Changes in Body Fatty Acid Composition of Rats Undergoing Different Modes of Food Restriction

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ABSTRACT

Obesity is a growing health concern in the developed countries and is believed to be one of the culprits of many life-threatening diseases. Putting off weight is recommended to improve and combat the related health problems. The present study was to examine the effect of food restriction, in another word, dieting, on fatty acid composition. Normal young adult Sprague Dawley rats were used as an animal model. They received different modes of food restrictions, namely (1) yo-yo dieting with a 50% chow diet restriction, (2) different levels of food restriction with a medium fat diet, from 50%, 40%, 30%, 20%, 10% and no food restriction, and (3) a 50% food restriction with different amount of fat in diets, from 0%, 5%, 15%, 30% to 45% fat.

All these modes of food restriction did not induce central obesity, but a weight loss. However, the results showed that undergoing a 2-cycled yo-yo dieting, receiving food with half of the required or taking high fat meal during a dieting programme can cause a significant depletion of the n-3 fatty acids in the body. This fatty acid profile remodellation can be explained by the preferential synthesis and accumulation of saturated fatty acids as well as mobilisation and oxidation of linoleic acid, α-linolenic acid and other n-3 poly-unsaturated fatty acids in the adipose tissues and carcass during energy restriction, where n-3 PUFAs serve as good fatty acids in the prevention and treatment of coronary heart disease. Similar outcome can be observed in these three studies despite the fact

that the latter two experiments did not include an *ad libitum* refeeding period after food restriction. Significant and dramatic accumulation of saturated fatty acids and depletion of n-3 fatty acids were also observed in rats feeding with the fat-free diets.

By using the cholesterol-free canola oil as the source of fat together with restricting the food intake of the rats, the levels of serum triglyceride and cholesterol decreased in a similar fashion in the rats receiving limited food supply throughout the experiment. However, a sudden and significant drop of the serum triglyceride level was observed in rats receiving 70% or less food supply. From the correlation of serum triglyceride level and cardiovascular risk, this study supported the idea of having moderate food restriction, which has minimum effect on the body fatty acid imbalance.

The present study illustrated that food restriction promoted a disturbance in the balance of the individual fatty acids with a general depletion of good n-3 fatty acids and accumulation of saturated fatty acids in the adipose tissues and the remaining body. Such a change might elevate the risk of having cardiovascular diseases in the dieters pursuing a healthier life. Careful selection and monitoring must be carried out to enable a fruitful health improvement by dieting.

不同模式的飲食節制對身體脂肪酸成份的影響

摘要

過度肥胖在已發展國家中，已經引起越來越大的關注。它也同時被認爲是誘發多種致命疾病的要因。於是減肥便成爲改善身體狀況及對付這些疾病的手段。這次研究的重點是在於飲食節制，或節食減肥，對身體脂肪酸成份的影響。體重正常的白老鼠被選擇作爲這次的活體實驗對象，接受不同模式的飲食節制：1)以體重波動形式的百分之五十的飲食節制、2)中脂肪度食物的不同程度(百分之五十、四十、三十、二十、十和零)的節制、3)食物中不同脂肪比例(零、百分之五、十五、三十和四十五) 的百分之五十的節制。

以上所有情況的飲食節制均未能形成過度肥胖，相反，導致體重下降。但是，實驗結果顯示兩次體重波動、近乎百分之五十的飲食節制、和進行節食時採用高脂肪度的食物，皆導致體內奧米加三脂肪酸比例的明顯下降。這個脂肪酸比例的調整可能是由於脂肪組織及身體內有選擇性的飽和脂肪酸的合成和積聚，與及亞麻油酸、亞麻脂酸和其他奧米加三脂肪酸的動用和氧化作用。雖然實驗二和三不包括節制後的隨意進食，但在三個實驗中均可以找到類似的結果。而且，在進行零脂肪飲食節制的老鼠體內，飽和脂肪酸的積聚及奧米加三脂肪酸的消耗更有大幅的變化。

