University of Alberta

The Potential Effects of n-3 Polyunsaturated Fatty Acid on Lipid Metabolism, Ischemic Myocardial Lesions and Glomerulosclerosis in a Rodent Model of Obesity and Insulin Resistance

by

Jing Lu

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science

in

Nutrition and metabolism

Department of Agriculture, Food and Nutritional Science

Edmonton, Alberta
Spring 2009

Your file Votre référence
ISBN: 978-0-494-54966-7
Our file Notre référence
ISBN: 978-0-494-54966-7

Abstract

The metabolic syndrome (MetS) and its components are associated with a high incidence of macro- and micro-vascular disease. N-3 polyunsaturated fatty acids (PUFA) are proposed to modulate lipid metabolism and improve insulin resistance, but the mechanisms remain equivocal. This thesis assessed the long term effects of n-3 PUFA on biochemical pathways associated with macro- and micro-vascular diseases in a model of MetS (JCR:LA-*cp*). Animals were randomized to receive a control diet, or 5% or 10% n-3 PUFA diets for 16 weeks. N-3 PUFA significantly lowered body weight gain as well as improved both fasting and postprandial dyslipidemia, together with lower levels of fasting insulin, glucose and adipokines. Ischemic myocardial lesions and glomerulosclerosis were significantly improved by n-3 PUFA supplementation. Lower levels of lipogenic-related enzymes and prostanoid production were observed in n-3 PUFA groups. In conclusion, long term n-3 PUFA supplementation comprehensively improves risk factors and end-stage vascular complications associated with MetS in the obese JCR:LA-*cp* rat.

Acknowledgments

Firstly, I would like to express my deepest gratitude to my supervisor, Dr. Spencer Proctor. He is always responsible and supportive for my graduate study. It has been great pleasure to work with him on the academic research. I truly appreciate his help, encouragement and financial support.

Secondly, I would like to thank Dr. Catherine J Field, Dr. Vera Mazurak, Dr Harold Aukema for being my MSc committee members and providing valuable comments to this thesis.

Thirdly, I want to thank my technicians, Kris, Sharon and Sandy in the Metabolism and Cardiovascular Disease Laboratory (MCVD) for their enlightening helps and priceless friendship.

Fourthly, I wish to thank my fellow graduated in AIHN including Rabban, Ugo, Megan, Jennifer, Jenny, Zahra and so on. Particularly, I want to express my appreciation to Rabban and Ugo, who are wonderful persons with great hearts.

Additionally, I highly appreciate the staff in Department of Agricultural, Food and Nutritional Science at U of A, especially Jody Forslund, Lynn Elmes and Francine Hodder for your patience and wonderful work ethnic in helping me achieve both my academic and career goals.

Finally, my special thanks go to my family and friends for their deep love and strong supports on the pursuit of my career. My husband Bo (Boaman) Liu is such wonderful person to live with. My twin sister Fan (Ocean) Lu is my strongest supporter, thank you standing by my side all the time. I highly appreciate my parents, especially my mother, Dr. Min Li, who is the one always make me proud. To all my friends in China including Dr. Xizhi Wang, Dr Tingshu Wang, Dr Beibei Wang, Chen Chen, Xuejing Zhang and so on, thank you for everything.

TABLE OF CONTENTS

Chapter 1	**Literature Review**	**1**
1.1	**Brief Introduction to Metabolic syndrome and n-3 Polyunsaturated Fatty Acids**	1
1.1.1	Metabolic Syndrome	1
1.1.2	Brief Introduction to Insulin Resistance and Pre-Diabetes	1
1.1.3	Metabolic Syndrome, Macro- and Micro-vascular Disease	2
1.1.4	N-3 Polyunsaturated Fatty Acids	3
1.2	**N-3 polyunsaturated fatty acids and obesity**	5
1.2.1	Metabolic Syndrome and Obesity	5
1.2.2	N-3 Polyunsaturated Fatty Acids, Body Weight and Adipose Tissue	6
1.2.2.1	*Animal Studies*	6
1.2.2.2	*Human Studies*	7
1.3	**N-3 Polyunsaturated Fatty Acids and Dyslipidemia**	9
1.3.1	Metabolic Syndrome and Dyslipidemia	9
1.3.1.1	*Low density lipoprotein and Metabolic Syndrome*	9
1.3.1.2	*Non-fasted and Postprandial dyslipidemia*	10
1.3.2	Metabolic Syndrome and Postprandial Dyslipidemia	10
1.3.2.1	*Postprandial Hypertriglyceridemia and Apolipoprotein B48*	11
1.3.3	N-3 Polyunsaturated Fatty Acids and Dyslipidemia	12
1.3.3.1	*N-3 Polyunsaturated Fatty Acids and Plasma Triglyceride and High Density Lipoprotein Cholesterol*	12
1.3.3.2	*N-3 Polyunsaturated Fatty Acids and Low Density Lipoprotein Cholesterol*	13
1.3.3.3	*N-3 Polyunsaturated Fatty Acids and Lipogenic-related Enzymes*	14
1.3.3.4	*N-3 Polyunsaturated Fatty Acids and Postprandial Dyslipidemia*	15
1.4	**N-3 Polyunsaturated Fatty Acids and Type 2 Diabetes**	**18**
1.4.1	Metabolic Syndrome and Type 2 Diabetes	18
1.4.2	N-3 Polyunsaturated Fatty Acids and Type 2 Diabetes	18
1.4.2.1	*Clinical Evidence*	19
1.4.3	Leptin and Metabolic Syndrome	20
1.4.4	N-3 Polyunsaturated Fatty Acids and Leptin	22
1.4.5	Adiponectin and Metabolic Syndrome	22
1.4.6	N-3 Polyunsaturated Fatty Acids and Adiponectin	23
1.5	**N-3 Polyunsaturated Fatty Acids, Atherosclerosis and Myocardial Damage**	25
1.5.1	Introduction of Metabolic Syndrome, Atherosclerosis and Myocardial Lesion	25
1.5.1.1	*Atherosclerosis*	25

1.5.1.2	*Myocardial Damage*	26
1.5.1.3	*Metabolic Syndrome and Cardiovascular Disease*	27
1.5.2	N-3 Polyunsaturated Fatty Acids, Atherosclerosis and Myocardial Lesions	28
1.6	**N-3 Polyunsaturated Fatty Acids, Glomerulosclerosis and Renal Prostanoids**	30
1.6.1	Metabolic Syndrome, Glomerulosclerosis and Renal Prostanoid Production	30
1.6.1.1	*Albuminuria*	32
1.6.1.2	*Renal Prostanoids*	32
1.6.2	N-3 Polyunsaturated Fatty Acids, Glomerulosclerosis and Renal Prostanoid Production	34
1.6.2.1	*Mechanisms of Diabetic Glomerulosclerosis*	35
1.6.2.2	*Prostanoids, Glomerulosclerosis and Impact of n-3 Polyunsaturated Fatty Acids*	36
1.7	**General Conclusion**	39
Chapter 2	**Introduction**	**40**
2.1	**Rationale**	40
2.2	**Thesis Aim**	43
2.3	**Specific Hypothesis**	43
2.4	**Specific Objectives**	44
Chapter 3	**Study design**	**47**
3.1	**Introduction and Brief Rationale**	47
3.2	**JCR:LA-*cp* rat**	49
3.3	**Study Diet**	51
3.4	**Animal care and Metabolic Assessments**	53
3.4.1	Husbandry	53
3.4.2	Fat Challenge and Postprandial Response	53
3.4.3	Sample Collection	55
Chapter 4	**Long-term Effects of n-3 Polyunsaturated Fatty Acids Supplementation in the JCR:LA-*cp* Rat: Effects on Insulin Resistance, Dyslipidemia and Myocardial Lesions**	**56**
4.1	**Introduction**	56
4.2	**Method**	58
4.2.1	Animals and Diet	58
4.2.2	Fat Challenge and Postprandial Response	58
4.2.3	Plasma Biochemical and Lipid Profile	59
4.2.3.1	*Biochemical Profile*	59
4.2.3.2	*Lipid Profile*	59

4.2.3.3	*Quantitation of Plasma Apolipoprotein B48*	59
4.2.3.4	*Analysis of Postprandial Response*	60
4.2.4	Immunoblotting for Lipogenic-related Enzymes	61
4.2.5	Heart Histology and Myocardial Analysis	62
4.2.6	Statistical Analysis	62
4.3	**Results**	61
4.3.1	Food Intake, Body Weight and Fat Pads	62
4.3.1.1	*Food Intake and Body Weight*	62
4.3.1.2	*Inguinal Fat Pads*	63
4.3.1.3	*Heart and Liver Weight*	63
4.3.2	Fasting Biochemical and Lipid Profile	64
4.3.2.1	*Biochemical Profile*	64
4.3.2.2	*Lipid Profiles*	64
4.3.2.3	*Postprandial Response of Triglyceride, ApoB48 and Total Cholesterol*	64
4.3.3	Lipogenic-related Enzymes	66
4.3.3.1	*Lipogenic-related Enzymes in Liver*	66
4.3.3.2	*Lipogenic-related Enzymes in Adipose Tissue*	66
4.3.4	Myocardial Lesions	67
4.4	**Discussion**	79
4.4.1	Effects of n-3 Polyunsaturated Fatty Acids on Glucose, Insulin Resistance and Adipokines	79
4.4.2	Effects of n-3 Polyunsaturated Fatty Acids on Body Weight, Fat Deposition and Lipogenic-related Enzymes	80
4.4.3	Effects of n-3 Polyunsaturated Fatty Acids on Fasting and Postprandial Dyslipidemia	82
4.4.4	Effects of n-3 Polyunsaturated Fatty Acids on Myocardial Lesions	85
4.4.5	Optimal Dose of n-3 Polyunsaturated Fatty Acids in Humans	86
4.5	**Conclusion**	87
Chapter 5	**Long term Effects of Dietary n-3 PUFA Supplementation on Glomerulosclerosis and Renal Prostanoid Production in the JCR:LA-*cp* Rat, a Model of the Metabolic Syndrome**	**88**
5.1	**Introduction**	88
5.2	**Method**	92
5.2.1	Animals and Treatments	92
5.2.2	Urine Samples	92
5.2.3	Renal Histology	92
5.2.4	Prostanoid Production and Cyclooxygenase Activity	93
5.2.5	Statistical Analysis	94
5.3	**Results**	94
5.3.1	Kidney Weight and Peri-renal Fat Pad	94

5.3.2	Urinary Biochemical Profile	97
5.3.3	Glomerulosclerosis	98
5.3.4	Renal Prostanoid Production and Cyclooxygenase Activity	101
5.4	**Discussion**	107
5.4.1	Effects of n-3 Polyunsaturated Fatty Acids on Albuminuria and Glomerulosclerosis	107
5.4.2	N-3 Polyunsaturated Fatty Acids and Renal Prostanoid Production	108
5.4.3	N-3 Polyunsaturated Fatty Acids and Cyclooxygenase Activity	110
5.5	**Conclusion**	111
Chapter 6	**Overall Discussion and Conclusion**	**112**
6.1	**Overall Discussion**	112
6.2	**Study Limitations**	116
6.3	**Future Directions**	117
6.4	**Conclusion**	118
Chapter 7	**Literature Cited**	119

List of Tables

Table 3-1.	The fat acids composition of the three dietary treatment groups including lipid balanced diet (LBD), 5% fish oil diet (FO) and 10% fish oil diet (FO) from GC analysis	51
Table 3-2.	The ingredients of 1 kg basis diet in three dietary treatment groups including lipid balance diet (LBD), 5% fish oil (FO) and 10% fish oil (FO)	52
Table 3-3.	The macronutrients composition and energy yield of three dietary treatment groups including	52
Table 4-1.	Heart and liver weights in JCR:LA-*cp* rats	67
Table 4-2.	Fasting plasma concentration of the risk factors associated with MetS	70
Table 4-3	Sample calculations of converting 5% and 10% fish oil of rodent diet to human diet	87
Table 5-1.	Kidney weights pre- and post-lyophilization	95
Table 5-2.	In vitro levels (ng/45mg kidney) of PGD_2, PGE_2, TXB_2, 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ in 0-min and 10-min incubation in kidneys pf *cp/cp* rats; and the total potential renal COX activity in JCR:LA-*cp cp/cp* rats	102

List of Figures

Figure 1-1.	The risk factors and complications of metabolic syndrome	3
Figure 1-2.	The chemical structure of EPA and DHA	4
Figure 1-3.	Three general ways in which alterations of the leptin regulatory loop could lead to obesity	21
Figure 1-4.	Representative ischemic lesions of the heart	26
Figure 1-5.	Diabetic nephropathy	31
Figure 1-6.	Cyclooxygenase (COX) pathway of arachidonic acid metabolism	33
Figure 1-7.	Pathways involved in the metabolism and function of prostanoids derived from arachidonic acid (AA) and eicosapentaenoic acid (EPA)	38
Figure 3-1.	Study Flow chart and Timeline	48
Figure 3-2.	The sample postprandial curve (AUC) and iAUC of plasma triglyceride (inset).	54
Figure 4-1.	Body weight of JCR:LA-*cp* rats	68
Figure 4-2.	The weight of inguinal fat pads in 24 week old JCR:LA-*cp* rats	68
Figure 4-3.	The ratio of inguinal fat pads weight to body weight in 24 week old JCR:LA-*cp* rats	69
Figure 4-4.	The fasting adiponectin concentration in 24 week old JCR:LA-*cp* rats	69
Figure 4-5.	The postprandial response of plasma TG (AUC) and iAUC in four treatment groups with different quantity of fish oil following an oral fat challenge in the male JCR:LA-*cp* rat.	71
Figure 4-6.	The postprandial response in plasma apolipoprotein B48 (AUC) and iAUC following an oral fat challenge in *cp/cp* (obese) and +/? (lean) controls	72
Figure 4-7.	The postprandial response in plasma total cholesterol (AUC) and iAUC following an oral fat challenge in *cp/cp* (obese)	73

	and +/? (lean) controls assigned to treatment groups with varying quantities of fish oil in the diet	
Figure 4-8.	The AUC of postprandial response of TG:B48 ratio following an oral fat challenge in obese (*cp/cp*) and Lean (+/?) rats	74
Figure 4-9.	The lipogenic-related enzymes in liver	75
Figure 4-10.	The lipogenic-related enzymes in adipose tissue	76
Figure 4-11.	Frequency of myocardial lesions in 24 weeks old rats	77
Figure 4-12.	Representative micrographs of ischemic lesions of the heart of JCR:LA-*cp* rats (24 weeks of age)	78
Figure 5-1.	Peri-renal fat pad	96
Figure 5-2.	Urinary albumin and creatinine	97
Figure 5-3.	Proportion of sclerotic glomeruli in 24 weeks old JCR:LA-*cp* rats	99
Figure 5-4.	Kidney H&E stained sections	100
Figure 5-5.	Renal PGD_2 production and COX activity	103
Figure 5-6.	Renal PGE_2 production and COX activity	104
Figure 5-7.	Renal TXB_2 production and COX activity	105
Figure 5-8.	Renal $PGF_{2\alpha}$ production and COX activity	106
Figure 6-1.	Possible mechanisms of the effects of n-3 PUFA on metabolic syndrome	114

List of Abbreviations

AA	arachidonic acid
ACC	acetyl CoA carboxylase
Adipokines	Adipose-derived cytokines
ApoB48	apolipoprotein B48
AUC	area under the curve
BMI	body mass index
CHD	coronary heart disease
CM	chylomicron
CMr	chylomicron remnant
CKD	chronic kidney disease
CVD	cardiovascular disease
CETP	cholesterol ester transfer protein
COX	cyclooxygenase
$cPLA_2\alpha$	cytosolic phospholipase $A_2\alpha$
DGAC	Dietary Guidelines Advisory Committee
DHA	docosahexaenoic acid
DXA	dual energy X-ray absorptiometry
ECL	enhanced chemiluminescence
ELISA	enzyme linked immunosorbent assays
EPA	eicosapentaenoic acid
ER	endoplasmic reticulum
FAS	fatty acid synthase
FFA	free fatty acid
FO	fish oil
GBM	glomeular basement membrane
GFR	glomerular filtration rate
GC/MS	gas chromatography-mass spectrometry
H&E	hematoxylin and eosin stain

HDL	high density lipoprotein
iAUC	incremental area under the curve
IL-6	interleukin-6
IR	insulin resistance
LA	linoleic acid
LBD	lipid Balance diet
LDL	low density lipoprotein
MetS	metabolic syndrome
MNS	Milan normotensive rats strain
NHANES	National Health and Nutrition Examination Survey
NPY	neuropeptide Y
ObR	leptin receptor gene
PAS	periodic acid Schiff stain
PG	prostaglandin
PGH_2	prostaglandin H_2
PGD_2	prostaglandin D_2
PGE_2	prostaglandin E_2
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
PGI_2	prostaglandin $I_{2\alpha}$
PN	prostanoid
PP	postprandial
PPAR	peroxisome proliferators-activated receptor
P:S ratio	polyunsaturated: saturated ratio
PUFA	polyunsaturated fatty acid
SCAP	SREBP-activating protein
SCD	sudden cardiac death
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SF	subcutaneous fat
SFA	saturated fatty acid

$sPLA_2s$	secretory phospholipase A_2s
SRD	sucrose-rich diet
SREBP	sterol regulatory element binding protein
SREs	activate sterol-regulatory enzymes
STZ	streptozotocin
STZ-DM	streptozotocin induced insulin-dependent diabetes mellitus
TG	triglyceride
TNF-α	tumor necrosis factor-alpha
TX	thromboxane
TXA_2	thromboxane A_2
TXB_2	thromboxane B_2
VF	visceral fat
VLDL	very low density lipoprotein

Chapter 1: Literature review

1.1 Brief Introduction to Metabolic Syndrome and n-3 Polyunsaturated Fatty Acids

1.1.1 Metabolic Syndrome

Metabolic syndrome (MetS) or syndrome X was initially described as by Reaven in 1988 (Reaven *et al.* 1988). MetS comprises a cluster of cardiovascular risk factors including dysglycemia, hypertension, dyslipidemia, and albuminuria (Reaven *et al.* 1988). More recently, MetS was redefined by the National Cholesterol Education Program's Adult Treatment Panel III report (Bonow 2002, Crundy et al. 2004). Using these criteria, MetS is currently characterized by a multitude of factors including abdominal obesity, serum lipid profile alterations such as hypertriglyceridemia, low HDL (high density lipoprotein) and high cholesterol levels, hypertension, and fasting hyperglycemia (Alberti *et al.* 2006). In addition, cardiovascular disease (CVD) and chronic kidney disease (CKD) have become two major complications associated with MetS (Alberti *et al.* 2006, Iseki 2008) (Figure 1-1). MetS is a multi-factorial disease with multiple complications. At present, MetS is common in developed countries, especially in North America and Europe. For example, according to the 1999 to 2004 National Health and Nutrition Examination Survey (NHANES), the unadjusted prevalence of MetS was 13.6% for normal weight individuals and highest among obesity class 3 individuals, at a rate of 39.2% (Nguyen et al. 2008). In recent years, China, an economically developing country has also experienced an increased prevalence of MetS, at rate of 9.8% in men and 17.8% in women (Gu *et al.* 2005).

1.1. 2 Brief Introduction to Insulin Resistance and Pre-Diabetes

Recently, obesity is considered as a major etiological factor for the development of insulin resistance (IR) in some patients, although some obese patients do not

develop significant IR. Obesity was the independent predictor of hyperglycemia and coronary atherosclerosis (Włodarczyk *et al.* 2008). Impaired glucose metabolism is commonly observed in patients with acute coronary syndrome (Okosieme *et al.* 2008). Together with rapidly increasing obesity, the prevalence of Mets is expected to be higher in the future (Lottenberg *et al.* 2007) (Figure 1-1). For more than a decade, IR has been proposed to be the key linking factor for a cluster of MetS associated conditions: glucose intolerance, hypertension, dyslipidemia, obesity, CVD and kidney disease (Geronimo *et al.* 2005). Moreover, IR is an important risk factor for the progression and incidence of heart attack, stroke, polycystic ovary syndrome and cancer (Giallauria *et al.* 2008, Hsu *et al.* 2007, Ash-Bernal *et al.* 2006). It has been reported that elevated blood glucose concentrations also exist in overweight and obese populations (de Lusignan *et al.* 2006). Importantly, we also consider the condition of pre-diabetes to be when blood glucose levels are higher than normal but not high enough for a diagnosis of frank diabetes (Reisin *et al.* 2005). Several studies have shown that subjects diagnosed with pre-diabetes develop type 2 diabetes within ten years (Kaufman 2002). The development of type 2 diabetes occurs when insulin cannot control glucose concentration adequately, then hyperinsulinemia and IR (impaired insulin sensitivity) start (Bloomgarden 2008, Danish *et al.* 2005).

1.1.3 Metabolic Syndrome, Macro- and Micro-vascular Disease

Individuals with MetS are at increased risk for the development of pre-diabetes, IR and the subsequent development of type 2 diabetes, hypertension, dyslipidemia, macro- and micro-vascular disease including CVD and CKD (Figure 1-1) (Bagby *et al.* 2004). Ninomiya *et al.* reported that in using NHANES III data, an approximate two-fold increase was found in myocardial infarction and stroke risk in the presence of MetS (Ninomiya *et al.* 2004). Furthermore, data also from the NHANES III survey demonstrated that the prevalence of CKD and microabluminuria were associated with every risk factor of MetS. In addition, there was a graded relationship between the number of risk factors present and the corresponding prevalence of CKD or microalbuminuria (Chen *et al.* 2004).

Unfortunately, a clear understanding of the progression of MetS and its complications are not fully elucidated, specifically with respect to myocardial damage and CKD. MetS is an epidemic that requires increased attention for intervention. Vascular complications are becoming more common and public heath costs are rising. MetS remains one of the biggest public heath risks for the western world. Indeed there has been increased interest in the potential beneficial effects of dietary n-3 fatty acids on MetS and its complications and forms the rationale for nutritional investigation in this thesis.

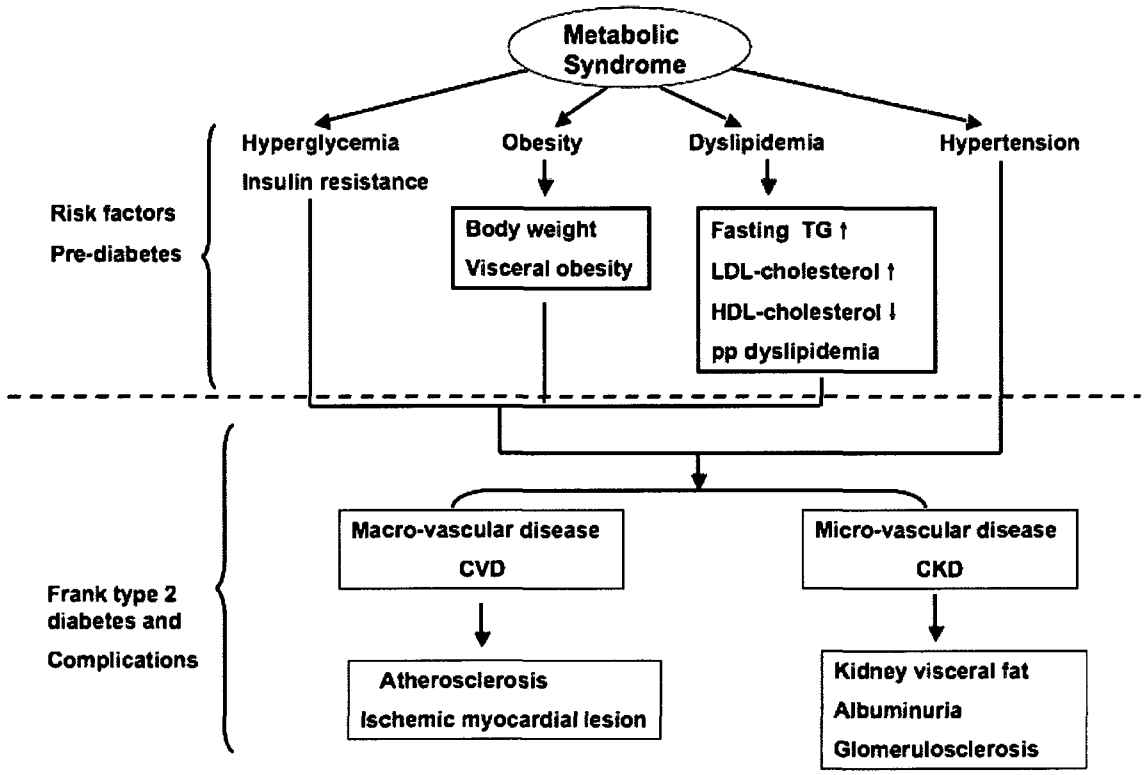


Figure 1-1. The risk factors and complications of metabolic syndrome. MetS: metabolic syndrome; CVD: cardiovascular disease; CKD: CKD.

1.1.4 N-3 Polyunsaturated Fatty Acids

The increased consumption of n-3 polyunsaturated fatty acids (PUFA) has been demonstrated to contribute to improved risk factors of MetS in animals and humans with diabetes and CVD (Nettleton *et al.* 2005, Salmerón *et al.* 2001,

Grundt et al. 1995, Hartweg *et al.* 2007). By way of definition, this thesis will refer to n-3 PUFA as eicosapentaenoic acid (EPA, 20:5,n-3) and docosahexaenoic acid (DHA, 22:6,n-3), which are fatty acids found mainly in fatty fish, such as salmon, tuna and herring (Rice 1996) (Figure 1-2). Benefits of increasing consumption of n-3 PUFA include reduced plasma triglyceride (TG) concentration, increased high-density lipoprotein (HDL) concentration, and improved IR and glucose metabolism, and reduced cardiovascular mortality, particularly sudden cardiac death (Sirtori *et al.* 1998, Leaf *et al.* 2003). This chapter focuses on the current understanding of the role of n-3 PUFA in the risk factors and complications of MetS and will be discussed in further detail in the following sections.

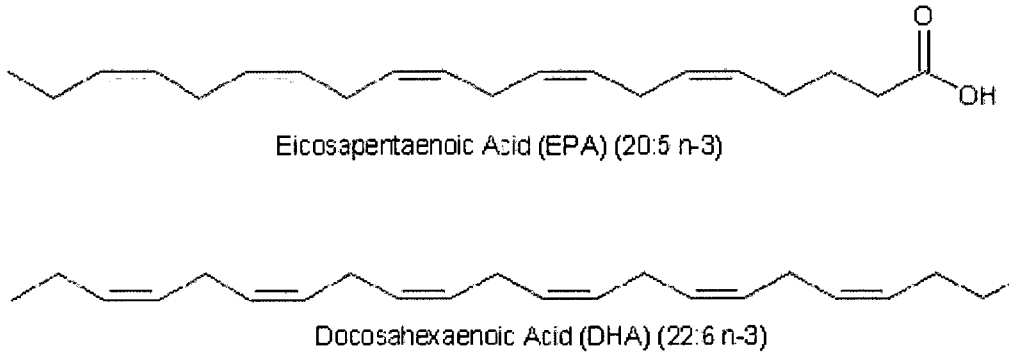


Figure 1-2 The chemical structure of EPA and DHA.

1.2 N-3 Polyunsaturated Fatty Acids and Obesity

1.2.1 Metabolic Syndrome and Obesity

Numerous studies in diabetic and non-diabetic populations have addressed the importance of obesity and adipose tissue deposition and distribution (Kopelman *et al.* 1997, Björntorp 1991). Specifically, increasing abdominal body fat and distribution of body fat were associated with higher concentrations of plasma glucose, insulin and TG (Roman *et al.* 2004). Although several studies show that visceral, but not subcutaneous abdominal fat volume can be associated with IR, other studies show that the volume of subcutaneous fat also relates to glucose tolerance, hyperinsulinemia, hypertriglyceridemia and arterial hypertension (Hayashi *et al.* 2008, Goodpaster *et al.* 1997).

Subcutaneous fat (SF) refers to body fat that is close to the surface of skin. However, visceral fat (VF), also called as intra-abdominal fat, refers to the fat that surrounds the internal organs such as liver and kidney (Singh *et al.* 2007). VF is considered to be a more important risk factor than SF to MetS, because of its anatomic location and unique metabolic and hyperlipolytic activity (Després 2006, Smith *et al.* 2001). It is known that visceral adipose tissue produces more pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), as well as less adiponectin relative to subcutaneous adipose tissue (Rodríguez *et al.* 2007, Li *et al.* 2008). Changes in these cytokines induce IR and play a major role in the pathogenesis of endothelial dysfunction and subsequent atherosclerosis. However, there have been inconsistent findings regarding the relationship between SF and CVD associated with MetS. The association between visceral adiposity and accelerated incidence of atherosclerosis was shown to be independent of overall adiposity (or the volume of SF in humans) (Eguchi *et al.* 2007). However, in contrast, some studies have indicated that peripheral fat volume is negatively correlated with MetS risk factors. The term "Metabolic Obesity" has emerged to reflect VF accumulation in either obese or non-obese individuals, and may be used to better identify those

at risk for MetS than the currently used definitions of obesity (Hamdy *et al.* 2006). Collectively, it is important to further consider the role of VF and SF on obesity and CVD during conditions of MetS.

1.2.2 N-3 Polyunsaturated Fatty Acids, Body Weight and Adipose Tissue

There are relatively few studies supporting increased intake of n-3 PUFA as a useful adjunct to exercise programs for improving body composition and decreasing CVD risk (Russell *et al.* 1991a). Mori *et al.* reported that combined effects of weight-loss regimens and consumption of n-3 PUFA in daily meals of 63 overweight subjects was more effective for weight loss, serum lipids and glucose-insulin metabolism than either measure alone (Mori *et al.* 1999). Thorsdottir *et al.* also showed that in young, overweight men, the inclusion of either fish or fish oil as part of an energy-restricted diet (1600 kcal/day) resulted in approximately 1 kg more weight loss after 4 weeks than a similar diet without seafood or any supplement of marine origin (Thorsdottir *et al.* 2007). However, there are limited studies that support an increased n-3 PUFA intake can lower body weight or body weight gain per se.

1.2.2.1 Animal Studies

In 1993, Belzung *et al.* found that diets rich in n-3 PUFA selectively limited hypertrophy of retroperitoneal and epididymal fat depots in rats (Belzung *et al.* 1993). This effect of n-3 PUFA on hypertrophy of fat depots was dose dependent and was possibly mediated by a reduction in the circulating concentration of TG. Interestingly, other major fat depots (subcutaneous and mesenteric), as well as total adiposity remained unaffected. In another animal study, Lombardo *et al.* examined the effectiveness of n-3 PUFA in reversing or improving the dyslipidemia, IR and adiposity induced in rats by long-term feeding of a sucrose-rich diet (SRD) (Lombardo *et al.* 2007). In this study, rats were fed a SRD for 8 months to produce a stable dyslipidemia and IR. Then, the source of fat in the diet (corn oil) was replaced by an isocaloric amount of n-3 PUFA for 2 months. N-3 PUFA reduced the cell size of adipocytes. Moreover, there was a significant

decrease in TG content and cell volume in isolated adipocytes from epididymal fat tissue in n-3 PUFA treated group. Dietary n-3 PUFA markedly reduced epididymal fat pad mass and the hypertrophy of fat cells (decreased cell volume and TG contents). However, only a slight decrease in total body weight was observed. Consistent with this finding, several other studies have revealed that the lipid content in adipose and muscle tissues, and the distribution of intra-abdominal fat were also decreased by fish oil feeding (Soria *et al.* 2002, Soriguer *et al.* 2003).

1.2.2.2 Human Studies

Some human studies have also demonstrated positive effects of n-3 PUFA on adiposity. Garaulet *et al.* observed that abdominal fat (determined by computed tomography-CT), as well as BMI (body mass index) and percentage body fat (determined from sum of skinfold thickness), were negatively correlated with the n-3 PUFA content of adipose tissue in 84 obese adults. These data suggest that n-3 PUFA may be protective against obesity, particularly abdominal adiposity (Garaulet *et al.* 2001). A study done by Matsumura evaluated the effect of feeding EPA on the volume of VF determined by CT. A comparison was made between the control group (n=74 subjects; conventional therapy) and those taking EPA (n=91 subjects; conventional therapy plus EPA 1800 mg/day) over a 6-month period. Increased dietary EPA significantly reduced blood pressure and diminished VF area (Matsumura 2007). Kunesova *et al.* recently reported that significantly greater loss of both BMI and hip circumference in obese women treated with 3 weeks of a very low-calorie diet supplemented with n-3 PUFA versus placebo (Kunesová *et al.* 2005). Further, a significant decrease in fat mass was reported by Couet *et al*, as measured by dual energy X-ray absorptiometry (DXA), observed in 6 normal weight men and women (despite no changes in total body mass or energy intake) when saturated fatty acid was replaced by 6 g/day of n-3 PUFA for 3 weeks (Couet *et al.* 1997).

In summary, the evidence suggests that n-3 PUFA may play a beneficial role on body composition and obesity, including decreasing central adiposity, even in the absence of significant weight loss. However, the effects of n-3 PUFA on body weight gain and loss as well as SF depot volume in MetS populations complicated with diabetes and CVD, are not clearly indicated. Consequently, more studies are required to further confirm and explore the effects of n-3 PUFA on adiposity, specifically visceral and subcutaneous adiposity.

1.3 N-3 Polyunsaturated Fatty Acids and Dyslipidemia

1.3.1 Metabolic Syndrome and Dyslipidemia

Dyslipidemia refers to an abnormality within lipid profile, including a variety of disorders relating to elevations of; total cholesterol, the 'bad" low density lipoprotein (LDL) cholesterol, and TG concentrations, or a decrease in the "good" high density lipoprotein (HDL) cholesterol concentration in the blood (Kingsbury *et al.* 2003). Elevated plasma TG and total cholesterol concentrations are found most commonly in high MetS risk individuals (Cortez-Dias *et al.* 2007). Reduced plasma levels of HDL are also often associated with an increased risk for MetS (Zheng *et al.* 2002, Williams *et al.* 1995). A high plasma cholesterol concentration has been increasingly acknowledged as a major risk factor for CVD, especially coronary heart disease (CHD) (Volk 2007). IR associates more closely with apolipoprotein B (apoB) concentration than with TG and chylomicron (CM) metabolism, and thus may be an independent predictor of future myocardial infarction (Lind *et al.* 2006). As a result, Sniderman *et al.* 2007 have suggested that the definition of dyslipidemia of MetS should include apoB (Sniderman *et al.* 2007). Amore inclusive definition of dyslipidemia of MetS would also include raised TG concentration, small LDL particle size, low HDL concentration, potential elevation of LDL cholesterol concentration, and abnormal apoB levels.

1.3.1.1 Low density lipoprotein and Metabolic Syndrome

Elevated LDL cholesterol can also be found in those with MetS but the data remains inconsistent. The absence of consistently high elevations of LDL cholesterol in those with MetS is of key importance to this thesis, as these subjects have increased risk of CVD in the absence of abnormal plasma LDL concentration. LDL cholesterol has been regarded as the predominant atherogenic lipogenic lipoprotein and designated as the primary target for cholesterol-lowering therapy (Isley 2006). Indeed evidence suggests that LDL lowering therapy reduces risk for subsequent coronary events, even in patients with advanced atherosclerotic disease (Grundy *et al.* 2004). Moreover, some

recent clinical trials found that statins, (ie HMG-CoA reductase inhibitors), produce significant reductions in heart ischemia incidence in MetS patients, with the effects mainly on lowering LDL levels (Laufs 1997). Despite this convincing data, a large proportion of those with MetS do not develop abnormal LDL concentrations, suggesting that other fractions of cholesterol (such as non-fasted and postprandial lipids) may also play a large role in the increasing the risk of CVD during MetS.

1.3.1.2 Non-fasted and Postprandial Dyslipidemia

In hypertriglyceridemia, LDL cholesterol particles are TG-enriched, small and dense. In the liver of insulin resistant patients, free fatty acid (FFA) flux is high, TG synthesis and storage are increased, and TG is secreted as VLDL (very low density lipoprotein) (Lewis *et al.* 1996, Cornier *et al.* 2008). Blackburn *et al.* and Carmena *et al.* both have reported that the atherogenic effect of postprandial dyslipidemia may be due to the production of large, TG rich VLDL particles formed when the flux of fatty acids to the liver is increased (Blackburn *et al.* 2003, Carmena *et al.* 2004).

1.3.2 Metabolic Syndrome and Postprandial Dyslipidemia

Despite abundant evidence linking fasting dyslipidemia and atherosclerosis, it has been reported that there exists a population of patients whose fasting TG levels remain found to be within a normal range, but who present only with postprandial TG concentration (Poppitt 2005). Postprandial dyslipidemia refers to an abnormality of lipid metabolism occurring following ingestion of a lipid load (Madhu *et al.* 2008). Interestingly, in both human and animal studies, postprandial TG concentration is regarded as the most accurate predictor of the presence and progression of atherosclerosis (Patsch *et al.* 1993). Moreover, some studies have reported that when compared to healthy subjects, MetS subjects had a higher total and incremental TG response and higher total FFA response during the postprandial period (Carstensen *et al.* 2004). Some clinical studies have also shown that postprandial dyslipidemia is an important factor in

the pathogenesis and progression of CVD (Sullivan *et al.* 2004). Therefore, postprandial dyslipidemia is becoming recognized as a risk factor for CVD that now needs a greater understanding in the context of MetS.

1.3.2.1 Postprandial Hypertriglyceridemia and Apolipoprotein B48

Multiple studies have shown that postprandial hypertriglyceridemia is closely related to postprandial TG levels (Halkes *et al.* 2001, Sharrett *et al.* 2001). However, postprandial TG response in MetS remained elevated after correction for baseline TG, which suggesting delayed clearance of exogenous TG rich lipoproteins (van Oostrom *et al.* 2007). It has been shown that patients with CVD have delayed and marked postprandial dyslipidemia (increased concentration of chylomicrons) compared to control subjects (Groot *et al.* 1991). Chylomicrons (CMs) are secreted by the intestine and function to transport exogenous lipids from intestine to liver, adipose, cardiac and skeletal muscle tissues (Mamo *et al.* 1998). Once secreted into the plasma, large native CMs are rapidly converted to chylomicron remnants (CMr) by the action of lipoprotein lipase. The liver clears CMr from the circulation (Denis 2008). ApoB48, a major lipoprotein found in CMs, is regarded as a constructive marker for the metabolism of CMs and their remnants (Smith *et al.* 1997). It is known that there is only one apoB48 molecule for each CM particle and that it remains with the particle and is not transferred. Karpe *et al.* investigated the postprandial dyslipidemic response in male post-infarction patients and reported that the concentration of postprandial chylomicron remnant apoB48 was directly related to the rate of progression of coronary lesions (Karpe *et al.* 1994). It has been shown that CMr can be taken up by aorta (Proctor *et al.* 1996). It has also been demonstrated that delayed clearance of CMr from circulation is highly correlated with the progression of atherosclerotic and myocardial lesions (Mahley *et al.* 1991). Moreover, in several large epidemiological studies, including the NHANES III database, high apoB concentrations were associated with low HDL cholesterol concentrations, high TG concentrations, high waist circumference, high glucose concentrations, and high blood pressure (Sierra-Johnson *et al.* 2006). In recent years, researchers

have found that VF accumulation, postprandial dyslipidemia, and decreased early insulin response often account for the single concept of "postprandial metabolic disorders", which has important implications as an early therapeutic target for prevention of CVD. However, a limitation to the clinical evaluation of postprandial dyslipidemia in MetS and CVD patients is not fully elucidated. There is currently a lack of standard protocols to both measure and evaluate postprandial dyslipidemia.

1.3.3 N-3 Polyunsaturated Fatty Acids and Dyslipidemia

Over decades, the dietary supplementation of marine n-3 PUFA (fish oil) has proven to be effective in lowering both TG and VLDL-TG concentration in experimental animals and normal and hypertriglyceridemic patients (Connor 2000). For example, in rats and hamsters fed a cholesterol-free diet, plasma cholesterol, TG and VLDL-TG levels in n-3 PUFA supplemented group were significantly lower than control group (Lin *et al.* 2005). Anandan *et al. also* concluded that the overall cardioprotective effect of n-3 PUFA on isoproterenol-induced myocardial infarction in male rats is probably related to its ability to inhibit lipid accumulation including reducing cholesterol, TG and FFA in plasma and heart tissue (Anandan *et al.* 2007).

1.3.3.1 N-3 Polyunsaturated Fatty Acids and Plasma Triglyceride and High Density Lipoprotein Cholesterol

In human studies it is also well established that intake of n-3 PUFA lowers plasma TG levels in type 2 diabetes or hypertriglyceridemic subjects, and increases HDL cholesterol levels. Rivellese *et al.* reported that in MetS and hypertriglyceridemic patients, after 2 months of 2.7 g/day and then 4 months of 1.7 g/day EPA and DHA consumption, a 25% reduction in plasma TG (Rivellese *et al.* 1996). Furthermore, Petersen *et al.* 2002 reported that in 42 subjects with type 2 diabetes, 4/g day fish oil compared with corn oil supplementation had significantly reduced TG concentration and raised HDL cholesterol concentration after 8 weeks (Petersen *et al.* 2002). Moreover, in a 24-week randomized study,

142 overweight subjects were assigned to a control group, n-3 PUFA intervention group including oily fish (4.5 g EPA + DHA), or white fish intervention group (0.7 g EPA + DHA) (Moore *et al.* 2006). The oily fish diet significantly reduced plasma TG concentration compared to the white fish diet, but not to the control diet. In addition, it has been reported that in 34 type 2 diabetes patients being treated with anti-diabetic drugs, supplementation of 1.8 g/day n-3 PUFA for two months significantly reduced TG levels and increased HDL cholesterol levels (Kesavulu *et al.* 2002). It has been reported that EPA enrichment in platelet phospholipids to be independently associated with lower serum TG levels (Leigh-Firbank *et al.* 2002). Collectively, most studies concluded that n-3 PUFA supplementation can improve hypertriglyceridemia in type 2 diabetes and overweight subjects.

1.3.3.2 N-3 Polyunsaturated Fatty Acids and Low Density Lipoprotein Cholesterol

Some studies have found elevated LDL cholesterol levels to be associated with the consumption of n-3 PUFA, however other studies report inconsistent findings. For example, in a review of 44 intervention studies, after supplementation with a range of 0.5 to 25 g n-3 PUFA/day (for an average of 6 weeks), hypertriglyceridemic patients had no significant changes in plasma LDL- and HDL-cholesterol concentrations, but had consistently and significantly reduced plasma TG concentrations (Harris 1989). Similarly, the US government's evidence-based review of 13 randomized trials of subjects with type 2 diabetes concluded that there is strong evidence that n-3 PUFA reduces serum TG, but has no effect on total, LDL or HDL cholesterol levels (MacLean *et al.* 2004). However, in contrast, a systematic review conducted by Montori *et al.* 2000 by analysis of 10 studies found that n-3 PUFA was associated with a slight increase in LDL cholesterol levels (Montori *et al.* 2000). Another meta-analysis included 18 trials and 823 subjects, and the doses of fish oil used ranged from 3 to 18 g/day. This meta-analysis of pooled data demonstrated a statistically significant effect of fish oil on lowering TG, and raising LDL cholesterol (Friedberg *et al.* 1998). It is noteworthy that the different metabolic characteristics and lipid metabolic status of the subjects, as well as different treatment doses and time of n-3 PUFA

supplementation may be critical reasons for inconsistent findings of the effects of n-3 PUFA supplementation on LDL and HDL cholesterol. Therefore, in this thesis, we considered a design to utilise two different doses of long-term feeding of n-3 PUFA in a rodent model of MetS in order to clarify the effects of n-3 PUFA on dyslipidemia and postprandial dyslipidemia.

