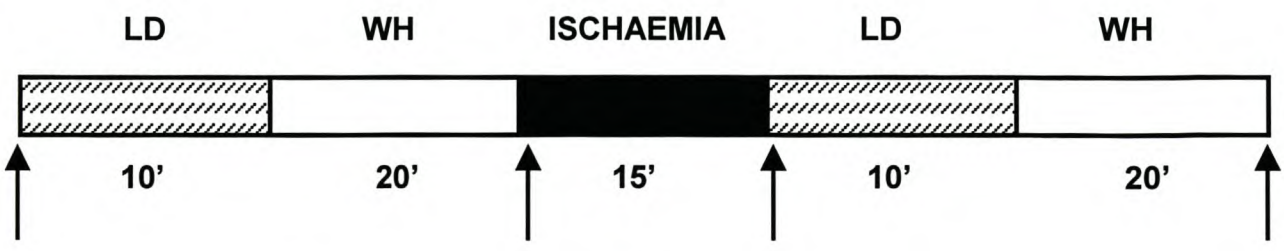
Hearts were perfused under standard conditions, 10 minutes in Langendorff mode and 20 minutes in working heart mode. The hearts were freeze-clamped and stored at -64^{0}C.

3.6.3 Perfusion Protocol

LD = Langendorff mode

WH = Workheart mode

↑ = Time points at which hearts were freeze-clamped



3.7 Myocardial Function

In order to compare the pre-ischaemic and reperfusion function of the hearts in the different groups, the heart rate (beats/min), coronary flow (ml/min), aortic output (ml/min), aorta diastolic and systolic pressure and temperature (^{0}C) were monitored at 5 minute intervals before and after ischaemia.

Coronary flow (ml/min) and aortic output (ml/min) were measured manually by collecting the coronary effluent and aortic output over a known time using a measuring cylinder.

Aorta diastolic and systolic pressures (mmHg), and total work (mW) were recorded on the Powerlab data collection system connected to a pressure transducer linked to the aortic cannula of the working heart perfusion apparatus (AD Instruments, Castle Hill, Australia).

3.8 Exclusion Criteria

Hearts with heart rates less than 180 beats/min or more than 330 beats/min, aortic output of less than 30 ml/min and coronary flow of more than 25 ml/min were excluded from the study at the beginning of the perfusion experiment. These values were adapted for this study as the obese animals had poor heart functions and often had high coronary flows due to their extreme heart size.

3.9 Biochemical analysis

3.9.1 Insulin determination

The assay for insulin determination is based on the procedure wherein ^{125}I labeled insulin competes for a fixed time with insulin in the serum for sites on insulin-specific

antibody. Since the antibody is immobilized to the wall of a polypropylene tube, simply decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radiolabeled insulin. Counting the tube in a gamma-counter then yields a value, which is converted by way of a calibration curve to measure the insulin present in the sample in micro International Units/ml (μIU/ml) (Paternostro *et al.*, 1999).

.

Assay procedure for insulin determinations

Four polypropylene tubes were labeled for total counts (T) and nonspecific binding (NSB) in duplicate. Fourteen insulin Ab-Coated Tubes A were labeled (maximum binding) and B through G in duplicate (polypropylene tubes coated with antibodies to insulin). Additional antibody-coated tubes were labeled for samples, also in duplicate. 200μl of the zero calibrator A (lyphilized processed human serum diluted in 6ml distilled water) was pipetted into the NSB and A tubes, and 200μl of each remaining calibrator and unknowns were added to each tube. Then 1ml of ^{125}I Insulin was added to every tube and vortexed. The tubes were incubated for 18-24 hours at room temperature, and following this step the tubes were decanted thoroughly. All tubes were counted for 1 min in a gamma counter.

3.9.2 TNFα determination

Serum preparation

Blood was collected from the animals and centrifuged at 1000g for 10 minutes at 4^{0}C within 30 minutes. The supernatant was removed and stored in a freezer at -64 ^{0}C. Prior to use, serum samples were thawed and equilibrated with room temperature.

Myocardial tissue homogenization procedure for TNF α determination

Freeze-clamped hearts were homogenized in 5 volumes of cold lysis buffer containing in mM/L: imidazole acetate 50, magnesium acetate 10, KH_2PO_4 4, EDTA 2, in μM/L: N-acetylcysteine 50, Sulphur 12,5 (pH 7.6) (Meldrum *et al.*, 1998).

The homogenates were spun down in a microfuge at 4°C for 15 minutes to remove particulate matter. The lysate protein content was determined using the Bradford technique.

Adipose tissue homogenization procedure for TNF_α determination

Peri-renal adipose tissue was removed and immediately frozen in liquid nitrogen for storage and later processing. 200mg of frozen adipose tissue was homogenized in 400 μl of homogenization buffer containing 10 mmol/l TRIS-HCl, 250 mmol/l sucrose and 5μg/5ml protease inhibitor (general). The homogenates were spun down in a microfuge at 4°C for 15 minutes to remove particulate matter and the supernatant was recovered for cytokine level determinations (Bastard *et al.*, 2000).

TNF_α Assay Procedure

The levels of TNF_α were determined using the OptEIA ™ – ELISA kit (PharMingen USA, Lot number M065951). The Cytokine sandwich ELISA is a sensitive enzyme immunoassay that can specifically detect and quantitate the concentration of soluble cytokine.

Microwells were coated with 100μl per well of Capture Antibody *(Anti-rat TNF_α)* diluted in coating buffer (1:250) containing 0.1M Carbonate, pH 9.5. The plate was incubated overnight at 4°C. During this period the coating antibody is adsorbed onto

the plastic microwells because of hydrophobic interactions. The following day the wells were aspirated and washed 5 times with 300 µl/well wash buffer (Phosphate-buffered saline with 0.05% Tween-20) and after the last wash inverted and blotted on absorbent paper to remove any residual buffer. This washing procedure was followed throughout the experiment. After the plate washings, the immobilized antibodies serve to specifically capture soluble cytokine proteins present in the samples.

The plates were then blocked with 200µl/well Assay diluent (animal serum in buffered solution with 0.15% ProClin-150 as preservative, PharMingen, USA) and incubated for one hour at room temperature. During this period of incubation the standards and samples were prepared. After the incubation period wells were washed again with PBS-Tween 20 for 5 times. 100µl of each standard and sample were pipetted carefully into the appropriate wells, the plate was sealed and finally incubated at room temperature for two hours after which the wells were aspirated and washed 5 times.

After washing away unbound material, the captured cytokine was detected by biotin-conjugated anti-cytokine antibodies (termed Detection antibodies), therefore in the next step 100µl Detection Antibody *(Biotinylated anti-rat TNFα)* diluted in Assay diluent (1:250) was added to each well, the plate was sealed and incubated for one hour at room temperature. The plate was again washed 5 times with wash buffer.

100µl of Enzyme Reagent *(Avidin-horseradish peroxidase conjugate)* diluted in Assay diluent (1:250) was added to each well and the plate was sealed and incubated for 30 min at room temperature. After this the plate was washed 7 times

with wash buffer and each well was allowed to soak in the buffer for 30 seconds to 1 min.

100μl of TMB (equal volumes of Substrate Reagent A containing hydrogen peroxide in a buffered solution and Substrate Reagent B containing 3,3',5,5' tetramethylbenzidine in organic solvent, BD PharMingen USA) substrate solution, which is a chromogenic substrate containing solution, was added to each well and allowed to incubate in the dark, unsealed, for 30 min. Finally 50μl of Stop solution (2 N H_2SO_4) was added to each well. The intensity of the coloured product generated by the bound, enzyme-linked detection reagent was measured spectrophotometrically using an ELISA-plate reader at a wavelength of 450 nm within 30 minutes of adding the Stop solution. The cytokine concentrations of the samples were interpolated from the standard curve obtained from the standards used in the assay. The levels of TNF_α in each sample are expressed in pg/gram ww for adipose and myocardial tissue (Meldrum *et al.*, 1998).

3.9.3 cGMP determination

The myocardial cGMP levels were measured using the cGMP (^{125}I) assay system with Amerlex – M^{TM} magnetic separation (Amersham International plc, Amersham UK). The assay is based on the competitive binding between the unlabelled cGMP and a fixed quantity of ^{125}I-labelled cGMP for a limited number of binding sites on a cGMP-specific antibody.

With known amounts of antibody and reactive ligand, the amount of radioactive ligand bound by the antibody will be inversely proportional to the concentration of added non-radioactive ligand. The cGMP that is bound to the antibody is then

reacted with the Amerlex-M secondary antibody reagent, which contains secondary antibody that is bound to magnetizable polymer particles. Separation of this bound fraction is affected by either magnetic separation, or centrifugation of the suspension and decantation of the supernatant. The amount of the cGMP in the bound fraction can then be calculated once the radioactivity in the pellet is measured. The concentration of the unlabelled cGMP in the sample is determined by interpolation of the standard curve obtained (Du Toit *et al.*, 1998).

Myocardial tissue homogenization procedure for cGMP determination

100 mg of frozen tissue was homogenized in cold 6% trichloroacetic acid at 4^0C to give a 10% w/v homogenate. This homogenate was then centrifuged at 2000g for 15 minutes at 4^0C. The supernatant was recovered and washed three times with 5 volumes of water saturated diethyl ether. The upper ether layer was removed by aspiration after each wash. The samples were then stored at 4^0C overnight for the remaining ether to evaporate.

cGMP Assay Procedure

On day 1 the standards and the Assay buffer containing 0.5M acetate buffer, pH5.8 with 0.01% w/v sodium azide was used to reconstitute all other reagents involved in the assay. Glass tubes (12x75mm) were labeled for the zero standard tube and the unknowns, collectively termed acetylation tubes. Polypropylene tubes were also labelled for total counts, zero standard, each standard dilution and unknowns.

The acetylation reagent was prepared by mixing 1 volume of acetic anhydride with 2 volumes of triethylamine in a glass vessel. 500μl of Assay buffer was pipetted into the zero standard acetylation tube, and 500μl of each unknown or standard was

added to the tubes. Following this step, 25μl of acetylation reagent was added carefully to all the acetylation tubes containing the standards and the unknowns. All tubes were immediately vortexed after addition of the reagent. Acetylated samples were diluted 1:6 with assay buffer.

In the following step, 100μl from each diluted acetylated sample was pipetted into the corresponding polystyrene assay tubes in duplicate. 100μl of antiserum containing rabbit anti-cGMP serum in 0.05M acetate buffer (1% w/v bovine serum albumin and 0.01% w/v sodium azide) was added to each assay tube except the tube for total counts. This procedure was followed by vortexing all the tubes thoroughly. Finally all the tubes were covered with foil and incubated at room temperature for one hour. After this incubation period, 100μl of [^{125}I]cGMP (guanosine 3',5'-cyclic phosphoric acid 2'-0-succinyl-3-[^{125}I] iodotyrosine methyl ester in 0.05M acetate buffer) was added to each assay tube, and all tubes were vortexed. The total count tubes were stoppered. Finally, the tubes were covered and incubated overnight (18hours) at 4^{0}C. The following day, 500μl of Amerlex-M secondary antibody reagent (donkey anti-rabbit Amerlex-M) containing sodium azide, was added to each tube except the total counts tube and vortexed thoroughly. These tubes were allowed to stand for 10 minutes at room temperature.

After this process all tubes were centrifuged for 10 minutes at 1500g, supernatant liquids carefully discarded and finally the tubes were placed inverted on absorbent tissues for 5 minutes to drain the remaining supernatants. The counts per minute of each tube was counted over one minute in a gamma scintillation counter (Du Toit *et al.*, 1998).

3.10 Statistical Methods

For the determination of statistical significance, the Unpaired Students t-test was used. Data are presented as means +/- standard error of the mean (SEM). $P< 0.05$ was considered as significant. Microsoft Excel '97 and GPIS statistical programs were used to perform all the tests.

The percentage aortic output recovery was measured as the post-ischaemic aortic output divided by pre-ischaemic aortic output and multiplied by one hundred. These values were obtained after 20 minutes reperfusion in the working heart mode.

Chapter 4

RESULTS

4.1 Biometric Data

There was no significant difference in the weekly body weight gain when comparing the HCD fed rats with the control rats. Weekly food consumption was significantly higher in the HCD fed animals when compared with the control animals. The total weight gain (over 12 weeks) of the HCD fed animals was significantly higher than that of the controls (Table 4.1.1, Figure 4.1.2).

Table 4.1.1: Biometric data for control and HCD fed animals

	Control	**HCD**
Body weight gain (g)/week	29.0 ± 3.4	39.1± 4.2
Food consumption (g)/week	173.2 ± 4.9	365.5 ± 5.0 *
Total weight gain (g)	271.5 ± 102.6	459.9 ± 173.8 *

*$p<0.05$, n=7-10

Blood glucose levels were higher in the HCD fed animals when compared to the controls (Table 4.1.2). The plasma cholesterol and HDL cholesterol levels were lower in the HCD fed animals compared with the control group, while the plasma TAG was significantly higher (Table 4.1.2). There was no difference in the non-fasted plasma insulin levels of control animals compared with HCD fed animals. Because blood glucose levels were higher in the HCD group compared with controls (Table 4.1.2), the HCD fed animals may have developed mild insulin resistance.

Table 4.1.2: Plasma lipid profiles and glucose and insulin levels after 12 weeks on the feeding program for control and HCD animals

	Control	**HCD**
Plasma chol. (mmol/l)	2.082 ± 0.062	1.794 ± 0.058 *
HDL chol. (mmol/l)	1.451 ± 0.050	1.207 ± 0.031 *
Plasma TAG (mmol/l)	0.503 ± 0.043	0.664 ± 0.062 *
Glucose (mmol/l)	7.8 ± 0.3	9.2 ± 0.3 *
Insulin (μIU/ml)	12.42 ± 5.06	12.87 ± 1.02

*$p<0.05$, n=7-10

HDL = high density lipoprotein

TAG = triacylglyceride

chol = cholesterol

HCD = high caloric diet

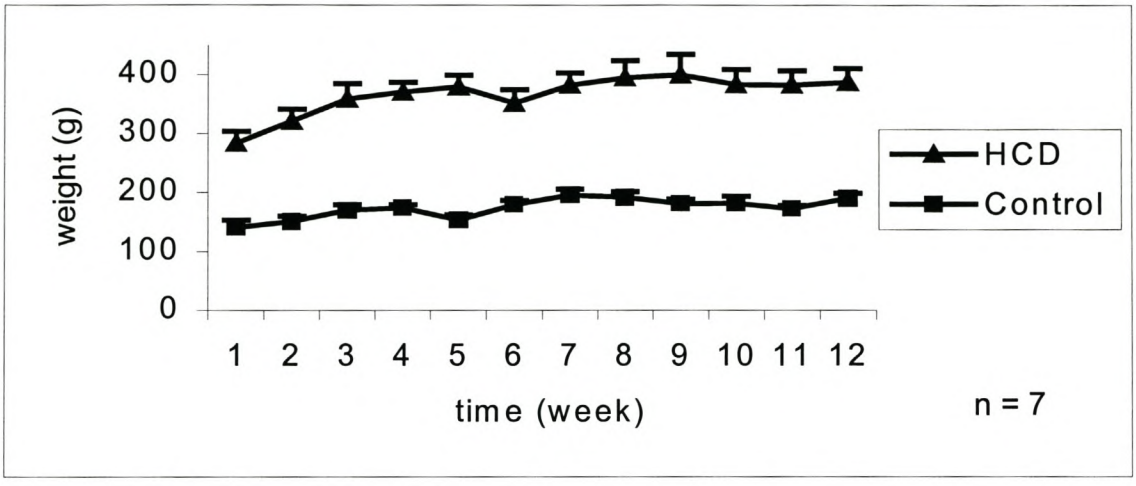


Figure 4.1.2: Weekly food consumption of control and HCD fed animals over 12 weeks.

The animals fed the high caloric diet consumed more food per week when compared with their concurrent controls. The food given to the HCD group was however in a wet form, while that of the control group was a dry pellet. The comparison of the quantity of food consumed should therefore be interpreted with caution.

4.2 Indices Used to Identify Cardiac Hypertrophy

When comparing the heart weight to body weight ratios of the control animals with that of the HCD animals, there was a significant difference between the two groups, suggesting the development of cardiac hypertrophy in the HCD animals. When employing heart weight to tibia length as an index of cardiac hypertrophy (Yin et al, 1982), there was no difference between the groups.

Table 4.2.1: Heart weight to body weight and heart weight to tibia length ratios for control and HCD fed animals after 12 weeks on the diet

	Control	**HCD**
HW/BW (mg/g)	3.7 ± 0.1:1	4.2 ± 0.1 :1*
HW/TL (mg/cm)	367.94 ± 3.58 :1	406.53 ± 17.42 :1

*$p<0.05$, n=7

HW = heart weight

BW = body weight

TL = tibia length

4.3 Cardiac Mechanical Function Data

a) Pre-ischaemic function

There was a significant difference between pre-ischaemic aorta systolic pressure (ASP) and heart rate when comparing control hearts with the HCD hearts. There was however no significant difference in the aorta diastolic pressures (Table 4.3.1, Figure 4.3.3, Figure 4.3.4 and Figure 4.3.5).