採取不含膽固醇的芥花籽油及飲食節制，老鼠的血三酸甘油脂和血膽固醇均發現有下降的結果。然而，在進食少於百分之七十的老鼠血液樣本中，發現了三酸甘油脂突然的大幅下降。這個結果驗證了中度的飲食節制有助減低患上心血管病的機會，和對身體脂肪酸的不平衡，有最輕微的影響。

是次研究引證了飲食節制導致了脂肪組織及體內的脂肪酸比例不平衡，有選擇性的飽和脂肪積聚，和奧米加三脂肪酸的消耗。文獻記載奧米加三脂肪酸有助預防及治療心血管等致命疾病，所以這次研究發現了飲食節制可能會提高節食者患上心血管等疾病的機會。因此，小心的選擇和監察對一個有效的節食療程是不可缺少的。

TABLE OF ABBREVIATIONS

5HT	Serotonin
AA	Arachidonic acid
ACC	Acetyl-CoA carboxylase
ACP	Acyl carrier protein
AQP	Aquaporin
AT III	Antithrombin III
BED	Binge eating disorder
BMI	Body mass index
BMR	Basal metabolic rate
CHD	Coronary heart disease
CRP	C-reactive protein
CTL	Control
DART	Diet and Reinfarction Trial
DGLA	Dihomo- γ-linoleic acid
DHA	Docosaheptaenoic acid
DPA	Docosapentaenoic acid
EDRF	Endothelium-derived relaxing factor
EPA	Eicosapentaenoic acid
FAS	Fatty acid synthase
FFA	Free fatty acids
FR	Food restriction, food-restricted
GLA	γ-linolenic acid
HDL	High-density lipoproteins
HF	High fat
IHD	Ischaemic heart disease
LA	Linoleic acid
LDL	Low-density lipoproteins
LDL-RD	LDL-receptor-deficient
LF	Low fat
LNA	α-Linolenic acid
LPL	Lipoprotein lipase
ME	Malic enzyme
MF	Medium fat
MUFA	Monounsaturated fatty acid
NES	Night eating syndrome
NIDDM	Non-insulin-dependent diabetes mellitus
NO	Nitric oxides
OA	Oleic acid
PA	Palmitic acid
PAI	Plasminogen activator inhibitor

PDRF	Platelet-derived growth factor
PG	Prostaglandins
PK	Pyruvate kinase
POA	palmitoleic acids
PUFA	Polyunsaturated fatty acid
REE	Resting energy expenditure
RLP	Remnant lipoprotein
SA	Stearic acid
SD	Sprague-Dawley
SFA	Saturated fatty acid
SMC	Smooth muscle cell
TG	Triglycerides/Triacylglycerols
tPA	Tissue plasminogen activator
VLCDs	Very-low-calorie diets
VLDL	Very-low-density lipoproteins
WC	Weight cycling
WHR	Waist-to-hip circumference ratio

TABLE OF CONTENT

1 GENERAL INTRODUCTION 1

1.1 Classes of Fatty Acids 3

1.2 Polyunsaturated Fatty Acids (n-6 & n-3) 4

1.2.1 High Fish Oil Content in Diet, High n-3 PUFAs Intake, Fight against Cardiovascular Risk 4

1.2.2 n-3 Fatty Acids Improve Hypertension 7

1.2.3 n-3 Fatty Acids Protect from Atherosclerosis 8

1.2.4 PUFAs are Beneficial in Inflammation 11

1.2.5 n-3 PUFAs Help to Control Tumour Growth 13

1.3 Obesity and Eating Disorder 14

1.3.1 Obesity, a Companion of the Modern World 14

1.3.2 Health Risks Related to Obesity 16

1.3.3 Management of Obesity 19

1.3.4 Care Must be Taken to Prevent the Development of Eating Disorder or Other Psychological Disturbances during Weight Loss Programme 21