1.3.3.3 N-3 Polyunsaturated Fatty Acids and Lipogenic-related Enzymes

Sterol regulatory element binding proteins (SREBP) including SREBP-1a, SREBP-1c and SREBP-2 are transcription binding factors that control the transcription of genes involved in cholesterol and fatty acid synthesis (Xu *et al.* 2001). SREBP-1a and SREBP-1c are produced from the same gene through the use of alternate promoters, and SREBP-2 is transcripted from a separate gene (Nogalska *et al.* 2005). SREBP-1a is the main isoform found in spleen and cell lines (Felder *et al.* 2005). SREBP-1c and SREBP-2 are predominant in most organs of adult animals and humans including white adipose tissue (Shimomura *et al.* 1997).

SREBPs are synthesized as large precursors (~125 KDa) anchored to the endoplasmic reticulum. With sterol depletion both SREBP and SREBP-cleavage activating protein move to the Golgi, where proteases cleave the protein to release a mature, transcriptionally active form (~65 KDa) that travels to the nucleus to bind to sterol regulatory elements in promoters of specific genes (Jump 1999, Field *et al.* 2002). Some mouse studies indicate that SREBP-1 isoforms are very selective for activating genes involved in fatty acid synthesis, such as acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS). FAS catalyzes all the reaction steps in the conversion of acetyl-CoA and malonyl-CoA to palmitate. Mitochondrial glycerol-3-phospate acyltransferase catalyzes the first acylation step in glycerophospholipid synthesis (Sul *et al.* 2000). The expression of the lipogenic transcription factors, SREBP-1c, parallel the expression of lipogenic enzymes of ACC and FAS in both liver and white adipose tissue (Gallardo *et al.* 2007).

The release of the mature form of SREBP-1 appears to be regulated by both the sterol content of the endoplasmic reticulum and the amount of n-3 PUFA in the membrane phospholipids (Price *et al.* 2000). It was reported that n-3 PUFA might regulate SREBP-1 gene expression by accelerating the rate of SREBP-1 mRNA decay in liver (Price *et al.* 2000, Xu *et al.* 1999 and 2001). N-3 PUFA has also been shown to down-regulate lipogenic gene expression by reducing the hepatic precursor and mature SREBP-1, whereas n-3 PUFA has also been shown to up-regulate genes of fatty acid oxidation and thermogenesis by functioning as ligand activators for peroxisome proliferators-activated receptor-α (PPARα) (Price *et al.* 2000). PPARα are membrane-bound proteins and activate sterol-regulatory enzymes (SREs) containing promoters, as well as some E-boxes, which makes SREBPs eligible to regulate a wide range of lipid genes, including lipogenic-related enzymes (Griffin *et al.* 2004).

It has been shown in cell culture as well as *in vivo* that n-3 PUFA can inhibit the transcription and expression of SREBP, ACC and FAS (Ntambi *et al.* 2001, Suchankova *et al.* 2005, Botolin *et al.* 2006). In a dietary obesity-susceptible (DOS) Sprague-Dawley (SD) rat study, three diets containing 160 g maize oil, beef tallow or fish oil/kg were fed for 9 weeks. A lower ACC mRNA expression in the liver was observed in the DOS-fish oil group, whereas there were no differences in ACC expression in epididymal fat of the DOS rats consuming the three different diets. In addition, the DOS-fish oil rats had significantly ($p<0.05$) reduced weight gain and abdominal and epididymal fat-pad mass than the DOS-maize oil and DOS-beef tallow rats. The authors concluded that the diet rich in fish oil played a potential role in down-regulation of adiposity by altering hepatic lipogenic genes (Jang *et al.* 2003). Furthermore, another animal study revealed that mice fed fish oil were less obese, with reductions in liver TG synthesis, relative to other dietary oils, along with decreases in mature SREBP-1 (Kim *et al.* 1999). A decrease of mature SREBP-1 protein by n-3 PUFA supplementation may be due to either inhibition of SREBP-1 proteolytic cascade or by decreases

in its mRNA (Nakatani *et al.* 2003). Few studies have examined the effects of n-3 PUFA on the production of SREBP-1, ACC and FAS in adipose tissue. Therefore, this thesis explores the long-term effects of n-3 PUFA on the production of these lipogenic-related enzymes in both liver and adipose tissue in a model of MetS, the JCR:LA-*cp* rat.

1.3.3.4 N-3 Polyunsaturated Fatty Acids and Postprandial Dyslipidemia

It is well established that n-3 PUFA supplementation decreases fasting triglyceride concentrations (Roche *et al.* 2000). However, the effect of n-3 PUFA on postprandial triglyceride (TG) concentration is inconsistent and not well understood. For example, Rivellese *et al.* reported that a moderate supplementation of n-3 PUFA (3.6 g/day) in healthy individuals reduced postprandial TG concentration (Rivellese *et al.* 2003). Similarly, Weintraub *et al.* reported that a high n-3 PUFA diet (30 % of dietary fat was from n-3 PUFA) significantly reduced postprandial lipoproteins, including CM and non-CM fraction levels in healthy subjects, when compared with an isocaloric high SFA diet (Weinbraub *et al.* 1988). In addition, a low dose of n-3 PUFA (1 g/day) was also found to reduce postprandial TG concentrations significantly, to a degree similar to that seen in studies using a much higher dose of n-3 PUFA (24 g/day) (Harris *et al.* 1988, Roche *et al.* 1996).

However in contrast, a study with normolipidemic subjects, Lovegrove *et al.* 1997 reported that consumption of n-3 PUFA (1.4 g/d) for 3 weeks had no effect on the postprandial TG response (Lovegrove *et al.* 1997). Similarly, a placebo-controlled, parallel study involving 150 moderately hyperlipidemic subjects showed that the change in fasting or postprandial lipid was not significantly different after either 0.8 or 1.7 g EPA+DHA/day intervention compared with the n-6 PUFA control (Finnegan *et al.* 2003). Furthermore, in a single-meal, randomized crossover study, twelve healthy males consumed two test meals with ^{13}C-labelled cholesterol (45 mg), and either an inter-esterified butter blend with fish oil (352 mg n-3 PUFA) or a commercial butter blend (Roche *et al.* 1996). No

significant difference in the postprandial plasma fatty acid composition was observed between groups from this study. In the same study by Roche *et al*, there was also no difference in cholesterol absorption, plasma cholesterol or the cholesterol contents of plasma lipoproteins, despite the fact that incorporation of fish oil in the butter resulted in a significant lowering of TG concentration in the plasma 2 hours after the meal in comparison with the commercial butter blend (p = 0.02). Roche *et al* concluded that fish oil-enriched butter blend had no acute effect on cholesterol absorption and plasma cholesterol concentration in human. In addition, Trinker *et al.* 1999 reported that in moderately hypertriglyceridemic adults treated with a standardized test meal, n-3 PUFA significantly suppresses the hepatic, as well as the intestinal apoB secretion or synthesis (Tinker *et al.* 1999). Clearly is seems that further studies are necessary to fully elucidate the short term and long-term effects of n-3 PUFA feeding on postprandial dyslipidemia, particularly for the apoB response. It also remains to be verified as to whether n-3 PUFA affects postprandial cholesterol response in a pre-existing condition of hypercholesterolemia. These later points of issue help to form the premise for the rationale for the key objectives of my thesis.

1.4 N-3 Polyunsaturated Fatty Acids and Type 2 Diabetes

1.4.1 Metabolic Syndrome and Type 2 Diabetes

We know that the MetS is a complex constellation of multiple risk factors that predispose to diabetes and CVD. Impaired glucose metabolism, IR, and obesity are essential criterion for MetS. We also know that IR corresponds to a decreased efficacy of insulin to stimulate glucose uptake in adipose tissues and skeletal muscles (Yvon *et al.* 2006). For example, in the liver of insulin-resistant animals, glucose-6-phosphatase is over-produced and hepatic glucose output is less efficiently inhibited by insulin. Impairment in glucose metabolism has been correlated with molecular alterations of insulin signalling pathway(s), particularly in the skeletal muscle (Hotamisligil *et al.* 1996). In early stages of MetS, the combination of these factors results in increased insulin secretion. Whereas, in more advanced stages of MetS, these factors result in alterations in glucose homeostasis and type 2 diabetes (Yvon *et al.* 2006). Consistent with this Hanson *et al.* found that compensatory hyperinsulinemia associated with IR was the strongest predictor of diabetes incidence (Hanson *et al.* 2000). Hence the development of IR and compensatory hyperinsulinemia can progressively shift the balance of insulin signaling towards a mitogenic state that may contribute to atherosclerosis.

1.4.2 N-3 Polyunsaturated Fatty Acids and Type 2 Diabetes

It has been reported that in rodents, n-3 PUFA has a protective effect against fat-induced IR. Pérez-Matute *et al.* reported that EPA administration in rats fed a standard or a high-fat (cafeteria) diet significantly reduced retroperitoneal adipose tissue weight ($p<0.05$), which could potentially explain the beneficial effects of EPA on IR under certain conditions (Pérez-Matute *et al.* 2007). In an ob/ob mouse study (an obesity model of IR and fatty liver disease), dietary intake of n-3 PUFA (6% n-3 PUFA of the total lipid content) had insulin-sensitizing actions in both adipose tissue, and liver, leading to a overall improved insulin tolerance (González-Périz *et al.* 2009). Similarly, Lombardo *et al.* reported that in

rats fed with a sucrose-rich diet (SRD), dietary fish oil (what was the dose?) completely normalized glucose induced insulin secretion. Moreover, dietary fish oil enhanced the first and second phase of insulin secretion, which was greater ($p<0.05$) than those in the SRD group, and comparable to the control group (Lombardo *et al.* 2007).

1.4.2.1 Clinical Evidence

Although animal, ecological and epidemiological studies have shown associations between n-3 PUFA intake and insulin sensitivity (Waite *et al.* 2008, Thorsdottir *et al.* 2004), there is relatively little evidence from intervention studies that n-3 PUFA supplementation can improve insulin sensitivity in humans (Vessby 2000). For example, an intervention study involving 116 overweight insulin-resistant women showed independent effects from weight loss via n-3 PUFA supplementation on TG and adiponectin levels, but not insulin sensitivity (Krebs *et al.* 2006). Similarly, an isoenergetic dietary intervention study in 162 subjects, there was no effect of fish oil supplementation on insulin sensitivity, despite reduced plasma TG (Vessby *et al.* 2001). A more recent study investigating 29 Asian Indians (a group particularly susceptible to MetS and type 2 diabetes), did not find an effect of n-3 PUFA supplementation on IR, even though supplementation was associated with improved fasting and postprandial plasma TG concentration (Brady *et al.* 2004). Browning *et al.* has also recently reported that subjects who received n-3 PUFA supplements for 12 weeks showed no change in postprandial insulin response, despite the lower plasma TG concentration (Browning *et al.* 2007).

There is some limited evidence to support the hypothesis that increased dietary n-3 intake can improve insulin-resistance under certain conditions in humans. For example, a 8 week dietary intervention, 324 participants (20-40 years, BMI 27.5-32.5 kg/m^2, from Iceland, Spain and Ireland) were randomised into one of four energy-restricted diets (30%, energy percent) of identical macronutrient composition but different n-3 PUFA content, including; control (no seafood); lean

fish (150 g cod, 3 times/week); fatty fish (150 g salmon, 3 times/week) and fish oil (6 × 500 mg EPA + DHA capsules/day). This study reported that the fish oil diet reduced fasting insulin and improved IR to a significantly greater extent than the control diet, and to an extent similar to that observed with weight loss (Ramel *et al.* 2008). Similarly, Waite *et al.* reported that after 60 days of supplementing 440 mg DHA and 660 mg EPA for 60 days, overweight subjects had an increase in insulin sensitivity as demonstrated by a reduction in the plasma glucose response, and a reduction in serum TG and total cholesterol (Waite *et al.* 2008). Another study, investigating the combined effects of fish consumption and weight loss on cardiovascular risk factors in 69 overweight patients, found that the greatest decreases in fasting insulin and glucose occurred in the fish and weight-loss groups (Mori *et al.* 1999). Collectively, there is conflicting data regarding the potential beneficial properties of n-3 fatty acids on insulin sensitivity in humans and it maybe important to carefully consider the pre-existing lipid and metabolic abnormalities.

1.4.3 Leptin and Metabolic Syndrome

Several adipose-derived cytokines (adipokines), including leptin and adiponectin have been suggested to decrease muscle lipid content and increase rates of fatty acid oxidation (Dyck *et al.* 2006). Leptin is an adipocyte-derived cytokine and hormone that regulates energy balance through a wide range of functions including providing feedback to signal the key regulatory centers of the hypothalamus to inhibit food intake and regulate energy homeostasis and body weight (Klok *et al.* 2007). Hyperleptinemia, a marker of leptin resistance, is common in obesity and independently associated with IR and CVD in humans (Martin *et al.* 2008). In most cases of human obesity, there is development of central and/or peripheral resistance to leptin. Evidence suggests that central leptin resistance results in obesity (Figure 1-3). The obesity-induced leptin resistance affects numerous peripheral tissues and may cause a complex pathophysiological complication. For example, Paolisso *et al.* reported that fasting plasma leptin levels are associated with increased myocardial wall

thickness independent of body composition and blood pressure levels in hypertensive subjects (Paolisso *et al.* 1999). Human studies indicated that increased leptin is highly associated with hyperinsulinemia and IR (Ahrén *et al.* 1997, Havel *et al.* 1996). It has been reported that insulin and glucose appear to stimulate leptin secretion in adipocytes (Boden *et al.* 1997, Koopmans *et al.* 1998, Sonnenberg *et al.* 2001). Increased concentration of Leptin responsively decreases insulin secretion through direct action on leptin receptors in pancreatic β cells (Seufert *et al.* 1999). In addition, leptin may enhance skeletal muscle glucose uptake and oxidation, as well as suppress hepatic glucose production (Kamohara *et al.* 1997, Wang *et al.* 1999).

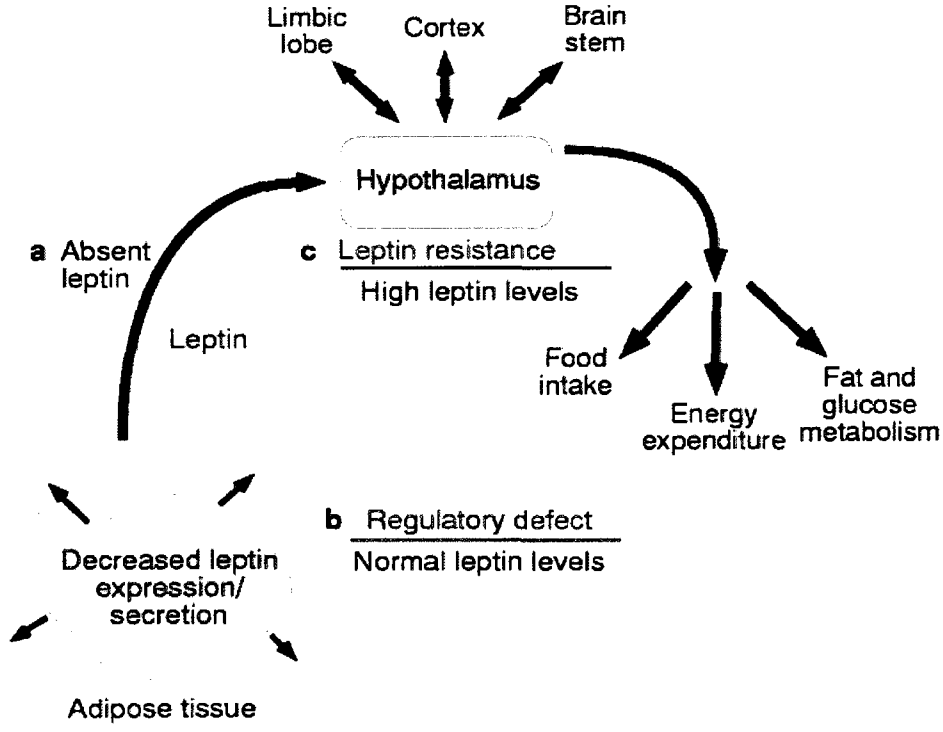


Figure 1-3. Three general ways in which alterations of the leptin regulatory loop could lead to obesity. **a**: Failure to produce leptin; **b**: Low leptin secretion for a given fat mass. In the latter case, the fat mass would expand to secret more leptin until "normal levels" are reached, which results in obesity. **c**, Finally, obesity could result from relative or absolute insensitivity to leptin at its site of action. Such resistance would be associated with increased circulating leptin, analogous to the increased insulin levels seen with MetS and type 2 diabetes. In general, high plasma leptin levels are evident in obese rodents and humans (from Friedman *et al.* 1998).

1.4.4 N-3 Polyunsaturated Fatty Acids and Leptin

Although the role of n-3 PUFA on leptin production is not fully elucidated, Ukropec *et al.* observed that n-3 PUFA lowered plasma leptin concentration as well as reduced leptin mRNA expression (Ukropec *et al.* 2003). Moreover, a 3-week n-3 PUFA-enriched diet, as compared to a 3-week lard-enriched diet, induced lower plasma leptin concentrations and reduced leptin mRNA expression in rat epididymal adipose tissue (Rayner *et al.* 1996). *In vitro*, n-3 PUFA had both dose- and time-dependent effects on leptin expression in the human trophoblast cell line (BeWo). Treatment of human trophoblast (BeWo cells) with 1 mM EPA or DHA reduced leptin expression by 71% and 78%, respectively, as compared to the control after 72 h. Additionally, n-3 PUFA may reduce the promoter activity in BeWo cells transfected with the human leptin promoter (Reseland *et al.* 2001). Evidence from these studies support that n-3 PUFA may decrease leptin gene expression both *in vivo* and *in vitro*, resulting in decreased the leptin production. However, in a human study, Reseland *et al.* 2001 observed that supplementation with n-3 PUFA for 6 weeks did not alter plasma leptin concentrations in male smokers (Reseland *et al.* 2001). Therefore, further studies are needed to clearly describe the effects of n-3 PUFA on leptin synthesis, turnover and/or metabolism.

1.4.5 Adiponectin and Metabolic Syndrome

Adiponectin is another important adipokine, which is an adipocyte-derived hormone that stimulates glucose utilisation and fatty acid oxidation in muscle, decreases hepatic gluconeogenesis, and plays an important role in regulating energy homeostasis and insulin sensitivity (Fruebis *et al.* 2001). It has been reported that adiponectin concentration is reduced in both humans and rodents with obesity and type 2 diabetes (Drevon 2005). Plasma adiponectin concentration is negatively correlated with the incidence of IR, obesity, MetS and type 2 diabetes (Bacha *et al.* 2004, Hotta *et al.* 2000). It is becoming recognised that a low plasma level of this protein is an independent risk factor for the development of type 2 diabetes (Zietz *et al.* 2003). Administration of recombinant adiponectin to rodents results in increased glucose uptake and fat oxidation in

muscle, reduced hepatic glucose production, and improved insulin sensitivity (Rabe *et al.* 2008, Berg *et al.* 2001, Yamauchi *et al.* 2001). In contrast, adiponectin-deficient mice exhibited glucose intolerance and IR (Kubota *et al.* 2002, Nwrocki *et al.* 2006). Several studies found that adiponectin may stimulate pancreatic insulin secretion in *vivo* (Okamoto *et al.* 2008). In addition, adiponectin increases food intake and reduces energy expenditure during fasting via its effects in the central nervous system (Kubota *et al.* 2007).

1.4.6 N-3 Polyunsaturated Fatty Acids and Adiponectin

An association between circulating adiponectin and plasma n-3 PUFA was recently found in healthy humans (Fernandez-Real *et al.* 2005). Based on this evidence, it has been hypothesized that possible protective effects of n-3 PUFA may involve induction of adiponectin. In animal research, the intake of diets rich in EPA and DHA (5.3% of total energy intake) leads to elevated systemic concentrations of adiponectin, largely independent of food intake or adiposity (Flachs *et al.* 2006). However, there are limited human studies clearly demonstrating the effects of supplementation of n-3 PUFA on adiponectin production. Sneddon *et al.* 2008 reported that n-3 PUFA combined with CLA significantly raised plasma adiponectin levels (Sneddon *et al.* 2008). Moreover, a ten-week dietary intervention study in 17 healthy subjects concluded that nutritional intervention to reduce the n-6/n-3 fatty acid ratio increased adiponectin concentration and fatty acid oxidation in healthy subjects (Guebre-Egziabher *et al.* 2008). Additionally, an intervention study involving 116 overweight insulin-resistant subjects showed that n-3 PUFA supplementation increased adiponectin concentrations independently from weight loss (Krebs *et al.* 2006). However, Kratz *et al.* reported that dietary n-3 PUFA consumed at levels of 3.5% of energy intake does not significantly increase plasma or high molecular weight adiponectin concentrations in overweight to moderately obese healthy men and women over the course of 14 weeks (Kratz *et al.* 2008). Hence, it seems that positive effects of increase n-3 intake on circulating adiponectin may play an important role in both humans and animal models of MetS.

In summary, the effects of n-3 PUFA on insulin sensitivity, on glucose control and adipokines in human are still inconsistent. This inconsistency could be because the different doses of n-3 PUFA may produce varied response under different metabolic conditions. Some studies have postulated that the deleterious effects of n-3 PUFA on adiponectin maybe largely attributable to the high doses used. Furthermore, compared to the animal studies, most of the human studies were conducted within a short duration. It is possible that n-3 PUFA needs to be supplemented for a longer duration in order to see an overall effect on IR, hyperleptinemia and/or adiponection production.

1.5 N-3 PUFA, Atherosclerosis and Myocardial Damage

1.5.1 Introduction of Metabolic Syndrome, Atherosclerosis and Myocardial Lesion Damage

Cardiovascular disease (CVD) is one of the major complications for end-stage MetS, and is highly associated with type 2 diabetes. The risk factors for MetS predispose to the development of macro-vascular disease i.e. atherosclerosis. Atherosclerotic complications, such as myocardial infarction, are rising in incidence modern society. Seventy to eighty percent of patients with type 2 diabetes will die of some form of macro-vascular disease, making it the most prominent complication leading to mortality (Snow *et al.* 2003). Several risk factors have been proposed to explain the increased risk of CVD with MetS including hyperglycemia and dyslipidemia (Renard *et al.* 2006).

1.5.1.1 Atherosclerosis

Atherosclerosis is a progressive and chronic disease of the arterial wall characterized by accumulation of lipids, fibrous elements, and macrophages. Because of differences in blood flow dynamics, low or oscillatory shear stress occurs in the aortic root and the arterial branching points, leading to an alteration of endothelial permeability. These areas are preferred sites for lesion formation (Renard *et al.* 2006). Plaques of cholesterol are gradually deposited in the damaged endothelia, causing hardening of the arterial walls and narrowing of the inner lumen. Arteries that are narrowed by atherosclerosis fail to deliver sufficient blood to maintain normal function of the parts of the body they supply (Hadi *et al.* 2007). For example, disease caused by the reduced blood supply to the heart muscle from coronary atherosclerosis is called coronary heart diseases (CHD). Coronary heart complications include myocardial ischemia and infarction, heart attacks, sudden death, and heart failure due to the progressive ischemic myocardial lesions. We have chosen to focus on the characteristics of myocardial damage that is of particular relevance for the JCR:LA-*cp* rat model.

1.5.1.2 Myocardial Damage

In humans, ischemic myocardial lesions are highly associated with acute myocardial infarction. Unfortunately, few animal models are able to spontaneously develop atherosclerosis and ischemic damage in the heart. However, Russell *et al.* 1991b and 1998a reported that the JCR:LA-*cp* rat is unique in the development of a frank vasculopathy with atherosclerotic lesions and associated ischemic myocardial lesions (Russell *et al.* 1991 and 1998a). The JCR:LA-*cp* rats carry the mutant autosomal recessive corpulent (*cp*) polygenic trait (or phenotype). Animals of all corpulent strains that are homozygous (*cp/cp*) are obese, insulin resistant, and with both fasting and postprandial dyslipidemia. Heterozygotes or homozygous normal rats (+/+) are lean and metabolically normal (Russell *et al.* 1998b). The ischemic myocardial damage in the heart of JCR:LA-*cp* rats suggest 'clinical' evidence of myocardial infarction episodes (Russell *et al.* 1991b). It has been documented that occasional spontaneous deaths do occur in this model, especially during or immediately following stressful procedures. Hence, JCR:LA-*cp* rats have become an animal model of choice for the risk factors and vascular complications of MetS.

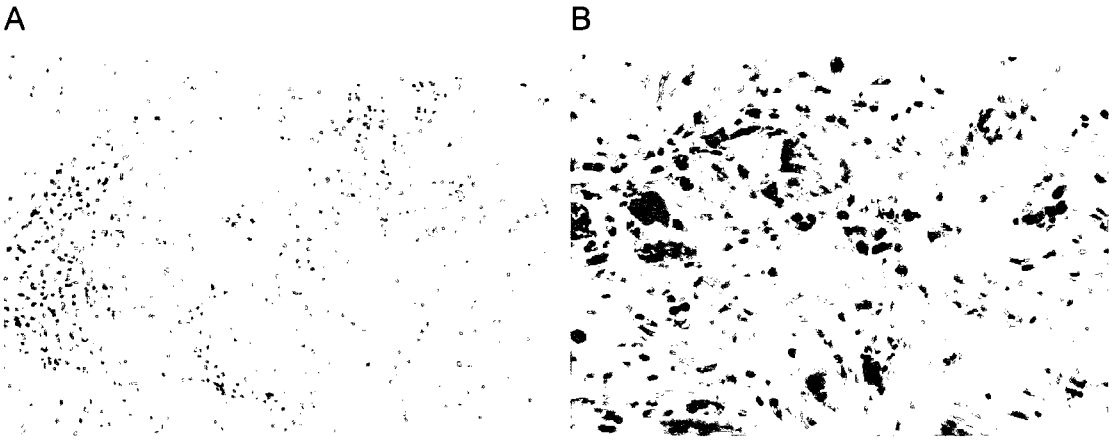


Figure 1-4. Representative ischemic lesions of the heart. These lesions are from 9-month-old male *cp/cp* rats. **A**: Stage 2 lesion showing three different areas of varying ages, all with chronic inflammatory cell infiltration and cell lysis. Hematoxylin and eosin stain, ×100. **B**: an old, mature stage 4 lesion, showing striated collagen bands and some isolated surviving myocytes. Masson's trichrome stain, × 40. (From Russell *et al.* 1998a)

1.5.1.3 Metabolic Syndrome and Cardiovascular Disease

Several risk factors have been proposed to explain the increased risk of CVD with MetS including hyperglycemia and dyslipidemia (Gupta *et al.* 2006). Indeed, it is difficult to separate hypercholesterolemia, hypertriglyceridemia, and hyperglycemia, factors that are all characteristic of the MetS pathology. A recent study by Johansson *et al* attempted to address this issue. A high fat diet was used to induce type 2 diabetes in mice, and then a low fat diet was used to avoid dyslipidemia (Johansson *et al.* 2008). After fourteen weeks of diabetes induction and in the absence of dyslipidemia, hyperglycemia did not accelerate plaque progression. This suggests that in addition to hyperglycemia, dyslipidemia plays an important role in the development of atherosclerosis (Renard *et al.* 2006). A basal increase in cholesterol and triglyceride levels, together with hyperglycemia, is likely needed to induce atherosclerotic lesion initiation. Some studies observed that arterial influx of CMs occurs once the particles are converted to smaller cholesterol-rich remnants supporting a causal role in atherogenesis per se (Proctor *et al.* 2002). Interestingly, several studies have found that a slight increase in total cholesterol and triglyceride concentration is also required in diabetic mouse models to observe diabetes with accompanying atherosclerosis development (Renard *et al.* 2004). However, very high cholesterol and triglyceride concentrations may mask the effect of hyperglycemia (Reaven *et al.* 1997). In humans, high LDL cholesterol and low HDL cholesterol are associated with accelerated atherogenesis (Steinberg *et al.* 1989). A combination of treatment increasing HDL by 36% and decreasing LDL by 26% was associated with a significant reduction in angiographic obstruction (Gurfinkel *et al.* 2006). However, we also acknowledge that many human conditions of MetS do not have dramatic increases in blood lipids.

Many studies have recognized elevated levels of lipoproteins containing apoB as a risk factor for atherosclerosis (Glass *et al.* 2001). VLDL and LDL are the major cholesterol and triglyceride transporters in human plasma. In human apoB transgenic mice, STZ-induced diabetes and atherosclerosis presented with

hypertriglyceridemia and hypercholesterolemia (Kako *et al.* 2002). Proctor *et al.* has advocated that apoB48-containing lipoproteins (i.e. postprandial CMr) may be atherogenic in their own right. Moreover, it was reported that both plasma concentrations of both fasting and postprandial apoB48 are substantially elevated in CAD individuals (Mamo *et al.* 1998).

In summary, CVD are multi-factorial macro-vascular complications. CVD is highly associated with all the components of MetS. As atherosclerosis is a common pathway to acute myocardial infarction and ischemic stroke, modifying associated known risk factors, including hyperglycemia and dyslipidemia, is required for primary and secondary prevention of both conditions.

1.5.2 N-3 Polyunsaturated Fatty Acids, Atherosclerosis and Myocardial Lesions

Multiple studies have shown that n-3 PUFA confers significant cardiovascular benefits. Both intervention studies and cohort studies reported that n-3 PUFA lowers the risk and incidence of cardiac death, with or without nonfatal myocardial infarction (Daviglus *et al.* 1991, Siscovick *et al.* 1995). In addition, case control studies have observed that the levels of n-3 PUFA in body, including the tissue and blood are associated with a 90% reduction in risk of sudden cardiac death (Albert *et al.* 2002). For example, Erkkilä *et al.* reported that the consumption of two or more fish servings a week over a 3-year period significantly reduced the narrowing of coronary arteries in postmenopausal women with diabetes and coronary artery disease, compared to similar women without diabetes (Erkkilä *et al.* 2004).

As discussed earlier in this chapter, n-3 PUFA plays an important role in the improvement of glucose metabolism, dyslipidemia and obesity, which are the associated risk factors for atherosclerosis and ischemic myocardial lesions (Kanter *et al.* 2007). Moreover, the protective effect of a PUFA concentrate prepared from fish oil on isoproterenol-induced myocardial infarction in male albino rats was investigated with respect to changes including: reduced plasma

diagnostic marker enzymes (i.e. a tendency to prevent the isoproterenol-induced phospholipids depletion), reduced the levels of lipid components in plasma and heart tissue, including cholesterol, TG and FFA, and reduced glutathione and lipid peroxides (Anandan *et al.* 2007). This study suggests that the cardioprotective effect of n-3 PUFA is probably related to its hypolipidemic property. In addition, Metcalf *et al.* conducted a human trial that showed dietary n-3 PUFA was rapidly incorporated into human myocardial phospholipids during high dose fish oil supplementation (6 g/d) (Metcalf *et al.* 2006). It was suggested that the beneficial effects of dietary n-3 PUFA are from its incorporation into cardiomyocyte phospholipids, with consequent effects on myocardial membrane function and release as FFA by the action of phospholipase A_2.

However, of relevance for my thesis, we note that the literature has not provided a comprehensive account of corresponding histological evidence regarding the improvement of n-3 PUFA on ischemic myocardial lesions. One of the reasons may be the lack of a suitable animal model that can develop ischemic heart damage. Another reason may be the short duration of treatment of n-3 PUFA that cannot fully indicate the role of n-3 PUFA in the histological changes. Hence this forms a major component to the primary objective of this thesis.

1.6 N-3 Polyunsaturated Fatty Acids, Glomerulosclerosis and Renal Prostanoids

1.6.1 Metabolic Syndrome, Glomerulosclerosis and Renal Prostanoid Production

Glomerulosclerosis is another pathological complication of the end stage disease for type 2 diabetes and MetS. It is characterized by diffuse or nodular glomerulosclerosis, afferent and efferent hyaline arteriosclerosis, and tubulointerstitial fibrosis and atrophy (Figure 1-5). Approximately 40% of patients with MetS and diabetes develop diabetic glomerulosclerosis, a major characteristic of CKD (Alsaad *et al.* 2007). Diabetic glomerulosclerosis is the single most common cause of end-stage renal failure worldwide, and a major indication for dialysis and transplantation. Coresh *et al.* reported that the prevalence of the MetS in United States is about 47 million and rising, as a result of the current obesity and diabetes epidemics (Coresh *et al.* 2003). Many aspects of MetS are associated with glomerulosclerosis. Particularly, individuals with hyperglycemia, dyslipidemia and visceral obesity are at increased risk for progressive loss of renal function. In addition, several observational, cross-sectional, and longitudinal studies document obesity as an independent risk factor for the onset, aggravated course, and poor outcomes of CKD. This association remains significant even after adjustment for the confounding co-morbidities of MetS, including hyperglycemia and dyslipidemia, the two causes of CKD (Bagby 2004). Unfortunately, because of the lack of appropriate animal models, that may spontaneously develop glomerulosclerosis associated with MetS and diabetes, current studies have yet to completely elucidate the etiology of diabetic glomerulosclerosis. We prose that in order to fully appreciate the potential metabolism benefit of chronic increased intake of n-3 PUFA on both macro and micro vascular disease, appreciating the impact on glomerulosclerosis is critical.

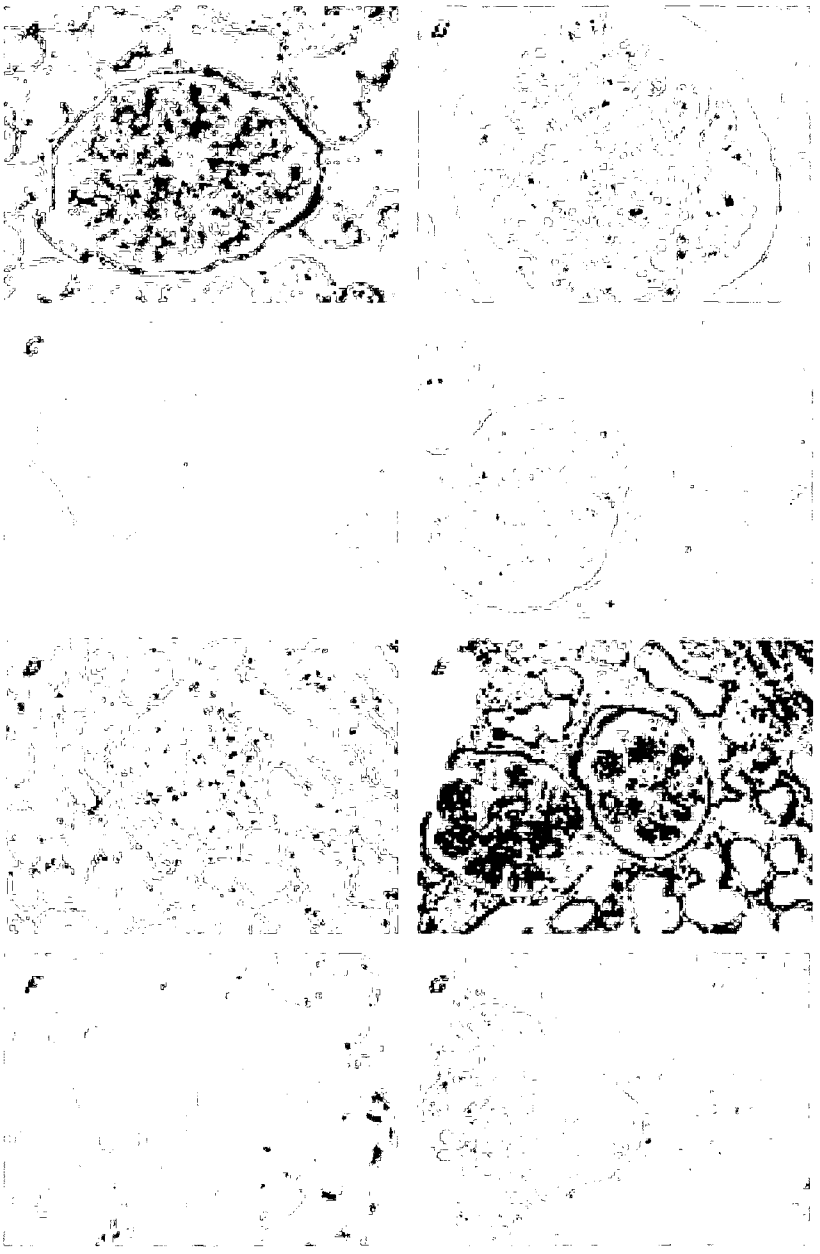


Figure 1-5. Diabetic nephropathy. **A:** Mild diffuse expansion of mesangium in early diabetic nephropathy (periodic acid Schiff (PAS) stain). **B:** Diffuse thickening of the glomerular basement membrane (GBM) (PAS stain). **C:** Sclerotic nodule (i.e. Kimmelstiel–Wilson nodules) in early nodular diabetic nephropathy (PAS stain). **D:** Marked glomerular lobular accentuation in advanced nodular diabetic nephropathy (PAS stain). **E:** The glomerular lobulation is highlighted by silver stain. **F**: Fibrin cap is characteristic for diabetic nephropathy. It is caused by insudation and accumulation of plasma proteins between the glomerular endothelium and the GBM (PAS stain). **G:** Afferent and efferent arteriolar hyalinosis is characteristic for diabetic nephropathy (PAS stain). (From Alsaad *et al.* 2007).

1.6.1.1 Albuminuria

The typical clinical presentation of CKD (CKD) associated with diabetes is albuminuria, a condition that may foreshadow the progression of diabetes to end-stage renal disease, and strong predictor of end-stage renal disease in itself. Chen *et al.* extracted data from the Third National Health and Nutrition Examination Survey database containing clinical information from over 6000 subjects. They found a statistical association between MetS and albuminuria, and a significant correlation between number of MetS factors and glomerular filtration rate (GFR) <60 mL/min. They concluded that, MetS might be an important factor in the cause of CKD (Chen *et al.* 2004).

1.6.1.2 Renal Prostanoids

Cyclooxygenase (COX)-derived prostanoids operate complex and diverse functions within the kidney. COXs are the enzymes responsible for the initial rate-limiting metabolism of arachidonic acid to prostanoids including prostaglandin (PG) and thromboxane (TX) (Figure 1-6) (Hao *et al.* 2008). Two isoforms of COX, including COX-1 and COX-2 have been identified. At present, the relative contribution of COX isoform (COX-1 and COX-2) activity for renal prostanoid production is unclear. COX-1 and COX-2 both appear to be responsible for maintaining basic physiological functions (Kim *et al.* 2004). COX-2 appears to play a key role in pathophysiological processes such as inflammation and glomerulosclerosis (Lim *et al.* 2008). Warford-Woolgar *et al.* showed that the majority of total COX activity is due to the COX-2 isoform and that its activity is increased in diseased kidneys (Warford-Woolgar *et al.* 2006).

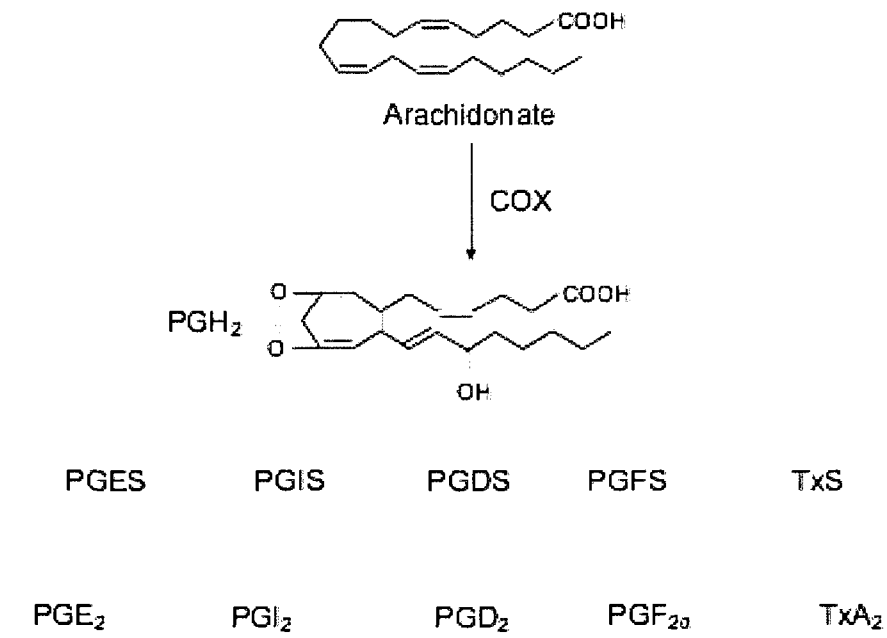


Figure 1-6. Cyclooxygenase (COX) pathway of arachidonic acid (AA) metabolism. COX first converts arachidonic acid to PGH_2 through cyclooxygenase and peroxidase activity. PGH_2 is subsequently metabolized to five major bioactive prostanoids—PGE_2, PGI_2, PGD_2, $PGF_{2\alpha}$, and TXA_2—through their respective synthases, PGES, PGIS, PGDS, PGFS, and TXs. (Adapted from Hao *et al.* 2008)

Because multiple PGs and TXs can be synthesized via the COX pathway, the biological effects of COX-derived prostanoids are diverse and complex. It has been reported that in diseased kidney, increased renal prostanoids, particularly PGI_2 and PGE_2, were observed and may play key roles in maintaining blood pressure and renal function in volume contracted states (Hao *et al.* 2007). Moreover, prostanoids are involved in glomerular filtration rate (GFR) and salt-water homeostatsis, as well as in inflammatory and proliferative processes in response to renal damage. In addition, COX-derived prostanoids also are involved in certain pathophysiological processes: PGD_2 and PGE_2 are considered to be a pro-inflammatory mediators, while PGE_2 and PGI_2 are vasodilators and important in maintaining normal kidney flow; PGE_2 is also involved in the regulation of sodium re-absorption and can maintain GFR by dilating the afferent arteriole (Lim *et al.* 2008, Hao *et al.* 2007, Breyer *et al.* 2001). Elevated concentrations of local PGE_2 could lead to renal cell over-proliferation, formation

of fibrous tissue and/or pro-inflammation (Remuzzi *et al.* 1991, Warford-Woolgar *et al.* 2006, Kitahara *et al.* 2002). In comparison, TXA_2 is a potent vasoconstrictor and platelet aggregator agonist, and involved in the pathogenesis of hypertension associated with chronic renal failure (Larivière *et al.* 2004). In addition to the direct vasodilator effect of PGs on preglomerular arterioles, the PGs, particularly PGE_2 synthesized from the macula densa can also increase rein secretion (Hao *et al.* 2008).

Of importance to the context of this thesis, is that diabetic glomerulosclerosis is reported to be associated with an overproduction of COX and prostanoids. In streptozotocin (STZ)-induced diabetic rats, renal synthesis of PGE_2, PGI_2, and TXB_2 were elevated (Schambelan *et al.* 1985, Wey *et al.* 1986). COX-2 expression is also reported to increase in the thick ascending limbs and macula densas in type II diabetic Zucker rats (Komers *et al.* 2005). It was also reported that human diabetic kidneys have enhanced macula densa COX-2 expression (Komers *et al.* 2005). Selective COX-2 inhibition significantly reduces glomerular hyperfiltration in STZ-induced diabetic rats, consistent with COX-2-derived prostanoids increasing renal blood flow in the diabetic kidney (Komers *et al.* 2001). However, the identity of these prostanoids involved in the pathogenesis of diabetic glomerulosclerosis has not been fully characterized. In addition, the role of these COX-derived prostanoids in the pathogenesis of diabetic nephropathy remains to be elucidated. Therefore, in this thesis we aimed to establish the effects of long-term feeding of n-3 PUFA in JCR:LA-*cp* rats on corresponding renal prostanoid production and glomerulosclerosis.