Table 4.3.1: Mechanical function for control and HCD hearts before ischaemia (after 20 minutes WH perfusion)

	Control	**HCD**
CF (ml/min)	20.0 ± 0.4	18.0 ± 1.0
ADP (mmHg)	68.68 ± 2.69	62.68 ± 1.17
ASP (mmHg)	100.75 ± 2.49	89.89 ± 2.36 *
HR (beats/min)	291 ± 7	352 ± 12 *
Wt (mW)	12.45 ± 0.70	10.12 ± 1.15
AO (ml/min)	38.14 ± 1.73	33.67 ± 3.12

*$p<0.05$, n=7

CF = coronary flow

ADP = aorta diastolic pressure

ASP = aorta systolic pressure

HR = heart rate

Wt = total work

AO = aortic output

b) Reperfusion Function

After ischaemia there was a reduction in aorta systolic pressure, total work and aortic output in HCD hearts compared with control hearts. The aorta diastolic pressures in the control group showed very little fluctuation during reperfusion. Although the heart rates remained significantly higher, there was a decrease in aortic output of the HCD hearts during reperfusion when compared with control hearts. The aortic output recovery (%) was lower in the HCD hearts compared with the controls (Table 4.3.2, Figure 4.3.4 and Figure 4.3.5).

Table 4.3.2: Mechanical function data for control and HCD hearts after ischaemia (after 20 min WH perfusion)

	Control	**HCD**
CF (ml/min)	18.0 ± 0.6	17.0 ± 1.0
ADP (mmHg)	68.29 ± 2.69	64.01 ± 1.50
ASP (mmHg)	93.93 ± 2.58	85.96 ± 1.59 *
HR (beats/min)	267 ± 12	316 ± 16 *
Wt (mW)	9.56 ± 0.59	6.06 ± 0.93 *
AO (ml/min)	26.29 ± 2.24	16.67 ± 3.74 *
%AO recovery	66.67 ± 3.76	47.86 ± 7.87 *

*$p<0.05$, n=7

CF = coronary flow

ADP = aorta diastolic pressure

ASP = aorta systolic pressure

HR = heart rate

Wt = total work

AO = aortic output

% AO = percentage AO recovery

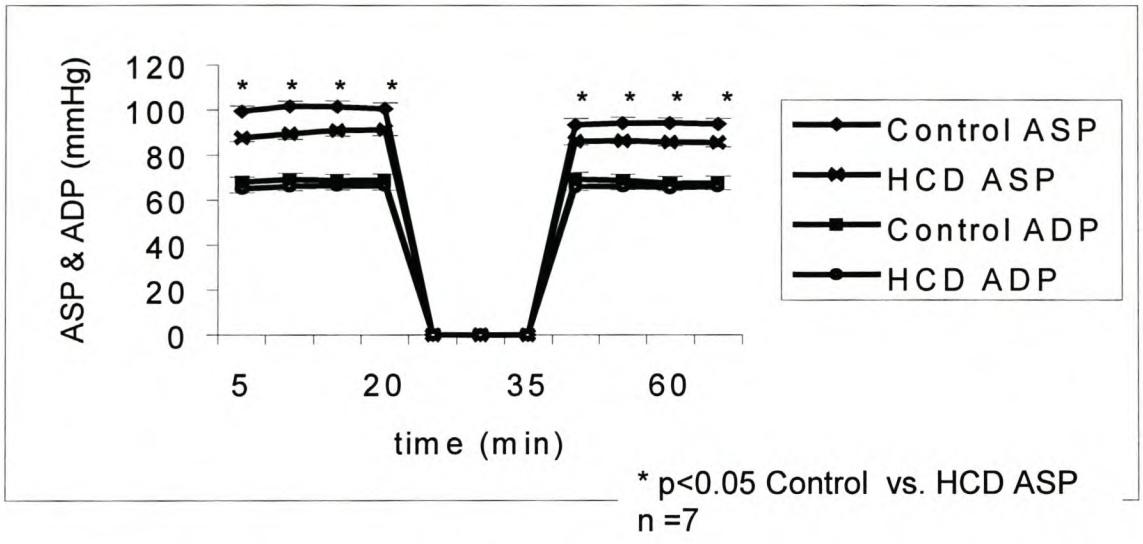


Figure 4.3.3: Aorta systolic and diastolic pressures for control and HCD before ischaemia and during reperfusion.

ADP= aorta diastolic pressure

ASP = aorta systolic pressure

HCD = high caloric diet

Aorta systolic and diastolic pressure was recorded at 5 minute intervals before ischaemia and during reperfusion. The aorta systolic pressure remained significantly higher in the control hearts compared with the HCD group before ischaemia (100.75 $\pm$ 2.49 mmHg and 89.89 $\pm$ 2.36 mmHg respectively after 20 minutes WH perfusion ($p<0.05$)), and during reperfusion (93.93 $\pm$ 2.58 mmHg and 85.96 $\pm$ 1.59 mmHg respectively after 20 minutes WH perfusion ($p<0.05$)). There was no significant difference in aorta diastolic pressure of control hearts after ischaemia compared with HCD hearts (68.29 $\pm$ 2.69 mmHg and 64.01 $\pm$ 1.50 mmHg respectively).

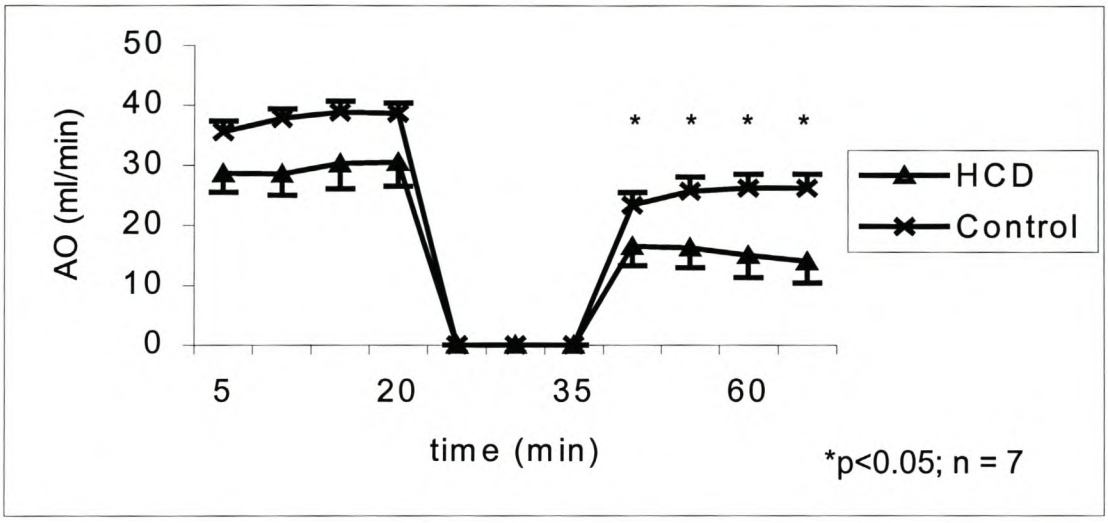


Figure 4.3.4: Aortic output for control and HCD hearts before ischaemia and during reperfusion.

AO = aortic output

HCD = high caloric diet

There was no significant difference between aortic outputs of the control hearts compared with the HCD group before ischaemia (38.14 $\pm$ 1.73 ml/min and 33.67 $\pm$ 3.12 ml/min respectively after 20 minutes WH perfusion). During reperfusion the aortic output of the HCD hearts decreased, reflecting a decline in cardiac function. The aortic output in the HCD hearts continued to decrease with reperfusion, while the aortic output in the control hearts stabilized (26.29 $\pm$ 2.24 ml/min and 16.67 $\pm$ 3.74 ml/min respectively after 20 minutes of WH perfusion ($p<0.05$)).

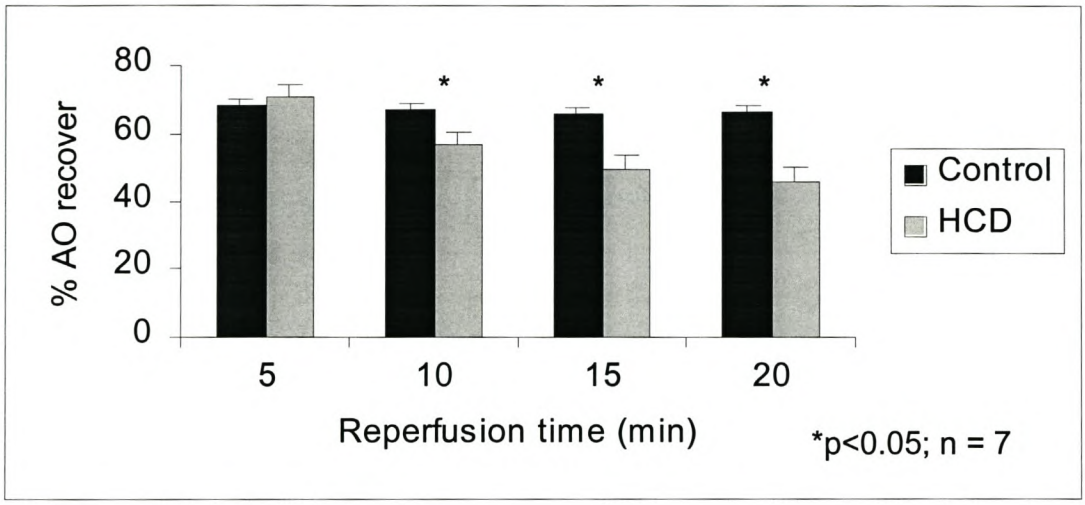


Figure 4.3.5: % AO recovery for control and HCD hearts during 20 min WH perfusion

AO = aortic output

HCD = high caloric diet

The aortic output recovery declined throughout reperfusion in the HCD hearts (47.86 $\pm$ 7.87% after 20 minutes WH reperfusion), while the aortic output recoveries for the control hearts remained more stable (66.67 $\pm$ 3.76% after 20 minutes WH reperfusion ($p<0.05$)).

4.4 Biochemical Parameters Measured

4.4.1 Serum TNFα levels

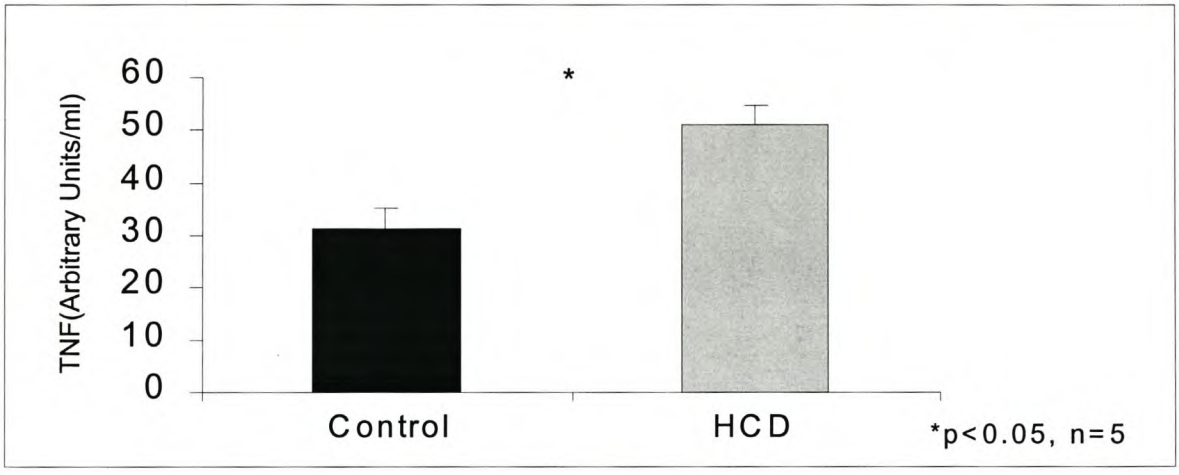


Figure 4.4.1: Serum TNFα levels in control and HCD fed animals after 12 weeks on the high caloric diet.

HCD = high caloric diet

Serum TNF_α levels were lower in the control group (31.46 $\pm$ 0.32 AU) compared with the HCD group (51.04 $\pm$ 5.14 AU). Depending on the relative concentrations of TNF_α in the serum, soluble TNF_α receptors (sTNFRs) can either enhance or inhibit TNF's biological activities. When TNF_α and its soluble receptor are present in the serum, they form complexes. The sTNFR can therefore bind with serum TNF_α and interfere with and compromise accurate ELISA measurements of total TNF protein levels. The ELISA assay kit we used could not measure bound (to the soluble TNF_α receptor) TNF_α levels in the serum. For this reason we chose to present serum TNF_α data in arbitrary units.

4.4.2 Myocardial TNFα levels

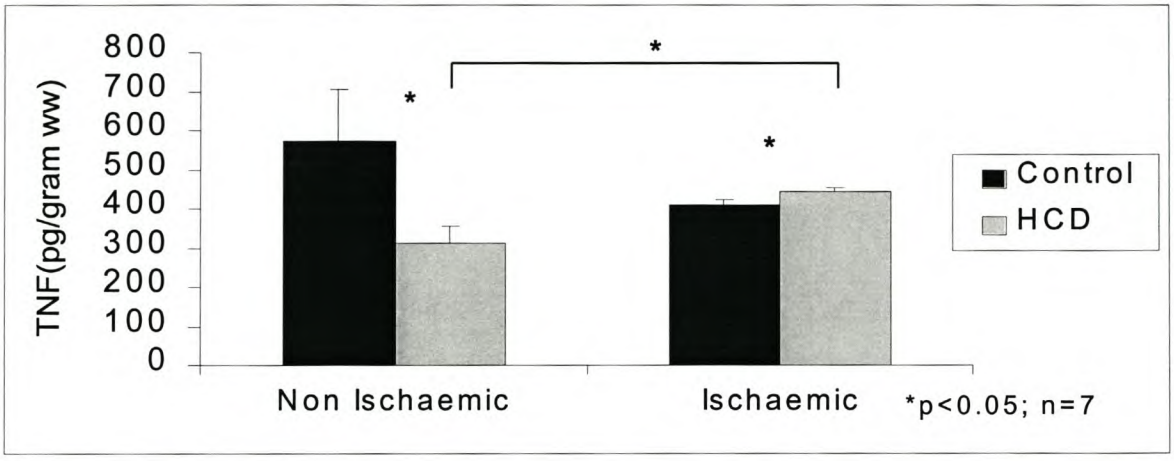


Figure 4.4.2: Myocardial TNFα levels in non-ischaemic and ischaemic control and HCD fed animals.

HCD = high caloric diet

Myocardial TNF_α levels in the control hearts were not significantly different before and during ischaemia (571.4 ± 132.9 pg/gram ww and 410.0 ± 12.5 pg/gram ww respectively). TNF_α levels before ischaemia were however considerably higher in the control compared with the HCD hearts (571.4 ± 132.9 pg/gram ww and 312.0 ± 44.7 pg/gram ww respectively ($p<0.05$)). There was a significant increase in the myocardial TNF_α levels of the HCD fed animals when comparing the pre-ischaemic with ischaemic TNF_α levels (312.0 ± 44.7 pg/gram ww and 442.9 ± 12.4 pg/gram ww ($p<0.05$)). TNF_α levels were higher in the HCD hearts during ischaemia (442.9 ± 12.4 pg/gram ww) when compared with their concurrent ischaemic controls (410.0 ±12.5 pg/gram ww ($p<0.05$)).

4.4.3 Adipose tissue TNFα levels

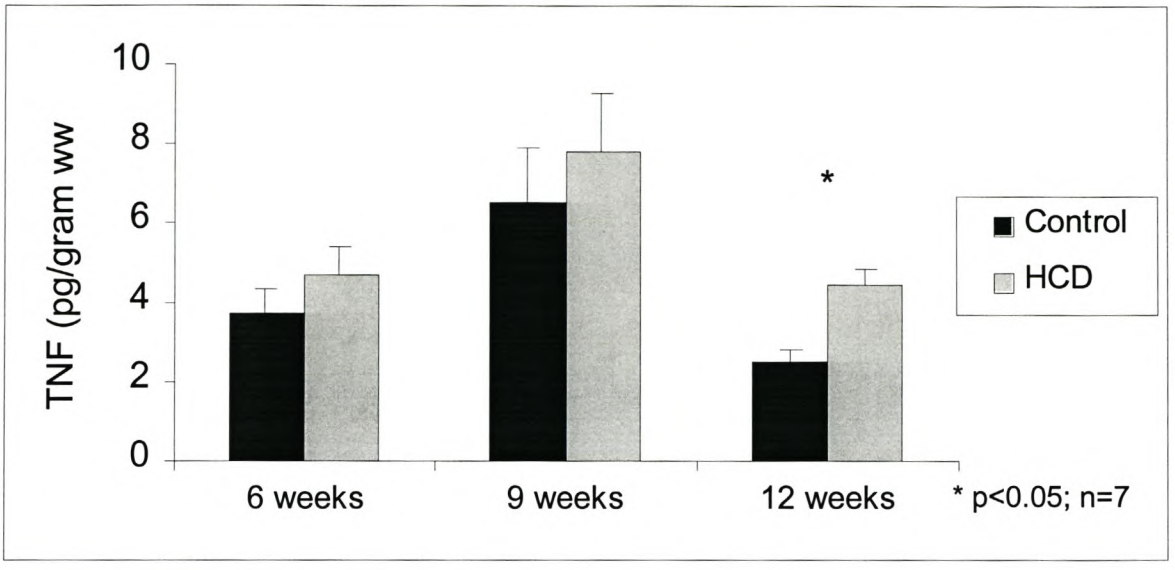


Figure 4.4.3: Adipose tissue TNFα of control and HCD fed animals after 6, 9 and 12 weeks on the respective diets

HCD = high caloric diet

The TNF_α levels in the adipose tissue of the control group and HCD were similar after 6 and 9 weeks. After 12 weeks there was an increase in adipose tissue TNF_α levels of the HCD group (4.4 $\pm$ 0.4 pg/gram ww) compared with the concurrent controls (2.5 $\pm$ 0.3 pg/gram ww ($p<0.05$)).