2 WEIGHT CYCLING WITH CHOW DIET 24

2.1 Introduction 24

2.1.1 Definition of Weight Cycling 25

2.1.2 Incentives Leading to Weight Cycling 26

2.1.3 Problems Aroused by Weight Cycling 26

2.1.3.1 Food Preference, Efficiency and Expenditure 27

2.1.3.2 Increased Overall and Central Adiposity 28

2.1.3.3 Increased Morbidity and Mortality of Cardiovascular Disease 29

2.1.3.4 Psychological Impact and Social Consequences 30

2.2 Objective 30

2.3 Materials and Methods 31

2.3.1 Animal Handling 31

2.3.2 Lipid Analysis 35

2.3.2.1 Adipose Tissues 35

2.3.2.2 Carcass 36

2.3.3 Proximate Analysis 37

2.3.3.1 Crude Fat 37

2.3.3.2 Crude Protein 38

2.3.3.3 Moisture 40

2.3.3.4 Ash 40

2.3.4 Serum Analysis 41

2.3.4.1 Serum Triglycerides 41

2.3.4.2 Serum Cholesterol 42

2.4 Results 44

2.4.1 Body Weight 44

2.4.2 Food Intake 44

2.4.3 Organ Weight 47

2.4.3.1 Liver 47

2.4.3.2 Adipose Tissues....47
2.4.4 Lipid Analysis....52
2.4.4.1 Adipose Tissues....52
2.4.4.2 Carcass....52
2.4.5 Proximate Analysis....60
2.4.5.1 Crude Fat....60
2.4.5.2 Moisture....60
2.4.5.3 Crude Protein and Ash....62
2.4.6 Serum Analysis....64
2.4.6.1 Serum Triglycerides....64
2.4.6.2 Serum Cholesterol....64
2.5 Discussion....66

3 DEGREES OF FOOD RESTRICTION ON BODY FATTY ACID COMPOSITION. 71
3.1 Introduction....71
3.1.1 Skipping Breakfast....71
3.1.2 Nibbling, Grazing vs Gorging....72
3.1.3 Reducing Food Intake in Meals....74
3.1.3.1 Anti-Aging Action....74
3.1.3.2 Effects on Other Health Issues....75
3.1.3.3 Energy Expenditure....77
3.2 Objective....78
3.3 Materials and Methods....79
3.3.1 Animal Handling....79
3.4 Results....81
3.4.1 Body Weight....81
3.4.2 Food Intake....81
3.4.3 Organ Weight....83
3.4.3.1 Liver....83
3.4.3.2 Adipose Tissues....83
3.4.4 Lipid Analysis....88
3.4.4.1 Adipose Tissues....88
3.4.4.2 Carcass....88
3.4.5 Proximate Analysis....102
3.4.5.1 Crude Fat....102
3.4.5.2 Moisture....102
3.4.5.3 Crude Protein and Ash....103
3.4.6 Serum Analysis....106
3.4.6.1 Serum Triglycerides....106
3.4.6.2 Serum Cholesterol....106
3.5 Discussion....108

4 FOOD RESTRICTION WITH DIETS CONTAINING VARIOUS AMOUNT OF FAT....112
4.1 Introduction....112
4.1.1 Adverse Effects of High-Fat Diets....113
4.1.2 Adverse Effects of Low-Fat Diets....114
4.2 Objective....116
4.3 Materials and Methods....117
4.3.1 Animal Handling....117

4.4 Results....120
4.4.1 Body Weight....120
4.4.2 Food Intake....120
4.4.3 Organ Weight....122
4.4.3.1 Liver....122
4.4.3.2 Adipose Tissues....122
4.4.4 Lipid Analysis....127
4.4.4.1 Adipose Tissues....127
4.4.4.2 Carcass....127
4.4.5 Proximate Analysis....147
4.4.5.1 Crude Fat....147
4.4.5.2 Moisture....147
4.4.5.3 Crude Protein and Ash....148
4.4.6 Serum Analysis....151
4.4.6.1 Serum Triglycerides....151
4.4.6.2 Serum Cholesterol....151
4.5 Discussion....153