1.6.2 N-3 Polyunsaturated Fatty Acids, Glomerulosclerosis and Renal Prostanoid Production

It has been reported that n-3 PUFA has protective and therapeutic effects on albuminuria. Urinary albumin is used clinically as an indicator of renal micro-vascular damage, and often reflects elevated glomerular permeability and inability to retain albumin (Hamano *et al.* 2008). For example, membranous

nephropathy in passive Heymann nephritis rats was induced by injecting anti-Fx1A, an antiserum identifying antigens on the glomerular epithelial cell. In the study by Hamano *et al,* rats were fed a diet containing 10% fish oil for four weeks before anitibody injection, resulting in the development 50% to 60% less proteinuria between two and six weeks post anti-Fx1A, than rats fed an equivalent diet containing 10% safflower oil diets. In addition, rats fed fish oil had substantial enrichment of glomerular phospholipids, associated with a 50% reduction in release of glomerular TXB_2 (Weise *et al.* 1993). Moreover, De Caterina *et al.* reported that dietary supplementation with n-3 PUFA reduced proteinuria in experimental models of renal diseases as well as in human renal disease. N-3 PUFA was treated in the form of triglycerides (EPA+DHA = 3 g/day for 4 patients) and of ethyl esters (EPA+DHA = 7.7 g/day) into 10 patients with membranous glomerulonephritis and focal glomerular sclerosis. Treatment lasted for periods of six weeks, followed by a prolonged follow-up for 27 weeks. Dietary supplementation with n-3 PUFA caused a reduction in platelet generation of TXB_2 and proteinuria, associated with significant reduction in total cholesterol (De Caterina *et al.* 1993). Additionally, in a study of 10-week-old obese Zucker rats that were pair-fed regular chow or chow containing either 20% fish oil rich in n-3 PUFA (Kasiske *et al.* 1991). At 34 weeks of age, fish oil caused comparable reductions in albuminuria, mesangial matrix expansion, and glomerulosclerosis, accompanied with a reduction of serum TG and cholesterol (Kasiske *et al.* 1991).

1.6.2.1 Mechanisms of Diabetic Glomerulosclerosis

Limited studies have addressed that supplementation with fish oil can reduce the severity of diabetic glomerulosclerosis. There is a particular lack of histological evidence in animals or humans. Mune *et al.* used subtotal nephrectomized cholesterol-fed rats as a model for progressive kidney disease (Mune *et al.* 1999). They examined the effect of 4 weeks treatment of 5% dietary fish oil, or a combination of 5% dietary fish oil with 500 IU/kg vitamin E diet, or 1% probucol on renal injury. The scores of glomerular segmental sclerosis and glomerular macrophage subpopulation were ameliorated by fish oil. Inclusion of probucol in

the fish oil diet group lowered the glomerular segmental sclerosis score by 73% and reduced glomerular macrophage subpopulation by 83% compared to the control group. These findings imply that progression of glomerular sclerosis in the rat remnant kidney model of progressive glomerulosclerosis can be significantly modulated with fish oil treatment (Mune *et al.* 1999). Furthermore, Hagiwara *et al.* observed that diabetic KKAy/Ta mice injected intraperitoneally with EPA ethyl ester (1 g/kg/day) showed attenuated glomerulosclerosis, mesangial matrix accumulation and tubulointerstitial inflammation (Hagiwara *et al.* 2005). These data suggest that dietary supplementation with n-3 PUFA may ameliorate chronic, progressive renal injury. However, further animal and human studies are still required to better define the benefits of n-3 PUFA on albuminuria and diabetic glomerulosclerosis, particularly under conditions of MetS.

1.6.2.2 Prostanoids, Glomerulosclerosis and Impact of n-3 Polyunsaturated Fatty Acids

Several studies demonstrated that the main action of n-3 PUFA is through its suppressive effect on the production of arachidonic acid (AA)-derived prostanoids, particularly PGE_2 (Horia *et al.* 2007). The administration of EPA has been shown to lower urinary PGE_2 excretion (Düsing *et al.* 1990). Neumayer *et al.* reported that n-3 PUFA (EPA 55 mg/kg/day, DHA 40 mg/kg/day) was given to eight female beagle dogs with ischemic acute renal failure for 6 weeks, while vehicle was given to seven control dogs. In dogs receiving fish oil, blood pressure, serum cholesterol and TG were decreased (Neumayer *et al.* 1992). The urinary excretion of TX metabolite TXB_2 and the excretion of PGE_2, as well as the excretion of the PGI_2 metabolite 6-keto $PGF_{1\alpha}$ was decreased.

Rats of the Milan normotensive strain (MNS) that spontaneously develop severe proteinuria, focal glomerulosclerosis and interstitial fibrosis, as well as excessive glomerular TXA_2 production, have been used to explore the benefits of n-3 PUFA (Bianchi *et al.* 1974). Goldstein *et al.* reported that MNS rats receiving fish oil for 10 months developed significantly less albuminuria than control groups

(Goldstein *et al.* 1995). Serum cholesterol and TG were also significantly lower in fish oil-fed MNS rats than control rats. Although, the extent of focal glomerulosclerosis was similar in the Goldstein *et al* study between the fish oil and control group, fish oil-fed rats had less interstitial injury than the control rats. More importantly, the Goldstein *et al* study showed that glomerular TXA_2 was significantly reduced in the fish oil group. However, these animal models do not necessarily develop some of the key metabolic abnormalities typically associated with clinical MetS such as diabetic glomerulosclerosis and albuminuria. As a result, while these studies indicated that n-3 PUFA may reduce renal prostanoid production in ischemic acute renal failure and focal glomerulosclerosis, intensive studies both in animals and humans are needed to explore the roles of n-3 PUFA on renal prostanoid production in diabetic glomerulosclerosis per se. One study has reported that fish oil-treated STZ-induced diabetes rats had a significant and greater than 50% decrease in all glomerular PGs (PGE_2, TXB_2, $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$) (Sinha *et al.* 1990). It was concluded that the reduction in proteinuria and the correction of hyperlipidemia and prostanoid production by fish oil may be beneficial in the long-term for improvement of diabetic glomerulopathy. Collectively these sets of data provide a formidable rationale for the latter objectives of my thesis.

Some cancer studies proposed that n-3 PUFA down-regulates COX-2 expression by affecting nuclear transcription factors (Narayanan *et al.* 2005). PGs are synthesized via three stages (Figure 1-7) (Smith *et al.* 2005): stage 1, mobilization of arachidonic acid (AA) from membrane phospholipids by cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$), sometimes in conjunction with secretory PLA_2s ($sPLA_2$s); stage 2, conversion of AA to the prostaglandin endoperoxide intermediate PGH_2 by COX-1 or COX-2; and stage 3, the specific synthase isomerizes PGH_2 to a '2-series' products, including PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 or thromboxane A_2 (TXA_2). Some studies have found that n-3 PUFA may serve as a substrate for the formation of the '3-series' PG products, including PGD_3, PGE_3, $PGF_{3\alpha}$, PGI_3 and TXA_3, which are believed to have less inflammatory effects on

the kidney. For example, EPA-derived PGE_3 is much less efficient compared to PGE_2 in inducing COX-2 expression and it is a weaker inflammatory agent. Increased EPA/AA ratios also appear to have significant effects on COX-2-mediated signalling. One major consequence is likely to be the increased production of PGE_3 relative to PGE_2. It will be essential to determine if these effects of fish oil involve enhanced production of PGE_3 mediated via COX-2, as well as what are the direct effects and mechanisms of fish oil on COX expression (Hagiwara *et al.* 2006). Currently, the mechanisms regarding the effects of n-3 PUFA on renal COX expression are not fully defined and help form the basis for my final series of objectives.

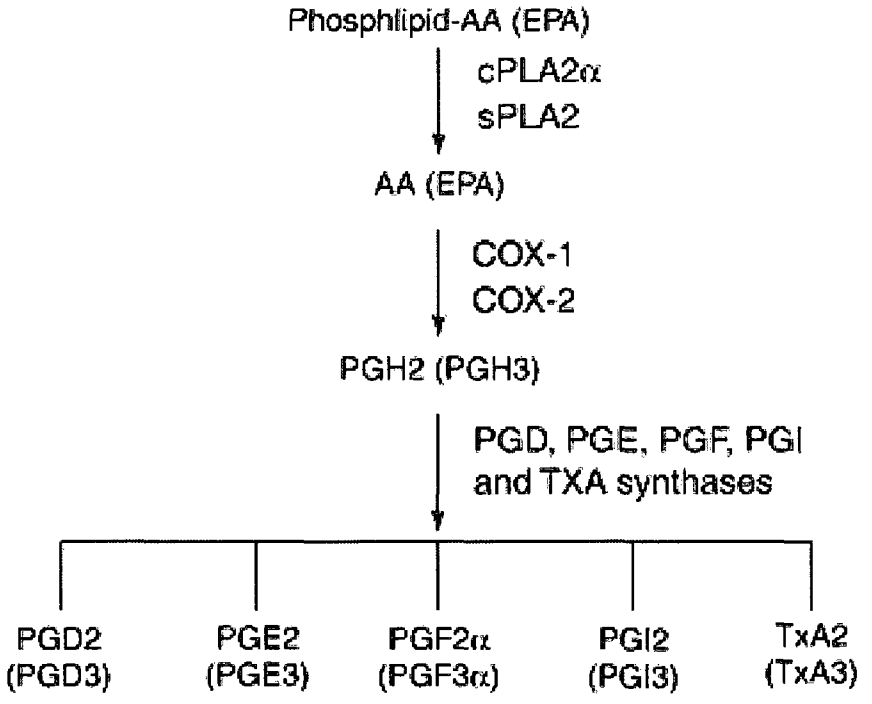


Figure 1-7. Pathways involved in the metabolism and function of prostanoids derived from arachidonic acid (AA) and eicosapentaenoic acid (EPA). COX: cyclooxygenases; cPLA2α: cytosolic phospholipase; sPLA2: secretory PLA2. (From Smith *et al.* 2005).

1.7 General Conclusion

In order to further study the effects of n-3 polyunsaturated fatty acids on the risk factors and complications of MetS, it is deemed necessary to have an appropriate animal with corresponding vascular complications. Among the numerous number animal models of MetS, available only the JCR:LA-*cp/cp* presents typical risk factors of MetS, as well as spontaneously develops end stage complications including macro-vascular and micro-vascular diseases. Although many studies have already observed the beneficial effects of n-3 PUFA on MetS, many have not encompassed comprehensive effects of n-3 PUFA on all MetS vascular components. In addition, the short duration of many of these studies may preclude observations of the chronic effects of n-3 PUFA on the development of atherosclerosis and glomerulosclerosis, because these end stage diseases are progressive, requiring longer time to develop. Therefore, a robust, reproducible, well characterized and, preferably spontaneous model is required to conduct a long-term n-3 PUFA feeding study, in order to explore comprehensive effects of n-3 PUFA on MetS.

Chapter 2: Introduction

2.1 Rationale

Chapter 1 outlines the premise that the metabolic syndrome (MetS) has become a common disorder in modern society. Further, that obesity is an underlying risk factor along with this syndrome and its complications such as CVD and type 2 diabetes (Gruny *et al.* 2004). With the high prevalence of obesity in North America, the MetS is predicted to be more common in the future. Indeed, one of the major observations is that MetS and/or its individual components including abdominal obesity, dyslipidemia, hypertension and hyperglycemia are associated with a high incidence of CVD (Hanley *et al.* 2003). Furthermore, more recent data indicate that MetS is associated with an increased risk for glomerulosclerosis (Abrass 2006).

In recent decades, an increasing accumulation of epidemiological and clinical studies have examined the relationship of the risk factors associated with MetS in order to delineate the putative mechanisms responsible for these complications (Ordovas *et al.* 2008). However, many studies have only explored 1 or 2 risk factors of MetS instead of studying the co-morbidities as independent entities. One reason may be the difficulty to find the appropriate animal models that present with cluster of co-morbidities associated with clinical MetS in humans. The JCR:LA-*cp* is a unique strain that exhibits obesity, hyperglycemia, hyperinsulinemia and dyslipidemia resulting from a defect in the leptin receptor gene (Vine *et al.* 2007). More importantly, the homozygous (*cp/cp*) JCR:LA-*cp* strain spontaneously develop pathological end-stage complications of MetS such as cardiovascular disease (CVD) and glomerulosclerosis, which are commonly observed in humans with MetS (Proctor *et al.* 2007, Russell *et al.* 2008). Accordingly, JCR:LA-*cp* rat is arguably the model-of-choice for studying disease risk indices associated with co-morbidities as a result of MetS (refer to chapter 3.2).

Notably, due to the increasing prevalence of MetS, there is a renewed interest in the capacity of nutritional treatments to reduce the corresponding risk factors and/or complications. Epidemiological studies have found n-3 long chain unsaturated polyunsaturated fatty acids (PUFA) supplementation or amount of fatty fish in the diet is associated with the improvement of insulin resistance, type 2 diabetes and hypertriglyceridemia (Calder 2004). Furthermore, recent clinical trials have shown that dietary n-3 PUFA is not only beneficial in reducing risk factors of MetS and CVD, but also decreasing the mortality from MetS and its complications (Kragelund *et al.* 2007). In addition, some studies suggest that supplementation with low-dose n-3 PUFA may have a favourable effect on kidney function and plasma lipid levels in subjects with glomerulosclerosis (Calviello *et al.* 1997). However, due to the lack of appropriate animal models, these current studies have been unable to demonstrate the comprehensive and systematic effects of n-3 PUFA on pathological facts observed during the conditions of MetS. In 1991a, Russell *et al.* reported that diets supplemented 10% w/w with red fish oil fed to JCR:LA-*cp* male rats with lipid disorders and atherosclerosis conferred protection against CVD (Russell *et al.* 1991a). However, because this diet contained lower proportion of cholesterol and lipid, it was not representative of a typical western diet recognised to exacerbate the severity of vascular complications in JCR:LA-*cp* animal. Our unpublished short term fish oil study also indicated that n-3 PUFA supplementation was beneficial to lipid metabolism, insulin resistance and obesity, however it was too short in duration (3 weeks) to demonstrate a significant effect of n-3 PUFA on myocardial and glomerular pathologic changes (Hassanali *et al.* 2005). Therefore, in order to explore the properties of n-3 PUFA in the progression of long-term end-stage pathological complications of MetS, we conducted a long-term intervention study in JCR:LA-*cp* rats.

As discussed previously (in section 1.2), dyslipidemia, including hypercholesterolemia, and hypertriglyceridemia, is an important component of

MetS. Postprandial dyslipidemia has been found to be a key risk factor in the development of dyslipidemia and atherosclerosis (Karpe *et al.* 1994). Increasing long chain n-3 PUFA in the diet can reduce postprandial plasma triglyceride (TG) and cholesterol (Roche *et al.* 2000). However, there are limited studies that have investigated the long-term effects of feeding n-3 PUFA on postprandial apoB48 response that is highly associated with chylomicron (CM) metabolism and CVD. Hence, in this study, we not only investigated long-term effects of n-3 PUFA on postprandial response of TG and total cholesterol, but also on postprandial response of apoB48.

Generally, it is well accepted that increased intake of n-3 PUFA either from natural sources or dietary supplementation is inversely associated with atherothrombosis and atherosclerosis (Baldassarre *et al.* 2006). Many studies investigate that n-3 PUFA may improve atherosclerosis and heart ischemia, however, the direct histological evidence of n-3 PUFA on reducing or preventing the incidence of ischemic myocardial lesions needs to be presented (Mozaffarian *et al.* 2008).

There is emerging evidence that suggests in diabetic and nondiabetic kidney diseases, dietary supplementation with n-3 PUFA may have beneficial effects on albuminuria, kidney function, as well as arterial blood pressure and dyslipidemia (Kasiske *et al.* 1991, Lu *et al.* 2003). In 1991, Sinha AK *et al.* reported that fish oil diet completely corrected the hypertriglyceridemia of diabetes and significantly reduced the mild and early proteinuria of diabetes. Sinha proposed that the decrease in proteinuria and the correction of hyperlipidemia of diabetes by a fish oil-enriched diet was beneficial in the long-term for the development of diabetic glomerulopathy (Sinha *et al.* 1990). In addition, other studies have showed that feeding n-3 PUFA may reduce prostanoid production and inhibit the COX activity as a result of improving kidney inflammation and alleviating glomerulosclerosis (Baggio *et al.* 2005). However, there have been limited studies determining the role of n-3 PUFA not only on glomerulosclerosis in longer studies. Also, most

current studies failed to fully elucidate whether n-3 PUFA supplementation can be chronically beneficial to improve kidney inflammation by providing histological evidence and prostanoid production and COX activity.

There is currently no good evidence to recommend an optimal dose range for n-3 PUFA to prevent and treat MetS and its complications. Mozaffarian indicated that modest consumption of fish or fish oil (1-2 servings/week of oily fish, or approximately 250 mg/d of EPA+DHA) substantially reduces the risk of coronary heart disease (CHD) and sudden cardiac death (Mozaffarian *et al.* 2008). The amount of EPA and DHA typically administered for the treatment of hypertriglyceridemia is 2-4 g/day (Mozaffarian 2008). Moreover, some studies have suggested there may be potential adverse effects due to high dose of n-3 PUFA or fish oil (Torres *et al.* 2008). However, there is no clear evidence of the adverse of effects of toxic dose of fish oil.

2.2 Thesis Aim

The overall aim of this study was to determine whether long-term n-3 PUFA dietary treatment could improve aetiological end points associated with the metabolic syndrome, including heart and kidney pathophysiology and provide possible insight into some of the putative beneficial mechanisms.

2.3 Specific Hypothesis

The general hypothesis is that feeding long chain polyunsaturated n-3 PUFA in an animal model of MetS will inhibit the pathogenesis of macro- and micro-vascular disease associated with obesity and insulin resistance:

Specifically I propose that:

I. Long-term n-3 PUFA feeding will improve fasting and postprandial (pp) dyslipidemia as well as other circulating metabolites in insulin resistant, obese JCR:LA-*cp* rodent model.

II. Modulating MetS by n-3 PUFA supplementation will improve lipogenic-related enzymes changes including fatty acid synthase and acetyl-CoA carboxylase.

III. Improvement from dyslipidemia, other circulating metabolites and lipogenic-related enzymes will result in corresponding improvements to macro-vascular disease such as ischemic myocardial lesions associated with MetS.

IV. Long-term n-3 PUFA supplementation in JCR:LA-*cp* rats will improve micro-vascular disease (glomerulosclerosis).

V. Improvements in glomerulosclerosis by n-3 PUFA in JCR:LA-*cp* rats will be associated with a decrease prostanoid production.

2.4 Specific Objectives

To test the above hypotheses, we used a single intervention study in JCR:LA-*cp* rats for 16 weeks (long-term) utilizing two specific doses of n-3 PUFA to:

I. Establish long-term effects of n-3 PUFA in JCR:LA-*cp* rats on fasting lipid and biochemical profile including measurements of fasting glucose, insulin, total cholesterol, TGs, LDL, HDL, apoB48, leptin and adiponectin.

II. Assess long-term effects of n-3 PUFA feeding in JCR:LA-*cp* rats on postprandial dyslipidemia including postprandial (pp) TG, apoB48 and total cholesterol.

III. Establish long-term effects of n-3 PUFA on lipogenic-related enzymes including protein levels of SREBP-1, ACC and FAS both in liver and white adipocyte tissue.

IV. Delineate long-term effects of n-3 PUFA feeding in JCR:LA-*cp* rats on corresponding ischemic heart damage by measuring the incidence of myocardial damage.

V. Determine the long-term effects of n-3 PUFA feeding in JCR:LA-*cp* rats on kidney function and glomerulosclerosis by assessing urinary albumin and creatinine, peri-renal fat pad and the corresponding frequency of glomerulosclerosis via histological techniques.

VI. Establish the effects of long-term feeding n-3 PUFA in JCR:LA-*cp* on corresponding in the kidney prostanoid production including renal PGD_2, PGE_2, TXB_2, 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$.

I have chosen to present these objectives in the subsequent chapters with the following outline:

Chapter 3: Study design - further discuss the rational of study design, animal model, diet and methodologies that are common to chapters 4 and 5.

Chapter 4: Long-term effects of n-3 polyunsaturated fatty acids (PUFA) supplementation in the JCR:LA-*cp* rat: effects on insulin resistance, dyslipidemia and myocardial lesions. This will address the first 4 objectives

Chapter 5: Long-term effects of dietary n-3 PUFA supplementation on glomerulosclerosis and renal prostanoid production in the JCR:LA-*cp* rat, a model of the metabolic syndrome. This will present objectives 5 and 6.

Chapter 6: Overall Discussion and Conclusion. This chapter will provide opportunities to discuss overall results and make comments relative to my hypothesis.

Chapter 7: Literature Cited

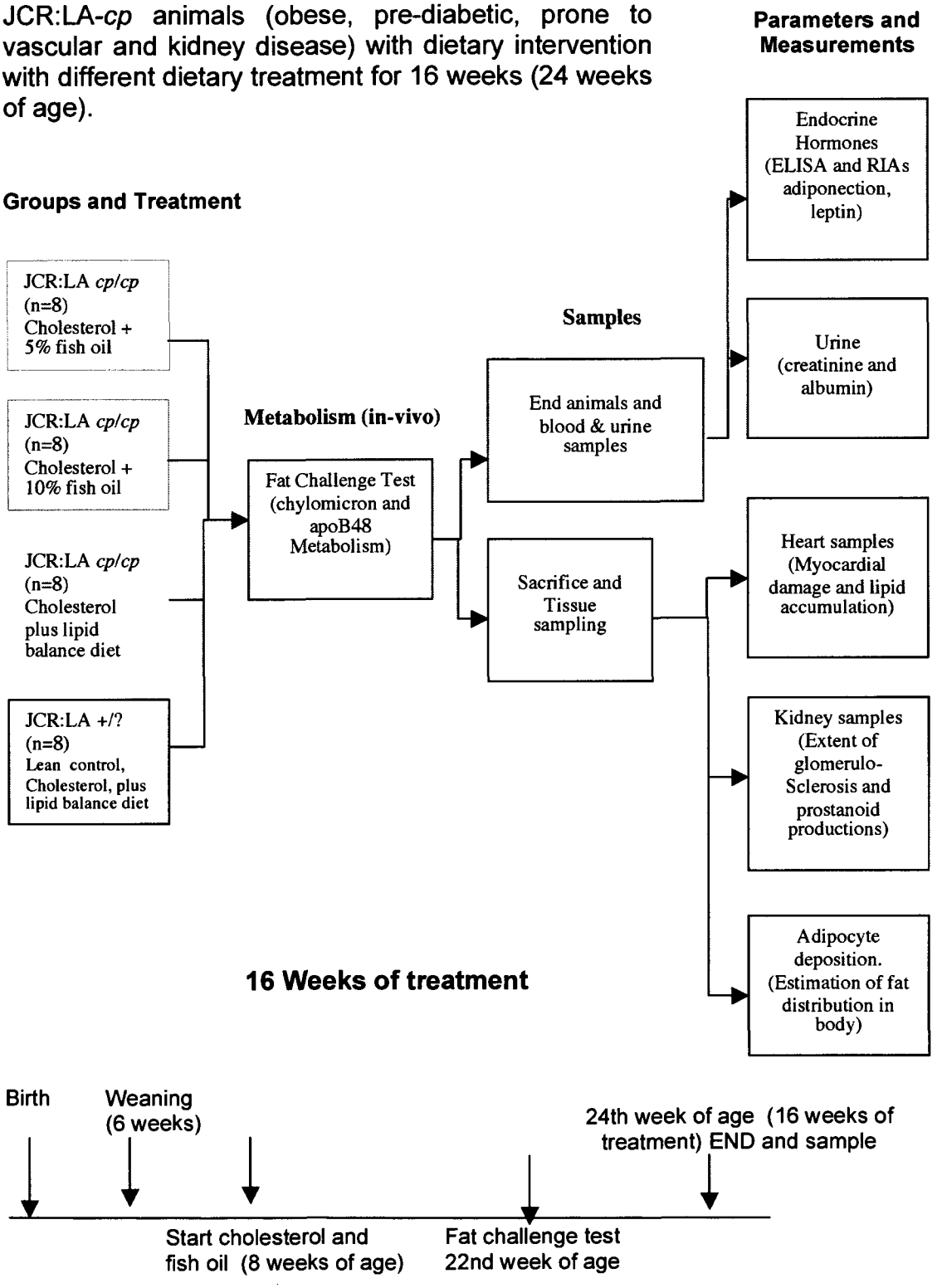
Chapter 3: Study design

3.1 Introduction and Brief Rationale

To test the hypothesis that feeding long chain polyunsaturated n-3 PUFA in an animal model of MetS will inhibit the pathogenesis of macro- and micro-vascular disease associated with obesity and insulin resistance, a long-term (16 week) dietary intervention was designed study with 5% w/w and 10% w/w (of fat) fish oil (n-3 PUFA) supplementation in JCR:LA-*cp* rats. The JCR:LA-*cp* rodent was chosen as a model of metabolic syndrome (MetS) because this model exhibits many of the biochemical characteristics and the corresponding end-stage pathological complications typically observed in humans (Russell *et al.* 1998a, Proctor *et al.* 2007). Obese JCR:LA-*cp* rats develop insulin resistance, fasting and postprandial dyslipidemia together with mild hyperglycemia (Vine *et al.* 2007). Importantly, the JCR:LA-*cp* rat spontaneously develops an array of significant pathological complications relevant to this study, including macro-vascular (presenting as early intimal and myocardial ischemia) and micro-vascular (present as glomerulosclerosis) pathophysiology (Russell *et al.* 1998a, Proctor *et al.* 2007).

To answer questions relevant to potential beneficial improvements to macro and micro vascular aetiology of n-3 supplementation, JCR:LA-*cp* rats were fed with control, 5% or 10% w/w fish oil supplemented diets for 16 weeks from age of 8 weeks to 24 weeks (see Figure 3-1). During this period, animals were fed with study diets from pre-diabetic stage (8 weeks of age) to a period where end-stage pathological complications manifest, including myocardial lesions and glomerulosclerosis (24 weeks of age). Accordingly, 16 weeks of feeding provide a long-term base to investigate the benefits of long-term n-3 PUFA supplementation on MetS and the associated macro and micro-vascular complications.

Figure 3-1. Study Flow chart and Timeline.
JCR:LA-*cp* animals (obese, pre-diabetic, prone to vascular and kidney disease) with dietary intervention with different dietary treatment for 16 weeks (24 weeks of age).



The control diet (labelled in this study as lipid-balanced diet or LBD) was designed to balance lipids and macronutrients contents corresponding to that formulated in the fish oil diet (Table 3-1). Table 3-1 shows the fatty acid composition for all three diets. Cholesterol (1% w/w) was also added to each of diets to further exacerbate hypercholesterolemia and corresponding severity of vascular complications (Table 3-2). In addition, to better show long tern effects of n-3 PUFA supplementation on kidney, a higher protein content (26% energy yield) was designed in this study to exacerbate renal morbidity (Table 3-3). The ability to synthetically formulate and custom prepare diets provide an added advantage from previous studies utilizing this animal model. For example, Russell *et al.* revealed that a diet supplemented with 10% w/w red fish oil in male JCR:LA-*cp* rats caused significant reduction in all circulating plasma lipid classes, including a marked 65% reduction in triglyceride (TG) and 35% reduction in cholesterol concentrations (Russell *et al.* 1991b). In this previous study, incidence of myocardial lesions was not significantly different between diets. For this study, a manufactured-prepared diet Lab Diet 5001 was supplemented with 10% w/w of weight with red fish oil (Russell *et al.* 1991b). The diet formulation used in this earlier study was not representative of a typical western diet recognised to exacerbate severity of vascular complications. The diet used in this previous study contained a lower proportion of cholesterol and lipid relative to the diet formulated for the current study. This, together with the length of feeding, may have limited potential with n-3 fatty acid supplementation to see an improvement on vascular function.

3.2 JCR:LA-*cp* rat

The JCR:LA-*cp* rat has the autosomal recessive corpulent (*cp*) trait first identified by Koletsky (Koletsky 1973 and 1975, Greenhouse *et al.* 1988). Similar to other strains incorporating the *cp* phenotype, homozygous (*cp/cp*) JCR:LA-*cp* rats are obese and insulin resistant, with resulting hyperinsulinemia and hyperlipidemia. Heterozygous (+/*cp*) or homozygous (+/+) animals are lean with normal

metabolism and not distinguishable from the parent LA/N strain (Dolphin *et al.* 1987, Russell *et al.* 1987, Russell *et al.* 1994 and 1995a). The *cp* trait is the result of a stop codon in the extracellular domain of the ObR gene (the leptin receptor gene) (Russell *et al.* 1998b). The absence of hypothalamic leptin action in the *cp/cp* rat results in elevated neuropeptide Y (NPY) levels (Williams *et al.* 1990), marked hyperphagia and obesity (Russell *et al.* 1995). The obese JCR: LA-*cp* (*cp/cp*) rat is arguably the model of choice for disease risk indices associated with complications of pre-diabetes and in particular, the MetS (Proctor *et al.* 2006). As a consequence, the JCR:LA-*cp* rat has proven to be a very useful animal for the study of MetS and dyslipidemia as well as myocardial and kidney damage associated with obesity as previously reviewed in chapter 1.1 and 1.3. In 1998, Russell *et al.* reported that JCR:LA-*cp* rats were unique among these strains in the development of both intimal vascular lesions and lesions in the myocardium that are evidently of ischemic origin (Russell *et al.* 1998a). In light of the vascular abnormalities seen in the *cp/cp* rat, it has also been reported that the presence of ischemic myocardial lesions in *cp/cp* rats develop spontaneously as early 3 months of age and increase in frequency up to 9 months of age (Brindley *et al.* 2002). The observation of ischemic damage to the heart suggests that the rats are exposed to frequent myocardial infarction episodes. Also of relevance to this study is the fact that JCR:LA-*cp* rats spontaneously develop glomerulosclerosis (micro-vascular damage), which is another important end stage complication of MetS found in humans. In humans, micro-vascular damage is evident physically as glomerulosclerosis and is a major cause of end-stage renal failure in diabetic patients.

Collectively, the JCR:LA-*cp* rat displays a unique phenotype associated with the risk factors and end-stage pathophysical macro- and micro- vascular complications often observed in clinical MetS. Accumulating evidence strongly suggests that the disease-prone character of the JCR:LA-*cp* rat is not just a consequence of the *cp* trait *per se*, but is also multifactorial and polygenetic. For the purposes of this current study, this unique animal model provides a very

appropriate MetS model to explore effects of long-term n-3 PUFA supplementation on MetS and its corresponding vascular complications.

3.3 Study Diet

The LBD diet was composed of 43.10% carbohydrate, 28.15% protein, 15% fat, 4.8% minerals, 0.95% vitamins and 8% fiber (w/w) (shown in Table 1). At 8 weeks of age, 24 *cp/cp* animals were randomized to one of three treatment diets containing either; (i) a hypercholesterolemic isocaloric lipid balanced diet (control) (15% w/w total fat, 1.0% cholesterol, P:S ratio 0.4); (ii) a hypercholesterolemic lipid balanced diet supplemented with 5% n-3 PUFA (fish oil derived EPA/DHA, 15% w/w total fat, 1.0% cholesterol, P:S ratio 0.4), or (iii) a hypercholesterolemic isocaloric lipid balanced diet supplemented with 10% n-3 PUFA (fish oil derived EPA/DHA, 15% w/w total fat, 1.0% cholesterol, P:S ratio 0.4) (Table 3-1 and 3-2). In addition, 8 lean (+/?) rats of the same age were also treated with a hypercholesterolemic isocaloric lipid balanced diet (control) (15% w/w total fat, 1.0% cholesterol, P:S ratio 0.4). All animals were fed the diets for 16 weeks. The feed was moistened, pelleted by extrusion through a die, and air-dried. Diet was sampled at regular time points during the feeding period to monitor fat composition using gas chromatography.

	LBD	5% FO Diet	10% FO Diet
Total Polyunsaturated	27.4	24.3	27.7
Total Saturated	64.5	66	65
P/S ratio	0.4	0.4	0.4
Total n-6	23.4	17.4	17.1
Total n-3	1.2	6.9	10.6
Total EPA + DHA	0	5.3	9.4

Table 3-1. The fat acids composition of the three dietary treatment groups including lipid balanced diet (LBD), 5% fish oil diet (FO) and 10% fish oil diet (FO) from GC analysis.

	LBD	5% FO Diet	10% FO Diet
Casein (g)	270	270	270
Starch (g)	214	214	214
Dextrose (g)	217	217	217
Non-nutritive Cellulose (g)	80	80	80
Vitamin Mixture (g)	9.5	9.5	9.5
Mineral Mix (g)	48	48	48
Choline (g)	2.75	2.75	2.75
Inositol (g)	6.25	6.25	6.25
L-methionine (g)	2.5	2.5	2.5
Linseed Oil (g)	3	3	3
Tallow (g)	91.71	91.88	94.73
Sunflower Oil (g)	55.29	40.13	24.27
Fish Oil (g)	0	15	28
Cholesterol (g)	10	10	10

Table 3-2. The ingredients of 1 kg basis diet in three dietary treatment groups including lipid balance diet (LBD), 5% fish oil (FO) and 10% fish oil (FO).

	g/kg Diet	% Energy Yield
Carbohydrate	430	41%
Protein	270	26%
Lipid	150	33%

Table 3-3. The macronutrients composition and energy yield of three dietary treatment groups including lipid balanced diet (LBD), 5% fish oil diet (FO) and 10% fish oil diet (FO) from GC analysis.

3.4 Animal care and Metabolic Assessments

3.4.1 Husbandry

Male rats of the JCR:LA-*cp* strain, obese (*cp/cp*) (n = 24) and lean [+/?, or a 2:1 mix of rats heterozygous (cp/+) and homozygous normal (+/+)] (n = 8), were raised and housed in the breeding colony at the University of Alberta as described previously (Russell *et al.* 1995b). Rats were weaned at 6 weeks of age and housed with a 12/12-hour reversed light cycle to allow for study and testing during the dark phase of the rats' diurnal cycle. The animal rooms were maintained at 21°C and 50% relative humidity. Food and water were available at all times. During the treatment period (16 weeks), all animals were weighed and food consumption measured once per week.

3.4.2 Fat Challenge and Postprandial Response

A fat challenge test was performed at 22 weeks of age as previously described (Vine *et al.* 2007). All animals were fasted overnight (16 h) and then offered a 5.0 g pellet made with 5001 laboratory chow consisting of 49% carbohydrate, 24.0% crude protein, 10% moisture, 6.5% minerals, 6.0% fiber and 4.5% fat, which were incorporated with 25% (w/w) of dairy fat from double cream (raising the total fat content of the 5.0 g meal to approximately 30% (w/w)) (Vine *et al.* 2007). Fat challenge experiments were performed using a standardized, conscious, non-restraint protocol to reduce variability (Russell *et al.* 2005). Blood samples were collected at times 0, 1, 2, 4, 5, 6, 8 and 10 h following consumption of the pellet meal via tail bleeding. Blood was collected into tubes containing Na_2EDTA. Plasma and serum were separated by centrifugation at 4 °C at 3000 rpm for 10 min. Aliquots of plasma were immediately stored at −80 °C for further analyses.

Area under the curve analysis created from fat challenge test provides an effective tool to evaluate postprandial dyslipidemia. Following an overnight (16 hours) fast, the concentration and proportions of plasma lipoproteins are often

used as a baseline state from which the effects of postprandial dyslipidemia can be evaluated. Figure 3-2 illustrates an example of the postprandial response of plasma TG (area under the curve, AUC). Zero hour represents the fasting level of plasma TG; the postprandial phase of 0–4 h predominantly represents plasma TG derived from the intestine, but it may also reflect minor contributions of TG and apoB48 from the liver; the concentration of TG typically reaches a peak during the 4-5 hour of the postprandial phase that declines at 5-6 h. However in *cp/cp* obese rats, the postprandial phase has been recorded to last as long as 10-12 hours (Vine *et al.* 2007).

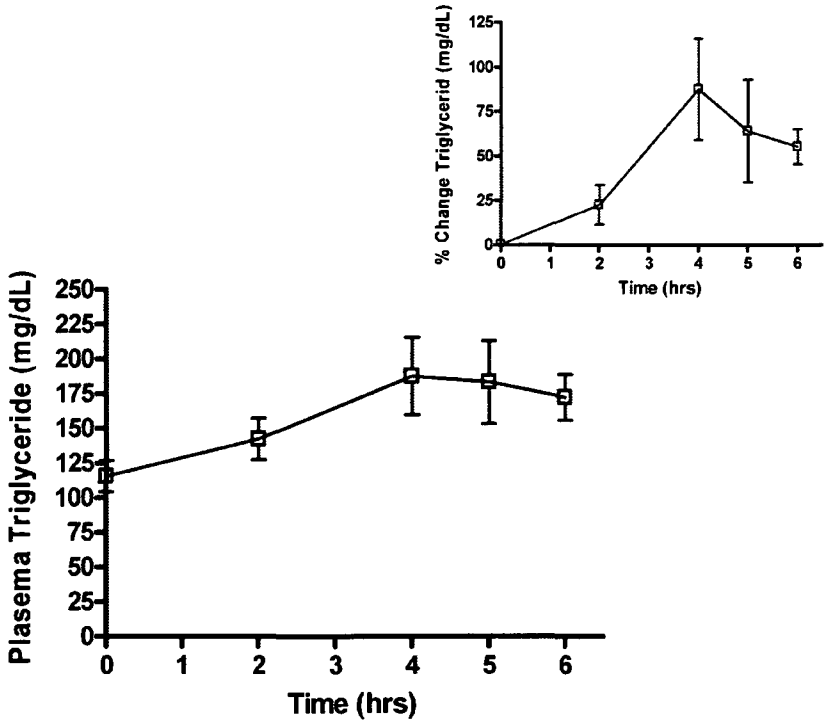


Figure 3-2. The sample postprandial curve (AUC) and iAUC of plasma triglyceride (inset).

Figure 3-2 inset shows an example of incremental area under the curve response of plasma TG. The iAUC represents the change in the postprandial response by compensating for the initial concentration of each parameter measured during time = 0 mins. iAUC is expressed as the percent change from baseline and

provide an accurate representation of contributions from intestine, irrespective of the baseline concentrations. The plasma content of TG during the postprandial phase is primarily due to the synthesis of CMs transporting dietary lipid. The characteristics of postprandial dyslipidemia associated with MetS including apoB48, total cholesterol and TG is further discussed in Chapter 4.4.3.

3.4.3 Sample Collection

Rats were euthanized in the fasted state using isoflurane/O_2 mixture anesthesia by cardiac exsanguination. Blood and urine were collected after animals were ended. Blood was collected into tubes containing Na_2EDTA. Plasma and serum were separated by centrifugation at 3000 rpm at 4 °C for 10 min. Aliquots of plasma were stored at −80 °C for further analyses. A urine sample (2 ml) were frozen at -80°C. The heart was removed and fixed in 10% v/v N.B. formalin. The left kidney was fixed via 10% v/v N.B. formalin for histology analysis, whereas, the right kidney was frozen at -80°C. Both inguinal and peri-renal fat pad were stored in -80°C freezer. The care of the animals and experimental procedures were in conformity with the guidelines of the Canadian Council on Animal care and subject to prior review and approval by the University of Alberta.

Format of Results and the following Chapters:

To clearly arrange the results of the study described in this chapter, the findings are presented in the following two Chapters (4 and 5) and are entitled:

Chapter 4: Long-term effects of n-3 polyunsaturated fatty acids (PUFA) supplementation in the JCR:LA-*cp* rat: effects on insulin resistance, dyslipidemia and myocardial lesions.

Chapter 5: Long-term effects of dietary n-3 PUFA supplementation on glomerulosclerosis and renal prostanoid production in the JCR:LA-*cp* rat, a model of the MetS.

Chapter 4: Long-term Effects of n-3 Polyunsaturated Fatty Acids Supplementation in the JCR:LA-*cp* Rat: Effects on Insulin Resistance, Dyslipidemia and Myocardial Lesions

4.1 Introduction

The metabolic syndrome (MetS) is a pre-diabetic state that is characterized by abdominal obesity, insulin resistance and dyslipidemia (Proctor *et al.* 2007). The long-term sequelae of MetS include macro-vascular diseases such as cardiovascular disease (CVD) and myocardial lesions (Russell *et al.* 2007). Postprandial dyslipidemia is known as a risk factor for macro-vascular and CVDs (Molitch 2006). Dietary cholesterol and fat are absorbed from the intestine, and then packaged into CMs and transported into circulation following a meal (Proctor *et al.* 2004). The arterial wall can retain lipoprotein-derived cholesterol, including that from apolipoprotein B48 (apoB48) containing CMs, supporting the notion that postprandial dyslipidemia can contribute to atherosclerosis (Hassanali *et al.* 2006. Proctor *et al.* 2003). Several studies suggest that intestinally derived apoB48-containing lipoproteins (i.e. CMs), particularly small-dense chylomicron-remnants (CMr) are atherogenic. The subsequent impairment of CM clearance from the plasma compartment is thought to be a contributor to whole body dyslipidemia and CVD risk (Proctor *et al.* 2003 and 2004). Accordingly, the effects of dietary fatty acids, especially fish oil on postprandial lipid metabolism have been extensively studied in recent years, especially in high risk individuals such as hypercholesterolemia, diabetes and obesity (Lopez-Miranda *et al.* 2007).

N-3 polyunsaturated fatty acids (PUFA), i.e., fish oil, especially eicosapentaenoic acid (EPA, 20:5,n-3) and docosahexaenoic acid (DHA, 22:6,n-3) have been reported to be of benefit for MetS and its complications such as CVD (Schwalfenberg 2006, Carpentier *et al.* 2006). N-3 PUFA is thought to play an important role in prevention and management of coronary heart disease, dyslipidemia, type 2 diabetes and insulin resistance. Some studies report that

fish oil supplementation may not only decrease plasma levels of insulin, glucose and cholesterol, but also attenuate blood pressure and decrease other cardiovascular risk factors, such as elevated LDL and triglyceride (TG) (Psota *et a* 2006, von Schakcy *et al.* 2007). Recently, a clinical study indicated that fish oil enriched products may reduce the serum concentrations of TG, total and LDL cholesterol, apoB, and glucose in patients with MetS (Benito *et al.* 2006).

Evidence from experimental and human studies suggest that n-3 PUFAs may decrease fatal acute infarct sizes and reduce the risks of fatal coronary heart disease (CHD) and sudden cardiac death (SCD) (Xiao *et al.* 2008, Mozaffarian 2008). However, long-term effects of n-3 PUFA on pathologic development of myocardial lesions in individuals with MetS have not been fully elucidated. Preliminary data from a short-term (3 week) fish oil study in JCR:LA-*cp* rats from our laboratory indicated that n-3 PUFA supplementation improved lipid metabolism, insulin resistance and obesity (Hassanali *et al.* 2006). However, due to the short term nature of this preliminary study we were unable to delineate any beneficial role of n-3 PUFA on myocardial lesion pathology. Accordingly, the long-term effects (longer than 6 weeks) of dietary n-3 PUFA supplementation on myocardial lesions using an animal model of MetS are still unclear, and form the premise of the primary objective of this study.