4.4.4 Myocardial cGMP levels

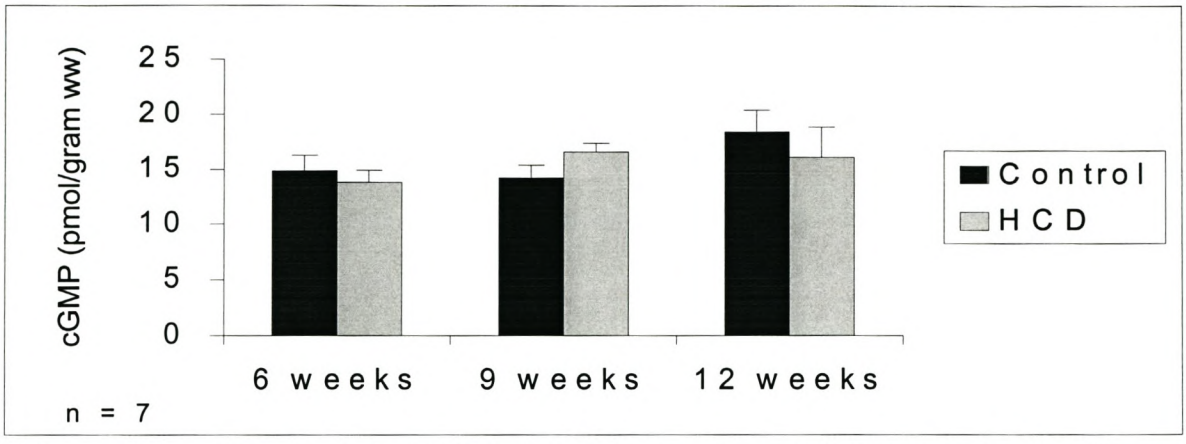


Figure 4.4.4: Myocardial cGMP levels of control and HCD fed animals after 6, 9 and 12 weeks.

HCD = high caloric diet

There were no differences in the cGMP levels in the control hearts compared with the HCD hearts after 6, 9 and 12 weeks on the respective diets.

Chapter 5
DISCUSSION

We investigated and characterized the effects of obesity induced by a high caloric diet and hyperphagia on myocardial hypertrophy, function and susceptibility to ischaemic/reperfusion injury. We also tried to determine whether there is a correlation between obesity, serum and myocardial TNF α levels, and cardiac hypertrophy.

Obesity is defined as the excessive storage of energy in the form of fat and plays a pivotal role in the pathophysiology of metabolic and cardiovascular diseases (Caro, 1991; Sowers and Farow, 1996). Cardiovascular diseases directly associated with obesity are cardiac hypertrophy and compromised ventricular function (Paulson and Tahiliani, 1992). Obesity has recently been reclassified as a major, modifiable risk factor for heart disease by the American Heart Association (Eckel and Krauss, 1998) and is currently one of the most common metabolic disorders in developing countries. Since the realization that obesity is a threat to the healthy individual, many models of experimental obesity have been developed. Examples of these are genetically obese animals and diet induced obese animals.

5.1 Development of a Model of Obesity and Cardiac Hypertrophy

In this study we wished to develop a model of diet induced obesity. This had to be achieved without major changes to the macronutrient composition of the diet. Pickavance and colleagues (1999) developed a model of hyperphagia-induced obesity, which also induced cardiac hypertrophy. This diet contained a slightly elevated carbohydrate content, which would be expected to have less severe effects on lipid profiles than would a high fat diet. For example, a high fat diet in dogs was accompanied by sympathetic overactivity, hyperglycemia, increased plasma insulin,

and dyslipidemia, with elevated triglycerides and total cholesterol levels (Rocchini *et al.*, 1989; Robles *et al.*, 1993; Villa *et al.*, 1998). High sucrose and fructose diets were primarily accompanied by insulin resistance and hypertension (Storlien *et al.*, 1993; Hulman and Falkner, 1994; Pagliosotti *et al.*, 1994; Daly *et al.*, 1997; Higashiura *et al.*, 1999). Interestingly a recent prospective study of 75 000 women in the USA performed over 10 years has indicated that a high dietary glycemic load from refined carbohydrates increased the risk of coronary heart disease, independent of other known coronary disease risk factors (Liu *et al.*, 2000).

We found that subjecting the rats to a diet consisting of 65% carbohydrates for 12 weeks resulted in an additional 60% weight gain in the HCD (high caloric diet) fed rats when compared with their concurrent controls (Table 4.1.1). As a result of hyperphagia and the high calory content of their diet, the HCD rats became obese and suffered from mild insulin resistance. These animals had increased blood glucose levels, but their insulin levels were similar to those of the control rats (Table 4.1.2). These results support the findings of previous studies employing this model of diet induced obesity (Wilding *et al.*, 1992; Pickavance *et al.*, 1999). In these latter studies the blood glucose levels and insulin levels were obtained in fasted animals. Due to the fact that the animals in our study were not fasted, comparisons with these studies should be done with caution. In the current study the animals were however subjected to the high caloric diet for 12 weeks compared with 8 weeks in the previous studies (Wilding *et al.*, 1992; Pickavance *et al.*, 1999).

5.2 Lipid Profiles

When comparing the lipid profiles of the HCD animals with control animals changes in the plasma cholesterol, HDL cholesterol and plasma TAG levels were observed

(Table 4.1.2). The plasma cholesterol and HDL cholesterol levels were lower in the HCD fed animals compared with the control rats, while plasma TAG levels were higher. The increased plasma TAG levels corresponded with the findings of Pickavance and colleagues (1999), using a similar model. To our knowledge most studies to date measured lipid profiles of rats subjected to high fructose, sucrose and fat diets (Matsui *et al.*, 1997; Morin *et al.*, 1997; Higashiura *et al.*, 1999). Subjecting rats to a high fructose and lard diet for 2 weeks resulted in increased plasma TAGs, but no differences were found in the plasma cholesterol levels. These findings were proposed to be closely related to the insulin resistance in the rats (Matsui *et al.*, 1997).

A short term (2 weeks) high caloric diet in humans caused increased plasma triglyceride levels, and this increase may be linked to increase in hepatic fatty acid availability resulting from lower splanchnic fatty acid oxidation (Mittendorfer and Sidossis, 2001). In the Quebec cardiovascular study performed on 2103 men over a period of 5 years, it was demonstrated that there is a highly significant negative relationship between plasma HDL-cholesterol concentrations and TAG levels, i.e. low HDL-cholesterol is associated with hypertriglyceridemia (Despres *et al.*, 2000). Coronary artery disease and congestive heart failure are risk factors associated with low HDL-cholesterol and high plasma TAG levels (Carroll *et al.*, 1996). Our rats may have therefore been more susceptible to CAD suggested by the low HDL-cholesterol and high TAG levels observed.

It has been suggested that dyslipidemia and insulin resistance coexist in obese rats, and that the abnormal lipid profiles of the HCD fed rats may have been due to the increased adiposity of the insulin resistant rats (Paulson and Tahiliani, 1992).

Visceral adipose tissue has greater lipolytic activity than subcutaneous adipose tissue, and the HCD fed rats had large visceral fat reserves. The subsequent release of free fatty acids into the portal blood supply has been shown to contribute to abnormal lipoprotein metabolism, including the presence of increased small, dense LDL-cholesterol particles, lower HDL-cholesterol concentrations and insulin resistance (Roust and Jensen, 1993; Kissebah and Krakower, 1994; Mauriege *et al.*, 1995; Hoffstedt *et al.*, 1996; Lamarche *et al.*, 1997; Hoffstedt *et al.*, 1997). As a result of technical difficulties associated with measuring LDL-cholesterol in rats, we did not measure the levels of this lipid in our study.

5.3 Obesity, TNFα and Cardiac Hypertrophy

Our results show that cardiac hypertrophy was induced in the HCD fed animals, since the heart weight to body weight ratios were increased compared with the control group (Table 4.2.1). This is particularly significant as this ratio increased despite the fact that there was a large increase in body weight of the HCD animals. Similarly, a high fat diet induced obesity in rabbits caused cardiac hypertrophy (Carroll *et al.*, 1996). Pickavance and colleagues (1999) found, that subjecting rats to a high caloric diet caused obesity, which is accompanied by cardiac hypertrophy. There are no further studies to our knowledge, where a high caloric diet induced obesity in rats resulted in cardiac hypertrophy.

Unfortunately, no significant differences were found when using heart weight to tibia length ratios as an index of cardiac hypertrophy (Table 4.2.1). This method for quantifying cardiac hypertrophy may only be reliable in fully-grown and old rats. Yin and colleagues (1982) only found a good correlation between heart weight and tibia length in rats that were 7 months and older. The rats in this study were between 2

and 4 months old, which may account for the inconclusive results we obtained using heart weight to tibia length as an index of cardiac hypertrophy. The reason we did not subject the animals to the high caloric diet for a longer period of time was because we hoped to avoid the development of hypertension and diabetes mellitus, which would further complicate the interpretation of our data.

It is well known that the heart weight to body weight ratio is increased in obese subjects. Autopsy findings on obese subjects revealed increased ventricular wall-thickness and diffuse myocardial hypertrophy, which correlated well with body weight (Amad *et al.*, 1965; Alexander and Pettigrove, 1967). The increased heart weight was mainly due to increased ventricular mass. The results from the Farmingham study also confirmed the correlation between heart weight, ventricular thickness and body weight (Gordon and Kannel, 1976). Obesity is therefore associated with left ventricular hypertrophy, which has been demonstrated to be an independent predictor of cardiovascular diseases (Lauer *et al.*, 1991).

In addition to establishing whether a HCD is linked to the development of cardiac hypertrophy, the aim of this study was also to determine whether there is a link between TNF_{α} secreted by adipose tissue and released into the circulating blood, and the induction of cardiac hypertrophy. We also wanted to determine whether the elevated serum TNF_{α} levels lead to myocardial dysfunction in the normoxic heart.

Since obesity is accompanied by excessive body weight, increased metabolic demands and increased blood volume, the chronic cardiovascular overload leads to cardiac hypertrophy (Messerli, 1982). It is known that TNF_{α} is over-expressed in adipose tissue of obese rats and humans (Hotamisligil and Spiegelman, 1994). In a

study done by Yudkin and colleagues (1999), it was shown that IL-6, TNF_{α} and C-reactive protein, arising in part from adipose tissue, might themselves be partly responsible for the hemodynamic and metabolic abnormalities observed in obese subjects. Yokoyama and colleagues (1997) concluded that physiologically obtainable concentrations of TNF_{α} provoked a hypertrophic growth response in the adult mammalian cardiac myocytes. Kapadia and colleagues (1997) demonstrated that hemodynamic pressure overloading *in vivo* under physiological conditions resulted in *de novo* intramyocardial TNF_{α} mRNA biosynthesis in the feline myocardium. These studies suggest that TNF_{α} may play an important role in the initiation and development of myocardial hypertrophy.

Studies performed on other cytokines, such as CT-1 showed that CT-1 secreted from cardiac nonmyocytes, was involved in the hypertrophic changes of cardiac myocytes in coculture with the nonmyocytes. They proposed that CT-1 is an important regulator in the process of cardiac hypertrophy (Kuwahara *et al.*, 1999; Latchman, 2000). It has also been suggested that CT-1 has a cardioprotective role in the pathophysiology of cardiac hypertrophy. There is therefore ample evidence supporting the role for cytokines in both cardiac hypertrophy and protection (Sack *et al.*, 2000; Ghosh *et al.*, 2000).

5.4 Obesity and Myocardial Function

Obesity is clearly linked to cardiovascular disease and congestive heart failure (CHF). In a study done by Berkalp and colleagues (1995), this group found that obesity caused ventricular relaxation and early filling abnormalities. Altered left ventricular compliance has also been shown by Wilcken (1968), where patients exhibited high end-diastolic ventricular and atrial pressures, which correlated with

body weight and reduced left ventricular compliance. Left ventricular function, as reflected by the ratio of stroke work index to left ventricular end-diastolic pressure was also reduced in a more recent study (Divitiis *et al.*, 1981). Data from the Farmingham study also suggest that obese subjects are at a greater risk of developing cardiomyopathy than their leaner counterparts (Gordon and Kannel, 1976).

Our results showed that before exposure to global ischaemia, hearts from the HCD animals generated lower aorta systolic pressures compared with the control hearts (Table 4.3.1, Figure 4.3.3). However, the heart rates were higher. This may explain why the normoxic aortic output values for the HCD group were not significantly lower than their concurrent controls (Figure 4.3.4). The exact cause of the increase in heart rate of the normoxic hearts in the HCD animals is unknown. The aortic output, total work, aorta diastolic pressures and coronary flow rates remained similar in the HCD hearts compared with their concurrent control hearts, suggesting normal cardiac function before ischaemia. This may be due to the young age of the animals used in this study. Older animals that have been obese for longer may display more severe functional abnormalities than seen in our study.

Garavaglia and colleagues (1988) found an increased cardiac output in obese subjects. They suggested that this might be due to an increased stroke volume caused by higher end-diastolic volumes accounted for by increased blood volumes in obesity. Crandall and colleagues (1988) on the other hand showed that cardiac output decreased proportionally with increased heart weight to body weight ratio. They proposed that obesity in combination with other possible cardiac complications resulted in greater cardiac dysfunction than either condition alone. It has also been

observed that left ventricular hypertrophy associated with obesity caused an abnormal ventricular filling pattern (Chakko *et al.*, 1991). Similarly, Stoddard and colleagues (1992) found a decreased peak diastolic filling rate in obese subjects with a normal left ventricular mass. This would suggest that these changes in ventricular filling in obese subjects may be independent of LV hypertrophy.

Our results showed an impaired systolic function and normal diastolic function (Table 4.3.1, Figure 4.3.3). This confirmed results obtained in studies on left ventricular function in obese subjects. It has been demonstrated that an impaired systolic function correlates with obesity and cardiac hypertrophy (Divitiis *et al.*, 1981; Alpert *et al.*, 1985; Scaglione *et al.*, 1992; Stoddard *et al.*, 1992), yet some studies have reported normal cardiac function (Nakajima *et al.*, 1985; Carabello and Giltens, 1987). The presence or absence of left ventricular function abnormalities may be strongly related to the degree and the duration of obesity.

5.5 Ischaemia and Reperfusion Injury

Reperfusion function was used as an indirect index of the severity of ischaemia/reperfusion injury in our study. We wished to determine whether the hypertrophied hearts from the obese animals were more prone to ischaemic injury compared with their lean counterparts. For the purpose of this study the aortic output, aorta systolic and diastolic pressures and total work were used as indices of cardiac function.

Ischaemia alone can cause injury and reperfusion in turn may inflict microvascular and myocyte damage thus compromising the return of normal coronary perfusion and cardiac function (Maxwell and Lip, 1997). Ischaemia, if sustained long enough,

causes changes in the cellular ultrastructure in the form of mitochondrial swelling, membrane disruption, shortening of sarcomeres and an overall loss of contractile function (Jennings and Ganote, 1976; Schaper *et al.*, 1979). These changes are brought about by complex mechanisms involving depletion of energy stores (Neely *et al.*, 1973; Jennings and Ganote, 1976; Jennings *et al.*, 1983), and accumulation of metabolic waste products including lipid metabolites (Corr *et al.*, 1984).

During ischaemia, there is an increased intracellular Ca^{2+} accumulation (Clusin *et al.*, 1983; Nayler *et al.*, 1988; Opie, 1989) and generation of oxygen derived free radicals (Hess and Manson, 1984; Kako, 1987). The generation of high levels of free radicals, such as ROI and NO, triggered by the expression of proinflammatory mediators such as cytokines, causes damage to the myocardium (Kilgore and Lucchesi, 1993),

Our results showed that after ischaemia, the aortic output, aorta systolic pressures and total work decreased significantly in the HCD hearts compared with the control hearts, suggesting that these hypertrophied hearts were more prone to ischaemia/reperfusion injury than their concurrent controls (Table 4.3.2, Figures 4.3.4 and 4.3.5). Aorta diastolic pressures and coronary flows however showed no differences. The decreased myocardial function may be due to an accelerated calcium overload during reperfusion of the hypertrophied hearts. The mechanisms involved in this acceleration are however not understood (Allard *et al.*, 1994). Another contributing factor in ventricular dysfunction of hypertrophied hearts may be the altered energy substrate use of these hearts, which may alter cardiac function. Studies to date support the hypothesis that low glucose oxidation rates and high glycolytic rates contribute to the exaggerated postischaemic dysfunction of hypertrophied hearts (Wambolt *et al.*, 2000). There are to date no other studies

evaluating the severity of ischaemic injury in models of diet induced cardiac hypertrophy. Post-ischaemic left ventricular dysfunction was greater in hypertrophied hearts than in nonhypertrophied hearts in various studies (Anderson *et al.*, 1990; Gaasch *et al.*, 1990; Allard *et al.*, 1994; Allard and Lopaschuk, 1996; Schonekess *et al.*, 1996; Wambolt *et al.*, 1997). The post-ischaemic cardiac functions obtained in the current study therefore support the findings of others using models of cardiac hypertrophy.