5 FUTURE PROSPECTS....159
5.1 Leptin....159
5.2 Enzymes....162

6 CONCLUSION....166

7 REFERENCES....170

Chapter ONE

GENERAL INTRODUCTION

Fat is an important component in a diet. Excluding fat entirely from a diet in vertebrates can result in retarded growth, dermatitis, kidney lesions and early death. Fatty acids are the major components of different fat and oil. They vary in structure and their composition or the profile is diverse and specific in every single kind of oil (Table 1.1). By specific analytical techniques, such as gas-liquid chromatography, it is possible to measure the fatty acid profile of oils with the identification of each component.

Table 1.1: Principal fatty acids in various dietary oils (Calder, 1998).

Oil	Principal Fatty Acids
Coconut	Medium chain saturated: capric (10:0), lauric (12:0), myristic (14:0)
Palm	Palmitic (16:0) comprises 50% fatty acids
Olive	Oleic (18:1n-9) comprises 55 to 85% fatty acids
Corn	Linoleic (18:2n-6) comprises 40 to 65% fatty acids
Sunflower	Linoleic (18:2n-6) comprises 50 to 75% fatty acids
Safflower	Linoleic (18:2n-6) comprises 70 to 85% fatty acids
Linseed	Linolenic (18:3n-3) comprises 35 to 65% fatty acids
Fish	EPA (20:5n-3) comprises 10 to 15% fatty acids
	DHA (22:6n-3) comprises 5 to 12% fatty acids

The differences in the fatty acid profiles in various oils give us some information to choose between the sources for a supply of this unreplaceable macronutrient in our diet. Cha and Jones (1997) have investigated the interaction of dietary fat source and energy intake level on tissue cholesterol and triglycerides synthesis. Different outcomes were found for different oils. Using a rat model with a 10-week *ad libitum* feeding, cholesterol and triglycerides synthesis rates are lower following safflower oil than olive oil treatment. This effect is not observed in 68% energy restricted animals. However, cholesterol synthesis in the small intestine is similar between fish oil and beef tallow feeding *ad libitum* (Cha and Jones, 1997). This results in different concentrations of serum cholesterol and triglycerides with the consumption of different oil in a diet.

1.1 *Classes of Fatty Acids*

Fatty acids are the simplest forms of lipids, which comprise of fatty acids, neutral lipids, waxes, phospholipids, glycolipids, lipoproteins and other lipid derivatives, e.g. sterols. A fatty acid is a molecule containing an aliphatic hydrocarbon chain terminating with a carboxylic acid group, which endows the molecule with a polar hydrophilic end, and a non-polar hydrophobic end.

The hydrocarbon chains span between 4 to about 24 carbon atoms in vertebrate. They can either be saturated or unsaturated, i.e. from having no double bond to 4 double bonds. The double bonds in the unsaturated fatty acids, further classified into monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), can be either in *cis* or *trans* geometric configuration. The *cis* configuration is more abundant in natural sources while the *trans* counterpart can only be seen in some natural and partially hydrogenated fats and oils. The *cis* configuration allows a bending of the molecule into a U-shaped orientation, which enables the PUFAs to play a role in the structure and function of cell membranes. The straight chain configuration of the *trans* fatty acids thereby cannot replace the function of its *cis* counterpart.

There are several ways to name a fatty acid. Common names such as palmitic acid (PA) and stearic acid (SA) are saturated fatty acids (SFAs) with 16 and 18 carbons respectively, while palmitoleic and oleic acids are fatty acids with 16 and 18 carbon atoms, respectively, with 1 double-bond on the ninth place from the carboxyl end.