N-3 PUFAs have been demonstrated to influence lipid metabolism via sterol regulatory element binding proteins (SREBPs) pathway (Xu *et al.* 2001). SREBPs are a family of transcription factors that regulate synthesis of cholesterol and fatty acids. SREBP-1 mainly regulates fatty acid synthesis and SREBP-2 mainly regulates cholesterol synthesis (Sul *et al.* 2000, Shimano 2001). Lipogenic-related enzymes include fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) which are key regulators of protein, fatty acid and glycerolipid synthesis. Over the long-term, inhibition of these latter events and increased fatty acid oxidation might be achieved by reducing the expression of SREBP-1 (Korczynska *et al.* 2004). N-3 fatty acids may inhibit transcription and expression

of genes for lipogenic enzymes such as FAS and ACC, and also down-regulate the mature form of SREBP-1 by decreasing SREBP-1 mRNA expression (Kim *et al.* 2002). Consequently, the second objective of this study is to assess the potential influence of n-3 PUFA supplementation these lipogenic pathways using the JCR:LA-*cp* rat model.

The JCR:LA-*cp* rat is a specific strain that develops marked hyperinsulinemia and obesity due to a defect in the leptin receptor gene (*cp*), The JCR:LA-*cp* rat model displays characteristics of the MetS including end stage complications such as ischemic myocardial lesions (Russell *et al.* 1995b). In a preliminary study, short term (3 weeks) fish oil supplementation reduced both postprandial dyslipidemia and pro-inflammatory status associated with the insulin resistance and the MetS (Hassanali *et al.* 2006). For my thesis, I proposed to study determined the long-term effects of n-3 PUFA at two physiological doses on body weight, hyperinsulinemia and dyslipidemia well as the effect of n-3 PUFA on expression of ACC, FAS and SREBP-1 in liver and adipose tissue. In addition, the effects of n-3 PUFA on ischemic myocardial lesions were investigated.

4.2 Method

4.2.1 Animals and Diet

The animal model, experimental design and dietary treatments have been detailed in chapter 3.2 and 3.3.

4.2.2 Fat challenge and Postprandial Response

Fat challenge and postprandial response have been detailed in chapter 3.4.2.

4.2.3 Plasma Biochemical and Lipid Profile

4.2.3.1 Biochemical Profile

The biochemical profile of fasting plasma from lean and obese JCR:LA-*cp* rats was assessed using commercially available homogenous, enzymatic colorimetric direct and indirect assays. Plasma glucose was assessed using the glucose oxidase method (Diagnostic Chemicals, Cat#220-32). Insulin was analyzed using a solid phase two-site enzyme immunoassay (Mercodia AB, Uppsala, Sweden, Cat#10-1137-01). Adiponectin (ALPCO Diagnostics Salem, NH, USA, Cat#44-ADPR-0434) and leptin (ALPCO, Cat#22-LEP-E06) was determined using commercially available enzyme immunoassays for rodents.

4.2.3.2 Lipid Profile

The biochemical lipid profile from lean and obese groups was assessed using commercially available assays. Triglyceride (TG) concentrations (Wako Pure Chemicals USA, Inc. Richmond, VA) Cat#998-40391/994-40491), total cholesterol (WAKO, Cat#439-17501), LDL (WAKO Cat#993-00404/999-00504) and HDL-cholesterol (Diagnostic Chemicals Ltd., Charlottetown, PEI, Canada Cat#258-20) were measured using direct colorimetric chemical enzymatic reactions.

4.2.3.3 Quantitation of Plasma Apolipoprotein B48

The isolation and quantitation of apoB48 in rodent plasma was performed by an adapted SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) and Western-blot technique coupled to enhanced chemiluminescemce procedure as described by Vine *et al.* 2007. Plasma samples and corresponding rodent apoB standard (1.006 g/ml) were denatured by mixing with a sample buffer/reducing agent (2-mercaptoethanol; Sigma-Aldrich, Oakville ON, Cat#60242, and NuPAGE LDS sample buffer; Invitrogen, Carisbad, CA, Cat# NP007). The rodent apoB standard was generated from the plasma of JCR:LA-*cp* rats. Plasma apolipoproteins were separated by NUPAGE SDS-PAGE 3-8% tris-acetate polyacrylamide gels (Invitrogen, Burlington ON,

Cat#EA0375BOX). Proteins were transferred to a polyvinylidene fluoride (PVDF) membrane (0.45 μm; ImmobilonPTM, Millipore, MA, USA, Cat# IPVH00010) by transfer buffer (Invitrogen, Burlington ON, Cat# NP0006-1) at 40 voltage for 3.5 hours. PVDF membrane was incubated overnight with a 2% (w/v) Amersham protein blocking solution (ECL Advance blocking agent, Amersham/GE Healthcare UK Ltd., Little Chalfont Buckinghamshire and TBST) at 4°C. Following incubation, membranes were washed and probed with primary antibody, an affinity-purified goat polyclonal antibody to apoB (Santa Cruz Biotech, CA, Cat#sc-11795). After a series of timed washes (55 minutes (min): 2 x 2 min; 2 x 3 min; 3 x 5 min; 2 x 10 min) with TBST, the PVDF membrane was incubated with a secondary donkey antibody raised against goat IgG having hydrogen peroxidase (Santa Cruz Biotech, Cat#sc2304). After a final series of washes (115 mins: 2 x 2 min; 2 x 3 min; 3 x 5 min; 6 x 10 min; 2 x 15min), the apoB48 band was visualized using ECL (ECL-Advance, Amersham Biosciences, UK) and the imaging of proteins was conducted using a Typhoon TRIO+ Variable Mode Imager (Amersham / GE Healthcare, Baie d'Urfé, QC). The mass of apoB48 from rodent plasma was quantified using linear densitometric comparison with a known mass of the purified rodent apoB48 protein standard. The comparison of band intensity was analyzed using computer program Image J (1.37a, National Institutes of Health, USA).

4.2.3.4 Analysis of Postprandial Response

The postprandial response of plasma apoB48, TG, cholesterol for +/? (n = 8) and *cp/cp* (n = 24) animals was determined using total area under the curve (AUC). Incremental area under the curve (iAUC) was calculated by subtracting the fasting concentration from the total AUC for the postprandial period. The iAUC is expressed as the percent change from baseline. iAUC represents the change in postprandial response (compensating for the initial concentration of each parameter measured during the fasting state) (Vine *et al.* 2007). The TG:apoB48 ratio is regarded as an indicator of particle size and was calculated using pair-matched AUC values, respectively, at each time point following the oral fat load.

4.2.4 Immunoblotting for Lipogenic-related Enzymes

Liver samples were collected at the end of the study and adipose tissue was taken from the peri-renal region. After homogenization, protein concentrations of liver and adipose tissue were determined by BCA™ Protein Assay Kit (PIERCE, USA, Cat#23225). Protein (40 µg) was separated by 3-8% tris-acetate polyacrylamide gels (NUPAGE) at 100 voltage for 2 hours. Tris-Acetate SDS Running Buffer was from Invitrogen (Invitorgen, Burlington ON, Cat#LA0041). Proteins were transferred to a PVDF membrane (0.45 µm; ImmobilonPTM, Millipore). ACC was probed using an affinity-purified goat polyclonal antibody to ACC (Santa Cruz Biotech, Santa Cruz, CA Cat#sc-11795); the actin protein was identified using goat polyclonal antibody to actin (Sigma). After incubating with a secondary donkey anti-rabbit antibody (New England BioLabs, Pickering ON, CA, cat#7074) and goat anti-mouse secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA cat#sc-2005), the ACC and actin bands were visualized using ECL-Advance and the imaging of proteins was conducted via a Typhoon TRIO+ Variable Mode Imager. The density of the ACC band from each liver and adipose tissue was quantified using linear densitometric comparison with the corresponding actin density, i.e. we compared the ratio of density of ACC to actin for each animal. Similarly, FAS protein (40µg from liver and adipose tissue) and SREBP-1 protein (40µg from liver and 15µg from adipose tissue) were probed by primary antibody for FAS and SREBP-1 used was goat monoclonal antibody IgG to FAS (New England BioLabs, Pickering ON, CA, Cat#3189) and mouse monoclonal antibody IgG to SREBP-1 (Santa Cruz Biotechnology, Santa Cruz, CA cat#sc-13551) respectively. After incubating with a secondary donkey anti-rabbit antibody (New England BioLabs, Pickering ON, CA, Cat#7074) and goat anti-mouse antibody (Santa Cruz Biotechnology, CA cat#sc-13551), the density of FAS and SREBP-1 were compared with the density of corresponding actin protein for each animal.

4.2.5 Heart Histology and Myocardial Analysis

Hearts were cut transversely into 4 blocks, fixed in formalin, and subjected to conventional processing, embedded in a single paraffin block and sectioned, followed by H&E staining (Russell *et al.* 1998a). Heart sections were examined blind by an experienced observer and the number of ischemic lesions identified in each of the sections summed for each heart. Myocardial lesions were categorized as stage 1 through 4 as previously described (Brindley *et al.* 2002, Russell *et al.* 1995 and 1998b): Stage 1, areas of necrosis; Stage 2, areas of cell lysis with long-term inflammatory infiltration; Stage 3, nodules of long-term inflammatory cell infiltration; and Stage 4, old, scarred lesions (Russell *et al.* 1990a and 1998a) (Figure 4-12). From an etiological perspective, stage 3 and 4 lesions are the most important, as they reflect the cumulative record of earlier stage lesions that were large enough to remain identifiable after the scarring and contraction of the repair process. The number of lesions in the sections from each heart was summed and the mean incidence for each group was calculated.

4.2.6 Statistical Analysis

Data were tested for normal distribution and differences between *cp/cp* LBD group and +/? group and n-3 PUFA treatment groups were analyzed using unpaired t-test and one-way ANVOA, and nonparametric tests for heart lesion frequency, with significance set at $p<0.05$ (Sigma Stat, Jandel Scientific, San Rafael, CA, USA and PRISM, Graphpad, San Diego, CA, USA). All results are shown as the mean±S.E.M.

4.3 Results

4.3.1 Food Intake, Body Weight, Fat Pads, Liver and Heart Weight

4.3.1.1 Food Intake and Body Weight

As reported in previous studies, obese (*cp/cp*) rats consumed significantly more food than lean (+/?) rats and the corresponding body weight curves differ from an

early age (Figure 4-1 and Table 4-1) (Russell 1998c). At 8 weeks of age (0 week of treatment), there was no detectable difference in body weight between the 5% and 10% fish oil-treated *cp/cp* rats and obese LBD *cp/cp* control rats. After one week, i.e. by 9 weeks of age (1 week of treatment), body weight of the fish oil-treated rats was lower than from *cp/cp* control rats. At 23 weeks of age (16 weeks of treatment), food intake of all obese groups was more than lean control rats (19.9±1.2 g/d). Further, food intake was not significantly reduced in either 5% fish oil (31.9±1.8 g/d) or 10% fish oil treated rats (24.5±3.1 g/d) compared to obese control rats (32.5±2.7 g/d). Surprisingly, despite no change in food intake, at 23 weeks of age, body weights of rats were significantly lower in 5% and 10% fish oil-treated *cp/cp* rats (584.0±11.8 and 552.4±10.0 g respectively, $p<0.001$) relative to *cp/cp* obese controls (668.5±8.2 g).

4.3.1.2 Inguinal Fat Pad

Compared to the *cp/cp* LBD group, 5% and 10% fish oil treated rats had significantly lower inguinal fat pad weight ($p<0.001$) (Figure 4-2). There was also significant reduction in the ratio of inguinal fat pad weight to body weight in both 5% and 10% fish oil groups ($p<0.001$) (Figure 4-3). Compared to the +/? LBD group, all obese groups had significantly higher inguinal fat pad weight and in the ratio of inguinal fat pad weight to body weight ($p<0.05$) (Figure 4-2 and 4-3). 10% fish oil treated rats had significantly lower inguinal fat pad weight and the ratio of inguinal fat pad weight to body weight than 5% fish oil treated rats ($p<0.05$) (Figure 4-2 and 4-3)

4.3.1.3 Heart and Liver Weight

LBD obese animals had higher heart weight than LBD lean animals (Table 4-1). There was no significantly different heart weight among LBD *cp/cp* control, 5% fish oil and 10% fish oil groups. All obese animals had significantly higher liver weight than LBD lean control (Table 4-1). Compared to LBD obese control, liver weight was significantly lower in 5% fish oil group.

4.3.2 Fasting Biochemical and Lipid Profile

4.3.2.1 Biochemical Profile

Fasting parameters of *cp/cp* controls, 5% and 10% fish oil animals and age-matched +/? animals are shown in Table 4-2. Fasting plasma insulin was significantly lower in both 5% and 10% n-3 fish oil dietary groups ($p<0.001$), compared to obese controls. There was no significantly different plasma insulin concentration among LBD lean control, 5% and 10% fish oil groups. Fasting plasma glucose level was significant lower in animals subjected to 10% fish oil ($p<0.01$), but not to 5% fish oil. Following 16 weeks of treatment, both 5% and 10% fish oil treated rats had lower fasting plasma leptin levels ($p<0.001$) (Table 4-2). All obese animals have higher fasting leptin concentrations than LBD lean controls ($p<0.001$). Additionally, fasting plasma adiponectin concentration was significantly higher in 5% fish oil (32.7±2.2 vs 24.4±1.9 mg/mL), but not in 10% fish oil (Figure 4-4).

4.3.2.2 Lipid Profiles

Compared to obese animals receiving the LBD, 5% fish oil but not 10% fish oil treatment significantly lowered fasting concentration of total cholesterol ($p<0.001$). All obese animals had higher fasting concentration of total cholesterol ($p<0.001$). In addition, 10% fish oil treatment reduced fasting plasma TG ($p<0.05$). Interestingly, fasting plasma apolipoprotein B48 (apoB48) was significantly decreased in both 5% and 10 % fish oil group ($p<0.001$) compared to *cp/cp* LBD controls (Table 4-1). There was no significantly different plasma apoB48 concentration among LBD lean control, 5% and 10% fish oil groups.

4.3.2.3 Postprandial Response of Triglyceride, Apolipoprotein B48 and Total Cholesterol

Relative to *cp/cp* LBD group, the postprandial response measured as area under the curve (AUC) was significant lower in both the 5% and 10% fish oil groups (AUC; 951.6±98.8, 974.2±114.7 area units respectively; $p<0.05$ vs LBD obese control: 1592±222.1 area units). All obese animals had higher postprandial TG

response than LBD lean (+/?) animals (p<0.05). Interestingly, the iAUC of 5% fish oil group, but not 10% fish oil group, was also significantly lower than *cp/cp* control group (iAUC; 251.1±85.75 area units. p<0.05 vs LBD obese control: 654.4±99.66 area units) (Figure 4-5). There was no significant difference for the TG iAUC response among LBD lean control, 5% and 10% fish oil groups.

The AUC for apoB48 was significantly lower in the 5% and 10 % fish oil group compared to obese LBD controls (AUC; 160.4±27.4 and 220.8±27.2 area units, respectively; p<0.001 vs 426.7±57.5 area units). Moreover, relative to the LBD control *cp/cp* animals (iAUC; 143.7±26.6 area units), both 5% and 10% fish oil significantly reduced and normalized incremental area under the curve for apoB48 (iAUC; 39.94±7.74, p<0.001 and 47.86±18.00 area units, p<0.01, respectively) (Figure 4-6). There was no significantly different apoB48 AUC and iAUC responses among LBD lean control, 5% fish oil and 10% fish oil.

The postprandial response of total cholesterol was significantly lower in both 5% and 10% fish oil relative to *cp/cp* LBD rats (AUC; 587.9±50.4 and 683.6±42.9 area units respectively; p<0.001 vs LBD obese control: 1083.0±50.4). The total cholesterol-iAUC is significantly reduced in 5% fish oil group (iAUC; 125.1±33.0 area units, p<0.01 vs LBD obese control: iAUC 293.7±29.9 area units) and 10% fish oil group (iAUC, 114.5±45.8 area units p<0.05) (Figure 4-7). There was no significant difference for total cholesterol AUC and iAUC responses among LBD lean control, 5% fish oil and 10% fish oil.

A rise of plasma TG and total cholesterol above fasting levels was observed up to 6 h in LBD obese animals. However, the 5% and 10% fish oil groups demonstrated a decline in TG and total cholesterol at 4-6 h (Figure 4-5 and 4-7). The TG:apoB48 ratio reflects the proportion of TG per particle during the postprandial period (for their respective incremental change over time). The AUC of TG:apoB48 ratio in 5% fish oil treated animals was significantly lower than obese LBD animals (Figure 4-8).

4.3.3 Lipogenic-related Enzymes

4.3.3.1 Lipogenic-related Enzymes in Liver

ACC protein concentration in liver was significantly reduced in 10% fish oil group ($p<0.05$), compared to LBD obese animals (Figure 4-9a). Relative to the LBD obese control animals, the amount of FAS protein in liver was significantly lower in 5% fish oil ($p<0.01$) but not 10% fish oil (Figure 4-9b). There was no significant difference in the amount of FAS protein in liver between LBD lean control and 5% fish oil groups. Both precursor and mature SREBP-1 protein in liver were significantly decreased in 5% fish oil obese animals ($p<0.01$), compared to LBD obese animals (Figure 4.9c & 4.9d). In contrast, the 10% fish oil only significantly decreased the amount of mature SREBP-1 (68-kDa), but not precursor SREBP-1 (125-kDa) (Figure 4-9c & 4.9d). There was no significant difference in the amount of precursor and mature SREBP-1 protein in liver between LBD lean control and 5% fish oil groups.

4.3.3.2 Lipogenic-related Enzymes in Adipose Tissue

A significant reduction of ACC in adipose tissue (peri-renal fat pad) was observed in 10% fish oil group, compared to LBD obese animals (Figure 4-10a). Both LBD obese and 5% fish oil animals had a significantly higher amount of ACC protein in adipose tissue than LBD lean animals ($p<0.05$). Interestingly, animals treated with 10% fish oil had significantly lower amount of ACC protein in adipose tissue than animals treated with 5% fish oil ($p<0.05$). Relative to LBD obese animals, the amount of FAS protein in adipose tissue was significantly reduced in 5% fish oil group (Figure 4-10b). There were no significant changes in the amount of FAS protein among LBD lean, 5% or 10% fish oil groups. There were no significant changes in the amount of SREBP-1 protein among LBD obese, LBD lean, 5% or 10% fish oil groups (Figure 4-10c).

4.3.4 Myocardial Lesions

Figure 4-11 and 4.12 show the frequency of the four stages of myocardial lesions for each of the treatment groups. There were relatively fewer Stage 1 myocardial lesions (areas of early ischemia or necrosis) than Stage 2 and 3 myocardial lesions in the hearts of all groups of animals. The frequency of Stage 1 lesions tended to be lower in n-3 PUFA groups, but was not significantly different, in hearts from n-3 PUFA treated obese rats, when compared to LBD obese rats. The frequency of Stage 2 lesions (foci of long-term inflammatory cells without cell dropout) was the highest across all four groups compared to other stages of lesions. Stage 3 lesions (areas with strong inflammatory cell infiltration and cell lysis) were infrequent in hearts of the LBD lean animals, but present at a significant frequency in the hearts of LBD obese rats. Interestingly, hearts of obese (*cp/cp*) rats treated with 5% PUFA had significantly fewer Stage 3 lesions than the LBD obese control animals (0.25 vs 1.5, $p = 0.02$). Stage 4 lesions were infrequent in the hearts of 24 weeks old rats with no significant differences between groups. Hearts of 5% PUFA obese animals had no Stage 4 lesions. There are no significant changes among LBD lean control, 5% or 10% fish oil groups at all stages.

Weights (g)	LBD +/?	LBD *cp/cp*	5% FO Diet *cp/cp*	10% FO Diet *cp/cp*
Heart (g)	1.0 ± 0.0^{a}	1.2 ± 0.0^{b}	1.2 ± 0.1	1.1 ± 0.0
Liver (g)	$9.9 \pm 0.5^{a, c, d}$	$20.0 \pm 0.6^{b, c}$	$14.3 \pm 1.8^{a, b}$	16.3 ± 0.4^{b}

Table 4-1. Heart and liver weights in JCR:LA-*cp* rats. All the values are from 24 weeks of age, and shown as mean $\pm$ S.E.M. [a]$p<0.05$, vs LBD *cp/cp* control; [b]$p<0.05$ vs LBD +/? control); [c]$p<0.05$ vs 5% fish oil (FO) *cp/cp* group; [d]$p<0.05$ vs 10% fish oil (FO) *cp/cp* group. There was no significant difference between 5% and 10% fish oil (FO) groups.

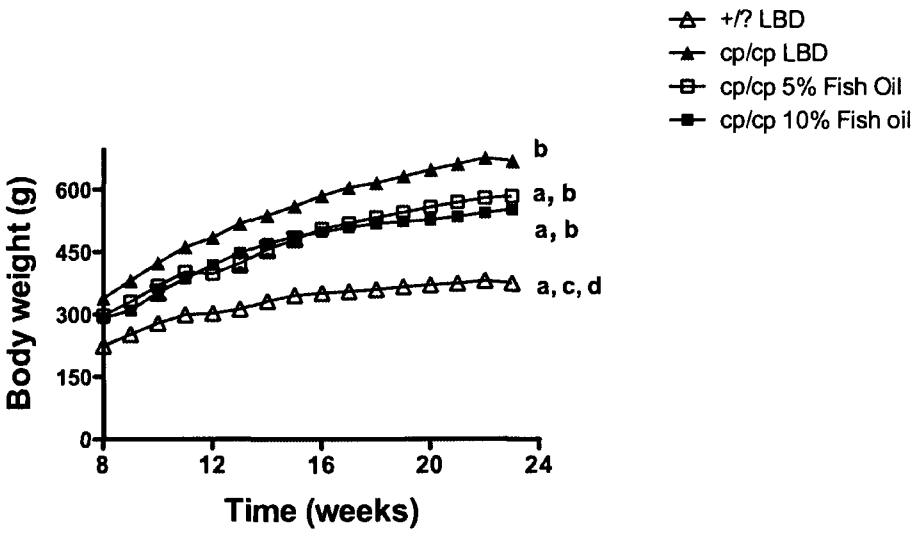


Figure 4-1. Body weight of JCR:LA-*cp* rats. Values are mean±S.E.M, 8 rats in each group. [a]$p<0.001$ vs LBD *cp/cp* control; [b]$p<0.001$ vs LBD +/? control; [c]$p<0.05$ vs 5% fish oil *cp/cp* group; [d]$p<0.05$ vs 10% fish oil *cp/cp* group. There was no significant difference between 5% and 10% fish oil group.

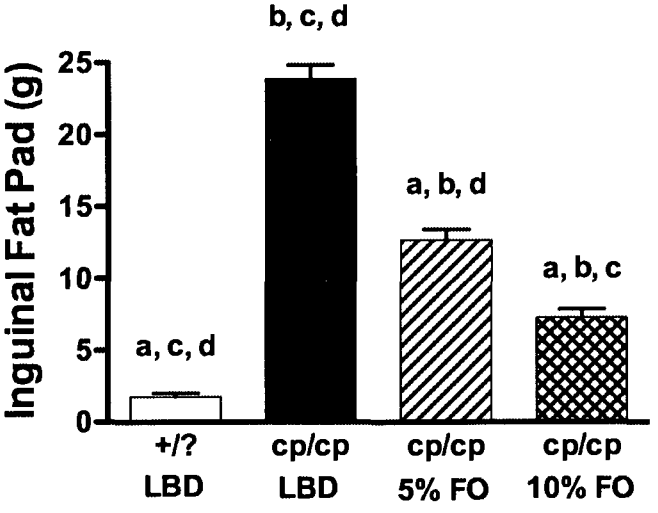


Figure 4-2. The weight of inguinal fat pads in 24 week old JCR:LA-*cp* rats. Values are mean±S.E.M. [a]$p<0.05$ vs LBD *cp/cp* control; [b]$p<0.05$ vs LBD +/? control; [c]$p<0.05$ vs 5% fish oil (FO) *cp/cp* group; [d]$p<0.05$ vs 10% fish oil *cp/cp* group.

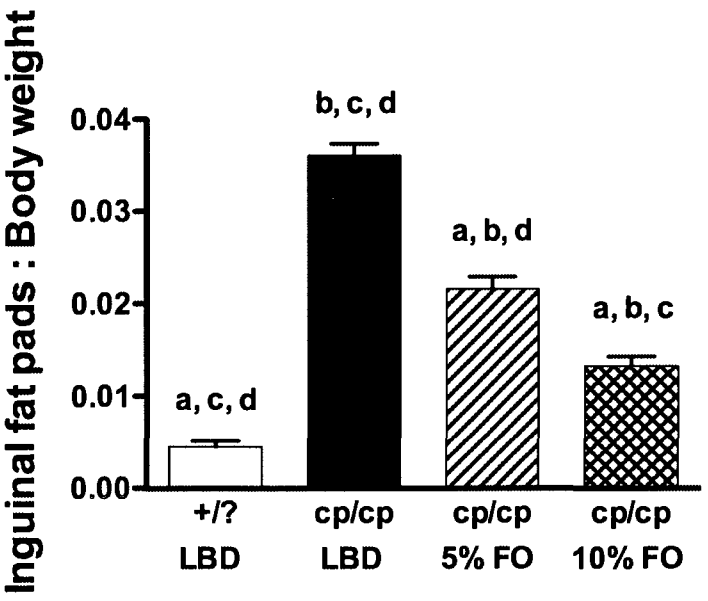


Figure 4-3. The ratio of inguinal fat pads weight to body weight in 24 week old JCR:LA-*cp* rats. Values are mean±S.E.M. [a]$p<0.05$ vs LBD *cp/cp* control; [b]$p<0.05$ vs LBD +/? control; [c]$p<0.05$ vs 5% fish oil *cp/cp* group; [d]$p<0.05$ vs 10% fish oil *cp/cp* group.

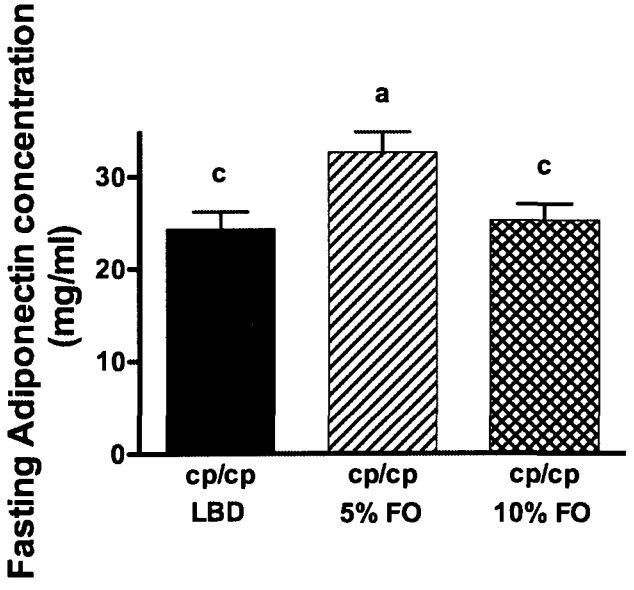


Figure 4-4. The fasting adiponectin concentration in 24 week old JCR:LA-*cp* rats. Values are mean±S.E.M. [a]$p<0.05$ vs LBD *cp/cp* control, [c]$p<0.05$ vs 5% fish oil (FO) *cp/cp* group.

Parameter	LBD +/?	LBD *cp/cp*	5% FO Diet *cp/cp*	10% FO Diet *cp/cp*
Food Consumptions (g)	19.9±1.2 [a]	32.5±2.7 [b]	31.9±1.8	21.5±3.1
Body Weight (g)	375.4±10.3 [a, c, d]	668.5±8.2 [b, d]	584.0±11.8 [a, b]	552.4±10.0 [a, b]
Fasting Glucose (mg/dL)	188.8±15.6 [a, d]	178.0±9.9 [b, c, d]	139.7±7.3 [b]	113.1±4.4 [a, b]
Fasting Insulin (mU/L)	21.8±2.1 [a]	450.1±38.9 [b, c, d]	66.1±7.6 [a]	78.2±12.7 [a]
Fasting Cholesterol (mg/dL)	87.1±2.7 [a, c, d]	158.3±6.4 [b, c]	124.9±4.4 [a, b]	152.3±3.2 [b]
Fasting Triglyceride (mg/dL)	42.7±2.5 [a, c, d]	156.3±22.4 [b, d]	119.1±10.3 [b]	99.4±9.5 [a, b]
HDL (mg/dL)	28.9±2.6 [a, c, d]	65.2±4.1 [b]	54.9±2.7 [b]	54.8±1.9 [b]
LDL (mg/mL)	26.8±4.4 [a, c, d]	46.0±1.6 [b]	43.1±3.0 [b]	41.4±2.2 [b]
Fasting ApoB48 (μg/mL)	12.7±1.7 [a]	57.1±7.5 [b, c, d]	20.1±3.6 [a]	29.3±2.6 [a]
Leptin (ng/mL)	2.2±0.3 [a, c, d]	120.3±9.1 [b, c, d]	90.7±5.2 [a, b]	69.8±5.1 [a, b]

Table 4-2. Fasting plasma concentration of the risk factors associated MetS. All the values are from 24 week old rats as mean±S.E.M, and compared to the lipid balanced obese diet (*cp/cp* control). [a]$p<0.05$ vs LBD obese (*cp/cp*) animals; [b]$p<0.05$ vs LBD +/? control animals; [c]$p<0.05$ vs 5% fish oil (FO) *cp/cp* group; [d]$p<0.05$ vs 10% fish oil *cp/cp* group.

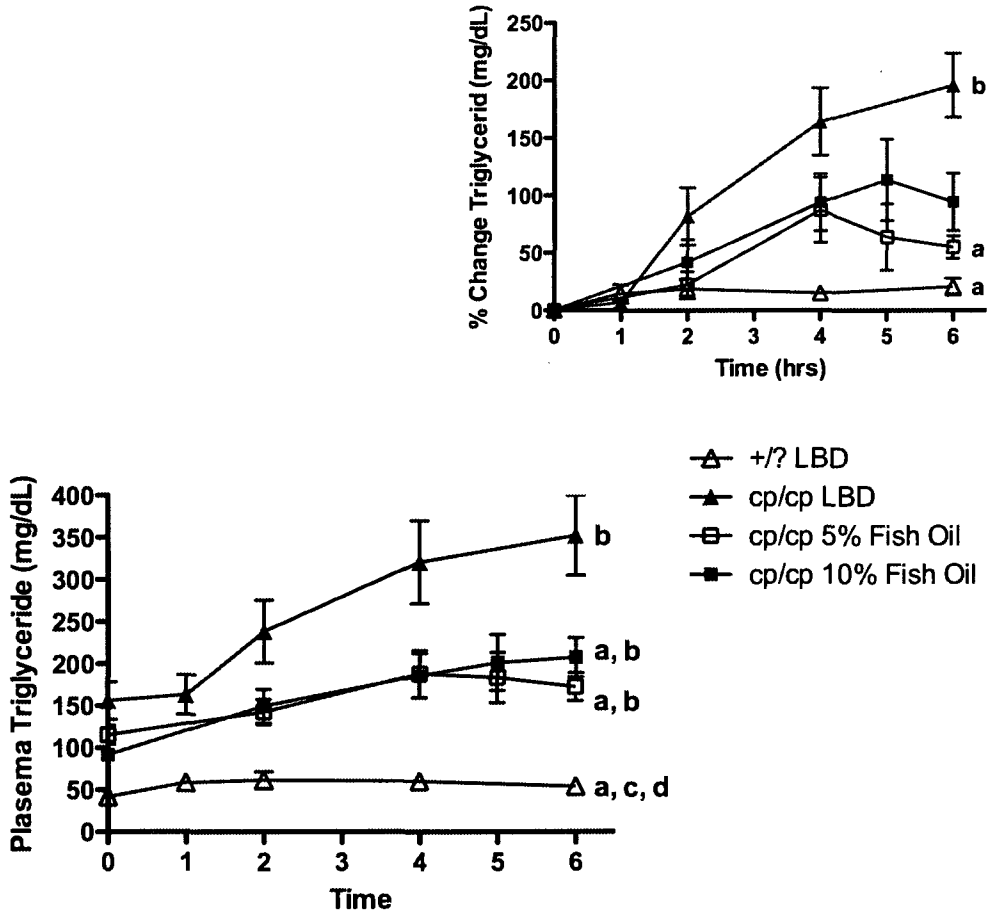


Figure 4-5. The postprandial response of plasma TG (AUC) and iAUC in four treatment groups with different quantity of fish oil following an oral fat challenge in the male JCR:LA-*cp* rat. The incremental area under the curve (iAUC) represents the change in TG from fasted concentrations (inset). The AUC for the *cp/cp* animals supplemented with 5% and 10% fish oil diet indicated a significant reduction in plasma TG levels as compared to the control group. 5% fish oil dietary intervention significantly decreased iAUC. $^{a}p<0.05$ vs LBD *cp/cp* control, $^{b}p<0.05$ vs LBD *cp/cp* control; $^{c}p<0.05$ vs 5% fish oil (FO) *cp/cp* group. $^{d}p<0.05$ vs 10% fish oil (FO) *cp/cp* group. There were no significant differences between 5% and 10% FO in postprandial response of plasma TG (AUC) and iAUC.

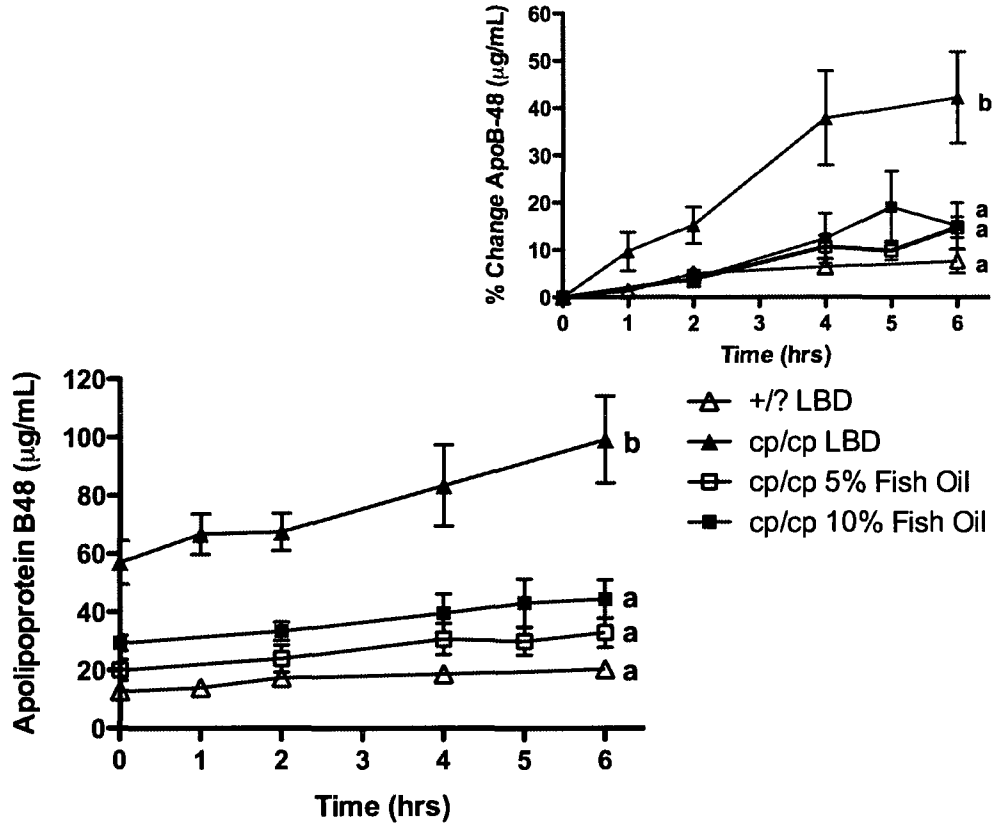


Figure 4-6. The postprandial response in plasma apolipoprotein B48 (AUC) and iAUC following an oral fat challenge in *cp/cp* (obese) and +/? (lean) controls assigned to treatment groups with varying quantities of fish oil in the diet. The change in apoB48 from fasted concentration is shown (inset) and represents the incremental area under the curve (iAUC). The 5% and 10% fish oil dietary interventions significantly decreased plasma apoB48 as compared the control (LBD) obese group. [a]$p<0.05$ vs LBD *cp/cp* control; [b]$p<0.05$ vs LBD +/? control. There were no significant differences between 5% and 10% FO in postprandial response of plasma apoB48 (AUC) and iAUC.

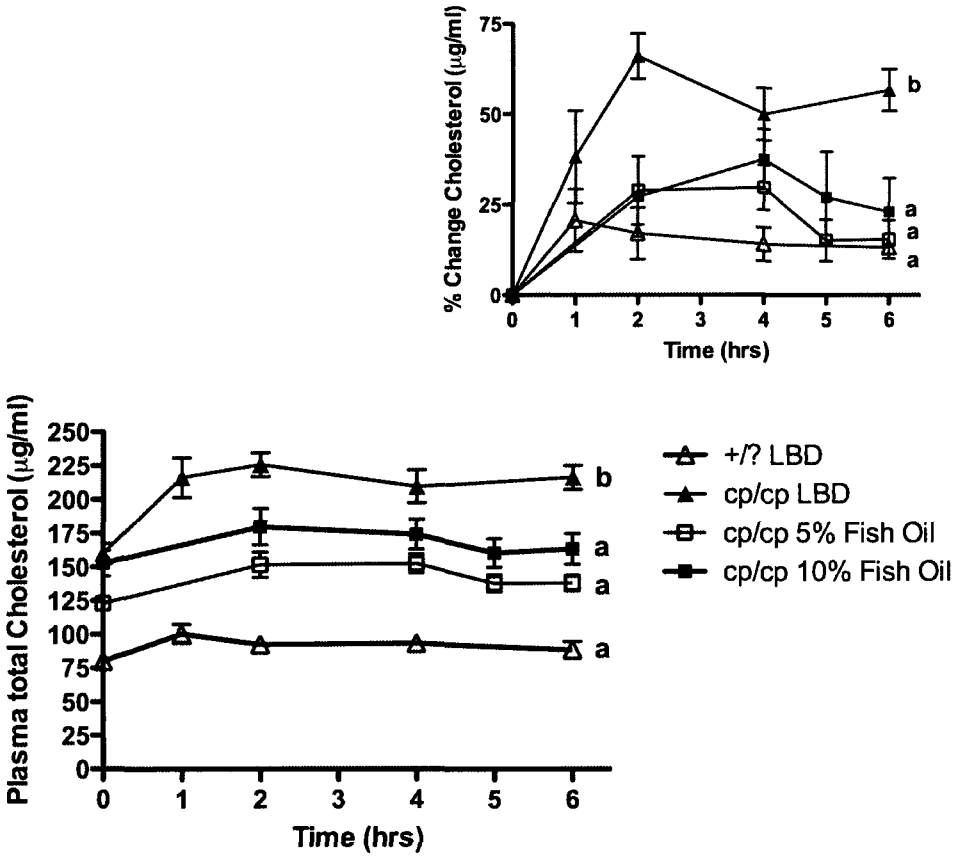


Figure 4-7. The postprandial response in plasma total cholesterol (AUC) and iAUC following an oral fat challenge in *cp/cp* (obese) and +/? (lean) controls assigned to treatment groups with varying quantities of fish oil in the diet. The change in total cholesterol from fasted concentration is shown (inset) and represents the incremental area under the curve (iAUC). The 5% and 10% fish oil dietary interventions significantly decreased plasma apoB48 as compared the control (LBD) obese group. $^{a}p<0.05$ vs LBD *cp/cp* control, $^{b}p<0.05$ vs LBD +/? control. There were no significant differences between 5% and 10% FO in postprandial response of plasma total cholesterol (AUC) and iAUC.

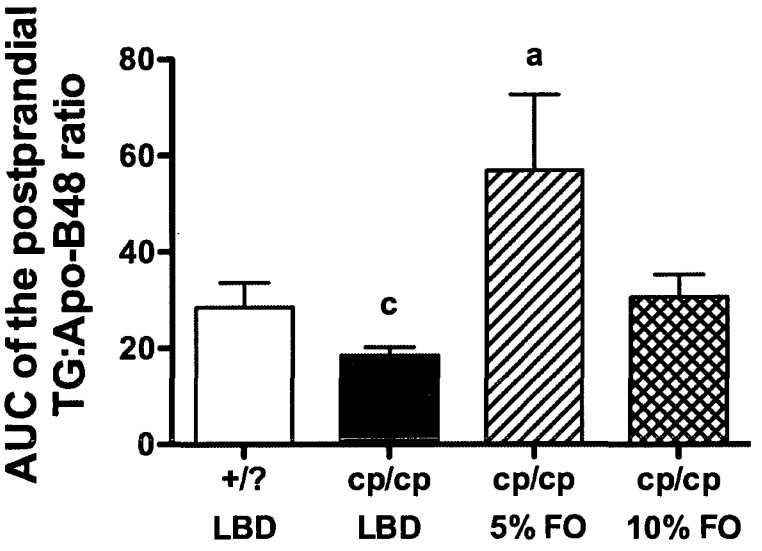


Figure 4-8. The AUC of postprandial response of TG:B48 ratio following an oral fat challenge in obese (*cp/cp*) and Lean (+/?) rats. $^{a}p<0.05$ vs LBD *cp/cp* control. $^{c}p<0.05$ vs 5% fish oil (FO) *cp/cp* group. There were no significant differences among other groups.

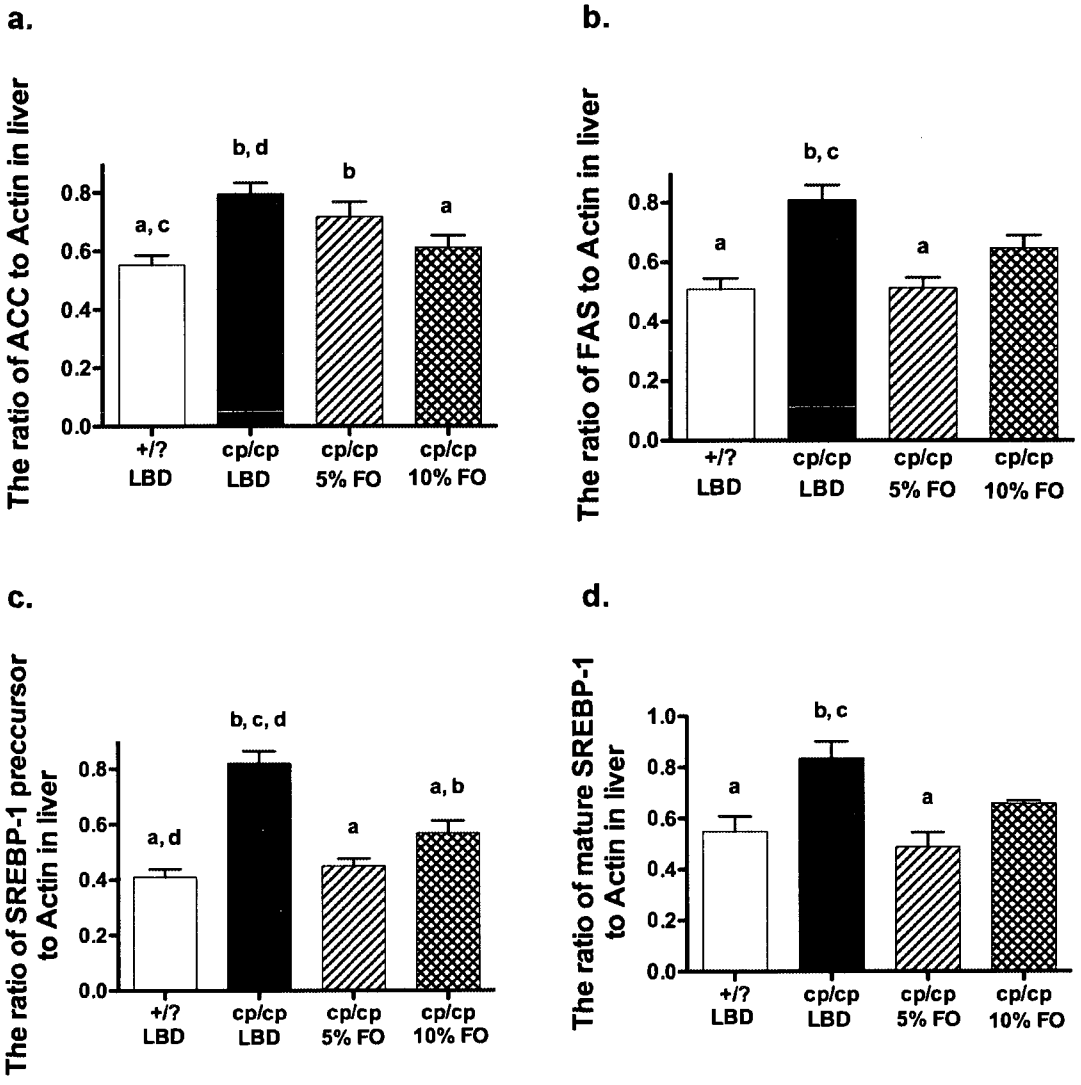


Fig 4-9. The lipogenic-related enzymes in liver. **a**: The ratio of mass of ACC to actin in liver; **b**: The ratio of FAS to actin in liver; **c**: The ratio of mass of SREBP-1 precursor to actin in the liver; **d**: The ratio of mass of mature SREBP-1 to actin in the liver. $^{a}p<0.05$ vs LBD *cp/cp* control animals; $^{b}p<0.05$ vs LBD +/? control animals; $^{c}p<0.05$ vs 5% fish oil (FO) *cp/cp* group; $^{d}p<0.05$ vs 10% fish oil (FO) *cp/cp* group.