The consequences of cardiac hypertrophy induced by other methods on cardiac function are however extensively documented. Studies in sponateously hypertensive obese rats have shown that the hypertrophied heart is extremely susceptible to ischaemic injury (Shimamoto *et al.*, 1982; Haneda *et al.*, 1986). Tang and Taylor (1995) found that isoproterenol-induced cardiac hypertrophy was associated with significantly altered myocardial contractile force at various concentrations of calcium, and an increased calcium influx via the sarcolemmal sodium-calcium exchanger may be one of the contributors for the altered myocardial contractility.

5.6 Correlation between Serum and Myocardial TNFα Levels and Ischaemia/Reperfusion Injury

TNFα is known to play a detrimental role in the pathophysiology of ischaemia and heart failure. This cytokine is released in response to an environmental stress such as pressure overload. Its expression occurs in almost all forms of cardiac injury where its gene regulation is tightly controlled (Kapadia *et al.*, 1995). TNFα is however also known to be a growth factor and mediator of inflammation, and can induce self amplification (Aggarwal and Natarajan, 1996). This cytokine is therefore proposed to have dual effects. On the one hand it allows the host self-protection by inducing

oxygen free radical scavenger enzymes and heat shock proteins, and on the other hand causes an increase in oxygen derivatives such as NO (Ferrari *et al.*, 1998).

There were elevated serum TNF_{α} levels and elevated adipose tissue TNF_{α} levels in the HCD fed, obese rats compared with the control rats after 12 weeks (Figures 4.4.1 and 4.4.3). Studies have shown that increased TNF_{α} levels are found in adipose tissue of obese subjects (Hotamisligil *et al.*, 1993). Elevated serum TNF_{α} in obesity has also been documented (Hube *et al.*, 1997; Winkler *et al.*, 1998; Hauner *et al.*, 1998). However it still remains unclear whether an increased adipose tissue TNF_{α} is reflected by elevated circulating TNF_{α} levels.

On the other hand Hotamisligil and colleagues (1995) observed increased TNF_{α} expression in adipose tissue, but very low and almost undetectable circulating TNF_{α} levels in human obesity while other studies conducted on human subjects showed no correlation between serum TNF_{α} levels and adipose tissue (Hauner *et al.*, 1998). These data suggest a primary local role for the cytokine. It has been postulated that increased TNF_{α} secretion by the adipose tissue has a local function in regulating the adipocyte size enlargement (Kern *et al.*, 1995), but since TNF_{α} has pleiotropic effects (Mann, 1996), the increased serum cytokine levels may also be detrimental to cardiac and other tissues. As proposed by Sack and colleagues (2000) (Figure 2.3.2.1 (2)), chronic elevated levels of TNF_{α} are possibly maladaptive and detrimental to the heart, and increased adipose tissue as seen in obesity may be a source of circulating cytokines. The adipose tissue of the HCD fed animals produced high levels of TNF_{α}, which may have been released into the serum, as reflected by the increased serum TNF_{α} levels. These chronic elevated levels of the cytokine may

account for the post-ischaemic myocardial dysfunction observed in the HCD fed animals when compared with their concurrent controls.

To investigate whether myocardial TNF_{α} levels changed in response to ischaemia, we measured its levels before and during ischaemia. During normoxia, the levels of myocardial TNF_{α} remained significantly lower in the hypertrophied HCD hearts compared with the control hearts (Figure 4.4.2). These results contradict other studies done on mycardial TNF α levels in cardiac hypertrophy and obesity. Shioi and colleagues (1997) demonstrated that increased proinflammatory cytokine expression in the heart plays a role in the pathogenesis of cardiac hypertrophy. During haemodynamic pressure overload due to isolated stretch, TNF_{α} expression and peptide production was also up-regulated in the adult myocytes (Kapadia *et al.*, 1997).

The reason for reduced myocardial TNF_{α} levels observed in the HCD hearts before ischaemia may therefore be due to increased TNF_{α} secretion from the myocardium into the serum. TNF_{α} released from the adipose tissue possibly induced its own amplification and release from the myocardium, which is possibly also reflected by the elevated serum TNF_{α} levels. Whether endogenous TNF_{α} directly induced cardiac hypertrophy remains unclear, since the levels remain low in the myocardium before ischaemia.

Interestingly, during ischaemia TNF_{α} levels were elevated in the myocardium of the HCD animals compared with the control animals (Figure 4.4.2). It has been documented that ischaemia/reperfusion induces myocardial TNF_{α} production (Lefer, 1970; Herskowitz *et al.*, 1995; Gurevitch *et al.*, 1996). This elevated TNF_{α} production

in response to ischaemia may therefore be an important contributor to post-ischaemic myocardial dysfunction (Yokoyama *et al.*, 1997). Post-ischaemic cardiac function of the HCD animals was significantly decreased compared to corresponding controls, which may be as a result of the increased myocardial TNF_{α} production rate in these animals. The fact that TNF_{α} mRNA expression was not measured in our study precludes us from being certain that TNF_{α} synthesis was elevated in these hearts.

The HCD animals showed elevated levels of serum triglycerides. We however have no data concerning tissue triglyceride levels in the hearts in our study. A previous study (Hendrickson *et al.*, 1996) has shown that intramyocardial lipolysis of triglycerides during ischaemia contributed to the increase of free fatty acids (FFA) and their intermediates, which are associated with increased reperfusion injury. Because TNF_{α} is also known to stimulate lipolysis (Beutler and Cerami, 1988; Grunfeld and Feingold, 1991; Spiegelman and Hotamisligil, 1993; Hotamisligil *et al.*, 1995), we believe that this mechanism may also have contributed to the exacerbation of myocardial ischaemic and reperfusion injury.

5.7 Myocardial cGMP

Because cytokines are thought to stimulate myocardial iNOS activity, we also measured cGMP levels in the hearts during the 12 week feeding program to determine whether the NO-cGMP pathway was possibly activated in the HCD fed animals.

Our results showed no differences in myocardial cGMP levels in the HCD fed animals compared with control animals after 6, 9 and 12 weeks (Figure 4.4.4). Studies have shown that TNF_{α} induces iNOS, which causes an increase in NO production from

cardiomyocytes and further impairs the ventricular function possibly by increasing myocardial cGMP levels or altering specific sarcolemmal ion channels (Gross *et al.*, 1996; Kelly and Smith, 1997). TNF_{α} induced desensitization of myofilaments to calcium is also thought to be mediated by NO (Gurevitch *et al.*, 1996; Goldhaber *et al.*, 1996). Several studies have demonstrated that myocardial infarction and allograft rejection was associated with increased iNOS in the myocardium (Yang *et al.*, 1994; Worrall *et al.*, 1995; Suzuki *et al.*, 1996; Feng *et al.*, 2001). Wang and Zweier (1996) observed increased NO and peroxynitrite release from isolated rat heart after 30 minutes of global ischaemia. We did not measure cGMP levels during ischaemia in our study and cannot comment on the activity of the NO-cGMP pathway during ischaemia in our HCD and control hearts.

However, there are two mechanisms suggested in TNF_{α} mediated contractile dysfunction. The first mechanism occurs early, i.e. within minutes where TNF α causes an increase in sphingosine, a stress induced second messenger, causing an early phase myocardial function depression (Oral *et al.*, 1997). The second mechanism involves the induction of NOS and delayed myocardial dysfunction (Kelly and Smith, 1997). The cGMP levels were not elevated in our HCD animals, and it would therefore appear that the NO-dependent mechanism was not involved, which is supported by the mechanical data obtained in the current study.

Chapter 6

CONCLUSION

The animals subjected to a 12 week high calory feeding program became obese and developed mild insulin resistance. These animals had abnormal lipid profiles, with increased TAG but lower plasma cholesterol and HDL cholesterol levels compared with their concurrent controls. High caloric diet induced obesity lead to cardiac hypertrophy.

There was a strong correlation between elevated adipose tissue and serum TNF_{α} levels, and cardiac hypertrophy. Elevated serum TNF_{α} levels did not lead to elevated myocardial cGMP levels in normoxic hearts. The latter findings suggest that the elevated serum TNF_{α} levels had no effect on myocardial iNOS and NO-cGMP pathway activity. It is therefore unlikely that the NO-cGMP pathway played a role in the myocardial function of the HCD hearts. The obesity induced hypertrophic hearts had poorer post-ischaemic myocardial functions when compared with their concurrent controls. These findings suggest that the hearts from obese animals may have been more prone to ischaemia/reperfusion injury than the non-hypertrophied hearts.

Study Reservations and Proposed Future Work

Although we attempted to develop a model of obesity devoid of other cardiovascular risk factors such as diabetes and hypercholesterolaemia, our animals had slightly elevated plasma glucose and triacylglyceride levels. These secondary cardiovascular risk factors may have contributed to the poorer reperfusion functions observed in the HCD fed animals in this study. These additional risk factors also make the interpretation of the data more complex. Another possible shortcoming in our model

may have been the age of the rats. Older rats would be expected to develop more severe cardiac abnormalities due to the prolonged obesity.

Based on our present data we cannot unequivocally state that adipose tissue was the source of TNF_{α} which lead to the elevated serum TNF_{α} levels. Similarly our data do not prove that the elevated serum TNF_{α} levels are the direct cause of the cardiac hypertrophy seen in our obese animals.

Future work should attempt to establish whether suppression of the elevation in serum TNF_{α} levels associated with obesity influences the degree of cardiac hypertrophy in obese animals. We propose that animal models such as obese TNF_{α} knock-out mice be included in a future study investigating the effects of TNF_{α} on myocardial morphology and function. Alternatively, TNF_{α} antibodies should be administered to the obese rats to determine whether this alters the degree of cardiac hypertrophy associated with obesity.

REFERENCES

Aggarwal BB, Natarajan K. Tumor necrosis factors: developments during the decade. Eur Cytokine Netw 1996;72:93-124

Albina JE, Cui B, Mateo RB, Reichner JS. Nitric oxide-mediated apoptosis in murine peritoneal macrophages. J Immunol 1993;150:5080-5085

Alexander JK, Pettigrove JR. Obesity and congestive heart failure. Geriatrics 1967;22:101-106

Allard MF, Flint JDA, English JC, Henning SL, Salamanca HC, Kamimuro CT, English DR. Calcium overload during reperfusion is accelerated in isolated hypertrophied rat hearts. J Mol Cell Cardiol 1994;26:1551-1563

Allard MF, Lopaschuk GD. Ischaemia and reperfusion injury in the hypertrophied heart. In: M.Karmazyn, Editor. Myocardial Ischaemia: mechanisms, reperfusion, Protection. Birkhauser Verlag, Basel, Switzerland 1996:423-452

Alpert MA, Terry BE, Kelly DL. Effect of weight loss on cardiac chamber size, wall thickness and left ventricular function in morbid obesity. Am J Cardiol 1985;55:783-786

Amad KH, Brennan JC, Alexander JK. The cardiac pathology of chronic exogenous obesity. Circulation 1965;32:740-745

Anderson KR, St John Sutton MG, Lie JT. Histopathological types of cardiac fibrosis in myocardial disease. J Pathol 1979;128:79-85

Anderson PG, Allard MF, Thomas GD, Bishop SP, Digerness SB. Increased ischaemic injury but decreased hypoxic injury in hypertrophied rat hearts Circ Res 1990;67:948-959

Anker SD, Egerer KR, Volk HD, Kox WJ, Poole-Wilson PA, Coats AJ. Elevated soluble CD14 receptors and altered cytokines in chronic heart failure. Am J Cardiol 1997;79:1426-30

Arai M, Yogushi A, Iso T, Takahashi T, Imai S, Murata K, Suzuki T. Endothelin-1 and its binding sites are upregulated in pressure overload cardiac hypertrophy. Am J Physiol 1995;268:H2084-2091

Arbustini E, Grasso M, Diegoli M, Bramerio M, Foglieni AS, Albertario M, Martinelli L, Gavazza A, Goggi C, Campana C. Expression of tumor necrosis factor in human acute cardiac rejection: an immunohistochemical and immunoblotting study. Am J Pathol 1991;139:709-715

Arstrall MA, Sawyer DB, Fukazawa R, Kelly RA. Cytokine-mediated apoptosis in cardiac myocytes. The role of inducible nitric oxide synthase induction and peroxynitrite generation. Circ Res 1999;85:829-840

Ayala A, Herndon CD, Lehman DL, Ayala CA, Chaudry IH. Differential induction of apoptosis in lymphoid tissue during sepsis: variation in onset, frequency, and the nature of the mediators. Blood 1996;87:4261-4275

Baines CP, Cohen MV, Downey JM. Signal transduction in ischaemic preconditioning: the role of kinases and mitochondrial K(ATP) channels. J Cardiovasc Electrophysiol 1999;10:741-754

Balligand JL, Cannon PJ. Nitric oxide synthase and cardiac muscle. Arterioscl Thromb Vasc Biol. 1997;17:1846-1858

Balligand JL, Ungureanu-Longrois D, Kelly RA, Kobzik L, Pimental D, Michel T, Smith TW. Abnormal contractile function due to induction of nitric oxide synthesis in rat cardiac myocytes follows exposure to activated macrophage-conditioned medium. J Clin Invest 1993; 91:2314-2319

Balligand JL, Ungureanu-Longrois D, Simmons WW, Kobzik L, Lowenstein CJ, Lamas S, Kelly RA, Smith TW, Michel T. Induction of NO synthase in rat cardiac microvascular endothelial cells by IL-1β and INF-γ. Am J Physiol 1995; 298:H1293-H1303

Banerji MA, Lebowitz J, Chaiken RL, Gordon D, Kral J. Relationships of visceral adipose tissue and glucose disposal in independent of sex in black NIDDM subjects. Am J Physiol 1997;273:E425-432

Basaran Y, Basaran MM, Babacan KF, Ener B, Ener B, Okay T, Gok H, Ozdemir M. Serum tumor necrosis factor levels in acute myocardial infarction and unstable angina pectoris. J Vascular Diseases 1993;332-337

Bastard JP, Jardel C, Bruckert E, Blondy P, Capeau J, Laville M, Vidal H, Ainique B. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. J Clin Endocrinol Metab 2000;85:3338-3342

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

Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly. Am J Physiol 1996(Cell Physiol 40): C1424-C1437

Beltrami CA, Finato N, Rocco M, Feruglio GA, Puricelli C, Cigola E, Sonnenblick EH, Olivetti G, Anversa P. The cellular basis of dilated cardiomyopathy in humans. J Mol Cell Cardiol 1995;27:291-305

Beltrami CA, Finato N, Rocco M, Feruglio GA, Puricelli C, Cigola E, Quaini F, Sonnenblick EH, Olivetti G, Anversa P. Structural basis of end-stage failure in ischaemic cardiomyopathy in humans. Circulation 1994;89:151-163

Berg B, Harmison CR. Growth, disease, and aging in the rat. J Gerontol 1958;13:370-377

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

Besse S, Robert V, Assayag P, Delcayre C, Swynghedauw B. Non synchronous changes in myocardial collagen mRNA and protein during aging. Effect of DOCA-salt hypertension. Am J Physiol 1994;267:H2237-H2244

Beutler B, Cerami A. Cachectin (tumor necrosis factor): a macrophage hormone governing cellular metabolism and inflammatory response. Endocr Rev 1988;9:57-66

Bhat GJ, Thekkumkara TJ, Thomas WG, Conrad KM, Baker KM. Angiotensin II stimulates sis-inducing factor-like DNA binding activity. J Biol Chem 1994;269:31443-31449

Bing OHL, Brooks WW, Robinson KG, Slawsky MT, Hayes JA, Litwin SE, Sen S, Conrad CH. The spontaneously hypertensive rat as a model of the transition from compensated left ventricular hypertrophy to failure. J Mol Cell Cradiol 1995;27:383-396

Bing OHL, Ngo HQ, Humphries DE, Robinson KG, Lucey EC, Carver W, Brooks WW, Conrad CH, Hayes JA, Goldstein RH. Localization of alpha 1(I) collagen mRNA in myocardium from spontaneously hypertensive rat during the transition from compensated hypertrophy to heart failure. J Mol Cell Cardiol 1997;29:2335-2344

Bogoyevitch MA, Gillespie-Brown J, Ketterman AJ, Fuller SJ, Ben R, Ashworth A, Marshall CJ, Sugden PH. Stimulation of the stress-activated mitogen-activated protein kinases and c-Jun N-terminal kinases are activated by ischaemia-reperfusion. Circ Res 1996; 79:162-173

Boluyt MO, Bing OHL. Matrix gene expression and decompensated heart failure: The aged SHR model. Cardiovasc Res 2000;46:239-249

Boluyt MO, Lakatta EG. Cardiovasular aging in health. In: EE Bittar, Ra Altschuld and RA Haworth Editors, Advances in organ biology: heart and metabolism Vol.4B J.A.I.Press, Stamford, ST (1998).

Boluyt MO, O'Neill L, Meredith AL, Bing OHL, Brooks WW, Conrad CH, Crow MT, Lakatta EG. Alterations in cardiac gene expression during the transition from stable hypertrophy to heart failure: marked upregulation of genes encoding extracellular matrix components. Circ Res 1994;75:23-32

Bonizzi G, Piette J, Merville MP, Bours V. Cell type-specific role for reactive oxygen species in nuclear factor-kappaB activation by Interleukin-1. Biochem Pharmacol 2000;59:7-11

Bounoutas GS, Kubota T, Miyagishima M. Adenoviral-directed overexpression of soluble tumor necrosis factor receptors reverse myocarditis in transgenic mice with congestive heart failure. Circulation 1998;98I-737

Bowie A, O'Neill LAJ. Oxidative stress and nuclear factor-$_K$B activation: a reassessment of the evidence in the light of recent discoveries. Biochem Pharmacol 2000;59:13-23

Bozkurt B, Kribbs SB, Clubb FJJ, Michael LH, Oral H, Spinale FG, Mann DL. Pathophysiologically relevant concentrations of tumor necrosis factor-alpha promote progressive left ventricular dysfunction and remodeling in rats. Circulation 1998;94(14):1382-1391

Braunwald E, Kloner RA. Myocardial reperfusion: a double-edged sword. J Clin Invest 1985;76:1713-1719

Bredt DS, Hwang PM, Glatt CE, Lowenstein C, Redd RR, Snyder SH. Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. Nature (London) 1991;351:714-718

Brilla CG, Matsubara L, Weber KT. Advanced hypertensive heart disease in spontaneously hypertensive rats: Lisiopril-mediated regression of myocardial fibrosis. Hypertension 1996;28:269-275

Brown JM, Terada LS, Grosso MA, Whitman GJ, Velasco SE, Patt A, Harken AH, Repine JE. Xanthine oxidase produces hydrogen peroxide which contributes to reperfusion injury of ischemic isolated rat heart. J Clin Invest 1998;81:1297-1303

Buerke M, Rupprecht HJ, Vom Dahl J, Terres W, Seyfarth M, Schultheiss HP, Richardt G, Sheehan FH, Drexler H. Sodium-Hydrogen exchange inhibition: novel strategy to prevent myocardial injury following ischaemia and reperfusion. Am J Cardiol 1999;83:19G-22G

Busby MJ, Bellantoni MJ, Tobin JD, Muller DC, Kafonek SD, Blackman MR. Glucose tolerance in women: the effects of age, body composition, and sex hormones. J Am Geriat Soc 1992;40:497-502

Cain BS, Harken AH, Meldrum DL. Therapeutic strategies to reduce TNF_{α} mediated cardiac contractile depression following ischaemia and reperfusion. J Mol Cell Cardiol 1999;31:931-947

Calderone A, Takahashi N, Izzo NJJ, Thaik CM, Colucci WS. Pressure-and volume-induced left ventricular hypertrophies are associated with distinct myocyte phenotypes and differential induction in peptide growth factor mRNAs. Circulation 1995;92:2385-2390

Campbell DL, Stamler JS, Strauss HC. Redox modulation of L-type calcium channels in ferret ventricular myocytes: mechanisms of dual indirect and direct modulation by nitric oxide and S-nitrosothiols. J Gen Physiol 1996;108:277-293

Carabello BA, Giltens L. Cardiac mechanics and function in obese normotensive persons with normal coronary arteries. Am J Cardiol 1987;59:469-473

Caro JF, Clinical review 26. Insulin resistance in obese and non-obese men. J Clin Endocrinol Metab 1991;73:691-695

Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV. Leptin. The tale of an obesity gene. Diabetes 1996;45:1455-1462

Carroll J, Dwyer TM, Grady AW, Reinhart GA, Montani JP, Cockrell K, Meydrech EF, Mizelle HL. Hypertension, cardiac hypertrophy, and neurohumoral activity in a new animal model of obesity. Am J Physiol 1996;271(Heart Circ Physiol 40):H373-H378

Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci USA 1975;72:3666-3670

Celentano A, Vaccara O, Tammaro P, Galderisi M, Crivaro M, Oliviero M, Imperatore G, Palmieri V, Iovino V, Riccardi G. Early abnormalities of cardiac function in non-insulin-dependent diabetes mellitus and impaired glucose tolerance. Am J Cardiol 1995;76:1173-1176

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

Cheung AT, Ree D, Kolls JK, Fuselier J, Coy DH, Bryer-Ash M. An in vitro model for the elucidation of the mechanism of tumor necrosis factor-α induced insulin resistance. Endocrinology 1998;139:4928-4935

Chiariello M, Perrone-Filardi P. Pathophysiology of heart failure. Miner Electrolyte Metab 1999;25(1-2):6-10

Cifone MG, De Maria R, Roncaioli P, Rippo MR, Azuma M, Lanier LL, Santoni A, Testi R. Apoptosis signaling through CD95 (Fas/Apo-1) activates an acidic sphingomyelinase. J Exp Med 1994;180:1547-52

Clerk A, Sugden PH. Activation of protein kinase cascades in the heart by hypertrophic G protein-coupled receptor antagonists. Am J Cardiol 1999;83:64H-69H

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

Coldritz GA, Willet WC, Rotsnitzky A, Manson JE. Weight gain as a risk factor for clinical diabetes in women. Ann Intern Med 1995;122:481-486

Conrad CH, Brooks WW, Hayes JA, Sen S, Robinson KG, Bing, OHL. Myocardial fibrosis and stiffness with hypertrophy and heart failure in the spontaneously hypertensive rats. Circulation 1995;91:161-170

Cooper G IV. Cardiocyte adaptation to chronically altered load. Annu Rev Physiol 1987;49:501-518

Cooper IVD, Kent RL, Uboh CE, Thompson EW, Marino TA. Hemodynamic versus adrenergic control of cat right ventricular hypertrophy. J Clin Invest 1985;75:1403-1414

Coroneos E, Martinez M, McKenna S, Kester M. Differential regulation of sphingomyelinase and ceramidase activities by growth factors and cytokines. J Bio Chem 1995;270:23305-9

Corr PB, Gross RW, Sobel BE. Amphipathic metabolites and membrane dysfunction in ischaemic myocardium. Circ Res 1993;72:214-218

Crandall DL, Goldstein BM, Lizzo FH, Lozito RJ, Cervoni P. Development of an animal model for investigating disparate myocardial effects of obesity and hypertension. J Appl Physiol 1988;64(3):1094-1097

Cui S, Reichner JS, Mateo RB, Albina JE. Activated murine macrophages induce apoptosis in tumor cells through nitric oxide-dependent or independent mechanisms. Cancer Res 1994;54:2462-2467

Curtis MJ, Pabla R. Nitric oxide supplementation or synthesis block- which is the better approach to treatment of heart disease? TIPS 1997;18:239-244

Dalby KN, Morrice N, Caudwell FB, Avruch J, Cohen P. Identification of regulatory phosphorylation sites in mitogen-activated protein kinase (MAPK)-activated protein kinase-1a/p90^{rsk} that are inducible by MAPK. J Biol Chem 1998;273:1496-1505

Daly ME, Vale C, Walker M, Littlefield A, Alberti KGMM, Mathers JC. Acute effects on insulin sensitivity and diurnal metabolic profiles of a high-sucrose compared with a high-starch diet. Am J Clin Nutr 1998;67:1186-1196

Dassouli A, Sulpice J, Roux S, Crozatier B. Stretch-induced inositol triphosphate and tetrakiphosphate production in rat cardiomyocytes. J Mol Cardiol 1993;25:973-982

Despres JP, Lemieux I, Dagenais GR, Cantin B, Lamarche B. HDL-cholesterol as a marker of coronary heart disease risk: the Quebec cardiovascular study. Atheroscl 2000;153:263-272

Dhalla AK, Hill MF, Singal PK. Role of oxidative stress in transition of hypertrophy to heart failure. J Am Coll Cardiol 1996;28:506-514

Ding B, Price RL, Borg TK, Weinberg EO, Holloran PF, Lorell BH. Pressure overload induces severe hypertrophy in mice treated with cyclosporine, an inhibitor of calcineurin. Circ Res 1999;84:729-734

Divitiis O, fazio S, Petitto M, Maddalena G, Contaldo F, Mancini M. Obesity and cardiac function. Circulation 1981;65:477-482

Doering CW, Jalil JE, Janicki JS, Pick R, Aghili S, Abrahams C, Wber KT. Collagen network remodeling and diastolic stiffness of the rat ventricle with pressure overload hypertrophy. Cardiovasc Res 1988;22:686-695

Du Toit EF, Miyashiro J, McCarthy J, Opie LH, Brunner F. effects of nitrovasodilators and inhibitors of nitric-oxide synthase in ischaemic and reperfusion functions in rat isolated hearts. Brit J Pharmacol 1998;123:1159-1167

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

Dutka DP, Elborn JS, Delamere F, Shale DJ, Morris GK. Tumor necrosis factor-alpha in severe congestive cardiac failure. Br Heart J 1993;70:141-143

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

Dzau VJ. Tissue renin-angiotensin system in myocardial hypertrophy and failure. Arch Intern Med 1993;153:937-942

Eckel RH, Krauss RM. American Heart Association call to action: obesity as a major risk factor for coronary heart disease. AHA Nutrition Committee. Circulation 1998;97(21):2099-2100

Engler RL, Dahlgreen MD, Morris DD, Peterson MA, Schmid-Schoenbein GW. Role of leukocytes in response to acute myocardial ischaemia and reflow in dogs. Am J Physiol Heart Circ Physiol 1986;251:H314-H323

Fabris R, Nisoli E, Lombardi AM, Tonello C, Serra R, Granzotto M, Cusin I, Rohner-Jeanrenaud, Curruba MO, Vettor R. Preferential channeling of energy fuels toward fat rather than muscle during high free fatty acid availability in rats. Diabetes 2001;50:601-8

Feldman AM, Weinberg EO, Ray PE, Lorell BH. Selective changes in cardiac gene expression during compensated hypertrophy and the transition to cardiac decompensation in rats with chronic aortic banding. Circ Res 1993;73:184-192

Feng Q, Lu X, Jones DL, Shen J, Arnold MO. Increased inducible nitric oxide synthase expression contributes to myocardial dysfunction and higher mortality after myocardial infarction in mice. Circulation 2001;104:700-704

Feron O, Salomone S, Godfraind T. Influence of salt loading on the cardiac and renal preproendothelin-1 mRNA expression in stroke-prone spontaneously hypertensive rats. Biochem Biophys Res Comm 1995;209:161-166

Ferrannini E, Barret EJ, Bevilacqua S, DeFrenzo RA. Effect of fatty acids on glucose production and utilization in man. J Clin Invest 1983;72:1737-1747

Ferrannini E, Natali A. Essential hypertension, metabolic disorders, and insulin resistance. Am Heart J 1991;121:1274-1282

Ferrari R, Agnoletti L, Comini L, Gaia G, Bachetti T, Cargnoni A, Ceconi C, Visioli. Oxidative stress induced myocardial ischaemia and heart failure. Eur Heart J 1998;19:B2-B11

Ferrari R. Tumor necrosis factor in CHF: a double facet cytokine. Cardiovasc Res 1998;37:554-559

Frederich RC, Lollmann B, Hamman A, Napolitano-Rosen A, Kahn BB, Lowell BB, Flier JS. Expression of the ob mRNA and its encoded protein in rodents. J Clin Invest 1995;986:1658-1663

Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763-770

Fuller SJ. Stimulation of gene expression in neonatal cardiac myocytes by raised extracellular pH. Biochem Soc Trans 1997;25:210S

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

Garavaglia GE, Messerli FH, Nunez BD, Schmieder RE, Grossman E. Myocardial contractility and LV function in obese patients with essential hypertension. Am J Cardiol 1988;62:594-597

Ghosh S, Ng LL, Talwar S, Squire IB, Galinanes M. Cardiotrophin-1 protects the human myocardium from ischaemic injury. Cardiovasc Res 2000;48:440-447

Gilbert EM, Bristow MR. Idiopathic dilated cardiomyopathy. In: Schlant RC, Alexander RW, eds. Hursts The Heart: Arteries and Veins. New York, NY: McGraw-Hill Inc; 1994:1609-19

Goldhaber JL, Kim KH, Natterson PD, Lawrence T, Yang P, Weiss JN. Effects on TNF-α on $[Ca^{2+}]_i$ and contractility in isolated adult rabbit ventricular myocytes. Am J Physiol 1996;271(Heart Circ Physiol 40):H1449-H1455

Goossens V, Grooten J, De Vos K, Fiers W. Direct evidence for tumor necrosis factor-induced mitochondrial reactive oxygen intermediates and their involvement in cytotoxicity. Proc Natl Acad Sci USA 1995;92:8115-8119

Gordon T, Kannel WB. Obesity and cardiovascular diseases: the Farmingham Study. Clin Endocrinol Metab 1976; 5:367-375

Gottlieb RA, Burleson KO, Kloner RA, Babior BM, Engler RL. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. J Clin Invest 1994;94:1621-1628

Gravanis MB, Kanter KR, Kassen M, Mayne A, Ansari AA. Semiquantitative assessment of apoptotic cells in cardiac tissue of patients with cardiomyopathies. Circulation 1994;90:584-589

Gross WL, Bak MI, Ingwall JS, Arstall MA, Smith TW, Balligand JL, Kelly RA. Nitric oxide regulates rat heart contractile reserve and inhibits creatine kinase. Proc Natl Acad Sci USA 1996;93:5604-5609

Grunfeld C, Feingold KR. The metabolic effects of tumor necrosis factor and other cytokines. Biotherapy 3 1991;143-158

Gulick TS, Chung MK, Pieper SJ, Lange LO, Schreiner GF: Interleukin 1 and timor necrosis factor inhibit cardiac myocyte beta-adrenergic responsiveness. Proc Natl Acad Sci USA 1989;86:6753-6757

Gurevitch J, Frolkis I, Yuhas Y, Paz Y, Matsa M, Mohr R, Yakirevich V. Tumor necrosis factor-alpha is released from the isolated heart undergoing ischaemia and reperfusion. J Am Coll Cardiol 1996;28:247-252

Gurevitch J, Frolkis I, Yuhas Y, Lifschitz-Mercer B, Paz Y, Matsa M, Kramer A, Mohr R. Anti-tumor necrosis factor alpha improves myocardial recovery after ischaemia and reperfusion. J Am Coll Cardiol 1997;30:1554-1561

Guyton KZ, Liu Y, Gorospe M, Xu Q, Holbrook NJ. Activation of mitogen-activated protein kinase by H_2O_2. Role in cell survival following oxidant injury. J Biol Chem 1996;271:4138-4142

Haimovitz-Friedman A, Kan CC, Ehleiter D, Persaud RS, McLoughlin M, Fuks Z, Kolesnick RN. Ionizing radiation acts on cellular membranes to generate ceramide and initiate apoptosis. J Exp Med 1994;180:73-80

Hall A. The cellular functions of small GTP-binding proteins. Science 1990;249:635-640

Han X, Shimoni Y, Giles WR. A cellular mechanism for nitric oxide-mediated cholinergic control of mammalian heart rate. J Gen Physiol 1995;106:45-65

Han X, Shimoni Y, Giles WR. An obligatory role of nitric oxide in autonomic control of mammalian heart rate. J Physiol 1994;476:309-314

Han X, Wiviott S, Balligand JL, Kelly RA, Smith TW. Nitric oxide regulation of L-type Ca^{2+} channel in rat ventricular myocytes is mediated by both cGMP-stimulated phosphodiesterase and cGMP-dependent protein kinase. Circulation 1996;94(suppl 1):I-1300

Haneda T, Ichihara K, Abiko Y, Onodera S. functional and metabolic responses to ischaemia in the perfused heart isolated from normotensive and spontaneously hypertensive rats. Jpn Circ J 1986;50:607-613

Hannun YA, Luberto C. Ceramide in the eukaryotic stress response. Trends Cell Biol 2000;10:73-80

Hannun YA. The shingomyelin cycle and the second messenger function of ceramide. J Biol Chem 1994; 269:3125-3128

Hansen CS. Myocardial reperfusion injury: experimental evidence and clinical relevance. Eur Heart J 1995;16:734-740

Hansen CS, Schroering AG, Carey DJ, Robishaw JD. Localization of heterotrimeric G protein γ subunit to focal adhesion and associated stress fibers. J Cell Biol 1994;126:811-819

Hasper D, Hummel M, Kleber FX, Reindl I, Volk HD. Systemic inflammation in patients with heart failure. Eur Heart J 1998;19:761-5

Hauner H, Bender M, Haastert B, Hube F. Plasma concentrations of soluble TNF-alpha receptors in obese subjects. Int J Obes Relat Metab Disord 1998;22(12):1239-1243

Hausladen A, Fridovich K. Superoxide and peroxynitrite inactiavtes aconitases, but nitric oxide does not. J Biol Chem 1994;269:29405-29408