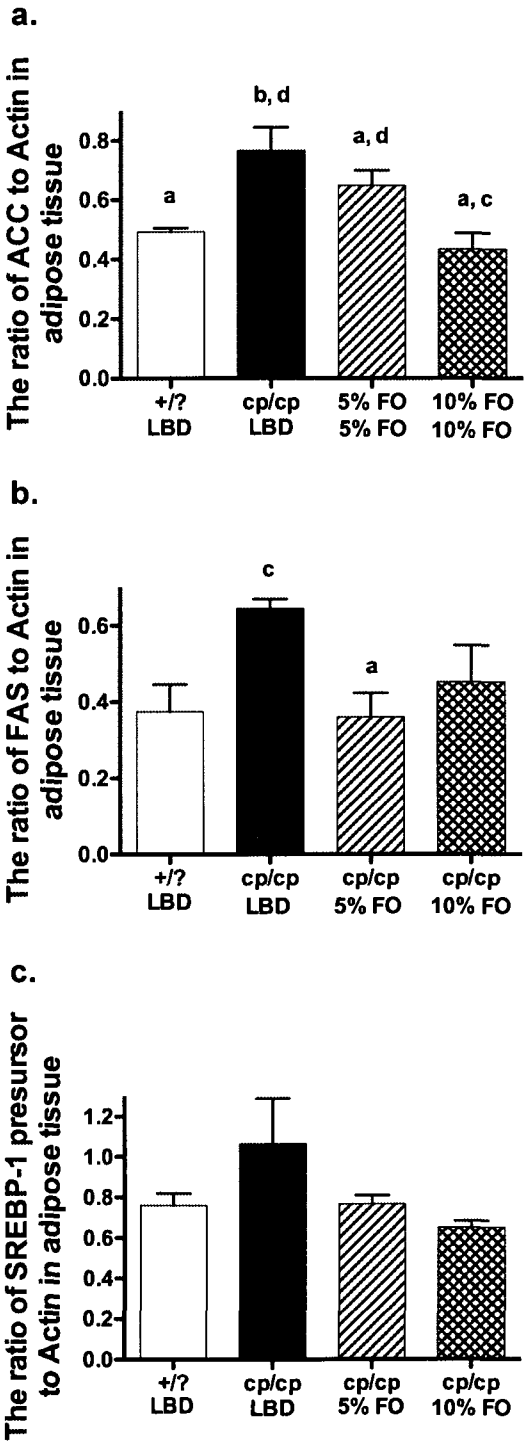


Figure 4-10. The lipogenic-related enzymes in adipose tissue. **a**: The ratio of mass of ACC to actin; **b**: The ratio of mass of FAS to actin; **c**: The ratio of mass of SREBP-1 precursor to actin. [a]$p<0.05$ vs LBD *cp/cp* control animals; [b]$p<0.05$ vs LBD +/? control animals; [c]$p<0.05$ vs 5% fish oil (FO) *cp/cp* group; [d]$p<0.05$ vs 10% fish oil (FO) *cp/cp* group.

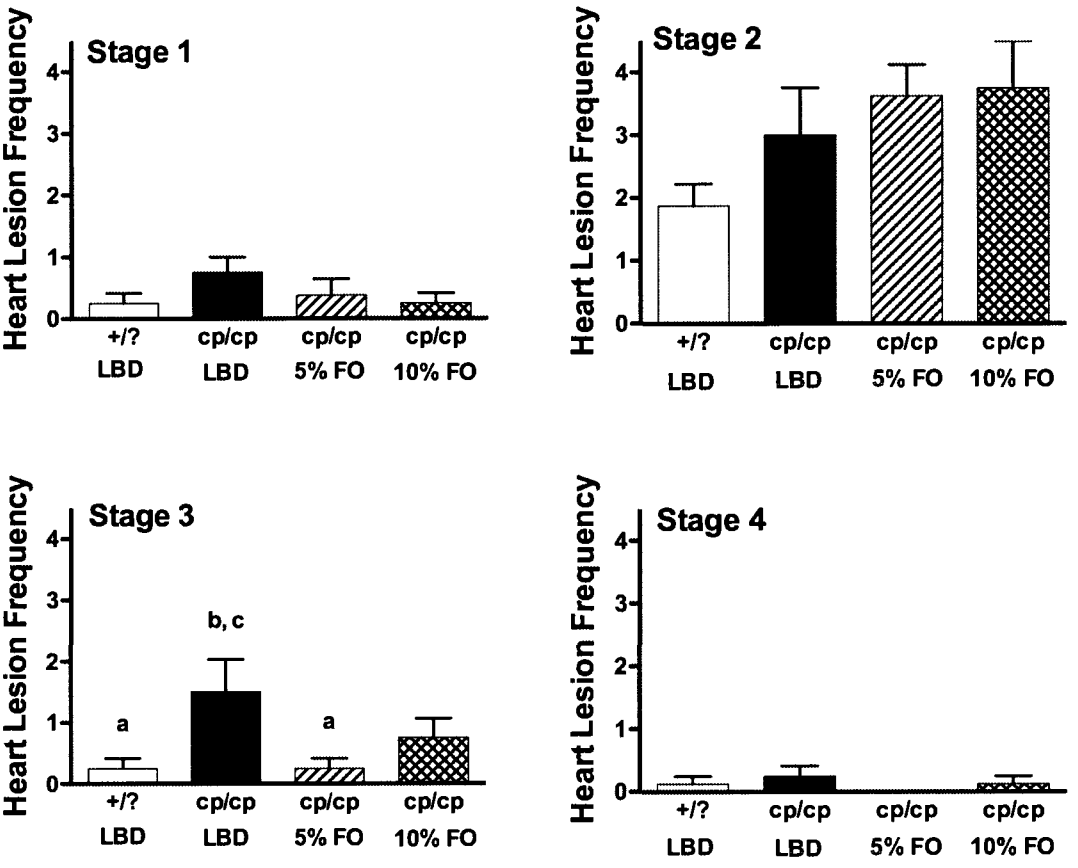


Figure 4-11. Frequency of myocardial lesions in 24 weeks old rats, as described in text. Values are mean±S.E.M, 8 rats per group. [a]$p < 0.05$ vs LBD *cp/cp* animals; [b]$p < 0.05$ vs LBD +/? control animals; [c]$p<0.05$ vs 5% fish oil (FO) *cp/cp* group. No Stage 4 lesions were detected in the hearts of 5% fish oil treated rats. There was no significant difference among LBD lean (+/?), 5% and 10% fish oil (FO) groups.

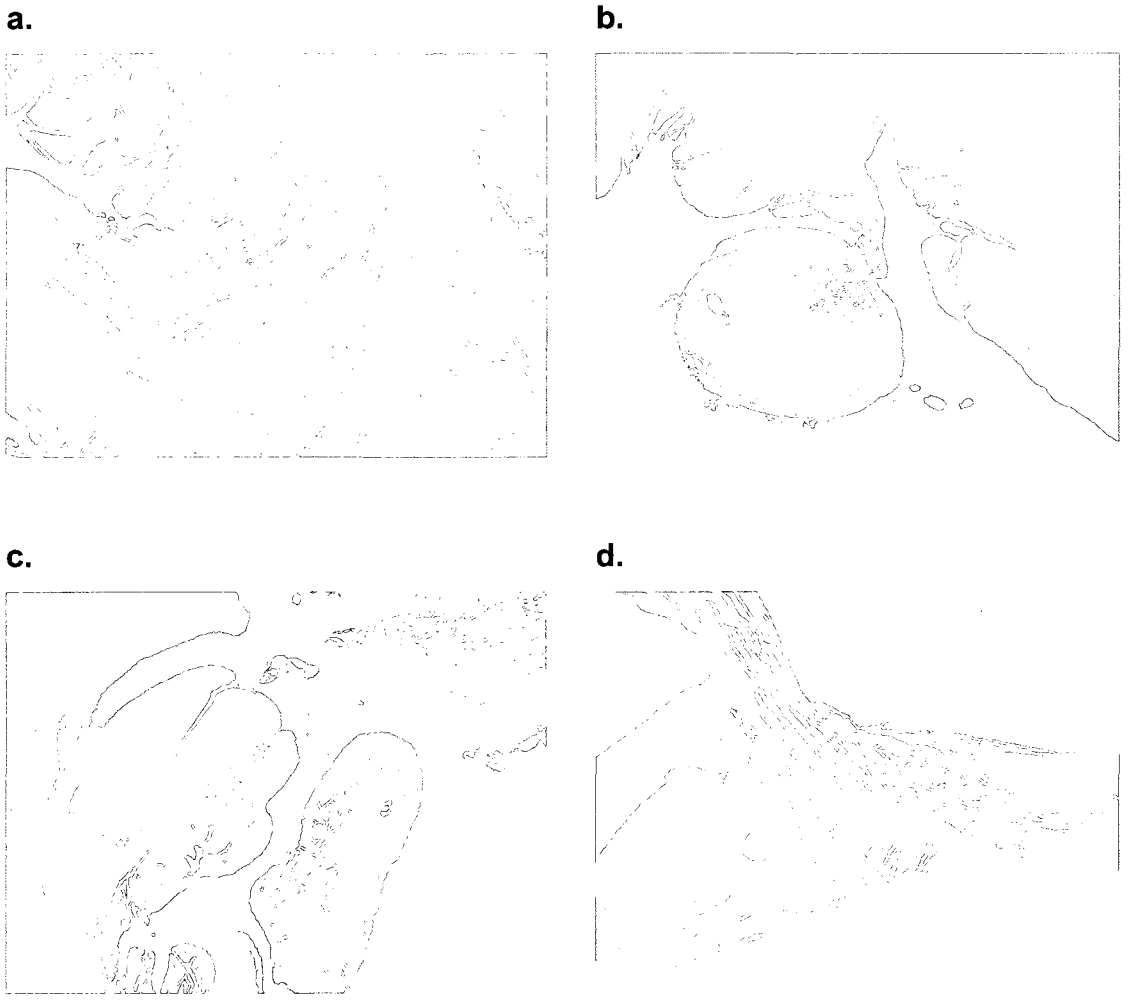


Figure 4-12. Representative micrographs of ischemic lesions of the heart of JCR:LA-*cp* rats (24 weeks of age). **a**: Stage 1 lesion, area of necrosis without long-term inflammatory cell infiltration, in left ventricle; **b**: Stage 2 lesion, area of long-term inflammatory cell infiltration, without visible cell lysis, in trabecular muscle; **c**: Stage 3 lesion, area of active inflammatory cell activity and cell lysis, in lower trabecular muscle; **d**: Stage 4, early scarred lesion with a small number of inflammatory cells or fibroblast, in upper perivalvular region of the heart. All images at X2, H & E stained sections.

4.4 Discussion

4.4.1 Effects of n-3 Polyunsaturated Fatty Acids on Glucose, Insulin Resistance and Adipokines

Several animal studies have reported that increased dietary n-3 PUFA can improve impaired glucose tolerance as well as insulin resistance (Simoncikova *et al.* 2002, Peyron-Caso *et al.* 2002). In this study, the obese JCR:LA-*cp* rat treated with fish oil significantly improved fasting plasma insulin and glucose levels (Table 4-1), which implies improved insulin sensitivity, consistent with earlier results (Russell *et al.* 1991). It has been reported that fish oil can reverse the impaired insulin stimulated glycogen storage and whole body insulin insensitivity, and return glucose to normal levels in animals (Lombardo *et al.* 2007).

Several adipose-derived cytokines (adipokines) including leptin and adiponectin have been suggested to decrease muscle lipid content and increase the rate of fatty acid oxidation (Dyck *et al.* 2006). Leptin is expressed in white adipose tissue, and functions to provide feedback to signal key regulatory centers in the hypothalamus to inhibit food intake and regulate both energy homeostasis and body weight (Klok *et al.* 2007). In addition, Schwartz *et al.* indicated that plasma leptin levels are higher in humans with a higher body mass index (BMI) and higher total body fat (Schwartz *et al.* 1996). In most cases of human obesity, there is development of central and/or peripheral resistance to leptin. Obese JCR:LA-*cp* rats have a defect of leptin receptor and leptin fails to bind to the corresponding receptors. Accordingly, leptin resistance and hyperleptinemia are characteristics of the JCR:LA-*cp* rat, that are similar to that observed in humans. Some other animal models of leptin resistance also observed hyperleptinemia in obese animals (Rahmouni *et al.* 2008, Correia *et al.* 2002).

Of relevance to this study, others have reported that n-3 PUFA feeding can reduce leptin gene expression (Ukropec *et al.* 2003). In humans and rats, the

concentration of leptin is associated with adipocyte size (Couillard et al 2000). Evidence shows that n-3 PUFA can decrease the abundance of fat tissue and reduce adipose cell size (Parrish *et al.* 1991 and Azain 2004). It has also been suggested that plasma leptin is reduced in rodents by dietary n-3 PUFA intake, and decreases in visceral adipose tissue weight are related to the reductions in circulating leptin (Higuchi *et al.* 2008). In this study, feeding 5% and 10% dietary n-3 PUFA significantly reduced plasma leptin levels, and reduced both inguinal fat pad weight and body weight gain.

Adiponectin is another adipokine that has anti-diabetic properties mediated by effects on muscle and liver fatty acid and glucose metabolism. Adiponectin concentration has been reported to be lower in both humans and rodents with obesity and type 2 diabetes including JCR:LA-*cp* rats (Drevon 2005). In this study, feeding 5% n-3 PUFA significantly improved plasma adiponectin concentration. One possible explanation for the action of n-3 PUFA on adiponectin secretion is via a direct effect on adipocytes, which influence the expression of adipokines like leptin and adiponectin directly by interaction with transcription factors, or indirectly via unknown mechanisms possibly linked to fatty acid oxidation, synthesis or storage (Côté *et al.* 2005, Lombardo *et al.* 2006). In addition, the impact of n-3 PUFA to improved glucose utilization and hypertriglyceridemia, may be a result of decreased fatty acid and/or TG storage in the adipocyte and in turn a lower fat pad weight (i.e., smaller fat cells).

4.4.2 Effects of n-3 Polyunsaturated Fatty Acids on Body Weight, Fat Deposition and Lipogenic-related Enzymes

Studies have reported that obesity can increase the risk of developing type 2 diabetes and CVD (Giorda *et al.* 2008). Dietary intervention including reduction of total dietary fat and types of dietary fat is an important topic in obesity and MetS research. However, longer term effects of modifying fat intake and types of dietary fat on development of obesity, dyslipidemia and insulin resistance are still controversial. Mori *et al.* 1999 indicated that combined effects of weight-loss

regimens and consumption of n-3 PUFA in daily meals of 63 overweight subjects was more effective on weight loss, serum lipids and glucose-insulin metabolism than either treatment alone (Mori *et al.* 1999). Further, Thorsdottir *et al.* also reported that in young, overweight men, the inclusion of either fish, or fish oil as part of an energy-restricted diet (1600 kcal/day) resulted in approximately 1 kg more weight loss after 4 weeks, than a similar diet without seafood or supplement of marine origin (Thorsdottir *et al.* 2007). In addition, increasing intake of n-3 PUFA could also be a useful adjunct to exercise programs for improving body composition and decreasing CVD risks (Hill *et al.* 2007). However, there is still limited evidence to show that feeding n-3 PUFA can decrease body weight or body weight gain. In this study, 5% and 10% dietary n-3 PUFA reduced the body weight gain in the absence of changes in food intake (Figure 4-1 and Table 4-1). Contrastingly, *cp/cp* rats fed a chow diet supplemented with 10% fish oil did not show reduced weight gain (Russell *et al.* 1991a).

The weight of inguinal (subcutaneous) fat pad depots was significantly lower in both 5% and 10% n-3 PUFA dietary groups. These results are consistent with clinical data that report changes in those with a diet rich in n-3 PUFA improved insulin resistance and decreased abdominal subcutaneous fat in subjects with type 2 diabetes (Summers *et al.* 2002). Indeed, other animal studies have shown that dietary supplementation of a fish oil-enriched lipid can have effects on fatty acid proportions and distribution in adipose including subcutaneous and visceral fat tissue (Bilby *et al.* 2006, Soriguer *et al.* 2003). Some studies suggest that n-3 PUFA are less readily deposited in adipose tissue and more readily oxidized, which would imply that n-3 PUFA could have at least partially protective effects against weight gain (Storlien *et al.* 1997, Du *et al.* 2004, Neschen *et al.* 2002, Feskens *et al.* 1994). The results of this study indicate that dietary n-3 PUFA not only decreased body weight gain, but also decreased the weight of subcutaneous fat tissue.

It has been reported that increased dietary n-3 PUFAs can influence hepatic SREBP-1 gene expression by accelerating the rate of SREBP-1 mRNA decay (Price *et al.* 2000). In this study, both precursor and mature SREBP-1 protein in liver were significantly decreased in obese rats treated with 5% n-3 PUFA. Feeding n-3 PUFA to obese rats downregulated lipogenic gene expression by reducing the hepatic precursor and mature SREBP-1. In the liver of obese rats, n-3 PUFA inhibited the release of mature SREBP-1 from endoplasmic reticulum, and also reduced the amount of precursor SREBP-1 found in the membrane. The hypolipidemic effect of n-3 PUFA could be two-fold: 1) n-3 PUFA may reduce the expression of SREBP-1, hence decreasing lipogenesis and cholesterol synthesis; 2) n-3 PUFA may increase the PPAR-α activation, hence increasing fatty acid β-oxidation (Davidson 2006, Kim *et al.* 1999). It has been shown in a sucrose fed rats of insulin resistance that dietary fish oil was able to reverse pre-existing metabolic and morphological changes of visceral fat pad tissue, reduce hypertrophy of adipocytes and make them smaller. The reduction of lipogenic genes, FAS and ACC by n-3 PUFA might be another reason for the decrease in body weight and inguinal fat pad weight. This study showed that unlike hepatic SREBP-1, SREBP-1 expression in adipose tissue is unaffected by dietary PUFA in the JCR:LA-cp rat, which is consistent with other rodent animal studies (Price *et al.* 2000, Mater *et al.* 1999).

4.4.3 Effects of n-3 Polyunsaturated Fatty Acids on Fasting and Postprandial Dyslipidemia

The hypertriglyceridemia and hypercholesterolemia in LBD obese (*cp/cp*) rats is thought to develop as a result of an increased rate of lipoprotein secretion and/or a decreased rate of lipoprotein removal from the circulation (increased SREBP mRNA). In 1990, Russell *et al.* conducted a study on the rate of lipid clearance and the apparent hepatic secretion rate, and suggested that the rate of secretion of TG and cholesterol was markedly higher in the *cp/cp* rats than in the lean rats (Vance *et al.* 1990a). In the present study, 5% and 10% n-3 PUFA fed rats both have lower fasting levels of TG, cholesterol and apoB48, which suggest that n-3

PUFA may increase lipid clearance or decrease the hepatic secretion rate. Studies of lipoprotein secretion in animals are complicated by the complex metabolism of lipoproteins in the circulation and the secretion of lipoproteins from both liver and intestine (Vance *et al.* 1990b). Previous studies showed that the rate of intestinal lipoprotein secretion was increased in *cp/cp* rats compared to lean rats (Vine *et al.* 2007). Hence, the effects of n-3 PUFA on lowering plasma TG, cholesterol and apoB48 may also be the result of inhibition of intestinal lipid uptake and secretion.

In contrast, HDL concentration of LBD obese rats was significantly higher than in LBD lean rats. The explanation for this difference is that obese JCR:LA-*cp* rats have a greater concentration HDL particles, due to increased catabolism of VLDL as there is no cholesterol ester transfer protein (CETP) in rodents (Russell *et al.* 1993). In addition, the possibility must be considered that increased intestinal lipoprotein secretion might contribute to the hyperlipidemia observed in obese rats (Vine *et al.* 2008). Other studies have shown that the rate of VLDL secretion in JCR:LA-*cp* rats is probably not determined by the rate of apoB synthesis, but rather by the availability of lipid from intestine and liver (Russell *et al.* 1993). The significant reduction of TG levels in fasting and postprandial state in n-3 PUFA treated rats suggested the reduction of VLDL secretion via the SREBP pathway.

Increasing evidence suggests that hypolipidemic effects of n-3 PUFA can decrease the utilization of the lipid fuel within the skeletal muscle and restore glucose oxidation to normalize the insulin sensitivity (Lombardo *et al.* 2007). The effects of n-3 PUFA on insulin sensitivity have been reported to be associate with a reduction of plasma free fatty acid (FFA), TG and glycerol levels and the lipid content in liver and skeletal muscle (Simoncíkova *et al.* 2002, Peyron-Caso *et al.* 2002). In this study, insulin concentration was significantly lower in 5% and 10% fish oil group, which were accompanied by reduced plasma glucose, TG, total cholesterol and apoB48. In addition, these changes were also accompanied by reduced fat tissue and lipogenic-related enzymes. Lipogenic-related enzymes,

especially ACC and FAS are key enzymes for TG synthesis (Qiu *et al.* 2008). Therefore, lower concentration of lipogenic-related enzyme in liver in n-3 PUFA groups suggested that n-3 PUFA may decrease TG synthesis via reducing lipogenic-related enzyme production in liver.

Postprandial dyslipidemia is a potential risk factor for both obesity and MetS, and a significant contributor to CVD. Some studies have demonstrated that n-3 PUFA may decrease plasma TG and hepatic production of TG rich particles, i.e. VLDL, the lipoprotein responsible for transporting TG for subsequent lipid decreasing by lipoprotein and liver lipases by peripheral tissue and the liver, respectively. In addition, n-3 PUFA might increase the clearance rate of lipoprotein (Taskinen *et al.* 2003, Balk *et al.* 2006, Harris *et al.* 1990, Park *et al.* 2003). This is likely due to both the suppression of the transcription of gene-encoding *lipogenic-related* enzyme such as ACC and FAS combined with the increased fatty acid oxidation (Manco *et al.* 2004). In this study, n-3 PUFA improved both fasting and postprandial response of total cholesterol, TG and apoB48.

AUC represents the change in the postprandial response that involves contribution from liver and intestine. Changes in iAUC values for TG, apoB48 and total cholesterol from fasting values provide an accurate representation for contributions from the intestine (Vine *et al.* 2007). In present study, n-3 PUFA supplementation significantly decreased the intestinal responses (iAUC) of TG, apoB48 and total cholesterol. Fatty acids taken up by intestinal cells are rapidly and efficiently esterified to triglyceride and cholesterol esters and within hours are secreted in CM particles. It was recently reported that CMs were over-produced in JCR:LA-*cp* model (Vine *et al.* 2008, Mangat *et al.* 2007). Feeding n-3 PUFA may reduce CMs' over-production, which cause a decrease of postprandial lipidemia. Field *et al.* suggested that the intestinal lipoprotein production is driven by luminal fatty acid flux not by an increase in endogenous production of fatty acids (Field *et al.* 2002). We suggest that the n-3 PUFA might

probably decrease the intestinal CMs production via a decrease in both luminal fatty acid flux and endogenous production.

4.4.4 Effects of n-3 Polyunsaturated Fatty Acids on Myocardial Lesions

Ischemic lesions that develop secondary to vascular damage or dysfunction are the one of the major symptomatic end stages of CVD (Baldassarre *et al.* 2006). The *cp/cp* rat develops such lesions spontaneously and these are related to the hyperinsulinemic status and probably also the hyperlipidemia (Russell *et al.* 1998d and 2002). The animals in this study were ended at age of 24 weeks. Hence, they had a low frequency of advanced scarred lesions (Stage 4) that accumulate with age. In contrast, they showed a large number of the early inflammatory lesions (Stage 2), which have been rare in previous studies using Lab Diet 5001 (Russell *et al.* 1991a), which is a standard chow diet. This increase is probably due to higher concentration of cholesterol and fat in the LBD diet. The lipid balanced diet was designed with higher proportion of lipid and cholesterol as to represent the macronutrient content of western diet. Compared to LBD *cp/cp* rats, rats supplemented with 5% fish oil showed reduced incidence of ischemic lesions with less active inflammatory response and cell lysis. However, this effect was confined to the rats supplemented at 5% n-3 PUFA, suggesting that in the presence of LBD diet, there is an optimum intake of n-3 PUFA in terms of cardiovascular protection. The complete absence of Stage 4 lesions in the hearts of the 5% n-3 PUFA group is consistent with the reduced Stage 3 lesion frequency. The lower incidence of myocardial lesion and amelioration of myocardial histological performance in 5% fish oil group provide a direct evidence that fish oil may inhibit the progress of CVD. The possible mechanisms of n-3 PUFA regarding improvement of ischemic myocardial lesions are associated with: (i) the hypolipidemic effects of n-3 PUFA; (ii) control of hyperglycemia and hyperinsulinemia; As a result, the improvement of hyperlipidemia, hyperglycemia and hyperinsulinemia resulted in the lower risks of atherosclerosis and ischemia myocardial lesions.

4.4.5 Optimal Dose of n-3 Polyunsaturated Fatty Acids in Humans

At present, the amount of dietary n-3 PUFA required to decrease TG output and increase liver and skeletal muscle oxidation in humans is not well established. As little as 1 g/d of fish oil has been shown chronically to reduce plasma TG levels in humans (Grekas *et al.* 2001). The expression of genes encoding enzymes involved in hepatic fatty acid oxidation and synthesis was modulated by feeding 12-15% of its energy as fish oil (Jump 2002). Nakatani *et al.* indicated that in higher dose of 40% of total energy from fish oil decreased the expression of SREBP-1 protein (Nakatani *et al.* 2003). The results of these studies suggest that patients with type 2 diabetes or CVD may be advised to consume 1 g/d n-3 PUFA from fish or supplements. Two to four grams per day of n-3 PUFA supplements has been recommended for patients with hypertriglyceridemia (Nettleton *et al.* 2005). The Dietary Guidelines Advisory Committee (DGAC) Report 2005 recommended that the weekly consumption of 2 servings (approximately 8 oz total) of fish high in EPA and DHA could have potential cardioprotective effects (Psota *et al.* 2006). The results from our present study demonstrate that the optimal dose of n-3 PUFA to improve dyslipidemia, body weight, insulin resistance and decrease the expression of lipogenic-related enzymes in the JCR:LA-*cp* rats is between 5% and 10% fish oil.

In addition, the differences of species are still a concern in order to fully appreciate the findings from rodent studies and translate into humans. In this study, after calculation, the amount of n-3 PUFA from 5% fish oil diet was approximately 5 g/day for a 2500 calories human diet/day. 10% n-3 PUFA was approximately 10 g n-3 PUFA/day for a 2500 kcal diet (Figure 4-13). Ten grams of fish oil per day exceeds what is achievable in free-living humans. In 2007, Fritsche indicated that rodents and humans have different cell and tissue response to n-3 PUFA. According to his suggestion, in order to enhance the preclinical value of our study on n-3 PUFA relative to understanding how much n-3 PUFA supplementation may improve human health, it is important to

considerately convert amount of dietary n-3 PUFA from animal studies into human studies (Fritsche *et al.* 2007).

For a 2, 500 kcal/d human diet:
1) In LBD diet, 33% energy yield from lipid, so: 2, 500 kcal × 33% = 825 kcal (from lipid) 2) Since 1 g of lipid produce 9 kcal energy, so: $\frac{825 \text{ kcal}}{9 \text{ kcal/g}} = 92 \text{ g}$ 3) Since 5% fish oil is 5% of the total lipid in the diet, and 10% fish oil is 10% of the total lipid in the diet, so: 5% fish oil: 92 g × 5% = 5 g 10% fish oil: 92 g × 5% = 10 g

Table 4-3. Sample calculations of converting 5% and 10% fish oil of rodent diet to human diet.

4.5 Conclusion

In this study, we have provided strong evidence that long-term n-3 PUFA supplementation has beneficial effects on fasting and postprandial lipid and lipoprotein metabolism, insulin resistance and weight reduction in the JCR:LA-*cp* rodent. A possible mechanism of action for hypolipidemic effects is through the inhibition of hepatic expression of SREBP-1 and further a decrease in the expression of lipogenic-related enzymes, FAS and ACC in the liver and white adipose tissue. The LBD diet appears to have exacerbated vascular dysfunction of the *cp/cp* rat and enabled us to identify a cardioprotective effect of the fish oil. There is an indication that the optimal dose of fish oil for ameliorating dyslipidemia and/or hyperinsulinemia, probably lies between 5% to 10% fish oil supplementation for rodents, but further animal and clinical studies are needed to formulate the ideal dose for humans.

Chapter 5: Long-term Effects of Dietary n-3 Polyunsaturated Fatty Acids Supplementation on Glomerulosclerosis and Renal Prostanoid production in the JCR:LA-*cp* Rat, a Model of the Metabolic Syndrome

5.1 Introduction

The long-term sequelae of the metabolic syndrome (MetS) include clinical presentation of both macro-vascular and micro-vascular diseases, of which kidney damage and glomerulosclerosis are of particular interest (Russell *et al.* 2007). In recent years, interest in glomerulosclerosis as a micro-vascular complication of obesity and metabolic syndrome has increased (Chen *et al.* 2004). Mechanisms responsible for initiation and progression of diabetic kidney dysfunction and associated micro-vascular etiology are not completely understood. However, among the contributing factors proposed are alterations in lipid metabolism (i.e. lipotoxicity), prostanoid production (including prostaglandin and thromboxane production), and activity of cyclooxygenase (COX) (Schambelan *et al.* 1995, Sinha *et al.* 1990, Poole *et al.* 2007).

Prostanoids are a subclass of eicosanoids consisting of prostaglandins (PG) and thromboxane (TX) (De Caterina *et al.* 2007). COX enzymes form prostaglandin H_2 (PGH_2) from arachidonic acid (AA), which is released from renal membrane phospholipids. PGH_2 is then isomerized and converted to PGD_2, PGE_2, $PGF_{2\alpha}$ PGI_2 and thromboxane A_2 (TXA_2) (Figure 1-6) (Hao *et al.* 2008). Prostanoids that are relatively rich in the kidney such as PGE_2 and TXB_2 play different roles in regulating glomerular filtration rate (GFR), water and salt homeostasis, as well as in inflammatory and fibrotic processes in response to kidney damage (Klahr 1989, Remuzzi *et al.* 1997, Warford-Woolgar *et al.* 2006). Some studies have already shown that in the diabetic kidney with glomerulosclerosis, an increase in prostanoid production is associated with development and aggravation of the disease such as inflammation (Schambelan *et al.* 1983). Accordingly, inhibition of

prostanoid formation such as inhibition of COX activity has been proposed as a means of reducing renal damage via improving associated pro-inflammatory response (Griswold *et al.* 1996). However, at present, the relative contribution of COX isoform (COX-1 and COX-2) activity for renal prostanoid production is unclear. Interestingly, Warford-Woolgar *et al.* 2006 showed that the majority of total COX activity is due to the COX-2 isoform and that its activity is increased in diseased kidneys (Warford-Woolgar *et al.* 2006). COX-2 appears to play a key role in pathophysiological processes such as inflammation and glomerulosclerosis. Therefore, measurement of expression and activities of COX-2 are important for assessing renal pathophysiology.

The etiology of glomerulosclerosis is complex and multi-factorial. Some studies have demonstrated that dietary fatty acids composition and lipid metabolism and may be key factors in glomerulosclerosis development (Wang *et al.* 2005). Consistent with this hypothesis, other studies have found that supplementation of fish oil to rats with immune complex nephritis resulted in a beneficial effect (Donadio *et al.* 2004). For example, n-3 PUFA may serve as substrates for cyclooxygenase and lipoxygenase pathways to provide less potent inflammatory mediators than those produced via the n-6 PUFA substrate, arachidonic acid. Moreover, n-3 PUFA also suppress inflammatory responses through eicosanoid-independent mechanisms. In addition, potential benefits of n-3 PUFA relevant to renal disease progression could also be those involved in preventing the development of CVD by reducing serum lipid levels and decreasing insulin resistance (Wang *et al.* 2005). Alterations in lipid metabolism with n-3 PUFA, such as improving dyslipidemia, may represent a common mediating pathway of glomerular and interstitial susceptibility to progressive sclerosis in the kidney. In a complementary study, enrichment of the diet with n-3 PUFA given to rats with reduced kidney mass has been shown to lead to a reduction in renal damage (Aguila *et al.* 2005). Aguila *et al.* suggested that these n-3 effects may be modulated by corresponding changes in renal fatty acid composition (Aguila *et al.* 2005). Essential fatty acid deficiency may inhibit prostanoid production including

both prostaglandin E and thromboxane production, and potentially suppressing the protective and injurious components of arachidonate oxidation (Horrocks *et al.* 2004). Furthermore, there are some studies that suggest n-3 PUFA may have a role in eicosanoid metabolism, independent of COX activity that appears to be protective to renal structure and function, however, the mechanism is still not clear. The process of glomerulosclerosis development appears to be amenable to manipulation by dietary modulation of fatty acid metabolism. The reduction of TXB_2 and proteinuria, were associated with significant reduction of total cholesterol after feeding n-3 PUFA (De Caterina R *et al.* 1993).

Studies have demonstrated that fish oil, especially eicosapentaenoic acid (EPA, 20:5,n-3) and docosahexaenoic acid (DHA, 22:6,n-3) supplementation can attenuate blood pressure, glomerular enlargement and/or glomeruli loss (Koop *et al.* 2008, Lu *et al.* 2003). Sinha *et al.* reported that a fish oil enriched diet may not only improve proteinuria and hyperlipidemia of diabetes mellitus in rats with streptozotocin-induced DM (STZ induced insulin-dependent diabetes mellitus), but also decrease prostanoid production in kidney (Sinha *et al.* 1990). Unfortunately, most of the current studies that have explored the effects of fish oil on diabetic glomerulosclerosis were either of a relatively short-term period (i.e. less than 3 weeks), or did not examine an association between vascular complications in the kidney and prostanoid production per se (Sinha *et al.* 1990, Weise *et al.* 1993). Diabetic nephropathy is a chronic disease, thus an effect on regression may take longer to demonstrate. Therefore, long-term n-3 PUFA studies are required to fully observe n-3 PUFA on glomerulosclerosis progression.

Animal models of diabetic nephropathy are commonly drug-induced, such as using alloxan and streptozocin. Although, these animal models are commonly used, compared to the pathological characteristics of diabetic glomerulosclerosis in humans, interpretation can remain limited. For example, in humans, diabetic glomerulosclerosis is characterized by the development of microalbuminuria, which progresses to macroalbuminuria and an ensuing decline in renal function

(i.e. decreased glomerular filtration rate). Although, these clinical features are also seen in rodent models of streptozocin (STZ)-induced diabetic nephropathy, the level of albuminuria and the loss of renal function are less severe than in humans. STZ is extremely toxic for many organs such as the liver, and thus may not necessarily induce accurate micro-vascular damage. In addition, the major histological presentation of human diabetic nephropathy is thickened and scletoric glomerular basement membrane with or without development of nodular mesangial sclerosis (i.e. Kimmelstiel–Wilson nodules), tubulointerstitial fibrosis and arteriolar hyalinosis (Figure 1-5). While some of these histological attributes have been detected in rodent models of STZ-induced diabetic nephropathy, their severity is usually milder and have more prominent Kimmelstiel–Wilson nodules, further suggesting subtle differences in initiating etiology that may be important for clinical interpretation (Alsaad *et al.* 2007, Wada *et al.* 2001).

Recently, the obese JCR:LA-*cp* rodent has been established for the study of both macro- and micro-vascular complications of MetS, including glomerulosclerosis (Proctor *et al.* 2007). The JCR:LA-*cp* rat is a strain identified by the corpulent (*cp*) phenotype that develops marked hyperinsulinemia and obesity due to a defect in the leptin receptor gene (ObR). The JCR:LA-*cp* rat model displays characteristics of MetS including complications of end stage kidney disease such as rampant glomerulosclerosis with severe proteinuria and a decline of kidney function, consistent with human pathological complications. Data in chapter 4, demonstrated that long-term fish oil supplementation reduced postprandial dyslipidemia, hyperinsulinemia and obesity using this animal model. Consequently for this chapter, the objective of this study was to assess the potential for increased intake of fish oil to improve facets of micro-vascular disease in the JCR:LA-*cp* rat, in the form of kidney pathophysiology and corresponding renal prostanoid profile.

The specific objectives of these series of studies using the JCR:LA-*cp* model were to:

1) Determine the long-term effects of n-3 PUFA feeding on albuminuria and renal fat deposition;

2) Assess the long-term effects of n-3 PUFA feeding on corresponding glomerulosclerosis histo-pathology and associated risk markers of inflammation;

3) Delineate whether renal prostanoid production profile could be improved by n-3 PUFA enriched dietary treatment.

5.2 Method

5.2.1 Animals and Treatments

The animal model, experimental design and dietary treatments have been detailed in chapter 3.2 and 3.4.

5.2.2 Urine Samples

Urine albumin and creatinine concentrations were measured using immuno-turbidimetric and Jaffé methods, respectively, via University of Alberta Hospital (Carfray *et al.* 2000. Proctor *et al.* 2007).

5.2.3 Renal Histology

Kidneys from all animals were excised and fixed in formalin. After conventional processing, sections of kidney were examined to quantify glomerular sclerosis by the method of Schäfer (Heidbreder *et al.* 1986). Briefly, each kidney was divided along the long axis, fixed, sectioned and stained with H&E (hematoxylin and eosin stain) (Proctor *et al.* 2007). Ten random fields of view of each kidney were recorded digitally using a X4 objective. Eight complete glomeruli in each field of

view were blindly scored as sclerotic (mild to severe glomerular sclerosis) or normal (minimal sclerosis or normal). The fraction of glomeruli that were sclerotic was calculated for each kidney and data represented as the average incidence of sclerotic glomeruli in each kidney.

5.2.4 Prostanoid Production and Cyclooxygenase Activity

The results presented in chapter 4 describe that 5% fish oil supplementation in the JCR:LA-*cp* model represents a more efficacious dose to ameliorate dyslipidemia and/or hyperinsulinemia. In contrast, data seemed to suggest a deterioration of the beneficial effect in the JCR:LA-*cp* model with a 10% fish oil dose, particularly for the frequency of myocardial and renal lesions (see Figure 4-11, 4.12, 5.3, 5.4). On this basis, in the subsequent series of COX analysis we chose to focus on the activities of tissue from animals receiving either 5% fish oil and control (LBD) diets only.

Forty-five milligrams of lyophilized tissue from the right kidney were homogenized in 1.5 ml of freshly prepared Tyrodes buffer on ice (Warfor-Woolgar *et al.* 2006). After homogenizing each sample on ice for 30 second, Triton X-100 was added and mixed to achieve a final concentration of 0.01%. The homogenate was place on ice for a further 10 minutes with vortexing for 10 second. Endogenous prostanoid levels and COX activity was determined under the following conditions: 1) 0 min is for determination of endogenous levels of prostanoids; 2) 10 min at 37 ℃ for determination of prostanoid production by potential total COX activity (as per reference Warford-Woolgar *et al.* 2006, Bradford *et al.* 1976, Brater *et al.* 2001). At the end of the incubation period, reactions were quenched by adding 800 µl of fresh ice-cold acetylsalicylic acid (5 mmol/l). All samples were vortexed and centrifuged further at 1,1400 rpm at 4℃ for 5 minutes and the supernatant collected and stored at -80℃. Prostanoid production was measured using gas chromatography-mass spectrometry (GC/MS) of PGE_2, $PGF_{2\alpha}$, PGD_2 and the stable metabolites of TXA_2 (TXB_2) and PGI_2 (6-keto-$PGF_{1\alpha}$) were measured to evaluate the production of TXA_2 and PGI_2 (DuBois *et al.* 1994).

The following formula was used to determine relative COX activity:

$$\text{Total COX activity} = \frac{\text{ng PN (10 min)} - \text{ng PN (0 min)}}{\text{10 min} \times \text{0.045 g kidney}} = \text{ng PN/min•g kidney}$$

PN was prostanoid production including PGD_2, PGE_2, 6-keto $PGF_{1\alpha}$, $PGF_{2\alpha}$ and TXB_2 (Warford-Woolgar *et al.* 2006). This formula is adapted as "ng PN/min•g kidney" from "ng PN/min•μg total protein", since there is no significant difference in pre-lyophilization and post-lyophilization kidney weights between LBD obese animals and 5% fish oil group (Table 5-1).

5.2.5 Statistical analysis

Data were tested for normal distribution and differences between *cp/cp* LBD group and +/? group and n-3 PUFA treatment groups were analyzed using unpaired t-test and one-way ANVOA with significance set at $p<0.05$ (Sigma Stat, Jandel Scientific, San Rafael, CA, USA and GraphPAd PRISM). All results are shown as the mean±S.E.M.

5.3 Results

5.3.1 Kidney Weight and Peri-renal Fat Pad

Relative to LBD obese (*cp/cp*) rats, lean (+/?) LBD rats had significantly lower kidney weight both pre- and post-lyophilization. Similarly, there was no significant difference in the kidney weight (either before or after lyophilization) from either 5% or 10% fish oil treated *cp/cp* rats (Table 5-1) when compared to control *cp/cp* LBD rats. However, relative to control LBD *cp/cp* rats, both 5% and 10% fish oil treated rats had significantly lower peri-renal fat pad weight ($p<0.001$) (Figure 5-1a) and ratio of peri-renal fat pad weight to body weight ($p<0.001$) (Figure 5-1b). All obese animals had a significant higher peri-renal fat pad weight and the ratio of peri-renal fat pad weight to body weight than LBD lean animals ($p<0.05$).

Obese rats treated with 10% fish oil had a significant lower peri-renal fat pad weight and the ratio of peri-renal fat pad weight to body weight than obese animals treated with 5% fish oil ($p<0.05$).