Haywood GA, Tsao PS, von der Leyen HE. Expression of inducible nitric oxide synthase in human heart failure. Circulation 1996;93:1087-1094

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

Hendrickson SC, St.Louis JD, Lowe J, Abdel-aleem S. Free fatty acid metabolism during myocardial ischaemia and reperfusion. Mol Cell Biochem 1997;166:85-94

Hernandez OM, Discher DJ, Bishopric NH, Webster KA. Rapid activation of neutral sphingomyelinase by hypoxia-reoxygenation of cardiac myocytes. Circ Res 2000;86:198-204

Herskowitz A, Choi S, Ansari AA, Wesselingh S. Cytokine mRNA expression in the postischaemic/reperfused myocardium. Am J Pathol 1995;146:419-428

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

Higashiura K, Ura N, Takada T, Agata J, Yoshida H, Miyazaki Y, Shimamoto K. Alteration of muscle fiber composition linking to insulin resistance and hypertension in fructose-fed rats. Am J Hypert 1999;12:596-602

Hoffstedt J, Arner P, Hellers G, Lonnqvist F. Variation in adrenergic regulation of lipolysis between omental and subcutaneous adipocytes from obese and non-obese men. J Lipid Res 1997;38:795-804

Hoffstedt J, Wahrenberg H, Thorne A, Lonnqvist F. The metabolic syndrome is related to beta 3-adrenoreceptor sensitivity in visceral adipose tissue. Diabetologia 1996;39:838-844

Holbrook TL, Barret-Connor E, Wingard DL. The association of lifetime weight and weight control patterns with diabetes among men and women in an adult community. Int J Obesity 1989;13:723-729

Horvath CM, Darnell JEJ. The state of the STATs: recent developments in the study of signal transduction to the nucleus. Curr Opin Cell Biol 1997;9:233-239

Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. J Clin Invest 1995;95:2409-15

Hotamisligil GS, Budavari A, Murray D, Spiegelman BM. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-α. J Clin Invest 1994;94:1543-9

Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-α: Direct role in obesity-linked insulin resistance. Science 1993;259:87-91

Hotamisligil GS, Spiegelman BM: Tumor necrosis factor-α: a key component of the obesity-diabetes link. Diabetes 1994;43:1271-8

Hotamisligil GS. The role of TNFα and TNF receptors in obesity and insulin resistance. J Int Med 1999;245:621-625

Hsu H, Huang J, Shu HB, Baichwal V, Goeddel DV. TNF-dependent recruitment of the protein kinase RIP to the TNF receptor-1 signaling complex. Immunity 1996a;4:387-396

Hsu H, Shu HB, Pan MG, Goeddel DV. TRADD-TRAF2 and TRADD-FADD intercations define two distinct TNF receptor 1 signal transduction pathways. Cell 1996b;84:299-308

Hsu H, Xiong J, Goeddel DV. The receptor 1-associated protein TRADD signals cell death and NF$_K$B Activation. Cell 1995;81:495-504

Hu H, Sachs F. Stretch-activated ion channels in the heart. J Mol Cell Cardiol 1997;29:1511-1523

Hube F, Rohrig K, Hauner H. Overexpression of both TNF receptors in human obesity. Int J Obes Relat Metab Disord 1997;21:(Suppl 2): S130

Hulman S, Falkner B. The effect of excess dietary sucrose on growth, blood pressure, and metabolism in developing Sprague-Dawley rats. Pediatr Res 1994;36:95-101

Huot J, Houle F, Marceau F, Landry J. Oxidative stress-induced actin reorganization mediated by the p38 mitogen-activated protein kinase/heat shock pathway in vascula endothelial cells. Circ Res 1997;80:383-392

Huysman JAN, Vliegen HW, Van der Laarse A, Eulderinnk F. Changes in nonmyocyte tissue composition associated with pressure overload of hypertrophic human hearts. Pathol Res Pract 1989;184:577-581

Hynes RO. Integrins: versatility, modulation and signaling in cell adhesion. Cell 1992;69:11-25

Ignarro LJ. Biosynthesis and metabolism of endothelium-derived nitric oxide. Annu Rev Toxicol 1990;30:535-560

Ihle JN. Cytokine receptor signalling. Nature 1995;377:591-594

Ing DJ, Zang J, Dzau VJ, Webster KA, Bishopric NH. Modulation of cytokine-induced cardiac myocyte apoptosis by nitric oxide, Bak, and Bcl-x. Circ Res 1999;84:21-33

Irwin MW, Mak S, Mann DL. Tissue expression and immunolocalization of tumor necrosis factor-α in postinfarction dysfunctional myocardium. Circulation 1999;99:1492-1498

Izumo S, Nadal-Ginard B, Mahdavi V. Protooncogene induction and reprogramming of cardiac gene expression produced by pressure overload. Proc Natl Acad Sci USA 1988;85:339-343

Jayadev S, Liu B, Bielawska AE, Lee JY, Nazaire F, Pushkareva MY, Obeid LM, Hannun YA. Role of ceramide in cell cycle arrest. J Biol Chem 1995;270:2047-2052

Jennings RB, Ganote CE. Mitochondrial structure and function in acute myocardial ischaemic injury. Circ Res 1976;38:(suppl1)180-191

Jennings RB, Reiner KA, Jones RN, Peyton RB. High energy phosphates, anaerobic glycolysis and irreversibility in ischaemia. In: Myocardial Ischaemia. Ed: Spitzer. Plenum, New York. 1983;403-419

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

Jennings RB. Myocardial ischaemia-observations, definitions and speculations. J mol Cell Cardiol 1970;1:345-349

Jensen M, Haymond M, Rizza R, Cryer P, Mules J. Influence of body fat distribution on free fatty acid metabolism in obesity. J Clin Invest 1989;83:1168-1173

Jones SP, Girod WC, Palazzo AJ, Granger DN, Grisham MB, Jourd'heuil D, Huang PL, Lefer DJ. Myocardial ischaemia-reperfusion injury is exacerbated in absence of endothelial cell nitric oxide synthase. Am J Physiol Circ Physiol 1999;276:H1567-H1573

Juliano RL, Haskill S. Signal transduction from the extracellular matrix. J Cell Biol 1993;120:577-585

Kahn CR, Vicent D, Doria A. Genetics of non-insulin-dependent (type II) diabetes mellitus. Annu Rev Med 1996;47:509-531

Kajstura J, Cheng W, Reiss K, Clark WA, Sonnenblick EH, Krajewski S, Reed JC, Olivetti G, Anversa P. Apoptosis and necrotic myocyte cell deaths are independent contributing variables of infarct size in rats. Lab Invest 1996;74:86-107

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

Kannel WB, Belanger AJ. Epidemiology of heart failure. Am Heart J 1991;12:951-957

Kannel WB, Castelli WP, Gordon T, McNamara PM. Serum cholesterol, lipoproteins, and the risk of coronary heart disease: The Farmingham Study. Ann Int Med 1971; 74:1-12

Kapadia SR, Lee J, Torre-Amione G, Birdsall HH, Ma TS, Mann DL. Tumor necrosis factor gene and protein expression in adult feline myocardium after endotoxin administration. J Clin Invest 1995;96:1042-1052

Kapadia SR, Oral H, Lee J, Nakano M, Taffet GE, Mann DL. Hemodynamic regulation of tumor necrosis factor-alpha gene and protein expression in adult feline myocardium. Circ Res 1997;81:187-95

Kariya K, Karns LR, Simpson PC. Expression of constitutively activated mutant of the β-isozyme of protein kinase C in cardiac myocytes stimulates the promoter of the β-myosin heavy chain isogene. J Biol Chem 1991;266:10023-10026

Kelly RA, Balligand JL, Smith TW. Nitric oxide and cardiac function. Circ Res 1996;79:363-380

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

Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 1995;95:2111-2119

Kilgore KS, Lucchesi BR. Reperfusion injury after myocardial infarction: the role of free radicals and the inflammatory response. Clin Biochem 1993;26:359-370

Kim MY, Linardic C, Obeid L, Hannun YA. Identification of sphingomyelin turnover as an effector mechanism for the action of tumor necrosis factor alpha and gamma-interferon. Specific role in cell differentiation. J Biol Chem 1991;266:484-489

Kinugawa KI, Kohmoto O, Yao A, Serizawa T, Takahashi T. Cardiac inducible nitric oxide synthase negatively modulates myocardial function in cultured rat myocytes. Am J Physiol 1997; 272:H35-H47

Kira Y, Kochel PJ, Gordon EE, Morgan HE. Aortic perfusion as a determinant of cardiac protein synthesis. Am J Physiol 1984;246:C247-C258

Kira Y, Nakaoka T, Hashimoto E, Okabe F, Asano S, Sekine I. Effect of long-term cyclic mechanical load on protein synthesis and morphological changes in cultured myocardial cells from neonatal rat. Cardiovasc Drugs Ther 1994;8:251-262

Kishimoto T, Taga T, Akira S. Cytokine signal transduction. Cell 1994;76:253-262

Kissebah AH, Krakower GR. Regional adiposity and morbidity. Physiol Rev 1994;74:761-811

Knabb RM, Bergmann SR, Fox KAA, Sobel BE. The temporal pattern of recovery of myocardial perfusion and metabolism delineated by positron emission tomography after coronary thrombolysis. J Nucl Med 1987;28:1563-1570

Kojda G, Kottenberg K, Nix P, Schluter KD, Piper HM, Noack E. Low increase in cGMP induced by organic nitrates and nitrovasodilators improves contractile response of rat ventricular myocytes. Circ Res 1996;78:91-101

Kolesnick R, Golde DW. The sphingomyelin pathway in tumor necrosis factor and interleukin-1 signaling. Cell 1994;77:325-328

Komuro I, Kaida Y, Shibazaki Y, Kurabayashi M, Katoh Y, Hoh E, Takaku F, Yazaki Y. Stretching cardiac myocytes stimulates protooncogene expression. J Biol Chem 1990;265:3595-3598

Komuro I, Katoh Y, Kaida T, Shibazaki Y, Kurabayashi M, Hoh E, Takaku F, Yazaki Y. Mechanical loading stimulates cell hypertrophy and specific gene expression in cultured rat cardiac myocytes. J Biol Chem 1991;266:1265-1268

Komuro I, Kurabayashi M, Takaku F, Yasaki Y. Expression of cellular oncogenes in the myocardium during the developmental stage and pressure-overload hypertrophy of the rat heart. Circ Res 1988;62:1075-1079

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

Kronke M. Involvement of sphingomyelinases in TNF signaling pathways. Chem Phys Lipids 1999;102(1-2):157-166

Krown KA, Page MT, Nguyen C, Zechner D, Gutierrez V, Comstock KL. Tumor necrosis factor alpha-induced apoptosis in cardiac myocytes: involvement of the sphingolipid signaling cascade in cardiac cell death. J Clin Invest 1996;2854-2865

Kubota T, McTiernan CF, Frye CS, Demetris AJ, Feldman AM. Cardiac-specific overexpression of tumor necrosis factor-alpha causes lethal myocarditis in transgenic mice. J Card Fail 1997;3:117-124

Kumar G, Gupta S, Wang S, Nel AE. Involvement of Janus kinases, p52shc, Raf-1 and MEK-1 in the IL-6 -induced mitogen-activated protein kinase cascade of growth-response B cell line. J Immunol. 1994;152:4436-4447

Kunisada K, Hirota H, Fujio Y, Matsui H, Tani Y, Yamauchi-Takihara K, Kishimoto T. Activation of JAK-STAT and MAP kinases by leukemia inhibitory factor through gp130 in cardiac myocytes. Circulation 1996;94:2626-2632

Kuwahara K, Saito Y, Harada M, Ishikawa M, Ogawa E, Miyamoto Y, Hamanaka I, Kamitani S, Kajiyama N, Takahashi N, Nakagawa O, Masuda I, Nakao K. Involvement of Cardiotropin-1 in Cardiac Myocyte-Nonmyocyte Interactions During Hypertrophy of Rat Cardiac Myocytes in Vitro. Circulation 1999; 100:1116-1124

Lamarche B, Tchernof A, Moorjani S, Cantin B, Bernard PM, Dagenais GR, Lupien PJ, Depres JP. Small dense low-density lipoprotein particles as a predictor of risks of ischaemic heart disease. Circulation 1997;95:69-75

Lamas S, Marsden PA, Li GK, Tempst P. Michel T. Endothelial nitric oxide synthase: molecular cloning and characterization of a distinct constitutive enzyme isoform. Proc Natl Acad Sci USA 1992;89:6348-6352

LaRosa JC, Hunninghake D, Bush D, Criqui MH, Getz GS, Gotto AM, Grundy SM, Rakita L, Robertson RM, Weisfeldt ML. The cholesterol facts: serum cholesterol, and coronary heart disease. Circulation 1990; 81 (5):1721-1733

Latchman D. Cardiotrophin-1: a novel cytokine and its effects in the heart and other tissues. Pharma Therap 2000;85:29-37

Latini R, Bianchi M, Correale E, Dinarello CA, Fresco C, Maggioni AP, Mengozzi M, Romano S, Shapiro L. Cytokines in acute myocardial infarction: selective increase in circulating tumor necrosis factor, its soluble receptor, and interleukin-1 receptor agonist. J Cardiovasc Pharmacol 1994;23:1-6

Lauer MS, Anderson KM, Kannel WB, Levy D. The impact of obesity on left ventricular mass and geometry. The Farmingham Heart Study. J Am Med Assoc 1991;266:231-236

Lecarpentier Y, Waldenstrom A, Clergue M, Chemla D, Oliviero P, Martin JL, Swynghedauw B. Major alterations in relaxation during cardiac hypertrophy induced by aortic stenosis in guinea pig. Circ Res 1987;61(1):107-116

Lefer AM, Tsao P, Aoki N, Palladino MA Jr. Mediation of cardioprotection by transforming growth factor-alpha. Science 1990;249:61-64

Lefer AM. Origin of myocardial depressant factor in shock. Am J Physiol 1970;218:1423-1427

Leroy P, Dessolin S, Villageois P, Monn BC, Freidman JM, Ailhaud G, Dani C. Expression of ob gene in adipose cells. J Biol Chem 1996;271:2365-2368

Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. N Engl J Med 1990;323:236-41

Lewis JM, Schwartz AM. Mapping in vivo associations of cytoplasmic proteins with integrin β_1 cytoplasmic domain mutants. Mol Biol Cell 1995;6:151-160

Li J, Hampton T, Morgan JP, Simons M. Stretch-induced VEGF expression in the heart. J Clin Invest 1997a;100:18-24

Li S, Sedivy JM. Raf-1 protein kinase activates the NF_KB transcription factor by dissociating the cytoplasmic NF_KB-I_KB complex. Proc Natl Acad Sci USA 1993;90:9247-9251

Li Z, Bing OHL, Long X, Robinson KG, Lakatta EG. Increased cardiac myocyte apoptosis during the transition to heart failure in the spontaneously hypertensive rat. Am J Physiol 1997b;272:H2313-2319

Litwin SE, Katz SE, Weinberg EO, Lorell BH, Aurigemma GP, Douglas PS. Serial echocardiographic-Doppler assessment of left ventricular geometry and function in rats with pressure-overload hypertrophy: chronic angiotensin-converting enzyme inhibition attenuates the transition to heart failure. Circulation 1995;91:2642-2654

Liu S, Willett WC, Stampfer MJ, Hu FB, Franz M, Sampson L, Hennekens CH, Manson JE. A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women. Am J Clin Nutr 2000;71(6):1455-1461

Liu Z, Shu H, Goeddel DV, Karin M. Dissection of TNF receptor 1 effector functions: JNK activation is not related to apoptosis while NF_KB activation prevents cell death. Cell 1996;87:565-576

Lompre AM, Mercardier JJ, Schwartz K. Changes in gene expression during cardiac growth. Int Rev Cyto 1991;124:137-186

Long CS, Henrich CJ, Simpson PC. A growth factor for cardiac myocytes is produced by cardiac nonmyocytes. Cell Regul 1991;2:1081-1095

Lopez-Soriano J, Lopez-Soriano FJ, Bagby GJ, Williamson DH, Argiles JM. Anti-TNF treatment does not reverse the abnormalities in lipid metabolism of the obese Zucker rat. Am J Physiol 1997;272:E656-E660

Luberto C, Hannun YA. Sphingolipid metabolism in the regulation of bioactive molecules. Lipids 1999;34:S5-S11

Lyons CR, Orloff GJ, Cunningham JM. Molecular cloning and functional expression of an inducible nitric oxide synthase from a murine macrophage cell line. J Biol Chem 1992;267:6370-6374

MacDonald LJ, Moss J. Stimulation by nitric oxide of an NAD linkage to glyceraldehyde-3-phosphate dehydrogenase. Proc Natl Acad Sci USA 1993;90:6238-6241

MacKenna DA, Dolfi F, Vuori K, Ruoslahti E. Extracellular signal-regulated kinase and c-Jun NH_2-terminal kinase activation by mechanical stretch is integrin-dependent and matrix-specific in rat cardiac fibroblasts. J Clin Invest 1998;101:301-310

Mallinin NL, Boldin MP, Kovalenko AV, Wallach D. MAP3K-related kinase involved in NF_KB induction by TNF, CD95 and IL-1. Nature 1997;385:540-544