Parameter	LBD +/?	LBD *cp/cp*	5% FO Diet *cp/cp*	10% FO Diet *cp/cp*
Kidney Weight pre-lyophilization (g)	1.26 ± 0.07 [a]	1.60 ± 0.07 [b]	1.41 ± 0.05	1.49 ± 0.07
Kidney Weight post-lyophilization (g)	0.35 ± 0.02 [a, c]	0.45 ± 0.02 [b]	0.43 ± 0.02 [b]	0.42 ± 0.02

Table 5-1. Kidney weights pre- and post-lyophilization. All the values are from 24 week old rats expressed as mean±S.E.M, and compared to the lipid balanced obese diet (*cp/cp* control). [a]$p<0.05$ vs LBD *cp/cp* control; [b]$p<0.05$ vs LBD +/? control; [c]$p < 0.05$ vs 5% fish oil (FO) *cp/cp* group.

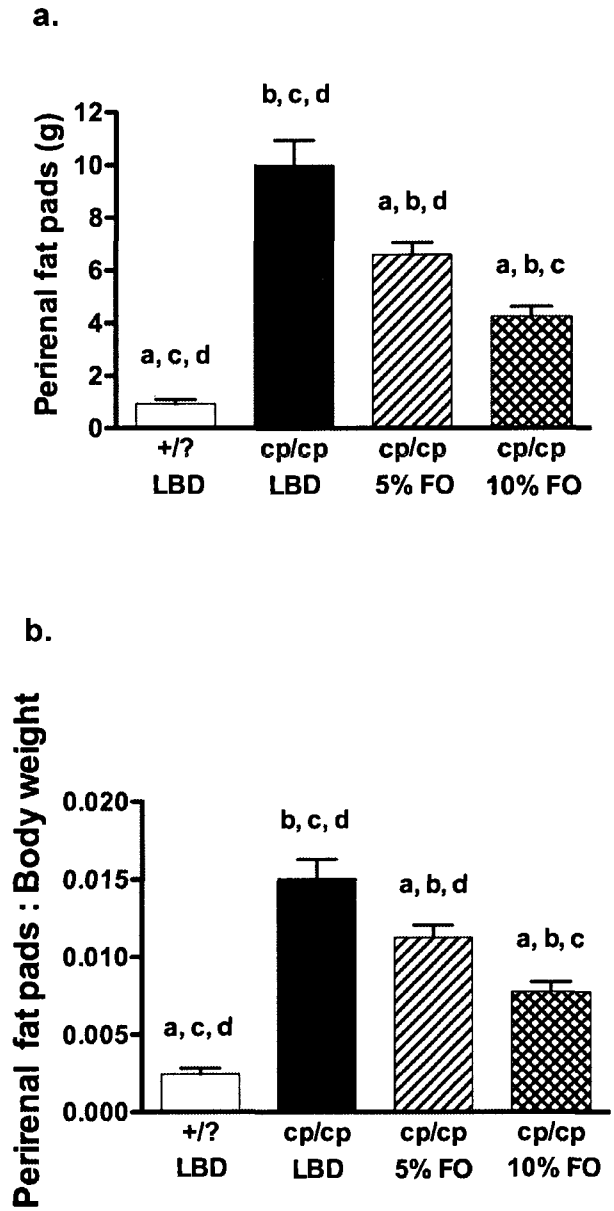


Figure 5-1. Peri-renal fat pad. **a**: The weights of peri-renal fat pad. **b**: The ratio of peri-renal fat pad weight to body weight. Values are expressed as mean±S.E.M. Eight rats were in each group. [a]$p<0.05$ vs LBD *cp/cp* control; [b]$p<0.05$ vs LBD +/? control; [c]$p<0.05$ vs 5% fish oil (FO) *cp/cp* group; [d]$p<0.05$ vs 10% fish oil (FO) *cp/cp* group.

5.3.2 Urinary Biochemical Profile

Urinary albumin concentrations were observed to be higher in *cp/cp* LBD animals compared to +/? lean animals (Figure 5-2a), consistent with previously published works by our group (Proctor *et al.* 2007). After 16 weeks of feeding, supplementation of either the 5% or the 10% fish oil diets resulted in a significant reduction in the urinary albumin levels compared to *cp/cp* LBD rats ($p<0.001$) (Figure 5-2a). Animals treated with 5% fish oil, not 10% fish oil had a significant higher urinary albumin concentration than LBD +/? (lean) controls ($p<0.001$).

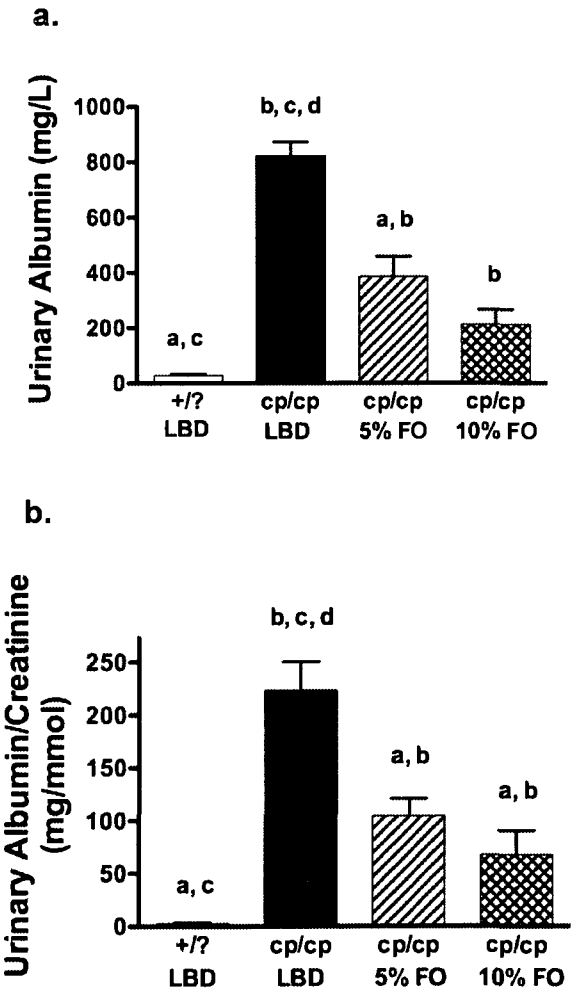


Figure 5-2. Urinary albumin and creatinine. **a**: Urinary albumin concentration; **b**: The ratio of urinary albumin to creatinine. Values are expressed as mean±S.E.M. Eight rats were in each group. [a]$p<0.05$ vs LBD *cp/cp* control; [b]$p<0.05$ vs LBD +/? control; [c]$p<0.05$ vs 5% fish oil (FO) *cp/cp* group; [d]$p<0.05$ vs 10% fish oil (FO) *cp/cp* group.

Urinary albumin concentration was not significantly different between animals fed with 5% and 10% fish oil. In contrast, there was a significant reduction in the ratio of urinary albumin to creatinine in 5% and 10% fish oil ($p<0.001$) (Figure 5-2b). All obese animals had significantly higher urinary albumin concentration than LBD +/? animals ($p<0.001$) (Figure 5-2b).

5.3.3 Glomerulosclerosis

The obese *cp/cp* rat exhibited a substantial increase in the frequency of glomerulosclerosis compared with +/? rats ($p<0.001$) (Figure 5-3 and 5-4) and is consistent with previous studies using this rat model (Proctor *et al.* 2007). Further, obese (*cp/cp*) LBD rats exhibited a greater severity of glomerulosclerosis and interstitial inflammation than the +/? rat (Figure 5-4a and 5-4b). JCR:LA-*cp* rats treated with 5% fish oil had a significant reduction of the fraction of sclerotic glomeruli (43% of reduction in 5% fish oil, $p<0.001$), relative to *cp/cp* LBD rats (see Figure 5-3 and 5-4). Animals treated with 5% fish oil had a significant lower fraction of sclerotic glomeruli than animals treated with 10% fish oil ($p<0.05$) (Figure 5-3).

In addition, we also observed that a 25% reduction of incidence of glomerulosclerosis in kidneys from animals supplemented with 10% fish oil compared to kidneys from the *cp/cp* LBD group. However, intriguingly and in contrast, we observed sporadic tubular damage in kidneys from the 10% fish oil group (Figure 5-4d).

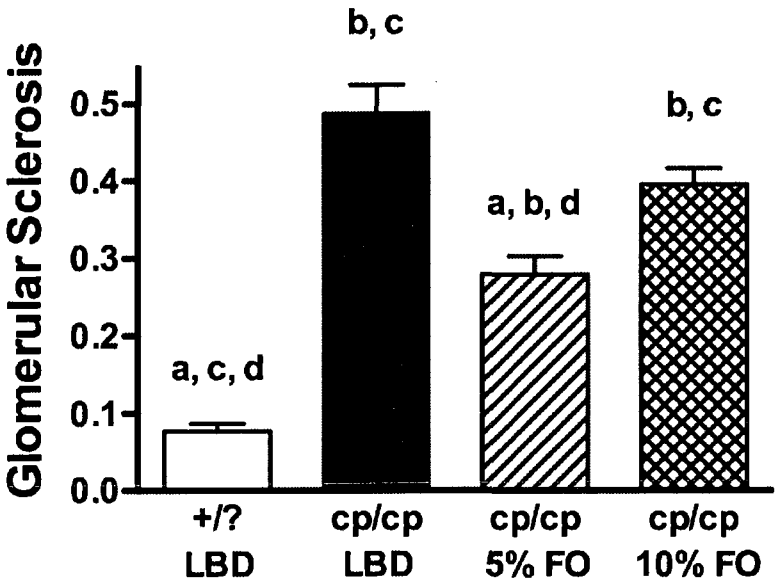


Figure 5-3. Proportion of sclerotic glomeruli in 24 weeks old JCR:LA-*cp* rats. Values are expressed as mean±S.E.M. Eight rats were in each group. [a]$p<0.05$ vs LBD *cp/cp* control; [b]$p<0.05$ vs LBD +/? control; [c]$p<0.05$ vs 5% fish oil (FO) *cp/cp* group; [d]$p<0.05$ vs 10% fish oil (FO) *cp/cp* group.

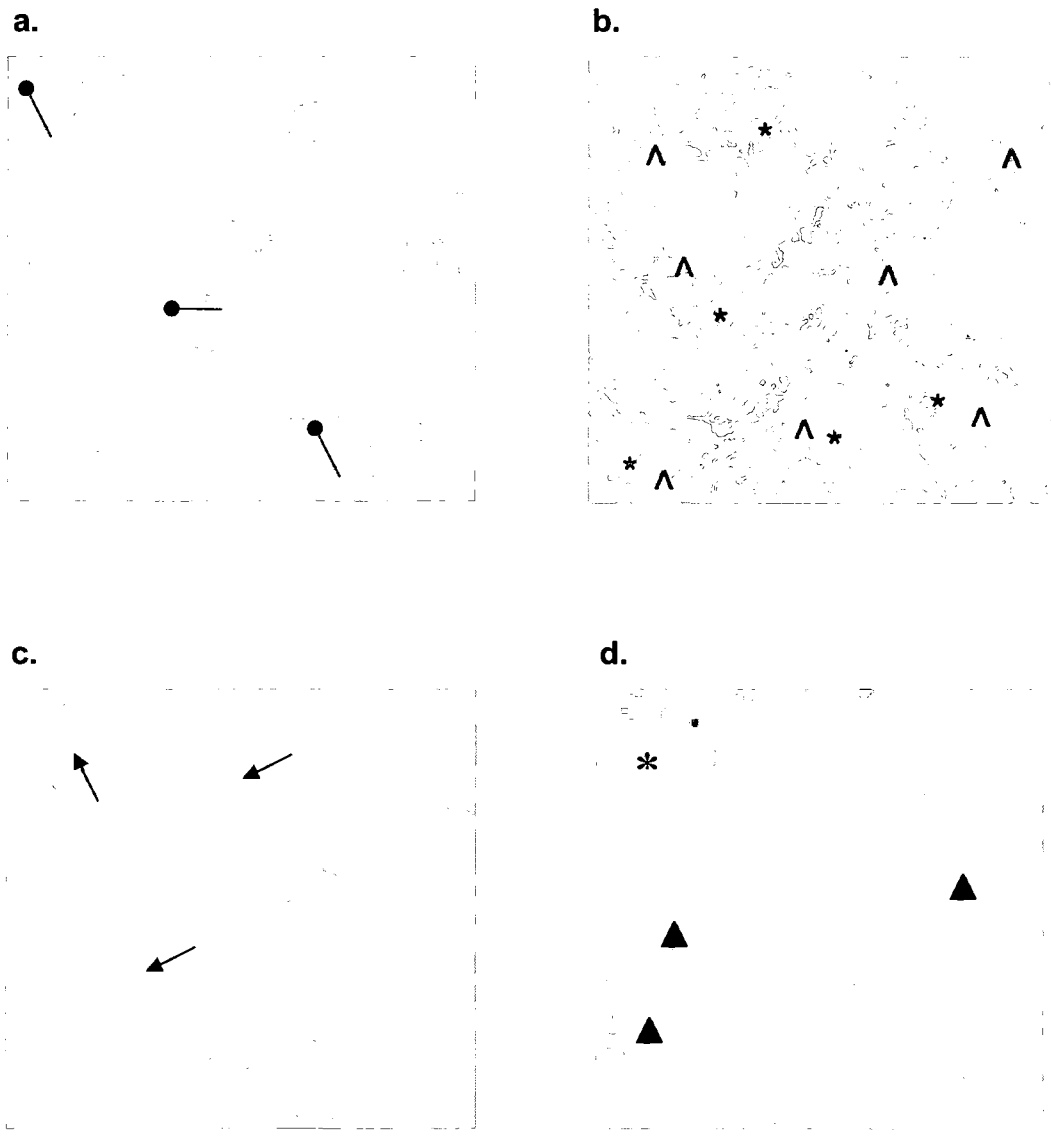


Figure 5-4. Kidney H&E stained sections. **a:** normal glomeruli from +/? LBD animals. The normal glomeruli are labeled as —●. **b**: representative micrographs of kidneys from *cp/cp* LBD animals illustrating some sclerotic glomeruli (*) including interstitial inflammation and malformed glomeruli (indicated by ∧). **c**: glomeruli from *cp/cp* animals treated with 5% fish oil showing relatively normal glomeruli (↑) (H&E staining, X4). **d**: glomeruli from *cp/cp* animals treated 10% fish oil showing malformed tubular structure (▲) and glomerulosclerosis (*) (H&E staining, X4). The tubule structures were malformed by fat vacuoles.

5.3.4 Renal Prostanoid Production and Cyclooxygenase Activity

In Figure 5-5 and Table 5-2, we show that feeding 5% fish oil significantly decreased PGD_2 levels in 0 and 10 min of incubation compared to *cp/cp* LBD control animals, Also, 5% fish oil supplementation reduced total COX activity by 60%, resulting in a corresponding decrease in the production of PGD_2 (from 9.9 ± 2.0 to 3.8 ± 1.5 ng/min•g kidney). Similarly, renal PGE_2 was reduced in 5% fish oil group at 0 min (representing endogenous levels) by 37% and by 58% with 10 min of incubation. The total potential COX activity resulting in PGE_2 production was significantly reduced in 5% n-3 PUFA group (10.3±4.8 ng/min•g kidney), compared to the obese LBD control group (35.1 ± 4.2 ng/min•g kidney) (Figure 5-6 and Table 5-2). Figure 5-7 and Table 5-2 show that 5% fish oil supplementation significantly reduced renal TXB_2 both at 0 min ($p<0.05$) and 10 min of incubation ($p<0.01$). In addition, the total potential COX activity resulting in TXB_2 activity was significantly decreased in 5% fish oil dietary group by 90% ($p<0.05$) (Figure 5-7 and Table 5-2).

Renal 6-keto-$PGF_{1\alpha}$ production COX activity was reduced by 37% in 5% fish oil *cp/cp* rats (Table 5-2). Furthermore, 5% fish oil significantly decreased the renal $PGF_{2\alpha}$ production at 10 min of incubation, relative to *cp/cp* LBD control group ($p<0.001$). Compared to *cp/cp* LBD rats, the total COX activity resulting in $PGF_{2\alpha}$ production was reduced by 87% in 5% fish oil group (Figure 5-8 and Table 5-2).

	Prostanoid levels (0-min) (ng/45mg kidney)		Prostanoid levels (10-min) (ng/45mg kidney)		COX Activity (ng /min•g kidney)	
	LBD (*cp/cp*)	5% FO (*cp/cp*)	LBD (*cp/cp*)	5% FO (*cp/cp*)	LBD (*cp/cp*)	5% FO (*cp/cp*)
PGD_2	8.2±1.4	2.4±0.1*	14.1±1.6	4.1±0.6**	9.9±2.0	3.8±1.5
PGE_2	17.2±5.4	10.8±1.6	32.9±4.7	13.9±2.5	35.1±4.2	10.3±4.8*
TXB_2	14.7±2.2	4.8±2.4*	22.6±2.1	5.9±2.7**	17.5±3.7	1.8±1.8*
6-keto-$PGF_{1\alpha}$	18.9±2.8	25.3±2.4	84.3±32.2	48.2±8.7	79.8±34.6	51.0±24.6
$PGF_{2\alpha}$	68.8±4.1	64.5±8.0	329.3±11.3	93.2±12.8***	402.9±153.9	51.3±50.0

Table 5-2. In vitro levels (ng/45mg kidney) of PGD_2, PGE_2, TXB_2, 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ in 0-min and 10-min incubation in kidneys pf *cp/cp* rats; and the total potential renal COX activity in JCR:LA-*cp cp/cp* rats. All the values are from 24 week old rats expressed as mean±S.E.M, and compared to the lipid balanced obese diet (*cp/cp* control): *p< 0.05, **p<0.01. ***p<0.001 vs corresponding *cp/cp* LBD control. FO=fish oil; LBD=lipid balance die; COX=cyclooxygenase.

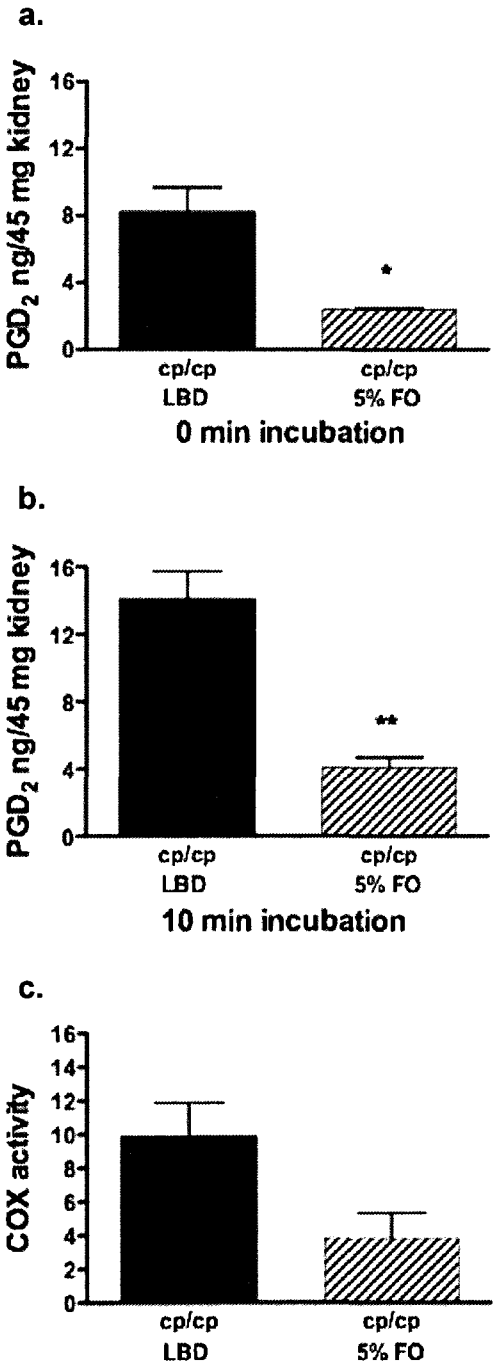


Figure 5-5. Renal PGD_2 production and COX activity. **a**: PGD_2 production in 0 min incubation; **b**: PGD_2 production in 10 min of incubation; **c**: Total potential COX activity resulting in the production of PGD_2. All the values are from 24 week old rats expressed as mean±S.E.M. Each group has 3 to 4 rats. $^{**}p<0.01$ vs *cp/cp* LBD control. Kidneys from JCR:LA-*cp* rats were lyophilized, homogenized in Tyrodes buffer, and incubated at 37℃ for 0-10min.

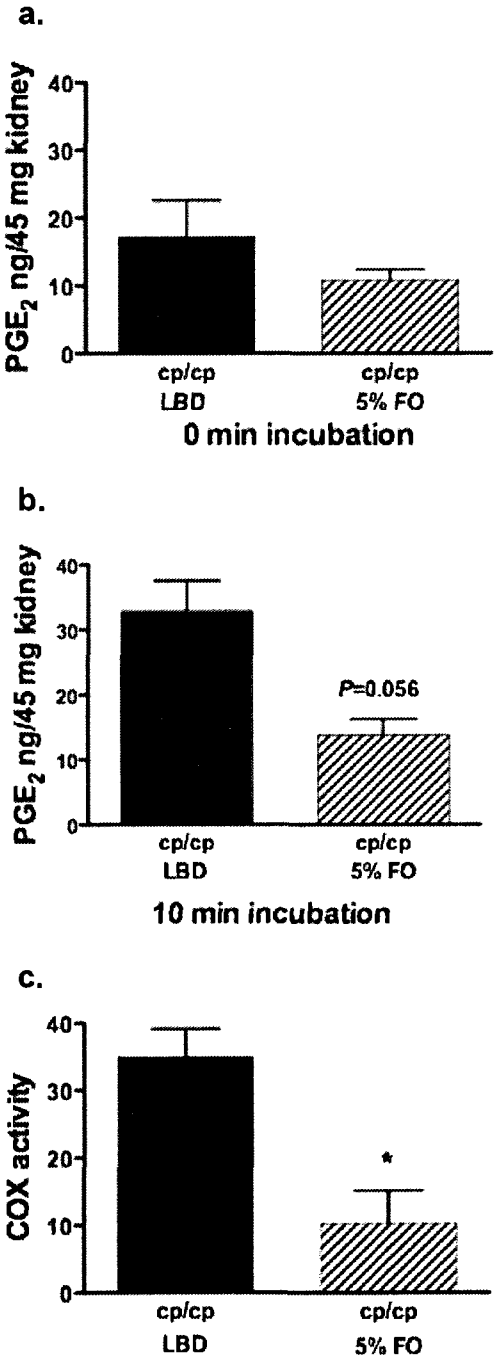


Figure 5-6. Renal PGE_2 production and COX activity. **a**: PGE_2 production in 0 min incubation; **b**: PGE_2 production in 10min incubation; **c**: Total potential COX activity resulting in the production of PGE_2. All the values are from 24 weeks old rats expressed as mean±S.E.M. Each group has 3 to 4 rats: *$p< 0.05$ vs *cp/cp* LBD control. Kidneys from JCR:LA-*cp* rats were lyophilized, homogenized in Tyrodes buffer, and incubated at 37℃ for 0-10min.

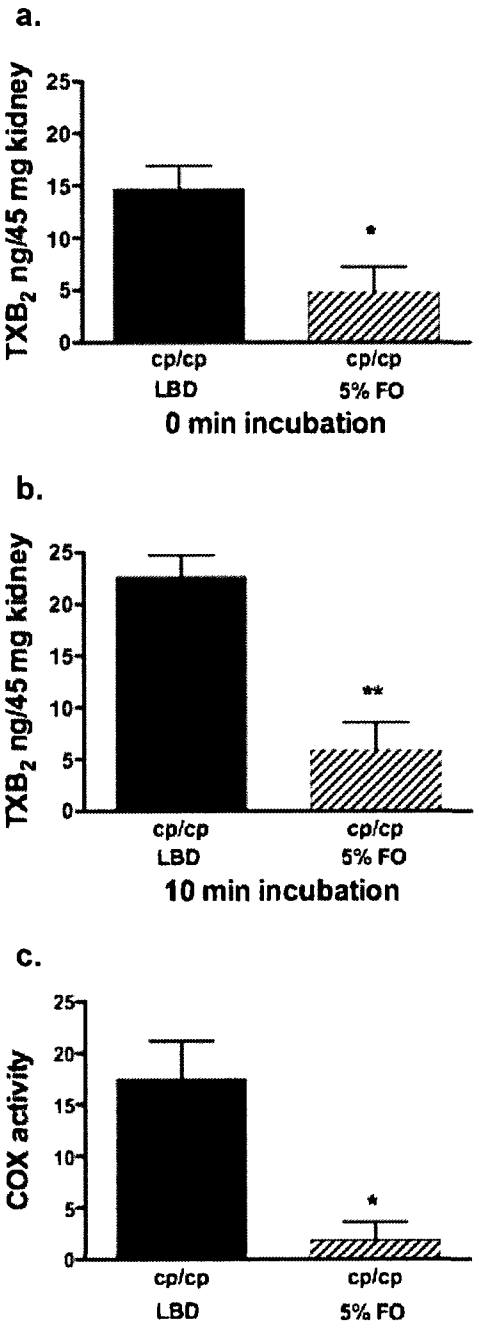


Figure 5-7. Renal TXB_2 production and COX activity. **a**: TXB_2 production in 0 min incubation; **b:** TXB_2 production in 10min incubation; **c**: Total potential activity resulting in the production of TXB_2. Expand on the experimental details. All the values are from 24 week old rats expressed as mean±S.E.M. Each group has 3 to 4 rats: *p<0.05, **p<0.01 vs *cp/cp* LBD control. Kidneys from JCR:LA-*cp* rats were lyophilized, homogenized in Tyrodes buffer, and incubated at 37℃ for 0-10min.

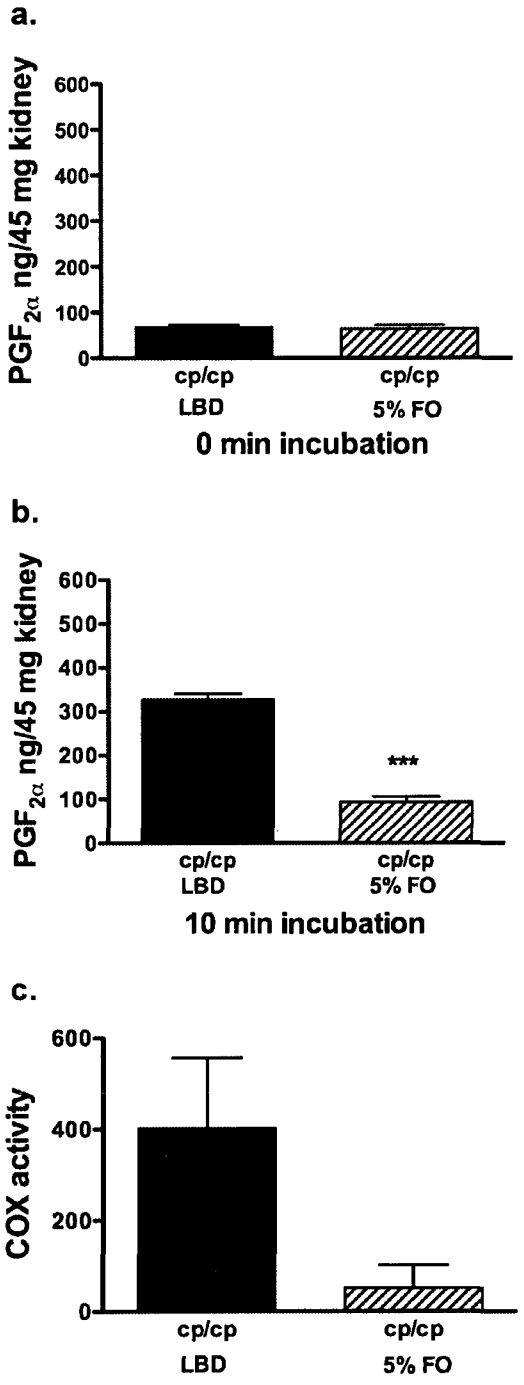


Figure 5-8. Renal $PGF_{2\alpha}$ production and COX activity. **a**: $PGF_{2\alpha}$ production in 0 min incubation; **b**: $PGF_{2\alpha}$ production in 10min incubation; **c**: Total potential activity resulting in the production of $PGF_{2\alpha}$. All the values are from 24 week old rats expressed as mean±S.E.M. Each group has 3 to 4 rats: ***$p<0.001$ vs *cp/cp* LBD control. Kidneys from JCR:LA-*cp* rats were lyophilized, homogenized in Tyrodes buffer, and incubated at 37℃ for 0-10min.

5.4 Discussion

5.4.1 Effects of N-3 Polyunsaturated Fatty Acids on Albuminuria and Glomerulosclerosis

The purpose of the present studies was to examine whether a fish oil-supplemented diet, rich in n-3 long chain polyunsaturated fatty acids, could improve renal function and glomerulosclerosis in the JCR:LA-*cp* rat model. A secondary objective was to investigate the corresponding prostanoid profile in this animal model. Our results indicate that 5% and 10% fish oil diets significantly lowered peri-renal fat pad (visceral) weight compared to *cp/cp* LBD group. In chapter 4, it was reported that n-3 PUFA supplementation lowered whole body weight gain as well as the inguinal fat pad weight. Collectively, the observation of lower peri-renal fat pad weight by n-3 PUFA supplementation further suggests robust effects on adiposity per se.

Urinary albumin is used clinically as an indicator of renal micro-vascular damage, and often reflects elevated glomerular permeability and inability to retain albumin (Hamano *et al.* 2008). Glomerulosclerosis is a major cause of end-stage renal failure in diabetic and obese individuals. One clinical symptom of glomerulosclerosis is proteinuria that is often a result of scarring within the glomeruli disturbing the filtering process and allows protein to leak into the urine (Eknoyan 2007). JCR:LA-*cp* rats fed with 5% and 10% fish oil had significant reduction in urinary albumin concentration (Figure 5-2). The significant reduction of the ratio of urinary albumin to creatinine indicated the improved ability of creatinine clearance (Figure 5-2). Consistent with this, treatment with 5% fish oil significantly reduced the incidence of glomerulosclerosis (Figure 5-3 & 5-4). Figure 5-4 showed the kidney of 5% fish oil had attenuated interstitial inflammation and fewer malformed glomeruli.

Few studies have demonstrated that supplementation with fish oil can markedly reduce the severity of glomerulosclerosis. Hagiwara *et al.* 2005 observed that

diabetic KKAy/Ta mice injected with EPA ethyl ester (1 g/kg/day) intraperitoneally attenuated glomerulosclerosis, mesangial matrix accumulation and tubulointerstitial inflammation (Hagiwara *et al.* 2005). A fish oil enriched diet has also been reported to substantially alleviate albuminuria and reduce interstitial injury (Goldstein *et al.* 1995). Current studies suggest that dietary supplementation with n-3 PUFA may ameliorate long-term, progressive renal injury (Kasiske *et al.* 1991). However, the effects of feeding n-3 PUFA in glomerulosclerosis are unknown, possibly due to the lack of an appropriate diabetic glomerulosclerosis animal. In addition, the mechanisms of kidney damage with respect to n-3 PUFA status have not been established. JCR:LA-*cp* rats can spontaneously develop diabetic glomerulosclerosis with insulin resistance and dyslipidemia. Significant benefits of n-3 PUFA on the progression of glomerulosclerosis were observed in this study.

Few studies have reported adverse effects of high dose fish oil on the kidney. Several studies state that the use of fish oil, even at levels as high as 12 g per day, carries a low probability of serious side effects (Kris-Etherton *et al.* 2003, Swails *et al.* 1993). However, these studies failed to the show the pathological complications in the subjects. Interestingly, in this study, 10% fish oil caused tubular damage in kidney, however, 10% fish oil lowered peri-renal fat pad and alleviated albuminuria. Future studies should examine the potential adverse effects of high dose fish oil on the diabetic kidney.

5.4.2 N-3 Polyunsaturated Fatty Acids and Renal Prostanoid Production

Some studies suggest that n-3 PUFA may have anti-inflammatory and anti-fibroic effects in the kidney mediated via 20-carbon fatty acid (Alexander 1998, Patten *et al.* 2005). The prostanoids including prostaglandins (PGs) and thromboxanes (TXs) are cyclic eicosanoids. They originate from linoleic acid (LA) and α-linolenic acid. The prostanoids are biologically active molecules and play different roles in kidney function. Of relevance to this study, most of prostanoids are also considered vasoactive factors and pro-inflammatory factors (Hao *et al.* 2008, Lim *et al.* 2008). PGD_2 and PGE_2 have been considered to be a pro-inflammatory

mediators while PGE_2 and PGI_2 are vasodilators and important in maintaining normal kidney flow (Kapoor *et al.* 2007, Mori *et al.* 2007). PGE_2 is also involved in the regulation of sodium re-absorption (Hodeify *et al.* 2007). Elevated concentrations of local PGE_2 have been shown to lead to renal cell over-proliferation, formation of fibrous tissue and/or pro-inflammation (Kitahara *et al.* 2002, Remuzzi *et al.* 1997). In comparison, TXA_2 is a potent vasoconstrictor and involved in the pathogenesis of hypertension associated with long-term renal failure (Larivière *et al.* 2004, Darlametsos *et al.* 2001). Therefore, the inhibition of prostanoid overproduction could be a potential mechanism by which to reduce renal damage and improve kidney function. Treatment with non-steroidal anti-inflammatory drugs has been reported to inhibit prostanoid formation and ameliorate kidney dysfunction (Norby *et al.* 1978, Brater *et al.* 2001). In this study, we observed that compared to *cp/cp* LBD control animals, 5% n-3 PUFA significantly decreased renal PGD_2, PGE_2, TXB_2, 6-keto-$PGF_{1\alpha}$, and $PGF_{2\alpha}$ production, when compared to *cp/cp* LBD rats. The significant reduction of renal prostanoid formation in 5% n-3 PUFA was consistent with the amelioration of inflammatory response and reduction of the severity and incidence of glomerulosclerosis.

A reduction in prostanoid formation is not only associated with amelioration of the disease in some types of renal disease, but are also thought to have some additional protective effects on kidney function (Warford-Woolgar *et al.* 2006, Weise *et al.* 1993). The evident amelioration of severity of glomerulosclerosis and inflammation in 5% fish oil rats may suggest that n-3 PUFA could prevent the progress of glomerulosclerosis; the reduction of albuminuria and the ratio of urinary albumin to creatinine indicats that fish oil could have protective effects on renal micro-vascular functions i.e. the glomerular permeability and ability to retain albumin. The amelioration of interstitial inflammation may be associated with reduced PGD_2 and PGE_2, which serve as pro-inflammatory mediators. Moreover, the excess of local PGE_2 could lead to renal cell over-proliferation and form fibrous tissue and inflammation (Hao *et al.* 2008, Remuzzi *et al.* 1997). The

reduction of overproduction of PGE_2 via n-3 PUFA may explain the improvement of glomerulosclerosis. In addition, the reductions of TXB_2 may cause the alleviation of hypertension, thus prevent the progress of glomerulosclerosis.

5.4.3 N-3 Polyunsaturated Fatty Acids and Cyclooxygenase Activity

Both cyclooxygenase isoforms (COX-1 and COX-2) are constitutively expressed in the adult mammalian kidney and contribute to biosynthesis of prostanoids. After being released from renal membrane phospholipids, arachidonic acid is converted to PGH_2 by COX enzymes. Importantly, COX-1 is responsible for the initial rate-limiting metabolism of arachidonic acid to prostaglandin G_2 and subsequently to prostaglandin H_2. COX-2 is believed to be an inflammatory mediated cyclooxygenase isoform. At present, the current understandings of the role of the two known COX isoforms in kidney remain inconclusive. Several studies showed that the higher COX activity in kidney is primarily due to COX-2, although not exclusively (Hao *et al.* 1999, Kitahara *et al.* 2002). The increased COX activity causes an increase of prostanoid profile and may contribute to the increased disease progression.

In present study, instead of specific activity of COX enzymes, we indirectly evaluated the total potential COX activity after 10 minutes at 37℃ incubation, which may activate the COX enzymes to cause prostanoid formation. Compared to *cp/cp* LBD rats, 5% fish oil reduced the potential total COX activity resulting in less production of PGD_2, PGE_2 and $PGF_{2\alpha}$. The reduction of COX activity in 5% fish oil group was consistent with the reduction of prostanoids formation. Therefore, it implicates that fish oil may reduce prostanoid production by inhibiting the activity of COX enzymes. Several studies have explored the possibility that long chain n-3 polyunsaturated fatty acids, EPA and DHA, could decrease the expression of COX-2 protein and mRNA in different tissues both *in vivo* and *vitro* (Mund *et al.* 2007, Sankaran *et al.* 2007, Calviello *et al.* 2004). Thomas *et al.* showed that the non-steroidal anti-inflammatory drug, ketoprofen, along with fish oil had greater inhibitory effects on COX-2 than ketoprofen alone

(Thomas *et al.* 2007). Several studies showed that EPA and DHA could accumulate in the plasma membrane and partially replace arachidonic acid (AA) as substrates for COX and LOX. As a result, the "2-series" prostanoid products of PGD_2, PGE_2, $PGF_{2\alpha}$ and TXB_2 that originate from AA were reduced (Needleman *et al.* 1979, LeBlanc *et al.* 2008), and the COX and LOX products of EPA such as TXA_3, PGD_3 and PGE_3 were formed in greater amounts (Smith *et al.* 2005). In this study, we observed that several rats treated with 5% fish oil, but not LBD obese control rats, produced some "3-series" prostanoid products. These "3-series" prostanoid products are considered to have much less vasoconstrictive and inflammatory action than "2-series" prostanoid products (Kulkarni *et al.* 1986, Darlametsos *et al.* 2001, Kulkarni *et al.* 1985). It is suggested that feeding n-3 PUFA may possibly improve renal inflammation via producing "3-series" prostanoid, which needs to be examined further in further studies.

5.5 Conclusion

In conclusion, the findings of this study offer strong evidence of the benefits of supplementation of n-3 PUFA derived from fish oil especially a dose of 5% fish oil in the JCR:LA-*cp* model. We propose that long-term n-3 PUFA feeding may offer benefits to improve glomerulosclerosis and kidney dysfunction associated with insulin resistance. Moreover, this study provides new evidence that long-term fish oil supplementation may ameliorate renal inflammation and glomerulosclerosis by reducing the prostanoid production and COX activity in a rodent model of diabetic glomerulosclerosis.

Chapter 6 Overall Discussion and Conclusion

6.1 Overall Discussion

The overall aim of this thesis was to determine whether long-term n-3 PUFA dietary treatment could improve macro- and micro-vascular complications of the Metabolic Syndrome (MetS), including heart and kidney pathophysiology in the insulin-resistant JCR:LA-*cp* rat. Collectively, our results support our hypothesis. For hypothesis I, the results described in chapter 4 suggest that after 16 weeks of treatment, fish oil may lower body weight and the deposition of adipose tissue in subcutaneous and visceral regions. The significant reduction of fasting glucose, insulin and leptin concentrations in 5% and 10% fish oil treated groups support improvement to hyperinsulinemia, hyperglycemia and hyperleptinemia respectively. Moreover, fasting and postprandial dyslipidemia as well as heart ischemia were all improved by fish oil dietary treatment. Similarly, these beneficial effects of long-term fish oil supplementation might be the consequence of the reduced expression of lipogenic-related enzymes including SREBP-1, ACC and FAS.

For specific hypothesis II, we document evidence of reduced liver and adipose SREBP-1, FAS and ACC, as well as the improvement of obesity, dyslipidemia and hyperinsulinemia, supported that modulating MetS by n-3 PUFA supplementation will improve a consequence of lipogenic-related enzymes changes including FAS and ACC.

With respect to specific hypothesis III, in this study, 5% n-3 PUFA significantly decreased the frequency of stage 3 myocardial lesions, which are highly associated with myocardial infarction. We also established that "the improvements to dyslipidemia, including other circulating metabolites and lipogenic-related enzymes through n-3 PUFA resulted in corresponding

improvements to macro-vascular disease such as ischemic myocardial lesions associated with MetS".

The findings of chapter 5 provided strong evidence of the benefits of supplementation of fish oil especially 5% fish oil in the JCR:LA-*cp* model of obesity and micro-vascular disease. Long-term n-3 fish oil feeding may prevent the progress of glomerulosclerosis and alleviate kidney dysfunction. In addition, the reduction of prostanoid production and COX activity by 5% fish oil dietary treatment is consistent with the amelioration of inflammation and glomerulosclerosis in histological changes.

Finally, for specific hypothesis V, a reduced level of renal prostanoid production and COX activity, with the amelioration of proteinuria, glomerulosclerosis and inflammation, established that improvements in glomerulosclerosis by n-3 PUFA in JCR:LA-*cp* rats were associated with a decrease prostanoid production.

MetS is clustering of risk factors and complex complications including cardiovascular disease (CVD), type 2 diabetes and kidney disease. This study evaluated the metabolic effects and possible mechanisms of n-3 PUFA on MetS and its complications. Collectively, we speculate that the hypolipidemic effects of n-3 PUFA may be explained several fold. Firstly, that n-3 PUFA may downregulate lipogenic gene expression by reducing the hepatic precursor and mature SREBP-1, hence reducing lipogenesis and cholesterol synthesis. For example, it is possible that decreased mature SREBP-1 may lead to a subsequent decrease in HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-CoA reductase), lowers total cholesterol synthesis in liver. The suppression of SREBP-1 synthesis has also been shown to decrease the expression of lipogenic-related enzymes such as FAS and ACC which would further result in a lower rate of fatty acid synthesis (Ramaprasad *et al.* 2006). The corresponding reduction of FAS and ACC by feeding n-3 PUFA would contribute to the lower concentration of plasma triglyceride (TG) and cholesterol as observed in this

study.

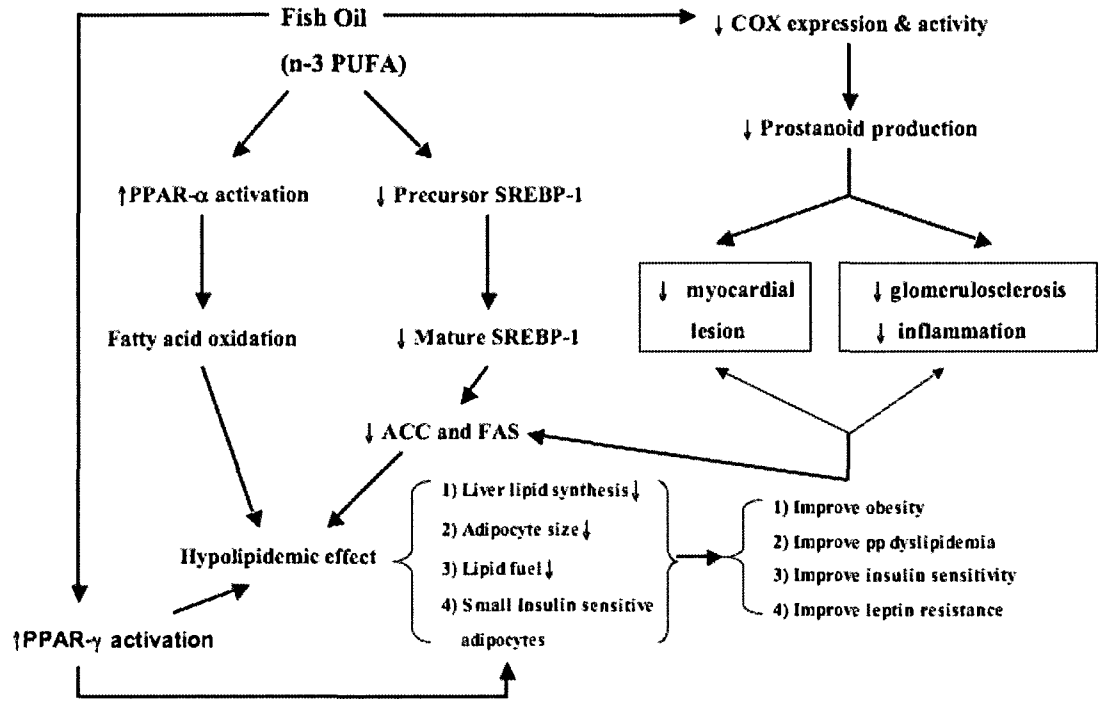


Figure 6-1. Possible mechanisms of the effects of n-3 PUFA on MetS.