Mamuya W, Chobanian WA, Brecher P. Age-realted changes in fibronectin expression in spontaneously hypertensive, Wistar-Kyoto, and Wistar rat hearts. Circ Res 1992;71:1341-1350

Mann DL, Kent RL, Cooper IVG. Load regulation of the properties of adult feline cardiocytes: growth induction by cellular deformation. Circ res 1989;65:1079-1090

Mann DL: The effect of tumor necrosis factor-alpha on Cardiac structure and Function: A tale of two cytokines. Journal of Cardiac Failure 1996; 2:S165-S172

Marui N, Offerman MK, Swerlick R, Kunsch C, Rosen CA, Ahmad M, Alexander RW, Medford RM. Vascular cell adhesion molecule-1(VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. J Clin Invest 1993;92:1866-1874

Mascareno E, Dhar M, MAQ Siddiqui. Signal transduction and activator of transcription (STAT) protein-dependent activation of angiotensinogen promoter: a cellular signal for hypertrophy in cardiac muscle. Proc Natl Sci USA 1998;95:5590-5594

Mathias S, Younes A, Kan CC, Orlow I, Joseph C, Kolesnick RN. Activation of the sphingomyelin signaling pathway in intact EL4 cells and in cell-free system by IL-1 beta. Science 1993;259:519-522

Matsui H, Okumura K, Kawakami K, Hibino M, Toki Y, Ito T. Improved insulin sensitivity by bezafibrate in rats. Relationship to fatty acid composition of skeletal-muscle triglycerides. Diabetes 1997;46:348-353

Matsumori A, Yamada T, Suzuki H, Matoba Y, Sasayama S. Increased circulating cytokines in patients with myocarditis and cardiomyopathy. Br Heart J 1994;72:561-66

Matthews N, Neale ML, Jackson SK, Stark JM. Tumor cell killing by tumor necrosis factor: inhibition by anaerobic conditions, free-radical scavengers and inhibitors of arachidonate metabolism. Immunology 1987;62:153-55

Mauriege P, Prud'homme D, Lemieux S, Tremblay A, Depres JP. Regional differences in adipose tissue lipolysis from lean and obese women: existence of postreceptor alterations. Am J Physiol 1995;269:E341-E350

Maury CPJ, Teppo AM. Circulating tumor necrosis factor-α (Cachectin) in myocardial infarction. J Intern Med 1989;225:333-336

Maxwell SR, Lip YH. Reperfusion injury: a review of the pathophysiology, clinical manifestations and therapeutic options. Internal J Cardiol 1997;58:95-117

McAinsh AM, Geyer M, Fandrey J, Ruegg JC, Wiesner RJ. Expression of vascular endothelial growth factor during the development of cardiac hypertrophy in spontaneously hypertensive rats. Mol Cell Biochem 1998; 187:141-146

MCormack JG, Dean HG, Jennings GS, Blundell JE. Effects of chronic low doses of d-Fenfluoramine on weight gain and caloric intake, brown adipose tissue thermogenic parameters and brain neurotransmitter content in rats fed chow or palatable food. Int J Obes 1989;13:633-635

McWhinney CD, Hunt RA, Conrad KM, Dostal DE Baker KM. The type I angiotensin II receptor couples to Stat and Stat3 actiavtion through Jak2 kinase in neonatal rat cardiac myocytes. J Mol Cell Cradiol 1997;29:2513-2524

Meerson FZ, Javich MP. Isoenzyme pattern and activity of myocardial creatine phosphokinase under heart adaptation to prolonged overload. Basic Res Cardiol 1982;77:349-358

Meerson FZ. The failing heart. In: Katz (eds). Adaptation and Deadaptation. New York:Raven, 1983

Meldrum DR, Cleveland JC, Sheridan BC, Rowland RT, Banerjee A, Harken AH. Cardiac surgical implications of calcium dyshomeostasis in the heart. Ann Thorac Surg 1996;61:1273-1280

Meldrum DR, Meng X, Dinarello CA. Human myocardial tissue TNF_{α} expression following acute global ischaemia in vivo. J Mol Cell Cardiol 1998;30:1683-1689

Meldrum DR. Tumor necrosis factor in the heart. Am J Physiol 1998;274(Regulatory Integrative Comp Physiol 43):R577-R595

Mende U, Kagen A, Meister M, Neer EJ. Signal transduction in atria and ventricles of mice with transient cardiac expression of activated G protein α_q. Circ Res 1999;85:1085-1091

Mercurio F, Manning AM. NF-kappaB as a primary regulator of stress response. Oncogene 1999;18:6163-6171

Mercurio F, Zhu H, Murray BW, Shevchenko A, Bennett BL, Li J, Young DB, Barbosa M, Mann M, Manning A, Rao A. IKK-1 and IKK-2: cytokine-activated IkappaB kinases essential for NF-kappaB activation. Science 1997;278:860-866

Mery PF, Pavoine C, Belhassen L, Pecker F, Fischmeister R. Nitric oxide regulates cardiac Ca^{2+} current: involvement of cGMP-inhibited and cGMP-stimulated phosphodiesterases through guanylyl cyclase activation. J Biol Chem 1993;268:26286-26295

Messerli FH. Cardiovascular effects of obesity and hypertension. Lancet 1982;1:1165-1168

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

Miyata S, Haneda T, Osaki J, Kikuchi K. Renin-angiotensin in stretch-induced hypertrophy of cultured neonatal rat heart cells. Eur J Pharmacol 1996;307:81-88

Mohamed-Ali V, Pinkey JH, Coppack SW. Adipose tissue as an endocrine and paracrine organ. Int J Obes Relat Metab Disord 1998;22:1145-1158

Molkentin JD, Dorn II GW. Cytoplasmic signaling pathways that regulate cardiac hypertrophy. Annu Rev Physiol 2001;63:391-426

Molkentin JD, Lu J, Antos CL, Markham B, Richardson J, Robbins J, Grant SR, Olson EN. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. Cell 1998;93:215-228

Moller DE. Potential role of TNF_α in the pathogenesis of insulin resistance and type 2 diabetes. Trends Endocrinol Metab 2000;11:212-217

Mondry A, Swynghedauw B. Biological adaptation of the myocardium to chronic mechanical overload. Molecular determinants of the autonomic nervous system. Eur Heart J 1995;16:64-73

Morin CL, Eckel RH, Marcel T, Pagliasotti MJ. High fat diets elevate adipose tissue-derived tumor necrosis factor-α activity. Endocrinology 1997;138:4665-4671

Morin CL, Gayles EC, Podolin DA, Wei Y, Xu M, Pagliasotti MJ. Adipose tissue derived tumor necrosis factor activity correlates with fat cell size but not insulin action in aging rats. Endocrinology 1998;139:4998-5005

Mulvagh SL, Michael LH, Perryman MB, Roberts R, Schneider MD. A hemodynamic load in vivo induces cardiac expression of the cellular oncogene, c-myc. Biochem Biophys Res Commun 1987;147:627-636

Nakafuku M, Satoh T, Kaziro Y. Differentiation factors, including nerve growth factor, fibroblast growth factor, and interleukin-6, induce an accumulation of an active RAS GTP complex in rat pheochromocytoma PC12 cells. J Biol Chem 1992;267:19448-19454

Nakajima T, Fujioka S, Tokunaga K, Hirobe K, Matsuzawa Y, Tarui S. Noninvasive study of left ventricular performance in obese patients: Influence of duration of obesity. Circulation 1985;71:481-486

Nakamura K, Fushimi K, Kouchi H, Mihara K, Miyasaki M, Ohe T, Namba M. Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor-α and angiotensin II. Circulation 1998;98:794-799

Nakanishi K, Vinten-Johansen J, Lefer DJ, Zhao Z, Fowler WC 3rd, McGee DS, Johnston WE.Intracoronary L-arginine during reperfusion improves endothelial function and reduces infarct size. Am J Physiol 1992;263(Heart Circ Physiol 32):H1650-H1658

Nakano M, Knowlton AA, Diblos Z, Mann D.Tumor necrosis factor alpha confers resistance to hypoxic injury in the adult mammalian cardiac myocyte Circulation 1998; 97:1392-1400

Narazaki M, Witthuhn BA, Yoshida K, Silvennoinen O, Yasukawa K, Ihle JN, Kishimoto T, Taga T. Activation of JAK2 kinase mediated by the interleukin 6 signal transducer gp130. Porc Natl Acad Sci USA 1994; 91:2285-2289

Nathan DM. Long-term complications of diabetes mellitus. New Eng J Med 1993;328:1676-1685

Natoli G, Costanzo A, Guido F, Moretti F, Levrero M. Apoptotic, non-apoptotic, and anti-apoptotic pathways of tumor necrosis factor signaling. Biochem Pharmacol 1998;56:915-920

Natoli G, Costanzo A, Lanni A, Templeton DJ Woodgett JR, Balsano C, Levrero M. Activation of SAPK/JNK by TNF receptor 1 through a nontoxic TRAF2-dependent pathway. Science 1997;275:200-203

Nayler WG, Panagiotopolous S, Elz JS, Daly MJ. Calcium mediated damage during post-ischemic reperfusion. J Mol Cell Cardiol 1988 20 (suppl II):41-54

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

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

Newton AC. Protein kinase C: structure, function, and regulation. J Biol Chem 1995;270:28495-28498

Newton AC. Regulation of protein kinase C. Curr Opin Cell Biol 1997;9:161-167

Nikolova-Karakashian M, Morgan ET, Alexander C, Liotta DC, Merrill AH. Bimodal regulation of ceramidase by interleukin-1β. Implication for the regulation of cytochrome P450 2C11 (CYP2C11). J Biol Chem 1997;272:18718-18724

Nishida K, Harrison DG, Navas JP, Fisher AA, Dockery SP, Uematsu M, Nerem RM, Alexander RW, Murphy TJ. Molecular cloning and characterization of the constitutive bovine aortic endothelial cell nitric oxide synthase. J Clin Invest 1992;90:2092-2096

Nishio E, Fukushima K, Shiozaki M, Watanabe Y. Nitric oxide donor SNAP induces apoptosis in smooth muscle cells through cGMP-independent mechanism. Biochem Biophys Res Commun 1996;221:163-168

Nishito H, Saitoh M, Mochida Y, Takeda K, Nakano H, Rothe M, Miyazono K, Ichijo H. ASK1 is essential for JNK/SAPK activation by TRAF2. Mol Cell 1998;2:389-95

Nishizuka Y. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 1992;29:2545-2559

Nutt LK, Goodwin GW, Taegtmeyer H. Tumor necrosis factor-α depresses contractile function while increasing oleate oxidation in the isolated working rat heart. Circulation 1998;98:I-213

Oddis CV, Finkel MS. Cytokine-stimulated nitric oxide production inhibits mitochondrial activity in cardiac myocytes. Biochem Biophys Res Commun 1995;213:1002-1009

Ofei F, Hurel S, Newkirk J, Soopwith M, Taylor R. Effects of an engineered human anti-TNF-α antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. Diabetes 1996;45:881-885

Olivetti GR, Abbi R, Quaini F, Kajstura J, Cheng W, Nitahara JA, et al. Apoptosis in the failing human heart. N Engl Med 1997;336:1131-1141

Opie LH. Reperfusion injury and its pharmacologic modification. Circulation 1989;80:1049-1062

Oral H, Dorn GW, Mann DL. Sphingosine mediates the immediate negative inotropic effects of tumor necrosis factor-α in the adult mammalian cardiac myocyte. J Biol Chem 1997;272:4836-4842

Packer M. Is tumor necrosis factor an important neurohormonal mechanism in chronic heart failure? Circulation 1995;92:1379-1382

Pagliosotti M, Shahrokhi K, Moscarello M. Involvement of liver and skeletal muscle in sucrose-induced insulin resistance: dose response studies. Am J Physiol 1994;266:R1637-1644

Pan J, Fukuda K, Kodama H, Makino S, Takahashi T, Sano M, Hori S, Ogawa S. Involvement of gp130 mediated signaling in pressure overload induced activation of the Jak/Stat pathway in rodent heart. Heart Vessels 1998;13:199-208

Patel VC, Yellon DM, Singh KJ, Neild GH, Woolfson RG. Inhibition of nitric oxide limits infarct size in the in situ rabbit heart. Biochem Biophys Res Commun 1993;194(1):234-238

Paternostro DJ, Pagano D, Gnecchi-Ruscone T, Bonser RS, Camici PG. Insulin resistance in patients with cardiac hypertrophy. Cardiovasc Res 1999;42:246-253

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

Pearlman ES, Weber KT, Janicki JS, Pietra G, Fishman AP. Muscle fiber orientation and connective tissue content in the hypertrophied human heart. Lab Invest 1982;46:158-164

Pickavance LC, Taddayon M, Widdowson PS, Buckingham RE, Wilding JPH. Therapeutic index for rosiglitazone in dietary obese rats: separation of efficacy and haemodilution. Brit J Pharmacol 1999;128:1570-76

Pinsky DJ, Yang Y, Aji W, Szabolcs M, Liao H, Sciacca RR, Cannon PJ. Nitric oxide induces apoptosis of adult cardiac myocytes. Circulation 1995;92 (suppl I):I-565

Pouliot MC, Despres JP, Nadeau A, Moorjani S, Prud'homme D, Lupien PJ, Tremblay A, Bouchard C. Visceral obesity in men. Associations with glucose tolerance, plasma insulin, and lipoprotein levels. Diabetes 1992;41:826-834

Puceat M, Vassort G. Signalling by protein kinase C isoform in the heart. Mol Cell Biochem 1996;157:65-72

Rao GN, Berk BC. Active oxygen species stimulate vascular smooth muscle cell growth and proto-oncogene expression. Circ Res 1992;70:593-599

Revis NW, Thomson RY, Cameron AJV. Lactate dehydrogenase isoenzymes in the human hypertrophic heart. Cardiovasc Res 1977;11:172-176

Robinson MJ, Cobb MH. Mitogen-activated protein kinase pathways. Curr Opin Cell Biol 1997;9:180-186

Robles RG, Villa E, Santirso R, Martinez J, Ruilope LM, Cuesta C, Sancho JM. Effects of Captopril on sympathetic activity lipid and carbohydrate metabolism in a model of obesity-induced hypertension in dogs. Am J Hypertens 1993;6:1009-15

Rocchini AP, Moorehead CP, DeRemer S, Bondie D. Pathogenesis of weight-related changes in blood pressure in dogs. Hypertension 1989;13:922-8

Rosen LB, Ginty DD, Greenberg ME. Calcium regulation of gene expression. Adv Sec Mess Phosphoprotein Res 1995;30:225-253

Rosenkranz ER, Buckberg GD. Myocardial protection during surgical coronary reperfusion. J Am Coll Cardiol 1983;1:1235-1246

Ross RS, Pham C, Shai SY, Goldhaber JI, Fenczik C, Glembotski CC, Ginsberg MH, Loftus JC. Beta 1 integrins participate in the hypertrophic response of rat ventricular myocytes. Circ Res 1998;82(11):1160-1172

Rothe M, Pan M-G, Henzel WJ, Ayres TM, Goeddel DV. The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. Cell 1995;83:1243-1252

Rothe M, Wong SC, Henzel WJ, Goeddel DV. A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor. Cell 1994;78:681-692

Roust LR, Jensen MD. Post prandial free fatty acids kinetics are abnormal in upper body obesity. Diabetes 1993; 42:1567-1573

Ruknudin A, Sachs F, Bustamante JO. Stretch-activated ion tissue-cultured chick heart. Am J Physiol 1993;264:H960-972

Ruwhof C, Van der Laarse A. Mechanical stress-induced cardiac hypertrophy: mechanisms and signal transduction pathways. Cardiovasc Res 2000;47:23-37

Ryan T, Tarver RD, Duerk JL, Sawada SG, Hollencamp NC. Distinguishing viable from infarcted myocardium after experimental ischaemia and reperfusion by using nuclear magnetic resonance imaging. J Am Coll Cardiol 1990;15:1355-1364

Sack MN, Smith RM, Opie LH. Tumor necrosis factor in myocardial hypertrophy and ischaemia-an anti-apoptotic perspective. Cardiovasc Res 2000;45:688-695

Sadoshima J, Izumo S. Mechanical stretch rapidly activates multiple signal transduction pathways in cardiac myocytes: potential involvement of an autocrine/paracrine mechanism. EMBO J 1993;12:1681-1692

Sadoshima J, Jahn L, Takahashi T, Kulik TJ, Izumo S. Molecular characterization of the stretch-induced adaptation of cultured cardia cells. An in vitro model of load-induced cardiac hypertrophy. J Biol Chem 1992;267:10551-10560

Sakobar A, Horton JD, Bowler K, Manning R, Meager A, dark JH. Raised levels of tumor necrosis-factor alpha (TNF-alpha) in cardiac transplant patients. Clin Transplantation 1993;7:459-466

Sawyer DB, Colucci WS. Nitric oxide in the failing myocardium. Clin Cardiol 1998;16:657-664

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

Schaper J, Mulch J, Winkler B, Schaper W. Ultrastructural, functional, and biochemical criteria for estimation of reversibility of ischaemic injury: a study on the effects of global ischaemia in the isolated dog heart. J Mol Coll Cardiol 1979;11:521-541