Moreover, Madsen *et al.* revealed that by binding and activation of PPAR-γ in white adipose tissue, n-3 PUFA could remodel the formation of small insulin sensitive cells (Madsen *et al.* 2005). Furthermore, other studies have considered that n-3 PUFA may increase the PPAR-α activation and elevate fatty acid β-oxidation (Kim *et al.* 1999). The hypolipidemic effect of n-3 PUFA supplementation could also potentially decrease the availability of lipid within skeletal muscle and as a result, normalize glucose oxidation during insulin resistance. A recent human study found that the replacement of 15% of the fat by n-3 PUFA could significantly decrease plasma TG (Lombardo *et al.* 2007, Turvey *et al.* 2005). Similarly, in cultured human skeletal muscle, 24 hours EPA

treatment could increase the absorption and oxidation of glucose, despite a marked increase in fatty acid uptake and synthesis of complex lipids (Aas *et al.* 2006). Moreover, because of smaller size of adipocytes, the reduced weight of inguinal and peri-renal fat pads may in turn produce less leptin secretion and improve hyperleptinemia (Li *et al.* 2008). The improvement of obesity and dyslipidemia by n-3 PUFA may also induce a decrease in expression of SREBP-1, ACC and FAS. Finally, the redistribution of cholesterol from the plasma membrane to endoplasmic reticulum after feeding n-3 PUFA could suppress proteolytic processing of the precursor of SREBP into it's mature form (Xu *et al.* 1999, Jump 2002, Kim *et al.* 1999).

MetS is highly associated with dyslipidemia, which is thought to contribute in part to the development of diabetic glomerulosclerosis. The growing epidemic of MetS contributes impaired glucose metabolism, hyperinsulinemia and dyslipidemia that conspire to increase the risk for progression of diabetic kidney disease. Epidemiologic, as well as experimental evidence, has established that an increase in plasma TG and a decrease in HDL accelerates the progression of diabetic glomerulosclerosis (Bays *et al.* 2008, Razani *et al.* 2008, Scott *et al.* 1997). There has been a particular interest in the lipid-mediated changes in glomeruli that lead to glomerulosclerosis and interstitial inflammation and fibrosis (Abrass 2006). Consequently, it is feasible that n-3 PUFA may improve glomerulosclerosis by reducing overall lipid deposition and oxidation as well as the migration of circulatory monocytes in the renal mesangium and minimising their transformation to foam cells (Shohat *et al.* 1993). Data from experimental studies suggest that alterations in lipid metabolism can significantly influence the development of renal disease (hanai *et al.* 2008, Shohat *et al.* 1993, Sawara *et al.* 2008). The hypolipidemic effects of n-3 PUFA observed in this study including the improvement of both fasting and postprandial dyslipidemia, as well as the effects of n-3 PUFA on inflammation via reducing COX and prostanoids in the kidney; are suggestive of the amelioration of progression of the renal disease.

6.2 Study Limitations

On reflection of the outcomes of the study we have identified a number of aspects that we were not able to conclude based on the experimental limitations of the work. Importantly, we were not able to elucidate the comprehensive effects of n-3 PUFA on postprandial glucose and insulin response. Additionally, the effects of n-3 PUFA on atherosclerosis in JCR:LA-*cp* rodent model was not evaluated per se. Moreover, the differences between rodents and humans will impose limitations on the direct translation of our findings to humans.

Humans with MetS have lower levels of HDL than healthy individuals. However, in JCR:LA-*cp* rodent model, HDL concentration of LBD obese rats was significantly higher than in LBD lean rats due to the absence of CETP (Russell *et al.* 1987). As a result, this study was not able to predict the effects of n-3 PUFA on human HDL concentration, which is often a useful clinical indicator of CVD risk.

Finally, we acknowledge that the amount of n-3 PUFA used in the 10% diet is more than what is achievable for free-living humans by food intake alone. The appropriate dose of fish oil for humans under certain conditions of chronic disease risk still requires further investigation. In addition, this study did not explore the effects of n-3 PUFA on the selectivity of COX isoforms.

Overall, this study comprehensively explored the effects of n-3 PUFA on key risk factors and complications of MetS in a relevant animal model. Although promising, more work is needed to establish the mechanisms for these effects, particularly the effects on lipogenic-related enzyme and COX gene regulation.

6.3 Future Directions

Some questions have been generated from this study and provide interesting avenues for future research. First, an ongoing study in our laboratory is evaluating intestinal production of CM in JCR:LA-*cp* rodent via lymph cannulation to directly explore the intestinal lipid response after a meal. Second, to explore the molecular mechanisms involved in the control of lipogenic-related enzymes by n-3 PUFA, future studies will assess the long-term effect of n-3 PUA on the lipogenic gene transcription and expression, as well as the corresponding cell signalling and enzyme activity. Third, the beneficial effects of fish oil supplementation found in this thesis are of potential clinical importance in the obese and diabetic population, especially those with CVD and CKD. The findings are sufficiently encouraging to support the design of a clinical trial of MetS with CVD and CKD using fish oil. However, the optimal dosage of fish oil still needs to be determined. Further, this study was the first to identify putative kidney tubular damage from high dose fish oil. Therefore, future studies should examine more closely the adverse effects and corresponding mechanisms for the effects of the high dose n-3 PUFA diet. Lastly, more in-depth studies should be designed to determine whether n-3 PUFA selectively decrease the gene expression and activity of COX-1 and/or COX-2.

6.4 Conclusion

Long-term n-3 PUFA feeding improves postprandial dyslipidemia, insulin resistance, and reduces obesity and heart damage in the JCR:LA-*cp* rodent fed a high cholesterol diet. These beneficial effects of a fish oil enriched diet may be the consequence of modulating the expression of lipogenic-related enzymes. Long-term n-3 PUFA feeding improved kidney function and decreased the incidence of glomerulosclerosis, together with a reduction of prostanoid production and COX activity in the JCR:LA-*cp* rodent. Collectively, this study suggests that feeding long chain n-3 polyunsaturated fatty acid (fish oil) may inhibit the pathogenesis of macro- and micro- sclerosis associated with obesity and insulin resistance.

Chapter 7: Literature Cited

Aas V, Rokling-Andersen H, Kase ET, Thoresen GH, Rustan AC. 2006. Eicosapentaenoic acid (20:5 n-3) increases fatty acid and glucose uptake in cultured human skeletal muscle cells. J Lipid Res. 47:366–37.

Abrass CK. 2006. Lipid metabolism and renal disease. Contrib Nephrol. 151:106-21.

Aguila MB, Pinheiro AR, Aquino JC, Gomes AP, Mandarim-de-Lacerda CA. 2005. Different edible oil beneficial effects (canola oil, fish oil, palm oil, olive oil, and soybean oil) on spontaneously hypertensive rat glomerular enlargement and glomeruli number. Prostaglandins Other Lipid Mediat. 76(1-4):74-85.

Ahrén B, Larsson H, Wilhelmsson C, Näsman B, Olsson T. 1997. Regulation of circulating leptin in humans. Endocrine. 7(1):1-8.

Albert CM, Hennekens CH, O'Donnell CJ, Ajani UA, Carey VJ, Willett WC, Ruskin JN, Manson JE. 1998. Fish consumption and risk of sudden cardiac death. JAMA. 279:23–8.

Albert CM, Campos H, Stampfer MJ, Ridker PM, Manson JE, Willett WC, Ma J. 2002. Blood levels of long-chain n–3 fatty acids and the risk of sudden death. N Engl J Med. 346:1113–8.

Alberti KG, Zimmet P, Shaw J. 2006. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med. 23(5):469-80.

Alexander JW. 1998. Immunonutrition: the role of omega-3 fatty acids. Nutrition. 14(7-8):627-33.

Alsaad KO, Herzenberg AM. 2007. Distinguishing diabetic nephropathy from other causes of glomerulosclerosis: an update. J Clin Pathol. 60(1):18-26.

Anandan R, Mathew S, Sankar TV, Viswanathan Nair PG. 2007. Protective effect of n-3 polyunsaturated fatty acids concentrate on isoproterenol-induced myocardial infarction in rats. Prostaglandins Leukot Essent Fatty Acids. 76(3):153-8.

Ash-Bernal R, Peterson LR. 2006. The cardiometabolic syndrome and cardiovascular disease. J Cardiometab Syndr. 1(1):25-8.

Austin MA, King MC, Vranizan KM, Krauss RM. 1990 Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. Circulation 82:495-506.

Axelrod L, Camuso J, Williams E, Kleinman K, Briones E and Schoenfeld D. 1994. Effects of a small quantity of omega-3 fatty acids on cardiovascular risk factors in NIDDM. A randomized, prospective, double-blind, controlled study. *Diabetes Care*.17: 37-44.

Azain MJ. 2004. Role of fatty acids in adipocyte growth and development. J Anim Sci. 82(3):916-24.

Azzout-Marniche D, Bécard D, Guichard C, Foretz M, Ferré P, Foufelle F. 2000. Insulin effects on sterol regulatory-element-binding protein-1c (SREBP-1c) transcriptional activity in rat hepatocytes. Biochem J. 350:389-93.

Bacha F, Saad R, Gungor N, Arslanian SA. 2004. Adiponectin in youth: relationship to visceral adiposity, insulin sensitivity, and beta-cell function. Diabetes Care. 27:547–52.

Bagby SP. 2004. Obesity-initiated metabolic syndrome and the kidney: a recipe for chronic kidney disease? J Am Soc Nephrol. 15(11):2775-91.

Baggio B, Musacchio E, Priante G. 2005. Polyunsaturated fatty acids and renal fibrosis: pathophysiologic link and potential clinical implications. J Nephrol. 18(4):362-7.

Baldassarre D, Amato M, Eligini S, Barbieri SS, Mussoni L, Frigerio B, Kozàkovà M, Tremoli E, Sirtori CR, Colli S. 2006. Effect of n-3 fatty acids on carotid atherosclerosis and haemostasis in patients with combined hyperlipoproteinemia: a double-blind pilot study in primary prevention. Ann Med. 38(5):367-75.

Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. 2006. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. Atherosclerosis. 189(1):19-30.

Bays HE, Tighe AP, Sadovsky R, Davidson MH. 2008. Prescription omega-3 fatty acids and their lipid effects: physiologic mechanisms of action and clinical implications. Expert Rev Cardiovasc Ther. 6(3):391-409.

Belzung F, Raclot T, Groscolas R. 1993. Fish oil n-3 fatty acids selectively limit the hypertrophy of abdominal fat depots in growing rats fed high-fat diets. Am J Physiol. 264(6 Pt 2):R1111-8.

Benito P, Caballero J, Moreno J, Gutiérrez-Alcántara C, Muñoz C, Rojo G, Garcia S, Soriguer FC. 2006. Effects of milk enriched with omega-3 fatty acid, oleic acid and folic acid in patients with metabolic syndrome. Clin Nutr. 25(4):581-7.

Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. 2001. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med. 7:947–53.

Bergman RN, Kim SP, Hsu IR, Catalano KJ, Chiu JD, Kabir M, Richey JM, Ader M. 2007. Abdominal obesity: role in the pathophysiology of metabolic disease and cardiovascular risk. Am J Med. 120(2 Suppl 1):S3-8; discussion S29-32.

Bianchi G, Fox U, Imbasciati E. 1974. The development of a new strain of spontaneously hypertensive rats. Life Sci. 14:339-347.

Biddinger SB, Hernandez-Ono A, Rask-Madsen C, Haas JT, Alemán JO, Suzuki R, Scapa EF, Agarwal C, Carey MC, Stephanopoulos G, Cohen DE, King GL, Ginsberg HN, Kahn CR. 2008. Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. Cell Metab. 7(2):125-34.

Bilby TR, Jenkins T, Staples CR, Thatcher WW. 2006. Pregnancy, bovine somatotropin, and dietary n-3 Fatty acids in lactating dairy cows: III. Fatty acid distribution. J Dairy Sci. 89:3386-99.

Björntorp P. 1991. Metabolic implications of body fat distribution. Diabetes Care. 14(12):1132-43.

Blackburn P, Lamarche B, Couillard C, Pascot A, Bergeron N, Prud'homme D, Tremblay A, Bergeron J, Lemieux I, Després JP. 2003. Postprandial hyperlipidemia: another correlate of the "hypertriglyceridemic waist" phenotype in men. Atherosclerosis. 171(2):327-36.

Bloomgarden ZT. 2008. Approaches to treatment of pre-diabetes and obesity and promising new approaches to type 2 diabetes. Diabetes Care. 31(7):1461-6.

Boden G, Chen X, Kolaczynski JW, Polansky M. 1997. Effects of prolonged

hyperinsulinemia on serum leptin in normal human subjects. J Clin Invest. 100(5):1107-13.

Bonow RO. 2002. Third Report of National Cholesterol Education Program (NCEP). Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment Panel Ⅲ) final report. Circulation. 106:3143-421.

Botolin D, Wang Y, Christian B, Jump DB. 2006. Docosahexaneoic acid (22:6,n-3) regulates rat hepatocyte SREBP-1 nuclear abundance by Erk- and 26S proteasome-dependent pathways. J Lipid Res. 47(1):181-92.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of proetin-dye binding. Anl Biochem. 72:248-54.

Brady LM, Lovegrove SS, Lesauvage SV, Gower BA, Minihane AM, Williams CM, Lovegrove JA. 2004. Increased n-6 polyunsaturated fatty acids do not attenuate the effects of long chain n-3 polyunsaturated fatty acids on insulin sensitivity or triacylglycerol reduction in Indian Asians. Am J Clin Nutr. 79: 983–991.

Brindley DN, Russell JC. 2002. Animal models of insulin resistance and cardiovascular disease: some therapeutic approaches using JCR:LA-*cp* rat. Diabetes Obes Metab. 4(1):1-10.

Brater DC, Harris C, Redfern JS, Gertz BJ. 2001. Renal effects of COX-2-selective inhibitors. Am J Nephrol. 21:1-15.

Breyer MD, Hao C, Qi Z. 2001. Cyclooxygenase-2 selective inhibitors and the kidney. Curr Opin Crit Care. 7(6):393-400.

Brown SA, Brown CA, Crowell WA, Barsanti JA, Kang CW, Allen T, Cowell C, Finco DR. 2000. Effects of dietary polyunsaturated fatty acid supplementation in early renal insufficiency in dogs. J Lab Clin Med. 135(3):275-86.

Browning LM, Krebs JD, Moore CS, Mishra GD, O'Connell MA, Jebb SA 2007. The impact of long chain n-3 polyunsaturated fatty acid supplementation on inflammation, insulin sensitivity and CVD risk in a group of overweight women with an inflammatory phenotype. Diabetes Obes Metab. 9(1):70-80.

Calder PC. 2004. n-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. Clin Sci (Lond). 107(1):1-11.

Calviello G, Palozza P, Franceschelli P, Bartoli GM. 1997. Low-dose eicosapentaenoic or docosahexaenoic acid administration modifies fatty acid composition and does not affect susceptibility to oxidative stress in rat erythrocytes and tissues. Lipids. 32(10):1075-83.

Calviello G, Serini S, Piccioni E. 2004. n-3 polyunsaturated fatty acids and the prevention of colorectal cancer: molecular mechanisms involved. Curr Med Chem. 14(29):3059-69.

Carfray A, Patel K, Whitaker P, Garrick P, Griffiths GJ, Warwick GL. 2000. Albumin as an outcome measure in haemodialysis in patients: the effect of variation in assay method. Nephrol Dial Transplant. 15(11):1819-22.

Carmena R, Duriez P, Fruchart JC. 2004. Atherogenic lipoprotein particles in atherosclerosis. Circulation. 109(23 Suppl 1):III2-7.

Carpentier YA, Portois L, Malaisse WJ. 2006. n-3 fatty acids and the metabolic syndrome. Am J Clin Nutr. 83(6 Suppl):1499S-1504S.

Carstensen M, Thomsen C, Gotzsche O, Holst JJ, Schrezenmeir J, Hermansen K. 2004. Differential postprandial lipoprotein responses in type 2 diabetic men with and without clinical evidence of a former myocardial infarction. Rev Diabet Stud. 1(4):175-84.

Chan DC, Watts GF, Barrett PH, Beilin LJ, Redgrave TG, Mori TA. 2002. Regulatory effects of HMG CoA reductase inhibitor and fish oils on apolipoprotein B-100 kinetics in insulin-resistant obese male subjects with dyslipidemia. Diabetes. 51(8):2377-86.

Chen J, Muntner P, Hamm LL, Jones DW, Batuman V, Fonseca V, Whelton PK, He J. 2004. The metabolic syndrome and chronic kidney disease in U.S. adults. Ann Intern Med. 140(3):167-74.

Coresh J, Astor BC, Greene T, Eknoyan G, Levey AS. 2003. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. Am J Kidney Dis. 41(1):1-12.

Connor WE. 2000. Importance of n-3 fatty acids in health and disease. Am J Clin Nutr. 71(suppl):171S–175S.

Cornier MA, Dabelea D, Hernandez TL, Lindstrom RC, Steig AJ, Stob NR, Van Pelt RE, Wang H, Eckel RH. 2008. The Metabolic Syndrome. Endocr Rev. 2008 Oct 29. [Epub ahead of print]

Correia ML, Haynes WG, Rahmouni K, Morgan DA, Sivitz WI, Mark AL. 2002. The concept of selective leptin resistance: evidence from agouti yellow obese mice. Diabetes. 2002 Feb;51(2):439-42.

Cortez-Dias N, Martins S, Fiuza M. 2007. Metabolic syndrome: an evolving concept. Rev Port Cardiol. 26(12):1409-21.

Côté M, Mauriège P, Bergeron J, Alméras N, Tremblay A, Lemieux I, Després JP. 2005. Adiponectinemia in visceral obesity: impact on glucose tolerance and plasma lipoprotein and lipid levels in men. J Clin Endocrinol Metab. 90:1434-9.

Couet C, Delarue J, Ritz P, Antoine JM, Lamisse F. 1997. Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults. Int J Obes Relat Metab Disord. 21(8):637-43.

Couillard C, Mauriège P, Imbeault P, Prud'homme D, Nadeau A, Tremblay A, Bouchard C, Després JP. 2000. Hyperleptinemia is more closely associated with adipose cell hypertrophy than with adipose tissue hyperplasia. Int J Obes Relat Metab Disord. 24(6):782-8.

Crundy SM, Brewer Jr HB, Cleeman JI, Smith Jr SC, Lenfant C. 2004. Definition of metabolic syndrome: report of the national heart, lung, and blood institute/American heart association conference on scientific issues related to definition. Circulation.109:433-8.

Robert K. Danish RK, West BB. 2005. Rapid progression from pre-diabetes to severely III diabetes while under "expert care": suggestions for improved screening for disease progression. Diabetes Spectrum. 18:229-39.

Darlametsos IE, Varonos DD. 2001. Role of prostanoids and endothelins in the prevention of cyclosporine-induced nephrotoxicity. Prostaglandins Leukot Essent Fatty Acids. 64(4-5):231-9.

Davidson MH. 2006. Mechanisms for the hypotriglyceridemic effect of marine omega-3 fatty acids. Am J Cardiol. 98:27i-33i.

Daviglus ML, Stamler J, Orencia AJ, Dyer AR, Liu K, Greenland P, Walsh MK, Morris D, Shekelle RB. 1991. Dietary polyunsaturated fatty acids and mortality in the Multiple Risk Factor Intervention Trial (MRFIT). World Rev Nutr Diet 66:205–16.

De Caterina R, Caprioli R, Giannessi D, Sicari R, Galli C, Lazzerini G, Bernini W, Carr L, Rindi P. 1993. n-3 fatty acids reduce proteinuria in patients with chronic glomerular disease. Kidney Int. 44(4):843-50.

De Caterina R, Madonna R, Bertolotto A, Schmidt EB. 2007. n-3 fatty acids in the treatment of diabetic patients: biological rationale and clinical data. Diabetes Care. 30(4):1012-26.

de Lusignan S, Hague N, van Vlymen J, Dhoul N, Chan T, Thana L, Kumarapeli P. 2006. A study of cardiovascular risk in overweight and obese people in England. Eur J Gen Pract. 12(1):19-29.

Després JP. 2006. Is visceral obesity the cause of the metabolic syndrome? Ann Med. 38(1):52-63.

Després JP. 2007. Cardiovascular disease under the influence of excess visceral fat. Crit Pathw Cardiol. 6(2):51-9.

Després JP, Lemieux I, Bergeron J, Pibarot P, Mathieu P, Larose E, Rodés-Cabau J, Bertrand OF, Poirier P. 2008. Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. Arterioscler Thromb Vasc Biol. 28(6):1039-49.

Dolphin PJ, Stewart B, Amy RM, Russell JC. 1987. Serum lipids and lipoproteins in the atherosclerosis-prone LA/N-corpulent rat. Biochim Biophys. 919:140-8.

Donadio JV, Grande JP. 2004. The role of fish oil/omega-3 fatty acids in the treatment of IgA nephropathy. Semin Nephrol. 24(3):225-43.
Drevon CA. 2005. Fatty acids and expression of adipokines. Biochim Biophys Acta. 1740:287-92.

DuBois, RN, Awad, J, Morrow, J, Roberts, LJ, Bishop, PR. 1994. Regulation of eicosanoid production and mitogenesis in rat intestinal epithelial cells by transforming growth factor-α and phorbol ester. J Clin Invest. 93:493-498.

Düsing R, Struck A, Göbel BO, Weisser B, Vetter H. 1990. Effects of n-3 fatty acids on renal function and renal prostaglandin E metabolism. Kidney Int. 38(2):315-9.

Dyck DJ, Heigenhauser GJ, Bruce CR. 2006. The role of adipokines as regulators of skeletal muscle fatty acid metabolism and insulin sensitivity. Acta Physiol (Oxf). 186:5-16.

Eguchi M, Tsuchihashi K, Saitoh S, Odawara Y, Hirano T, Nakata T, Miura T, Ura N, Hareyama M, Shimamoto. 2007. Visceral obesity in Japanese patients with metabolic syndrome: reappraisal of diagnostic criteria by CT scan. Hypertens Res. 30(4):315-23.

Eknoyan G. 2007. Obesity, diabetes, and chronic kidney disease. Curr Diab Rep. 7(6):449-53.

Elam MB, Wilcox HG, Cagen LM, Deng X, Raghow R, Kumar P, Heimberg M, Russell JC. 2001. Increased hepatic VLDL secretion, lipogenesis, and SREBP-1 expression in the corpulent JCR:LA-*cp* rat. J Lipid Res. 42(12):2039-48.

Erkkilä AT, Lichtenstein AH, Mozaffarian D, Herrington DM. 2004. Fish intake is associated with a reduced progression of coronary artery atherosclerosis in postmenopausal women with coronary artery disease. Am J Clin Nutr. 80(3):626-32.

Felder TK, Klein K, Patsch W, Oberkofler H. 2005. A novel SREBP-1 splice variant: tissue abundance and transactivation potency. Biochim Biophys Acta. 1731(1):41-7.

Fernandez R, Piechnik J, Fabris R, Malnic G, Fernandes LC. 2004. Effect of chronic fish oil supplementation on renal function of normal and cachectic rats. Braz J Med Biol Res. 37(10):1481-9.

Fernandez-Real JM, Vendrell J, Ricart W. 2005. Circulating adiponectin and plasma fatty acid profile. Clin Chem. 51:603–609.

Feskens EJ, Loeber JG, Kromhout D. 1994. Diet and physical activity as determinants of hyperinsulinemia: the Zutphen Elderly Study. Am J Epidemiol. 140:350-60.

Field FJ, Born E, Murthy S, Mathur SN. 2002. Polyunsaturated fatty acids decrease the expression of sterol regulatory element-binding protein-1 in CaCo-2 cells: effect on fatty acid synthesis and triacylglycerol transport. Biochem J. 368:855-64.

Finnegan YE, Minihane AM, Leigh-Firbank EC, Kew S, Meijer GW, Muggli R, Calder PC, Williams CM. 2003. Plant- and marine-derived n-3 polyunsaturated fatty acids have differential effects on fasting and postprandial blood lipid concentrations and on the susceptibility of LDL to oxidative modification in moderately hyperlipidemic subjects. Am J Clin Nutr. 77(4):783-95.

Flachs P, Mohamed-Ali V, Horakova O, Rossmeisl M, Hosseinzadeh-Attar MJ, Hensler M, Ruzickova J, Kopecky J. 2006. Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed a high-fat diet. Diabetologia. 49:394–397.

Föger B, Chase M, Amar MJ, Vaisman BL, Shamburek RD, Paigen B, Fruchart-Najib J, Paiz JA, Koch CA, Hoyt RF, Brewer HB Jr, Santamarina-Fojo S. 1999. Cholesteryl ester transfer protein corrects dysfunctional high density lipoproteins and reduces aortic atherosclerosis in lecithin cholesterol acyltransferase transgenic mice. J Biol Chem. 1999. 274(52):36912-20.

Friedberg CE, Janssen MJ, Heine RJ, Grobbee DE. 1998. Fish oil and glycemic control in diabetes. A meta-analysis. Diabetes care. Diabetes Care. 1998 Apr;21(4):494-500.

Friedman JM, Halaas JL. 1998. Leptin and the regulation of body weight in mammals. Nature. 395(6704):763-70.

Fritsche K. 2007. Important differences exist in the dose-response relationship between diet and immune cell fatty acids in humans and rodents. Lipids. 42(11):961-79.

Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF. 2001. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci U S A. 98(4):2005-10.

Gallardo N, Bonzón-Kulichenko E, Fernández-Agulló T, Moltó E, Gómez-Alonso S, Blanco P, Carrascosa JM, Ros M, Andrés A. 2007. Tissue-specific effects of central leptin on the expression of genes involved in lipid metabolism in liver and white adipose tissue. Endocrinology. 148:5604-10.

Garaulet M, Pérez-Llamas F, Pérez-Ayala M, Martínez P, de Medina FS, Tebar FJ, Zamora S. 2001. Site-specific differences in the fatty acid composition of abdominal adipose tissue in an obese population from a Mediterranean area: relation with dietary fatty acids, plasma lipid profile, serum insulin, and central obesity. Am J Clin Nutr. 74(5):585-91.

Griswold DE, Adams JL. 1996. Constitutive cyclooxygenase (COX-1) and inducible cyclooxygenase (COX-2): rationale for selective inhibition and progress to date. Med Res Rev. 16(2):181-206.

Geronimo FR, Abarquez Jr RF, Punzalan FE, Cabral EI. 2005. Clustering of risk factors, metabolic syndrome, and coronary heart disease risk in hypertensive patients. Asia Pac J Clin Nutr. 14 Suppl:S44.

Giallauria F, Orio F, Palomba S, Lombardi G, Colao A, Vigorito C. 2008. Cardiovascular risk in women with polycystic ovary syndrome. J Cardiovasc Med (Hagerstown). 9(10):987-9.

Glass CK, Witztum JL. 2001. Atherosclerosis. the road ahead. Cell. 104: 503-16.

Giorda CB, Avogaro A, Maggini M, Lombardo F, Mannucci E, Turco S, Alegiani SS, Raschetti R, Velussi M, Ferrannini E; Diabetes and Informatics Study Group. 2008. Recurrence of cardiovascular events in patients with type 2 diabetes: epidemiology and risk factors. Diabetes Care. 31(11):2154-9.

Goldstein DJ, Wheeler DC, Sandstrom DJ, Kawachi H, Salant DJ. 1995. Fish oil ameliorates renal injury and hyperlipidemia in the Milan normotensive rat model of focal glomerulosclerosis. J Am Soc Nephrol. 6(5):1468-75.

Goodpaster BH, Thaete FL, Simoneau JA, Kelley DE. 1997. Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. Diabetes. 46:1579–1585.

Greenhouse DD, Michaelis OE, Peterson RG. The development of fatty and corpulent rat strains. 1988. In: Hansen CT, Michaelis OE, eds. New models of genetically obese rats for studies in diabetes, heart disease, and complications of obesity. Bethesda: National Institutes of Health, 1988:3-6.

Grekas D, Kassimatis E, Makedou A, Bacharaki D, Bamichas G, Tourkantonis A. 2001. Combined treatment with low-dose pravastatin and fish oil in post-renal transplantation dislipidemia. Nephron. 88(4):329-33.

Griffin MJ, Sul HS. 2004. Insulin regulation of fatty acid synthase gene transcription: roles of USF and SREBP-1c. IUBMB Life. 56:595-600.

Groot PH, van Stiphout WA, Krauss XH, Jansen H, van Tol A, van Ramshorst E, Chin-On S, Hofman A, Cresswell SR, Havekes L. 1991. Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. Arterioscler Thromb. 11:653–62.

Grundt H, Nilsen DW, Hetland O, Aarsland T, Baksaas I, Grande T, Woie L. 1995. Improvement of serum lipids and blood pressure during intervention with n-3 fatty acids was not associated with changes in insulin levels in subjects with combined hyperlipidaemia. J Intern Med. 237(3):249-59.

Grundy SM. 1997. Small LDL, atherogenic dyslipidemia, and the metabolic syndrome. Circulation. 95(1):1-4.

Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C; American Heart Association; National Heart, Lung, and Blood Institute. 2004. Definition of

metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation. 109(3):433-8.

Gu D, Reynolds K, Wu X, Chen J, Duan X, Reynolds RF, Whelton PK, He J; InterASIA Collaborative Group. 2005. Prevalence of the metabolic syndrome and overweight among adults in China, Lancet. 365(9468):1398-405.

Guebre-Egziabher F, Rabasa-Lhoret R, Bonnet F, Bastard JP, Desage M, Skilton MR, Vidal H, Laville M. 2008. Nutritional intervention to reduce the n-6/n-3 fatty acid ratio increases adiponectin concentration and fatty acid oxidation in healthy subjects. Eur J Clin Nutr. 62(11):1287-93.

Gupta S, Gupta BM. 2006. Metabolic syndrome: diabetes and cardiovascular disease. Indian Heart J. 58(2):149-52.

Gurfinkel EP, Lernoud VS. 2006. Prevention of myocardial infarction. Curr Opin Cardiol. 21(5):503-9.

Hadi HA, Suwaidi JA. 2007. Endothelial dysfunction in diabetes mellitus. Vasc Health Risk Manag. 3(6):853-76.

Hagiwara S, Makita Y, Gu L, Tanimoto M, Zhang M, Nakamura S, Kaneko S, Itoh T, Gohda T, Horikoshi S, Tomino Y. 2006. Eicosapentaenoic acid ameliorates diabetic nephropathy of type 2 diabetic KKAy/Ta mice: involvement of MCP-1 suppression and decreased ERK1/2 and p38 phosphorylation. Nephrol Dial Transplant. 21(3):605-15.

Hajer GR, van Haeften TW, Visseren FL. 2008. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. Eur Heart J. 29(24):2959-71.

Horia E, Watkins BA. 2007. Complementary actions of docosahexaenoic acid and genistein on COX-2, PGE_2 and invasiveness in MDA-MB-231 breast cancer cells. Carcinogenesis. 28(4):809-15.

Hanley AJ, Wagenknecht LE, D'Agostino RB Jr, Zinman B, Haffner SM. 2003. Identification of Subjects with Insulin Resistance and β-Cell Dysfunction Using Alternative Definitions of the Metabolic Syndrome. Diabetes. 52(11):2740-7.

Halkes CJ, van Dijk H, de Jaegere PP, Plokker HW, van Der Helm Y, Erkelens DW, Castro Cabezas M. 2001. Postprandial increase of complement component 3 in normolipidemic patients with coronary artery disease: effects of expanded-dose simvastatin. Arterioscler Thromb Vasc Biol. 21(9):1526-30.

Hamdy O, Porramatikul S, Al-Ozairi E. 2006. Metabolic obesity: the paradox between visceral and subcutaneous fat. Curr Diabetes Rev. 2(4):367-73.

Hamano K, Nitta A, Ohtake T, Kobayashi S. 2008. Associations of renal vascular resistance with albuminuria and other macroangiopathy in type 2 diabetic patients. Diabetes Care. 31(9):1853-7.

Hanai K, Babazono T, Iwamoto Y. 2008. Renal manifestations of metabolic syndrome in type 2 diabetes. Diabetes Res Clin Pract. 79(2):318-24.

Hanson RL, Imperatore G, Bennett PH, Knowler WC. 2002. Components of the "metabolic syndrome" and incidence of type 2 diabetes. Diabetes. 51:3120-3127.

Hao CM, Kömhoff M, Guan Y, Redha R, Breyer MD. 1999. Selective targeting of cyclooxygenase-2 reveals its role in renal medullary interstitial cell survival. Am J Physiol. 277:F352-9.

Hao CM, Breyer MD. 2007. Physiologic and pathophysiologic roles of lipid

mediators in the kidney. Kidney Int. 71(11):1105-15.

Hao CM, Breyer MD. 2008. Physiological regulation of prostaglandins in the kidney. Annu Rev Physiol. 70:357-77.

Harris WS, Connor WE, Alam N, Illingworth RD. 1988. Reduction of postprandial triglyceridemia in humans by dietary n23 fatty acids. J Lipid Res. 29:1451–60.

Harris WS. 1989. Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. J Lipid Res. 30:785–807.

Harris WS, Connor WE, Illingworth DR, Rothrock DW, Foster DM. 1990. Effects of fish oil on VLDL triglyceride kinetics in humans. J Lipid Res. 31(9):1549-58.

Hartweg J, Farmer AJ, Perera R, Holman RR, Neil HA. 2007. Meta-analysis of the effects of n-3 polyunsaturated fatty acids on lipoproteins and other emerging lipid cardiovascular risk markers in patients with type 2 diabetes. Diabetologia. 50(8):1593-602.

Hayashi T, Boyko EJ, McNeely MJ, Leonetti DL, Kahn SE, Fujimoto WY. 2008. Visceral adiposity, not abdominal subcutaneous fat area, is associated with an increase in future insulin resistance in Japanese Americans. Diabetes. 57(5):1269-75.

Hassanali Z, Ruth MR, Gerdung CA, Goruk S, Jing Lu *et al.*. 2006. Acute n-3 PUFA supplementation in the JCR:LA-*cp* rodent model of insulin-resistance: Effects on postprandial lipid metabolism and associated inflammatory response. ISSFAL conference in England, Poster session B, Poster 97. London, England.

Havel PJ, Kasim-Karakas S, Mueller W, Johnson PR, Gingerich RL, Stern JS. 1996. Relationship of plasma leptin to plasma insulin and adiposity in normal

weight and overweight women: effects of dietary fat content and sustained weight loss. J Clin Endocrinol Metab. 81(12):4406-13.

Heidbreder E, Hüller U, Schäfer B, Heidland A. 1986. Fundus hypertonicus malignus. Increased incidence in renal parenchymal diseases. Dtsch Med Wochenschr. 111(11):411-6.

Higuchi T, Shirai N, Saito M, Suzuki H, Kagawa Y. 2008. Levels of plasma insulin, leptin and adiponectin, and activities of key enzymes in carbohydrate metabolism in skeletal muscle and liver in fasted ICR mice fed dietary n-3 polyunsaturated fatty acids. J Nutr Biochem. 19(9):577-86.

Hill AM, Buckley JD, Murphy KJ, Howe PR. 2007. Combining fish-oil supplements with regular aerobic exercise improves body composition and cardiovascular disease risk factors. Am J Clin Nutr. 85:1267-74.

Hodeify RF, Kreydiyyeh SI. 2007. PGE2 reduces net water and chloride absorption from the rat colon by targeting the Na+/H+ exchanger and the Na+ K+ 2Cl- cotransporter. Prostaglandins Leukot Essent Fatty Acids. 76(5):285-92.

Horrocks LA, Farooqui AA. 2004. Docosahexaenoic acid in the diet: its importance in maintenance and restoration of neural membrane function. Prostaglandins Leukot Essent Fatty Acids. 70(4):361-72.

Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. 1996. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science. 271:665– 8.

Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T,

Matsuzawa Y. 2000. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol. 20:1595–9.

Hsu IR, Kim SP, Kabir M, Bergman RN. 2007. Metabolic syndrome, hyperinsulinemia, and cancer. Am J Clin Nutr. 86(3):s867-71.

Iseki K. 2008. Metabolic syndrome and chronic kidney disease: a Japanese perspective on a worldwide problem. J Nephrol. 21(3):305-12.

Isley WL. 2006. Low-density Lipoprotein Cholesterol Lowering in the Prevention of CHD: How Low Should We Go? Curr Treat Options Cardiovasc Med. 8(4):289-97.

Jang IS, Hwang DY, Chae KR, Lee JE, Kim YK, Kang TS, Hwang JH, Lim CH, Huh YB, Cho JS. 2003. Role of dietary fat type in the development of adiposity from dietary obesity-susceptible Sprague-Dawley rats. Br J Nutr. 89(3):429-38.

Johansson F, Kramer F, Barnhart S, Kanter JE, Vaisar T, Merrill RD, Geng L, Oka K, Chan L, Chait A, Heinecke JW, Bornfeldt KE. 2008. Type 1 diabetes promotes disruption of advanced atherosclerotic lesions in LDL receptor-deficient mice. Proc Natl Acad Sci U S A. 105(6):2082-7.

Jump DB. 2002. Dietary polyunsaturated fatty acids and regulation of gene transcription. Curr Opin Lipidol. 13:155-64.

Kako Y, Massé M, Huang LS, Tall AR, Goldberg IJ. 2002. Lipoprotein lipase deficiency and CETP in streptozotocin-treated apoB-expressing mice. J Lipid Res. 43(6):872-7.

Kamohara S, Burcelin R, Halaas JL, Friedman JM, Charron MJ. 1997. Acute

stimulation of glucose metabolism in mice by leptin treatment. Nature. 389(6649):374-7.

Kanter JE, Johansson F, LeBoeuf RC, Bornfeldt KE. 2007. Do glucose and lipids exert independent effects on atherosclerotic lesion initiation or progression to advanced plaques? Circ Res. 100(6):769-81.

Kapoor M, Kojima F, Yang L, Crofford LJ. 2007. Sequential induction of pro- and anti-inflammatory prostaglandins and peroxisome proliferators-activated receptor-gamma during normal wound healing: a time course study. Prostaglandins Leukot Essent Fatty Acids. 76(2):103-12.

Karpe F, Steiner G, Uffelman K, Olivecrona T, Hamsten A. 1994. Postprandiallipoproteins and progression of coronary atherosclerosis. Atherosclerosis. 106:83–97.

Kasiske BL, O'Donnell MP, Lee H, Kim Y, Keane WF. 1991. Impact of dietary fatty acid supplementation on renal injury in obese Zucker rats. Kidney Int. 39(6):1125-34.

Kaufman F. 2002. Stop diabetes in its tracks. Nurs Manage. Suppl:2-6.

Kesavulu MM, Kameswararao B, Apparao Ch, Kumar EG, Harinarayan CV. 2002. Effect of omega-3 fatty acids on lipid peroxidation and antioxidant enayme status in type 2 diabetic patients. Diabetes Metab. 28(1):20-6

Kim HJ, Takahashi M, Ezaki O. 1999. Fish oil feeding decreases mature sterol regulatory element-binding protein 1 (SREBP-1) by down-regulation of SREBP-1c mRNA in mouse liver. A possible mechanism for down-regulation of lipogenic enzyme mRNAs. J Biol Chem. 274(36):25892-8.

Kim JW, Zou Y, Yoon S, Lee JH, Kim YK, Yu BP, Chung HY. 2004. Vascular aging: molecular modulation of the prostanoid cascade by calorie restriction. J Gerontol A Biol Sci Med Sci. 59(9):B876-85.

Kingsbury KJ, Bondy G. 2003. Understanding the essentials of blood lipid metabolism. Prog Cardiovasc Nurs. 18(1):13-8.

Kitahara M, Eitner F, Ostendorf T, Kunter U, Janssen U, Westenfeld R, Matsui K, Kerjaschki D, Floege J. 2002. Selective cyclooxygenase-2 inhibition impairs glomerular capillary healing in experimental glomerulonephritis. J Am Soc Nephrol. 13(5):1261-70.

Klahr S. 1989. Effects of protein intake on the progression of renal disease. Annu Rev Nutr. 9:87-108.

Klok MD, Jakobsdottir S, Drent ML. 2007. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. Obes Rev. 8:21-34.

Knopp RH, Paramsothy P, Atkinson B, Dowdy A. 2008. Comprehensive lipid management versus aggressive low-density lipoprotein lowering to reduce cardiovascular risk. Am J Cardiol. 101(8A):48B-57B.

Koletsky S. Obese spontaneously hypertensive rats: a model for the study of atherosclerosis. Exp Mol Pathol 1973;19:52-60.

Koletsky S. Pathological findings and laboratory data in a new strain of obese hypertensive rats. Am J Pathol 1975;80:129-42.

Komers R, Lindsley JN, Oyama TT, Schutzer WE, Reed JF, Mader SL, Anderson S. 2001. Immunohistochemical and functional correlations of renal

cyclooxygenase-2 in experimental diabetes. J Clin Invest. 107(7):889-98.

Komers R, Zdychová J, Cahová M, Kazdová L, Lindsley JN, Anderson S. 2005. Renal cyclooxygenase-2 in obese Zucker (fatty) rats. Kidney Int. 67(6):2151-8.

Koop K, Eikmans M, Wehland M, Baelde H, Ijpelaar D, Kreutz R, Kawachi H, Kerjaschki D, de Heer E, Bruijn JA. 2008. Selective loss of podoplanin protein expression accompanies proteinuria and precedes alterations in podocyte morphology in a spontaneous proteinuric rat model. Am J Pathol. 173(2):315-26.

Koopmans SJ, Frolich M, Gribnau EH, Westendorp RG, DeFronzo RA. 1998. Effect of hyperinsulinemia on plasma leptin concentrations and food intake in rats. Am J Physiol. 274(6 Pt 1):E998-E1001.

Kopelman PG, Albon L. 1997. Obesity, non-insulin-dependent diabetes mellitus and the metabolic syndrome. Br Med Bull. 1997;53(2):322-40.

Korczynska J, Stelmanska E, Nogalska A, Szolkiewicz M, Goyke E, Swierczynski J, Rutkowski B. 2004. Upregulation of lipogenic enzymes genes expression in white adipose tissue of rats with chronic renal failure is associated with higher level of sterol regulatory element binding protein-1. Metabolism. 53(8):1060-5.

Kragelund C, Køber L, Faber J, Steffensen R, Hildebrandt P. 2007. Metabolic syndrome and mortality in stable coronary heart disease: Relation to gender. Int J Cardiol. 121(1):62-7.

Kratz M, Swarbrick MM, Callahan HS, Matthys CC, Havel PJ, Weigle DS. 2008. Effect of dietary n-3 polyunsaturated fatty acids on plasma total and high-molecular-weight adiponectin concentrations in overweight to moderately obese men and women. Am J Clin Nutr. 87(2):347-53.