Schindler C, Darnell JEJ. Transcriptional responses to polypeptide ligands: The JAK-STAT pathway. Annu Rev Biochem 1995;64:621-651

Schluter KD, Weber M, Schraven E, Piper HM. NO donor SIN-1 protects against reoxygenation-induced cardiomyocyte injury by a dual action. Am J Physiol 1994; 267(Heart Circ Physiol 36):H1461-H1466

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

Schreck R, Rieber P, Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. EMBO J 1991;10:2247-2258

Schwartz K, de la Bastie D, Bouveret P, Oliviero , Alonso S, Buckingham M. α-Skeletal muscle actin mRNAs accumulate in hypertrophied adult rat hearts. Circ Res 1986;59:551-555

Schwartz MA, Schaller MD, Ginsberg MH. Integrins: emerging paradigms of signal transduction. Annu rev Cell Dev Biol 1995;11:549-599

Seko Y, Takahashi N, Shibuya M, Yazaki Y. Pulsatile stretch stimulates vascular endothelial growth factor (VEGF) secretion by cultured rat cardiac myocytes. Biochem Biophys Res Commun 1999;254:462-465

Sen S, Kundu G, Mekhail N, Castel J, Misono K, Healy B. Myotrophin: purofocation of a novel peptide from spontaneously hypertensive rat heart that influences myocardial growth. J Biol Chem 1990;265:16635-16643

Shakov AN, Collart MA, Vassali P, Nedospasov SA, Jongeneel CV. Kappa B-type enhancers are involved in lipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor-α gene in primary macrophages. J Exp Med 1990;171:35-47

Sharma R, Coats AJS, Anker SD. The role of inflammatory mediators in chronic heart failure: cytokines, nitric oxide, and endothelin-1. Int J Cardiol 2000;72:175-186

Shen W, Hintze TH, Wolin MS. Nitric oxide: an important signaling mechanism between vascular endothelium and parenchymal cells in the regulation of oxygen consumption. Circulation 1995;92:3505-3512

Shen W, Xu X, Ochoa M, Zhao G, Wolin MS, Hintze TH. Role of nitric oxide in the regulation of oxygen consumption in conscious dogs. Circ Res 1994;75:1086-1095

Shimamoto N, Goto N, Tanabe M, Imamoto T, Fujiwara S, Hirata M. Myocardial energy metabolism in the hypertrophied hearts of spontaneously hypertensive rats. Basic Res Cardiol 1982;77:359-371

Shimaoka M, Iida T, Ohara A, Tanaka N, Mashimo T, Honda T, Yshiya I. NOC, a nitric-oxide-releasing compound, induced dose-dependent apoptosis in macrophages. Biochem Biophys Res Commun 1995;209:519-526

Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL. Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. Nature 1999;401:73-76

Shioi T, Matsumori A, Kihara Y, Inoko M, Ono K, Iwanaga Y Yamada T, Iwasaki A, Matsushima K, Sasayama S. Increased expression of Interleukin-1β and monocyte chemotactic and activating factor/monocyte chemoattractant protein-1 in the hypertrophied and failing heart with pressure overload. Circ Res 1997;81:664-671

Shubeita HE, Martinson EA, Van Bilsen M, Chien KR, Brown JH. Transcriptional activation of the cardiac myosin light chain 2 and atrial natriuretic factor genes by protein kinase C in neonatal rat ventricular myocytes. Proc Natl Acad USA 1992;89:1305-1309

Singh K, Sirokman G, Communal C, Robinson KG, Conrad CH, Brooks WW, Bing OH, Colucci WS. Myocardial osteopontin expression coincides with the development of heart failure. Hypertension 1999;33(2):663-670

Skolnik EY, Marcusohn J. Inhibiting insulin receptor signaling by TNF: potential role of obesity and non-insulin-dependent diabetes mellitus. Cytokine Growth Factor Rev 1996;7:161-173

Smith JD, Bryant SR, Cooper LL, Vary CP, Gotwals PJ, Koteliansky VE, Lindner V. Soluble transforming growth factor-beta type II receptor inhibits negative remodeling, fibroblast transdifferentiation, and interstitial lesion formation but not endothelial growth. Circ Res 1999;84:1212-1222

Sowers JR, Farrow SL.1996. Treatment of elderly hypertensive patients with diabetes, renal disease, and coronary heart disease. Am J Geriat Cardiol 1996;5:57-70

Spiegelman BM, Flier JS. Adipogenesis and obesity: rounding out the big picture. Cell 1996;87:377-389

Spiegelman BM, Hotamisligil GS. Through thick and thin: wasting, obesity, and TNFα. Cell 1993;73:625-627

Spiegelman D, Israel RG, Bouchard C, Willet WC. Absolute fat mass, percent body fat, and body-fat distribution: which is the real determinant of blood pressure and serum glucose? Am J Clin Nutr 1992;55:1033-1044

Stanger BZ, Leder P, Lee TH, Kim E, Seed B. RIP: a novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death. Cell 1995;81:513-23

Steller H. mechanisms and genes of cellular suicide. Science 1995;267:1445-1449

Stephens JM, Pekala PH. Transcriptional repression of GLUT4 and c/EBP genes in 3T3-L1 cells by tumor necrosis factor-alpha. J Biol Chem 1991;266:21839-21845

Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA. The hormone resistin links obesity to diabetes. Nature 2001;409:307-312

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

Storlien LH, Oakes N, Pan D, Kusonoki M, Jenkins A. Syndromes of insulin resistance in the rat: inducement by diet and amelioration with benfluorex. Diabetes 1993;42:457-462

Stuehr DJ, Nathan CF. Nitric oxide: a macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. J Exp Med 1989;169:1543-1555

Sturgill TW, Wu J. Recent progress in characterization of protein kinase cascades for phosphorylation of ribosomal protein S6. Biochem Biophys Acta 1991;1092:350-357

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;321:280-7

Sugden PH, Clerk A. Cellular mechanisms of cardiac hypertrophy. Mol Med 1998;76:725-746

Sumii K, Sperelakis N. cGMP-dependent protein kinase regulation of L-type Ca^{2+} current in rat ventricular myocytes. Circ Res 1995;77:803-812

Suzuki M, Wildhirt SM, Dudek RR, Narayan KS, Bailey AH, Bing RJ. Induction of apoptosis in myocardial infarction and its possible relationship to nitric oxide synthase in macrophages. Tissue Cell 1996;28:89-97

Swedberg K, Eneroth P, Kjekshus J, Wilhelmsen L, CONSENSUS Trial Study Group. Hormones regulating cardiovascular function in patients with severe congestive heart failure and their relation to mortality. Circulation 1990;82:1730-36

Sweet MJ, Hume DA. Endotoxin signal transduction in macrophages. J Leukoc Biol 1996;60:8-26

Szaboles M, Michler RE, Yang X, Aji W, Roy D, Athan E, Sciacca R, Minanov OP, Cannon PJ. Apoptosis of cardiac myocytes during cardiac allograft rejection: relation to induction of nitric oxide synthase. Circulation 1996;94:1665-1673

Szalkowski D, White-Carrington S, Berger J, Zhang B. antidiabetic thiazolidinediones block the inhibitory effect of tumor necrosis factor-α on differentiation, insulin-stimulated glucose uptake, and gene expression in 3T3-L1 cells. Endocrinology 1995;136:1474-1481

Takahashi T, Fukuda K, Pan J, Kodama H, Sano M, Makino S, Kato T, Manabe T, Ogawa S. Characterization of insulin-like growth factor-1-induced activation of the JAK/STAT pathway in rat cardiomyocytes. Circ Res 1999;85:884-891

Tamura K, Umemura S, Nyui N, Hibi K, Ishigama T, Kihara M, Toya Y, Ishii M. Activation of angiotensinogen gene in cardiac myocytes by angiotensin II and mechanical stretch. Am J Physiol 1998;275:R1-R9

Tandia D, Krotkrewski, Smith U. Importance for the metabolic abnormalities associated with an abdominal fat distribution. Metabolism 1989;38:572-576

Tang L, Taylor PB. Altered contractile function in isoproterenol-induced hypertrophied rat heart. J Hypertension 1996;14:751-757

Tartaglia LA, Goeddel DV. Two TNF receptors. Immunol Today 1992;13:151-153

Tartaglia LA, Ayres TM, Wong GH, Goeddel DV. A novel domain within the 55Kd TNF receptor signal cell death. Cell 1993a;74:845-53

Tartaglia LA, Pennica D, Goeddel DV. Ligand passing: the 75-kDa tumor necrosis factor (TNF) receptor recruits TNF for signaling by the 55-kDa TNF receptor. J Biol Chem 1993b;268:18542-8

Taylor SI. Deconstructing type 2 diabetes. Cell 1999;97:9-12

Thompson CB. apoptosis in the pathogenesis and treatment of disease. Science 1995;267:1456-1462

Torre-Amione G, Kapadia S, Lee J, Bies RD, Lebovitz R, Mann DL. Expression and functional significance of tumor necrosis factor receptors in human myocardium. Circulation 1995;92:1487-1493

Torre-Amione G, Kapadia S, Lee J, Durand JB, Bies RD, Young JB, Mann DL. Tumor necrosis factor-alpha and tumor necrosis factor receptors in the failing human heart. Circulation 1996;93:704-11

Torres J, Darley-Usmar V, Wilson MT. Inhibition of cytochrome c oxidase turnover by nitric oxide: mechanism and implications for control of respiration. Biochem J 1995;212:169-173

Trede NS, Tsytsykova AV, Chatila T, Goldfield AE, Gehas RS. Transcriptional activation of the human TNF-alpha promoter by superantigen in human monocytic cells: role of NF_KB. Immunol 1995;155:902-908

Tschernof A, Lamarche B, Prud'homme A. The dense LDL phenomenon: association with plasma lipoprotein levels, visceral obesity, and hyperinsulinemia in men. Diabetes Care 1996;19:629-637

Ungureanu-Longrois D, Balligand JL, Kelly RA, Smith TW. Myocardial contractile dysfunction in the systemic inflammatory response syndrome: role of a cytokine-inducible nitric oxide synthase in cardiac myocytes. J Mol Cell Cardiol 1995;27:155-167

Vandenabeele P, Declercq W, Vanhaesebroek B, Grooten J, Fiers W. Both TNF receptors are rquired for TNF-mediated induction of apoptosis in PC60 cells. J Immunol 1995;154(6):2904-2913

Vandenburgh HH, Solerssi R, Shansky J, Adams JW, Henderson SA, Lemaire J. Response of neonatal rat cardiomyocytes to repetitive mechanical stimulation in vitro. Ann NY Acad Sci 1995;752:19-29

Vaux DL, Strausser A. The molecular biology of apoptosis. Proc Natl Acad Sci USA 1996;93:2239-2244

Vejlstrup NG, Bouloumi A, Boegarrd S, Anderson CB, Nielsen-Kudsk JE, Mortensen SA, Kent JD, Harrison DG, Busse R, Aldershivile J. Inducible nitric oxide synthase (iNOS) in the human heart: expression and localization in congestive heart failure. J Mol Cell Cardiol 1998;30:1215-1223

Vila-Petroff MG, Younes A, Egan J, Lakatta EG, Sollot SJ. Activation of distinct cAMP-dependent and cGMP-dependent pathways by nitric oxide in cardiac myocytes. Circ Res 1999;84:1020-1031

Villa E, Rabano A, Albarran OG, Ruilope LM, Garcia-Robles R. Effect of chronic combined treatment with Captopril and Pravastatin on the progression of insulin resistance and cardiovascular alterations in experimental model of obesity in dogs. Am J Hypertens 1998;11:844-51

Villarreal FJ, Dillmann WH. Cardiac hypertrophy-induced changes in mRNA levels for TGF-β_1, fibronectin, and collagen. Am J Physiol 1992;262:H1861-H1866

Von Harsdorf R, Lang RE, Fullerton M, Woodcock EA. Myocardial stretch stimulates phosphtidylinositol turnover. Circ Res 1989;65:494-501

Walter F, Addis T. Organ work and organ weight. J Exp Med 1939;69:467-83

Wambolt RB, Henning SL, English DR, Bondy GP, Allard MF. Regression of cardiac hypertrophy normalizes glucose metabolism and left ventricular function during reperfusion. J Mol Cell Cardiol 1997;29:939-948

Wambolt RB, Lopaschuk GD, Brownsey RW, Allard MF. Dichloroacetate improves postischaemic function of hypertrophied hearts. J Am Coll Cardiol 2000;36:1378-1385

Wang S, Zweier JL. Measurement of nitric oxide and peroxynitrite generation in the postischaemic heart: evidence for peroxynitrite-mediated reperfusion injury. J Biol Chem 1996;271:29223-29230

Wang YG, Lipsius SL. Acetylcholine elicits a rebound stimulation of Ca^{2+} current mediated by pertussis toxin-sensitive G protein and cAMP-dependent protein kinase A in atrial myocytes. Circ Res 1995;76:634-644

Weber KT, Brilla CG, Campbell SE, Guarda E, Zhou G, Sriram K,. Myocardial fibrosis: role of angiotensin II and aldosterone. Bas Res in Cardiol 1993;88(suppl 1):107-124

Weber KT, Brilla CG: Pathological hypertrophy and cardiac interstitium: fibrosis and rennin-angiotensin-aldosterone system. Circulation 1991; 83:1849-1865

Widdowson PS, Upton R, Buckingham R, Arch J, Williams G. Inhibition of food response to intracerebroventricular injection of leptin is attenuated in rats with diet-induced obesity. Diabetes 1997;46:1782-1785

Wilcken DE. Left ventricular volume in man: the relation to heart rate and to end-diastolic pressure. Ann Med Australian 1968;17:195-205

Wilding JPH, Gilbey M, Mannan M, Aslam N, Ghatei MA, Bloom SR. Increased neuropeptide Y content in individual hypothalamic nuclei, but not neuropeptide Y mRNA, in diet-induced obesity in rats. J Endocrinol 1992;132:299-304

Winkler G, Salomon F, Salomon D, Speer G, Simon K, Cseh K. Elevated serum tumor necrosis factor-alpha levels can contribute to the insulin resistance in type II (non-insulin-dependent) diabetes and in obesity. Diabetologia 1998;41:860-861

Wong GHW, Elwell JH, Oberley LW, Goeddel DV. Manganous superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. Cell 1989;58:923-31

Woolfson RG, Patel VC, Neild GH, Yellon DM. Inhibition of nitric oxide synthesis reduces infarct size by an adenosine-dependent mechanism. Circulation 1995;91(5):1545-1551

Worrall NK, Lazenby WD, Misko TP, Lin T-S, Rodi CP, Manning PT, Tilton RG, Williamson JR, Ferguson TB Jr. Modulation of in vivo alloreactivity by inhibition of inducible nitric oxide synthase. J Exp Med 1995;181:63-70

Yang X, Chowdhury N, Cai B, Brett J, Marboe C, Sciacca M, Michler RE, Cannon PJ. Induction of myocardial nitric oxide synthase by cardiac allograft rejection. J Clin Invest 1994;94:714-721

Yin FCP, Spurgeon HA, Rakusan K, Weisfeldt ML, Lakatta EG. Use of tibial length to quantify cardiac hypertrophy: application in the aging rat. Am J Physiol 1982;243(Heart Circ Physiol.12):H941-H947

Yokoyama T, Nakano M, Bednarczyk JL, McIntyre BW, Entman M, Mann DL. Tumor necrosis factor-alpha provokes a hypertrophic growth response in adult cardiac myocytes. Circulation 1997;95:1247-1252

Yokoyama T, Vaca L, Rossen RD, Durante W, Hazarika P, Mann DL. Cellular basis for the negative inotropic effects of tumor necrosis factor-α in the adult mammalian heart. J Clin Invest 1993;92:2303-12

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

Zahorska-Markiewicz, Janowska J, Olszanecka-Glinianowicz M, Zurakowski A. Serum concentrations of TNF-α and soluble TNF-α receptors in obesity. Int J Obesity 2000;24:1392-1395

Zhang B, Berger J, Hu E, Szalkowski D, White-Carrington S, Spiegelman BM, Moller DE. Negative regulation of perixisome proliferator-activated receptor-γ gene expression contributes to the anti-adipogenic effects of tumor necrosis factor-α. Mol Endocrinol 1996;10:1457-1466

Zhang DX, Fryer RM, Hsu AK, Zou A-P, Gross GJ, Campbell WB, Li P-L. Production and metabolism of ceramide in normal and ischaemic-reperfused myocardium of rats. Basic Res Cardiol 2001;96:267-74

Zhang J, Sun Y, Zhang JQ, FJ Ramires, Weber KT. Appearance and regression of rat pouch tissue. J Mol Cell Cardiol 1999a;31:10013-1013

Zhang W, Kowai RC, Rusnak F, Sikkink RA, Olson EN, Victor RG. Failure of calcineurin inhibitors to prevent pressure-overload left ventricular hypertrophy in rats. Circ Res 1999b;84:722-728

Zhang X, Dostal DE, Reiss K, Cheng W, Kajstura J, Li P, Hiang H, Sonnenblick EH, Meggs LG, Baker KM, Anversa P. Identification and activation of autocrine renin-angiotensin system in adult ventricular myocytes. Am J Physiol 1995;269:H1791-H1802

INDEX