Krebs JD, Browning LM, McLean NK, Rothwell JL, Mishra GD, Moore CS, Jebb SA. 2006. Additive benefits of long-chain n-3 polyunsaturated fatty acids and weight-loss in the management of cardiovascular disease risk in overweight hyperinsulinaemic women. Int J Obes (Lond). 30(10):1535-44.

Kris-Etherton PM, Harris WS, Appel LJ; Nutrition Committee. 2003. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Arterioscler Thromb Vasc Biol. 23(2):e20-30.

Kulkarni PS, Srinivasan BD. 1986. Eicosapentaenoic acid metabolism in human and rabbit anterior uvea. Prostaglandins. 31(6):1159-64.

Kunesová M, Braunerová R, Hlavatý P, Tvrzická E, Stanková B, Skrha J, Hilgertová J, Hill M, Kopecký J, Wagenknecht M, Hainer V, Matoulek M, Parízková J, Zák A, Svacina S. 2005. The influence of n-3 polyunsaturated fatty acids and very low calorie diet during a short-term weight reducing regimen on weight loss and serum fatty acid composition in severely obese women. Physiol Res. 55(1):63-72.

Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, Eto K, Yamashita T, Kamon J, Satoh H, Yano W, Froguel P, Nagai R, Kimura S, Kadowaki T, Noda T . 2002. Disruption of adiponectin causes insulin resistance and neointimal formation. J Biol Chem. 277:25863–6.

Kubota N, Yano W, Kubota T, Yamauchi T, Itoh S, Kumagai H, Kozono H, Takamoto I, Okamoto S, Shiuchi T, Suzuki R, Satoh H, Tsuchida A, Moroi M, Sugi K, Noda T, Ebinuma H, Ueta Y, Kondo T, Araki E, Ezaki O, Nagai R, Tobe K, Terauchi Y, Ueki K, Minokoshi Y, Kadowaki T. 2007. Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. Cell Metab. 2007;6:55–68.

Kulkarni PS, Srinivasan BD. 1985. Prostaglandins E3 and D3 lower intraocular pressure. Invest Ophthalmol Vis Sci. 26(8):1178-82.

Lairon D. 2008. Macronutrient intake and modulation on chylomicron production and clearance. Atheroscler Suppl. 9(2):45-8.

Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Després JP. 1997. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Québec Cardiovascular Study. Circulation. 95(1):69-75.

Laufs U, Custodis F, Böhm M. 2006. HMG-CoA reductase inhibitors in chronic heart failure: potential mechanisms of benefit and risk. Drugs. 66(2):145-54.

Lauretani F, Semba RD, Bandinelli S, Miller ER 3rd, Ruggiero C, Cherubini A, Guralnik JM, Ferrucci L. 2008. Plasma polyunsaturated fatty acids and the decline of renal function. Clin Chem. 54(3):475-81.

Larivière R, Moreau C, Rodrigue ME, Lebel M. 2004. Thromboxane blockade reduces blood pressure and progression of renal failure independent of endothelin-1 in uremic rats. Prostaglandins Leukot Essent Fatty Acids. 71(2):103-9.

Leaf A, Kang JX, Xiao Y-F, Billman GE. 2003. Clinical prevention of sudden cardiac death by n–3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n–3 fish oils. Circulation. 107:2646–52.

LeBlanc CJ, Horohov DW, Bauer JE, Hosgood G, Mauldin GE. 2008. Effects of dietary supplementation with fish oil on in vivo production of inflammatory mediators in clinically normal dogs. Am J Vet Res. 69(4):486-93.

Leigh-Firbank EC, Minihane AM, Leake DS, Wright JW, Murphy MC, Griffin BA, Williams CM. 2002. Eicosapentaenoic acid and docosahexaenoic acid from fish oils: differential associations with lipid responses. Br J Nutr. 7:435– 45.

Lewis GF, Steiner G. 1996. Acute effects of insulin in the control of VLDL production in humans. Implications for the insulin-resistant state. Diabetes Care 19:390-393.

Li M, Kim DH, Tsenovoy PL, Peterson SJ, Rezzani R, Rodella LF, Aronow WS, Ikehara S, Abraham NG. 2008. Treatment of obese diabetic mice with a heme oxygenase inducer reduces visceral and subcutaneous adiposity, increases adiponectin levels, and improves insulin sensitivity and glucose tolerance. Diabetes. 57(6):1526-35.

Lim K, Han C, Xu L, Isse K, Demetris AJ, Wu T. 2008. Cyclooxygenase-2-derived prostaglandin E2 activates beta-catenin in human cholangiocarcinoma cells: evidence for inhibition of these signaling pathways by omega 3 polyunsaturated fatty acids. Cancer Res. 68(2):553-60.

Lind L, Vessby B, Sundström J. 2006. The apolipoprotein B/AI ratio and the metabolic syndrome independently predict risk for myocardial infarction in middle-aged men. Arterioscler Thromb Vasc Biol. 26(2):406-10.

Lombardo YB, Chicco A. 2006. Effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. A Rev J Nutr Biochem 17:1–13.

Lombardo YB, Hein G, Chicco A. 2007. Metabolic syndrome: effects of n-3 PUFAs on a model of dyslipidemia, insulin resistance and adiposity. Lipids. 42(5):427-37.

Lopez-Miranda J, Williams C, Lairon D. 2007. Dietary, physiological, genetic and pathological influences on postprandial lipid metabolism. Br J Nutr. 98:458-73.

Lottenberg SA, Glezer A, Turatti LA. 2007. Metabolic syndrome: identifying the risk factors. J Pediatr (Rio J). 83(5 Suppl):S204-8.

Lovegrove J.A., Brooks, C.N., Murphy, M.C., Gould, B.J., and Williams, C.M. 1997. Use of manufactured foods enriched with fish oils as a means of increasing long-chain n-3 polyunsaturated fatty acid intake. Br. J. Nutr. 78: 223–236.

Lu J, Bankovic-Calic N, Ogborn M, Saboorian MH, Aukema HM. 2003. Detrimental effects of a high fat diet in early renal injury are ameliorated by fish oil in Han:SPRD-cy rats. J Nutr. 133(1):180-6.

MacLean CH, Mojica WA, Morton SC, Pencharz J, Hasenfeld Garland R, Tu W, Newberry SJ, Jungvig LK, Grossman J, Khanna P, Rhodes S, Shekelle P. 2004. Effects of omega-3 fatty acids on lipids and glycemic control in type II diabetes and the metabolic syndrome and on inflammatory bowel disease, rheumatoid arthritis, renal disease, systemic lupus erythematosus, and osteoporosis. Evid Rep Technol Assess (Summ). (89):1-4.

Madhu SV, Kant S, Srivastava S, Kant R, Sharma SB, Bhadoria DP. 2008. Postprandial lipaemia in patients with impaired fasting glucose, impaired glucose tolerance and diabetes mellitus. Diabetes Res Clin Pract. 80(3):380-5.

Madsen L, Petersen RK, Kristiansen K. 2005. Regulation of adipocyte differentiation and function by polyunsaturated fatty acids. Biochim Biophys Acta. 1740(2):266-86.

Mahley RW, Weisgraber KH, Innerarity TL, Rall SC Jr. 1991. Genetic defects in lipoprotein metabolism. Elevation of atherogenic lipoproteins caused by impaired catabolism. JAMA. 265(1):78-83.

Mamo JC, Proctor SD, Smith D. 1998. Retention of chylomicron remnants by arterial tissue; importance of an efficient clearance mechanism from plasma. Atherosclerosis. 1998 Dec;141 Suppl 1:S63-9.

Manco M, Calvani M, Mingrone G. 2004. Effects of dietary fatty acids on insulin sensitivity and secretion. Diabetes Obes Metab. 6(6):402-13.

Mangat R, Su J, Scott PG, Russell JC, Vine DF, Proctor SD. 2007. Chylomicron and apoB48 metabolism in the JCR:LA corpulent rat, a model for the metabolic syndrome. Biochem Soc Trans. 35(Pt 3):477-81.

Mater MK, Thelen AP, Pan DA, Jump DB. 1999. Sterol response element-binding protein 1c (SREBP1c) is involved in the polyunsaturated fatty acid suppression of hepatic S14 gene transcription. J Biol Chem. 274:32725-32.

Matsumura K. 2007. Effects of eicosapentaenoic acid on visceral fat and heart rate variability: assessment by power spectral analysis. J Cardiol. 50(4):243-51.

Martin SS, Qasim A, Reilly MP. 2008. Leptin resistance: a possible interface of inflammation and metabolism in obesity-related cardiovascular diseas. J Am Coll Cardiol. 52(15):1201-1.

Metcalf RG, James MJ, Gibson RA, Edwards JR, Stubberfield J, Stuklis R, Roberts-Thomson K, Young GD, Cleland LG. 2007. Effects of fish-oil supplementation on myocardial fatty acids in humans Am J Clin Nutr. 85(5):1222-8.

Molitch ME. 2006. Management of dyslipidemias in patients with diabetes and chronic kidney disease. Clin J Am Soc Nephrol. 1:1090-9.

Montori VM, Farmer A, Wollan PC, Dinneen SF. 2000. Fish oil supplementation in type 2 diabetes: a quantitative systematic review : Diabetes Care. 23(9):1407-15.

Mori TA, Bao DQ, Burke V, Puddey IB, Watts GF, Beilin LJ. 1999. Dietary fish as a major component of a weight-loss diet: effect on serum lipids, glucose, and insulin metabolism in overweight hypertensive subjects. Am J Clin Nutr. 70:817–825.

Mori A, Saito M, Sakamoto K, Narita M, Nakahara T, Ishii K. 2007. Stimulation of prostanoid IP and EP(2) receptors dilates retinal arterioles and increases retinal and choroidal blood flow in rats. Eur J Pharmacol. 2007 Sep 10;570(1-3):135-41.

Mozaffarian D. 2008. Fish and n-3 fatty acids for the prevention of fatal coronary heart disease and sudden cardiac death. Am J Clin Nutr. 87(6):1991S-6S.

Mune M, Meydani M, Gong J, Fotouhi N, Ohtani H, Smith D, Blumberg JB. 1999. Effect of dietary fish oil, vitamin E, and probucol on renal injury in the rat. J Nutr Biochem. 10(9):539-46.

Narayanan NK, Narayanan BA, Reddy BS. 2005. A combination of docosahexaenoic acid and celecoxib prevents prostate cancer cell growth in vitro and is associated with modulation of nuclear factor-kappaB, and steroid hormone receptors. Int J Oncol. 26(3):785-92.

Nakatani T, Kim HJ, Kaburagi Y, Yasuda K, Ezaki O. 2003. A low fish oil inhibits SREBP-1 proteolytic cascade, while a high-fish-oil feeding decreases SREBP-1 mRNA in mice liver: relationship to anti-obesity. J Lipid Res. 44:369-79.

Nawrocki AR, Rajala MW, Tomas E, Pajvani UB, Saha AK, Trumbauer ME, Pang Z, Chen AS, Ruderman NB, Chen H, Rossetti L, Scherer PE. 2006. Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor gamma agonists. J Biol Chem. 281:2654–60.

Needleman P, Raz A, Minkes MS, Ferrendelli JA, Sprecher H. 1979. Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. Proc Natl Acad Sci U S A. 76(2):944-8.

Nettleton JA, Katz R. 2005. n-3 long-chain polyunsaturated fatty acids in type 2 diabetes: a review. J Am Diet Assoc. 105(3):428-40.

Neschen S, Moore I, Regittnig W, Yu CL, Wang Y, Pypaert M, Petersen KF, Shulman GI. 2002. Contrasting effects of fish oil and safflower oil on hepatic peroxisomal and tissue lipid content. Am J Physiol Endocrinol Metab. 282:E395-401.

Neumayer HH, Heinrich M, Schmissas M, Haller H, Wagner K, Luft FC. 1992. Amelioration of ischemic acute renal failure by dietary fish oil administration in conscious dogs. J Am Soc Nephrol. 3(6):1312-20.

Ninomiya JK, L'Italien G, Criqui MH, Whyte JL, Gamst A, Chen RS. 2004. Association of the metabolic syndrome with history of myocardial infarction and stroke in the third national health and nutrition examination survey. Circulation. 109: 42–46.

Nguyen NT, Magno CP, Lane KT, Hinojosa MW, Lane JS. 2008. Association of hypertension, diabetes, dyslipidemia, and metabolic syndrome with obesity: findings from the National Health and Nutrition Examination Survey, 1999 to

2004. J Am Coll Surg. 207(6):928-34.

Nogalska A, Sucajtys-Szulc E, Swierczynski J. 2005. Leptin decreses lipogenic enzyme gene expression through modification of SREBP-1c gene expression in white adipose tissue of aging rats. Metabolism. 54(8):1041-7.

Norby LH, Weidig J, Ramwell P, Slotkoff L, Flamenbaum W. 1978. Possible role for impaired renal prostaglandin production in pathogenesis of hyporeninaemic hypoaldosteronism. Lancet. 2(8100):1118-22.

Nordøy A, Hansen JB, Brox J, Svensson B. 2001. Effects of atorvastatin and omega-3 fatty acids on LDL subfractions and postprandial hyperlipemia in patients with combined hyperlipemia Nutr Metab Cardiovasc Dis. 11(1):7-16.

Ntambi JM, Bené H. 2001. Polyunsaturated fatty acid regulation of gene expression. J Mol Neurosci. 16(2-3):273-8; discussion 279-84.

Okamoto M, Ohara-Imaizumi M, Kubota N, Hashimoto S, Eto K, Kanno T, Kubota T, Wakui M, Nagai R, Noda M, Nagamatsu S, Kadowaki T. 2008. Adiponectin induces insulin secretion in vitro and in vivo at a low glucose concentration. Diabetologia. 51:827–35.

Okosieme OE, Peter R, Usman M, Bolusani H, Suruliram P, George L, Evans LM. 2008. Can admission and fasting glucose reliably identify undiagnosed diabetes in patients with acute coronary syndrome? Diabetes Care. 31(10):1955-9.

Ordovas JM, Corella D. 2008. Metabolic syndrome pathophysiology: the role of adipose tissue. Kidney Int. 74(S111):S10-S14.

Paolisso G, Tagliamonte MR, Galderisi M, Zito GA, Petrocelli A, Carella C, de

Divitiis O, Varricchio M. 1999. Plasma leptin level is associated with myocardial wall thickness in hypertensive insulin-resistant men. Hypertension. 34(5):1047-52.

Park Y, Harris WS. 2003. Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. J Lipid Res. 44(3):455-63.

Parrish CC, Pathy DA, Parkes JG, Angel A. 1991. Dietary fish oils modify adipocyte structure and function. J Cell Physiol. 148(3):493-502.

Patsch JR, Miesenböck G, Hopferwieser T, Mühlberger V, Knapp E, Dunn JK, Gotto AM Jr, Patsch W. 1993. Relation of triglyceride metabolism and coronary artery disease. Arterioscler Thromb. 12:1336–45.

Patten GS, Adams MJ, Dallimore JA, Rogers PF, Topping DL, Abeywardena MY. 2005. Restoration of depressed prostanoid-induced ileal contraction in spontaneously hypertensive rats by dietary fish oil. Lipids. 40(1):69-79.

Pérez-Matute P, Pérez-Echarri N, Martínez JA, Marti A, Moreno-Aliaga MJ. 2007. Eicosapentaenoic acid actions on adiposity and insulin resistance in control and high-fat-fed rats: role of apoptosis, adiponectin and tumour necrosis factor-alpha. Br J Nutr. 97(2):389-98.

Petersen M, Pedersen H, Major-Pedersen A, Jensen T, Marckmann P. 2002. Effects of fish oil versus corn oil supplementation on LDL and HDL subclasses in type 2 diabetic patients. Diabetes Care. 25(10):1704-8.

Peyron-Caso E, Taverna M, Guerre-Millo M, Véronèse A, Pacher N, Slama G, Rizkalla SW. 2002. Dietary (n-3) polyunsaturated fatty acids up-regulate plasma leptin in insulin-resistant rats. J Nutr. 132(8):2235-40.

Poole EM, Bigler J, Whitton J, Sibert JG, Kulmacz RJ, Potter JD, Ulrich CM. 2007. Genetic variability in prostaglandin synthesis, fish intake and risk of colorectal polyps. Carcinogenesis. 28(6):1259-63.

Poppitt SD. 2005. Postprandial lipaemia, haemostasis, inflammatory response and other emerging risk factors for cardiovascular disease: the influence of fatty meals. Curr. Nutr. Food Sci. 1: 23–34.

Price PT, Nelson CM, Clarke SD. 2000. Omega-3 polyunsaturated fatty acid regulation of gene expression. Curr Opin Lipidol. 11:3-7.

Proctor SD, Vine DF, Mamo JC. 2002. Arterial retention of apolipoprotein B(48)- and B(100)-containing lipoproteins in atherogenesis. Curr Opin Lipidol. 13(5):461-70.

Proctor SD, Mamo JC. 1996. Arterial fatty lesions have increased uptake of chylomicron remnants but not low-density lipoproteins. Coron Artery Dis. 7(3):239-45.

Proctor SD, Mamo JC. 2003. Intimal retention of cholesterol derived from apolipoprotein B100- and apolipoprotein B48-containing lipoproteins in carotid arteries of Watanabe heritable hyperlipidemic rabbits. Arterioscler Thromb Vasc Biol. 23:1595-600.

Proctor SD, Vine DF, Mamo JC. 2004. Arterial permeability and efflux of apolipoprotein B-containing lipoproteins assessed by in situ perfusion and three-dimensional quantitative confocal microscopy. Arterioscler Thromb Vasc Biol. 24(11):2162-7.

Proctor SD, Dreher KL, Kelly SE, Russell JC. 2006. Hypersensitivity of prediabetic JCR:LA-*cp* rats to fine airborne combustion particle-induced direct and noradrenergic-mediated vascular contraction. Toxicol Sci. 90(2):385-91.

Proctor SD, Kelly SE, Stanhope KL, Havel PJ, Russell JC. 2007. Synergistic effects of conjugated linoleic acid and chromium picolinate improve vascular function and renal pathophysiology in the insulin-resistant JCR:LA-*cp* rat. Diabetes Obes Metab. 9(1):87-95.

Psota TL, Gebauer SK, Kris-Etherton P. 2006. Dietary omega-3 fatty acid intake and cardiovascular risk. Am J Cardiol. 98:3i-18i.

Qiu W, Federico L, Naples M, Avramoglu RK, Meshkani R, Zhang J, Tsai J, Hussain M, Dai K, Iqbal J, Kontos CD, Horie Y, Suzuki A, Adeli K. 2008. Phosphatase and tensin homolog (PTEN) regulates hepatic lipogenesis, microsomal triglyceride transfer protein, and the secretion of apolipoprotein B-containing lipoproteins. Hepatology. 48(6):1799-809.

Rabe K, Lehrke M, Parhofer KG, Broedl UC. 2008. Adipokines and insulin resistance. Mol Med. 14(11-12):741-51.

Rahmouni K, Fath MA, Seo S, Thedens DR, Berry CJ, Weiss R, Nishimura DY, Sheffield VC. 2008. Leptin resistance contributes to obesity and hypertension in mouse models of Bardet-Biedl syndrome. J Clin Invest. 118(4):1458-67.

Ramaprasad TR, Srinivasan K, Baskaran V, Sambaiah K, Lokesh BR. 2006. Spray-dried milk supplemented with alpha-linolenic acid or eicosapentaenoic acid and docosahexaenoic acid decreases HMG Co A reductase activity and increases biliary secretion of lipids in rats. Steroids. 71(5):409-15.

Ramel A, Martinéz A, Kiely M, Morais G, Bandarra NM, Thorsdottir I. 2008.

Beneficial effects of long-chain n-3 fatty acids included in an energy-restricted diet on insulin resistance in overweight and obese European young adults. Diabetologia. 51(7):1261-8.

Rayner DV, Trayhurn, P. 1996. Ob (obese) gene expression in white adipose tissue of obese Zucker (fa/fa) rats. Biochem. Soc. Trans. 24:156S.

Razani B, Chakravarthy MV, Semenkovich CF. 2008. Insulin resistance and atherosclerosis. Endocrinol Metab Clin North Am. 37(3):603-21, viii.

Reaven P, Merat S, Casanada F, Sutphin M, Palinski W. 1997. Effect of streptozotocin-induced hyperglycemia on lipid profiles, formation of advanced glycation endproducts in lesions, and extent of atherosclerosis in LDL receptor-deficient mice. Arterioscler Thromb Vasc Biol. 17: 2250-6.

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

Reisin E, Alpert MA. 2005. Definition of the metabolic syndrome: current proposals and controversies. Am J Med Sci. 330(6):269-72.

Remuzzi G, Ruggenenti P, Benigni A. 1991. Understanding the nature of renal disease progression. Kidney Int. 51(1):2-15.

Renard C, Van Obberghen E. 2006. Role of diabetes in atherosclerotic pathogenesis. What have we learned from animal models? Diabetes Metab. 32(1):15-29.

Renard CB, Kramer F, Johansson F, Lamharzi N, Tannock LR, von Herrath MG, Chait A, Bornfeldt KE. 2004. Diabetes and diabetes-associated lipid

abnormalities have distinct effects on initiation and progression of atherosclerotic lesions. J Clin Invest. 114: 659-68.

Reseland JE, Haugen F, Hollung K, Solvoll K, Halvorsen B, Brude IR, Nenseter MS, Christiansen EN, Drevon CA. 2001. Reduction of leptin gene expression by dietary polyunsaturated fatty acids. J Lipid Res. 42(5):743-50.

Ribeiro Filho FF, Mariosa LS, Ferreira SR, Zanella MT. 2006. Visceral fat and metabolic syndrome: more than a simple association. Arq Bras Endocrinol Metabol. 50(2):230-8.

Rice R. 1996. Fish and healthy pregnancy: more than just a red herring! Prof Care Mother Child. 6(6):171-3.

Rivellese AA, Maffettone A, Iovine C, Di Marino L, Annuzzi G, Mancini M, Riccardi G. 1996. Long term effects of fish oil on insulin resistance and plasma lipoprotein in NIDDM patients with hypertriglyceridemia. Diabetes Care. 19(11):1207-13.

Rivellese AA, Maffettone A, Vessby B, Uusitupa M, Hermansen K, Berglund L, Louheranta A, Meyer BJ, Riccardi G. 2003. Effects of dietary saturated, monounsaturated and n-3 fatty acids on fasting lipoproteins, LDL size and postprandial lipid metabolism in healthy subjects. Atherosclerosis. 167(1):149-58.

Roche HM, Gibney MJ. 1996. Postprandial triacylglycerolaemia: the effect of low-fat dietary treatment with and without fish oil supplementation. Eur J Clin Nutr. 50:617–24.

Roche HM, Gibney MJ. 2000. Effect of long-chain n-3 polyunsaturated fatty acids on fasting and postprandial triacylglycerol metabolism. Am J Clin Nutr. 71 (suppl):232S–7S.

Rodríguez A, Catalán V, Gómez-Ambrosi J, Frühbeck G. 2007. Visceral and subcutaneous adiposity: are both potential therapeutic targets for tackling the metabolic syndrome? Curr Pharm Des. 13(21):2169-75.

Roman G, Hâncu N. 2004. Metabolic syndrome--new insights into a growing entity. Rom J Intern Med. 42(2):257-66.

Russell JC, Amy RM. 1986. Myocardial and vascular lesions in the LA/N-corpulent rat. Can J Physiol Pharmacol. 64:1270-80.

Russell JC, Ahuja SK, Manickavel V, Rajotte RV, Amy RM. 1987. Insulin resistance and impaired glucose tolerance in the atherosclerosis prone LA/N-corpulent rat. Arteriosclerosis. 6:620-6.

Russell JC, Amy RM, Manickavel V, Dolphin PJ. 1989. Effects of chronic ethanol consumption in atherosclerosis-prone JCR:LA-corpulent rat. Arteriosclerosis. 9:122-8.

Russell JC, Koeslag DG, Dolphin PJ, Amy RM. 1990a. Prevention of myocardial lesions in JCR:LA-corpulent rats by nifedipine. Arteriosclerosis. 10:658-64.

Russell JC, Amy RM, Michaelis OE IV, McCune SM, Abraham AA. 1990b. Myocardial disease in the corpulent strains of rats. In: Shafrir E, ed. Frontiers in diabetes research: lessons from animal diabetes III. London: Smith-Gordon, 1990:402-7.

Russell JC, Amy RM, Dolphin PJ. 1991a. Effect of dietary n-3 fatty acids on atherosclerosis prone JCR:LA-corpulent rats. Exp Mol Pathol. 55:285-93.

Russell JC, Koeslag DG, Amy RM, Dolphin PJ. 1991b. Independence of

myocardial disease in the JCR:LA-corpulent rat on plasma cholesterol concentration. Clin Invest Med. 14(4):288-95.

Russell JC, Amy RM, Graham S, Wenzel LM, Dolphin PJ. 1993. Effect of castration on hyperlipidemic, insulin resistant JCR:LA-corpulent rats. Atherosclerosis. 100(1):113-22.

Russell JC, Graham S, Hameed M. 1994. Abnormal insulin and glucose metabolism in the JCR:LA-corpulent rat. .43:538-43.

Russell JC, Amy RM, Graham SE, Dolphin PJ, Wood GO, Bar-Tana J. 1995 a. Arterioscler Thromb Vasc Biol. 15(7):918-23.

Russell JC, Amy RM, Graham SE, Dolphin PJ, Bar-Tana J. 1995b. Inhibition of atherosclerosis and myocardial lesions in the JCR:LA-*cp* rat by [beta],[beta]'-tetramethylhexadecanedioic acid (MEDICA 16). Arterioscler Thromb Vasc Biol 15:918-23.

Russell JC. 1995c. The atherosclerosis-prone JCR:LA-corpulentrat. In Woodford FP, Davignon J, Sniderman A eds. Atherosclerosis X: Proceedings of the Tenth International Symposium on Atherosclerosis. Amsterdam: Elsevier Science, 1995: 121-125.

Russell JC, Graham SE, Richardson M. 1998a. Cardiovascular disease in the JCR:LA-*cp* rat Mol Cell Biochem. 188(1-2):113-26.

Russell JC, Dolphin PJ, Graham SE, Amy RM, Brindley DN. 1998b. Improvement of insulin sensitivity and cardiovascular outcomes in the JCR:LA-*cp* rat by D-fenfluramine. Diabetologia. 41(4):380-9.

Russell JC, Shillabeer G, Bar-Tana J, Lau DC, Richardson M, Wenzel LM, Graham SE, Dolphin PJ. 1998c. Development of insulin resistance in the JCR:LA-*cp* rat: role of triacylglycerols and effects of MEDICA 16. Diabetes. 47:770-8.

Russell JC, Ewart HS, Kelly SE, Kralovec J, Wright JL, Dolphin PJ. 2002. Improvement of vascular dysfunction and blood lipids of insulin-resistant rats by a marine oil-based phytosterol compound. Lipids. 37:147-52.

Russell JC, Proctor SD, Kelly SE, Löhn M, Busch AE, Schäfer S. 2005. Insulin-sensitizing and cardiovascular effects of the sodium-hydrogen exchange inhibitor, cariporide, in the JCR: LA-*cp* rat and db/db mouse. J Cardiovasc Pharmacol. 46(6):746-53.

Russell JC, Proctor SD. 2007. Increased insulin sensitivity and reduced micro and macro vascular disease induced by 2-deoxy-D-glucose during metabolic syndrome in obese JCR: LA-*cp* rats. Br J Pharmacol. 151:216-25.

Rustan AC, Klimes I, Drevon CA, Sebökova E. 2003. The hypotriglyceridemic effect of dietary n-3 FA is associated with increased beta-oxidation and reduced leptin expression. Lipids. 38(10):1023-9.

Salmerón J, Hu FB, Manson JE, Stampfer MJ, Colditz GA, Rimm EB, Willett WC. 2001. Dietary fat intake and risk of type 2 diabetes in women. Am J Clin Nutr. 73(6):1019-26.

Sankaran D, Lu J, Ogborn MR, Aukema HM. 2007. COX-2 expression in cystic kidneys is dependent on dietary n-3 fatty acid composition. J Nutr Biochem. 2007 Dec;18(12):806-12.

Sawara Y, Takei T, Uchida K, Ogawa T, Yoshida T, Tsuchiya K, Nitta K. 2008.

Effects of lipid-lowering therapy with rosuvastatin on atherosclerotic burden in patients with chronic kidney disease. Intern Med. 47(17):1505-10.

Schambelan M, Blake S, Sraer J, Bens M, Nivez MP, Wahbe F. 1985. Increased prostaglandin production by glomeruli isolated from rats with streptozotocin-induced diabetes mellitus. J Clin Invest. 75(2):404-12.

Schwalfenberg G. 2006. Omega-3 fatty acids: their beneficial role in cardiovascular health. Can Fam Physician. 52:734-40.

Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D Jr. 1996. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. Nat Med. 2:589-93.

Scott M. 1997. Grundy Small LDL, Atherogenic Dyslipidemia, and the Metabolic Syndrome. Circulation. 95:1-4.

Seufert J, Kieffer TJ, Leech CA, Holz GG, Moritz W, Ricordi C, Habener JF. 1999. Leptin suppression of insulin secretion and gene expression in human pancreatic islets: implications for the development of adipogenic diabetes mellitus. J Clin Endocrinol Metab. 84(2):670-6.

Sharrett AR, Heiss G, Chambless LE, Boerwinkle E, Coady SA, Folsom AR, Patsch W. 2001. Metabolic and lifestyle determinants of postprandial lipemia differ from those of fasting triglycerides: The Atherosclerosis Risk In Communities (ARIC) study. Arterioscler Thromb Vasc Biol. 21(2):275-81.

Shohat J, Boner G.1993. Role of lipids in the progression of renal disease in chronic renal failure: evidence from animal studies and pathogenesis. Isr J Med Sci. 29(4):228-39.

Shimano H. 2001. Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. Prog Lipid Res. 40:439-52.

Shimomura I, Shimano H, Horton JD, Goldstein JL, Brown MS. 1997. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. J Clin Invest. 99(5):838-45.

Shimomura I, Bashmakov Y, Ikemoto S, Horton JD, Brown MS, Goldstein JL. 1999. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. Proc Natl Acad Sci U S A. 96:13656-61.

Shin ES, Lee HH, Cho SY, Park HW, Lee SJ, Lee TR. 2007. Genistein downregulates SREBP-1 regulated gene expression by inhibiting site-1 protease expression in HepG2 cells. J Nutr. 137(5):1127-31.

Sierra-Johnson J, Somers VK, Kuniyoshi FH, Garza CA, Isley WL, Gami AS, Lopez-Jimenez F. 2006. Comparison of apolipoprotein-B/apolipoprotein-AI in subjects with versus without the metabolic syndrome. Am J Cardiol. 298(10):1369-73.

Simoncíkova P, Wein S, Gasperikova D, Ukropec J, Certik M, Klimes I, Sebokova E. 2002. Comparison of the extrapancreatic action of gamma-linolenic acid and n-3 PUFAs in the high fat diet-induced insulin resistance. Endocr Regul. 36:143-9.

Singh N, Singh H, Khanijoun HK, Iacobellis G. 2007. Echocardiographic assessment of epicardial adipose tissue - a marker of visceral adiposity. Mcgill J Med. 10(1):26-30.

Summers LK, Fielding BA, Bradshaw HA, Ilic V, Beysen C, Clark ML, Moore NR, Frayn KN. 2002. Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. Diabetologia. 45:369-77.

Sinha AK, Scharschmidt LA, Neuwirth R, Holthofer H, Gibbons N, Arbeeny CM, Schlondorff D. 1990. Effects of fish oil on glomerular function in rats with diabetes mellitus. J Lipid Res. 31(7):1219-28.

Sirtori CR, Crepaldi G, Manzato E, Mancini M, Rivellese A, Paoliett R, Pazzucconi F, Pamparana F, Stragliotto E. 1998. One-year treatment with ethyl esters of n-3 fatty acids in patients with hypertriglyceridemia and glucose intolerance: Reduced triglyceridemia, total cholesterol and increased HDL-C without glycemic alteration. Atherosclerosis. 137:419- 427.

Siscovick DS, Raghunathan TE, King I, *et al.* . Dietary intake and cell membrane levels of long-chain n–3 polyunsaturated fatty acids and the risk of primary cardiac arrest. JAMA 1995;274:1363–7.

Smith D, Proctor SD, Mamo JC. 1997. A highly sensitive assay for quantitation of apolipoprotein B48 using an antibody to human apolipoprotein B and enhanced chemiluminescence. Ann Clin Biochem. 34 (Pt 2):185-9.

Smith SR, Lovejoy JC, Greenway F, Ryan D, deJonge L, de la Bretonne J, Volafova J, Bray GA. 2001. Contributions of total body fat, abdominal subcutaneous adipose tissue compartments, and visceral adipose tissue to the metabolic complications of obesity. Metabolism. 50(4):425-35.

Smith WL. Curr Opin Cell Biol. 2005. Cyclooxygenases, peroxide tone and the allure of fish oil. 17(2):174-82.

Sneddon AA, Tsofliou F, Fyfe CL, Matheson I, Jackson DM, Horgan G, Winzell MS, Wahle KW, Ahren B, Williams LM. 2008. Effect of a conjugated linoleic acid and omega-3 fatty acid mixture on body composition and adiponectin. Obesity (Silver Spring). 16(5):1019-24.

Sniderman AD, Marcovina SM. 2006. Apolipoprotein A1 and B. ClinLab Med 26:733-750.

Sniderman AD, Faraj M. 2007. Apolipoprotein B, apolipoprotein A-I, insulin resistance and the metabolic syndrome. Curr Opin Lipidol. 18(6):633-7.

Snow V, Weiss KB, Mottur-Pilson C; Clinical Efficacy Assessment Subcommittee of the American College of Physicians. 2003. The evidence base for tight blood pressure control in the management of type 2 diabetes mellitus. Ann Intern Med. 138(7):587-92.

Sonnenberg GE, Krakower GR, Hoffmann RG, Maas DL, Hennes MM, Kissebah AH. 2001. Plasma leptin concentrations during extended fasting and graded glucose infusions: relationships with changes in glucose, insulin, and FFA. J Clin Endocrinol Metab. 86(10):4895-900.

Soria A,Chicco A, Eugenia D'Alessandro M, Rossi A, Lombardo YB. 2002. Dietary fish oil reverse epididymal tissue adiposity, cell hypertrophy and insulin resistance in dyslipemic sucrose fed rat model. J Nutr Biochem. 13(4):209-218.

Soriguer F, Moreno F, Rojo-Martínez G, Cardona F, Tinahones F, Gómez-Zumaquero JM, García-Fuentes E, Morcillo S. 2003. Redistribution of abdominal fat after a period of food restriction in rats is related to the type of dietary fat. Br J Nutr. 89:115-22.

Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. 1989. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989; 320: 915-24.

Storlien LH, Kriketos AD, Jenkins AB, Baur LA, Pan DA, Tapsell LC, Calvert GD. 1997. Does dietary fat influence insulin action? Ann N Y Acad Sci. 827:287-301.

Suchankova G, Tekle M, Saha AK, Ruderman NB, Clarke SD, Gettys TW. 2005. Dietary polyunsaturated fatty acids enhance hepatic AMP-activated protein kinase activity in rats. Biochem Biophys Res Commun. 326(4):851-8.

Sul HS, Latasa MJ, Moon Y, Kim KH. 2000. Regulation of the fatty acid synthase promoter by insulin. J Nutr. 130:315S-320S.

Sullivan DR, Celermajer DS, Le Couteur DG, Lam CW. 2004. The Vascular Implications of Post-prandial Lipoprotein Metabolism. Clin Biochem Rev. 25(1):19-30.

Swails WS, Bell SJ, Bistrian BR, Lewis EJ, Pfister D, Forse RA, Kelly S, Blackburn GL. 1993. Fish-oil-containing diet and platelet aggregation. Nutrition. 9(3):211-7.

Taskinen MR. 2003. Diabetic dyslipidaemia: from basic research to clinical practice. Diabetologia. 46(6):733-49.

Thomas CP, Davison Z, Heard CM. 2007. Probing the skin permeation of fish oil/EPA and ketoprofen-3. Effects on epidermal COX-2 and LOX. Prostaglandins Leukot Essent Fatty Acids. 76(6):357-62.

Thorsdottir I, Hill J, Ramel A. 2004. Omega-3 fatty acid supply from milk associates with lower type 2 diabetes in men and coronary heart disease in

women. Prev Med. 39:630–6348.

Thorsdottir I, Tomasson H, Gunnarsdottir I, Gisladottir E, Kiely M, Parra MD, Bandarra NM, Schaafsma G, Martinéz JA. 2007. Randomized trial of weight-loss-diets for young adults varying in fish and fish oil content. Int J Obes (Lond). 31:1560-6.

Tinker LF, Parks EJ, Behr SR, Schneeman BO, Davis PA. 1999. (n-3) fatty acid supplementation in moderately hypertriglyceridemic adults changes postprandial lipid and apolipoprotein B responses to a standardized test meal. J Nutr. 129(6):1126-34.

Torres DM, Tooley KL, Butler RN, Smith CL, Geier MS, Howarth GS. 2008. Lyprinol only partially improves indicators of small intestinal integrity in a rat model of 5-fluorouracil-induced mucositis. Cancer Biol Ther. 7(2):295-302.

Turvey EA, Heigenhauser JF, Parolin M, Peters SJ. 2005. Elevated n-3 fatty acids in a high-fat diet attenuate the increase in PDH kinase activity but not PDH activity in human skeletal muscle. J Appl Physiol. 98:350–355.

Ukropec J, Reseland JE, Gasperikova D, Demcakova E, Madsen L, Berge RK, Rustan AC, Klimes I, Drevon CA, Sebökova E. The hypotriglyceridemic effect of dietary n-3 FA is associated with increased beta-oxidation and reduced leptin expression. Lipids. 2003;38:1023-9.

Vance JE, Russell JC. 1990a. Hypersecretion of VLDL, but not HDL, by hepatocytes from the JCR:LA-corpulent rat. J Lipid Res. 31(8):1491-501.

Vance JE, Vance DE. 1990b. The assembly of lipids into lipoproteins during secretion. Experientia. 46(6):560-9.

van Oostrom AJ, Alipour A, Plokker TW, Sniderman AD, Cabezas MC. 2007. The metabolic syndrome in relation to complement component 3 and postprandial lipemia in patients from an outpatient lipid clinic and healthy volunteers. Atherosclerosis. 190(1):167-73.

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

Volk MG. 2007. An examination of the evidence supporting the association of dietary cholesterol and saturated fats with serum cholesterol and development of coron. Altern Med Rev. 12(3):228-45.

Vessby B. 1989. n-3 Fatty acids and blood glucose control in diabetes mellitus. J Int Med Suppl. 225:207- 210.

Vessby B. 2000. Dietary fat and insulin action in humans. Br J Nutr 83:S91–S96.

Vessby B, Unsitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, Nälsén C, Berglund L, Louheranta A, Rasmussen BM, Calvert GD, Maffetone A, Pedersen E, Gustafsson IB, Storlien LH; KANWU Study. 2001. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. Diabetologia. 44(3):312-9.

Vine DF, Takechi R, Russell JC, Proctor SD. 2007. Impaired postprandial apolipoprotein-B48 metabolism in the obese, insulin-resistant JCR:LA-*cp* rat: increased atherogenicity for the metabolic syndrome. Atherosclerosis. 190:282-90.

Vine DF, Glimm DR, Proctor SD. 2008. Intestinal lipid transport and chylomicron production: possible links to exacerbated atherogenesis in a rodent model of the metabolic syndrome. Atheroscler Suppl. 9(2):69-76.

Wada J, Zhang H, Tsuchiyama Y, Hiragushi K, Hida K, Shikata K, Kanwar YS, Makino H. 2001. Gene expression profile in streptozotocin-induced diabetic mice kidneys undergoing glomerulosclerosis. Kidney Int. 59(4):1363-73.

Waite N, Lodge J, Hart K, Robertson D, Badley E, Burton S. 2008. The impact of fish-oil supplements on insulin sensitivity. J Hum Nutr Diet. 21(4):402-403.

Wang JL, Chinookoswong N, Scully S, Qi M, Shi ZQ. 1999. Differential effects of leptin in regulation of tissue glucose utilization in vivo. Endocrinology. 140(5):2117-24.

Wang Z, Jiang T, Li J, Proctor G, McManaman JL, Lucia S, Chua S, Levi M. 2005. Regulation of renal lipid metabolism, lipid accumulation, and glomerulosclerosis in FVBdb/db mice with type 2 diabetes. Diabetes. 54(8):2328-35.

Warford-Woolgar L, Peng CY, Shuhyta J, Wakefield A, Sankaran D, Ogborn M, Aukema HM. 2006. Selectivity of cyclooxygenase isoform activity and prostanoid production in normal and diseased Han:SPRD-cy rat kidneys. Am J Physiol Renal Physiol. 290(4):F897-904.

Weintraub MS, Zechner R, Brown A, Eisenberg S, Breslow JL. 1988. Dietary polyunsaturated fats of the W-6 and W-3 series reduce postprandial lipoprotein levels. J Clin Invest. 82:1884–93.

Williams G, Cardoso HM, Domin J, Ghatei MA, Russell JC, Bloom SR. 1990. Hypothalamic regulatory peptide disturbances in the spontaneously obese JCR: LA-corpulent rat. Diabetes Res. 15(1):1-7.

Williams KJ, Tabas I. 1995. The response-to-retention hypothesis of early atherogenesis. Arterioscler Thromb Vasc Biol. 1995 May;15(5):551-61.

Weise WJ, Natori Y, Levine JS, O'Meara YM, Minto AW, Manning EC, Goldstein DJ, Abrahamson DR, Salant DJ. 1993. Fish oil has protective and therapeutic effects on proteinuria in passive Heymann nephritis. Kidney Int. 43(2):359-68.

Wey HE, Jakubowski JA, Deykin D. 1986. Effect of streptozotocin-induced diabetes on prostaglandin production by rat cerebral microvessels. Thromb Res. 42(4):527-38.

Włodarczyk A, Strojek K. 2008. Glucose intolerance, insulin resistance and metabolic syndrome in patients with stable angina pectoris. Obesity predicts coronary atherosclerosis and dysglycemia. Pol Arch Med Wewn. 118(12):719-26.

Xiao YF, Sigg DC, Ujhelyi MR, Wilhelm JJ, Richardson ES, Iaizzo PA. 2008. Pericardial delivery of omega-3 fatty acid: a novel approach to reducing myocardial infarct sizes and arrhythmias. Am J Physiol Heart Circ Physiol. 294(5):H2212-8.

Xu J, Nakamura MT, Cho HP, Clarke SD. Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. 1999. A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. J Biol Chem. 274:23577-83.

Xu J, Teran-Garcia M, Park JH, Nakamura MT, Clarke SD. 2001. Polyunsaturated fatty acids suppress hepatic sterol regulatory element-binding protein-1 expression by accelerating transcript decay. J Biol Chem. 276(13):9800-7.

Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T,

Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. 2001. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med. 7:941–6.

Zheng X, Rivabene R, Cavallari C, Napolitano M, Avella M, Bravo E, Botham KM. 2002. The effects of chylomicron remnants enriched in n-3 or n-6 polyunsaturated fatty acids on the transcription of genes regulating their uptake and metabolism by the liver: influence of cellular oxidative state. Free Radic Biol Med. 32(11):1123-31.

Zietz B, Herfarth H, Paul G, Ehling A, Müller-Ladner U, Schölmerich J, Schäffler A. 2003. Adiponectin represents an independent cardiovascular risk factor predicting serum HDL-cholesterol levels in type 2 diabetes. FEBS Lett. 545(2-3):103-4.