Since there can be many combinations on the length of the fatty acid chains, the number and the orientations of double bonds, common names are not as clear and systematic as notations. Linoleic acid (LA) is referred to as 18:2n-6. The first number denotes the number of carbon atoms; the number following the colon sign represents the number of double bonds; the last number refers to the place of the first double-bond from the carboxyl end. Other notation systems also exist. LA can thus be also named as 18:2ω6 or 18:2Δ9,12 . The latter system lists out the orientation of all the double-bonds one has.

1.2 Polyunsaturated Fatty Acids (n-6 & n-3)

1.2.1 High Fish Oil Content in Diet, High n-3 PUFAs Intake, Fights against Cardiovascular Risk

The n-3 PUFAs are abundant in fish, particularly in oily fish such as mackerel, herring, salmon, trout and sardine. Although no dietary habits of subjects were provided, Ehrstrom (1951) gathered information of blood pressure on Eskimos. Obviously, as the Eskimos consume large amount of fish and n-3 PUFAs, diastolic hypertension is rare in this population. After the first and inspiring study done by Bang and co-workers (1980) who compared the main differences of the Greenland Eskimo diet from the Western Scandinavian diet, it is noticed that the Eskimos have a strikingly high consumption of fish oil. A link between the high fish oil intake and the low mortality from coronary heart disease (CHD) has been well discussed in the literature ever since (Hirai *et al*, 1989).

The LA and α-linolenic acid, LNA (18:3n-3) are essential in a diet since they cannot be synthesised in the body of vertebrates. Other fatty acids with longer chains are synthesised by elongation and desaturation catalysed by the same enzyme (Sprecher, 1989). Linoleic acid has a much higher affinity for the n-6 desaturase enzyme. Therefore, dietary supplementation with 18:3n-3 is not sufficient *per se* to increase the membrane incorporation of the long chained n-3 derivatives (Dyerberg *et al*, 1980).

The two classes of PUFAs, n-6 and n-3, play an important role in modulating the response of cell function and cell activity to external stimuli (Willis, 1981). They are the precursors of eicosanoids, prostaglandins, thromboxanes and leukotrienes, which are very important intercellular mediators of a series of cellular reactions.

Numerous studies support the hypothesis that increasing consumption of n-3 fatty acids from fish oil will decrease cardiovascular morbidity and mortality (Dolecek and Grandits, 1991). In 1985, results of the Western Electric Study indicate a close relationship between fish consumption and coronary death. Increasing intake of fish from 0 to more than 35g per day leads to a decrease in reported coronary death from 20.5% to 13% (Shekele *et al*, 1985). The Zutphen study also supports this inverse correlation between fish intake and mortality by ischaemic heart disease (IHD) (Kromhout *et al*, 1985). Some of the studies suggest that a regular intake of fish such as, one or two fish meals per week (Siscovisk *et al*, 1995) or, in lieu of fish consumption, one to two fish-oil capsules

per day (Burr *et al*, 1989) can reduce sudden death caused by cardiovascular disease.

A more specific study, which lasted for 3.5 years, on the dosage of n-3 fatty acids on the cardioprotective effects has been conducted by the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardio (GISSI)-Prevenzione. An 850mg per day intake of n-3 fatty acids by patients with coronary artery disease reduces the overall mortality by 20% and the risk of sudden cardiac death by 45%. This study suggests that small consumption of n-3 fatty acids can be cardioprotective and it operates by stabilising the myocardium itself (Harris and Isley, 2001).

The protection against cardiovascular risk may be due to the fact that n-3 PUFAs can inhibit platelet function and vasodilation (Kristensen *et al*, 1989) and decrease serum triglycerides (Harris, 1989; Sanders, 1991), especially in the hyperlipidaemic population (Nordøy *et al*, 1989; 1991). Patients who had been given fish advice in the DART (Diet and Reinfarction Trial) conducted by Burr and associates in 1989 had higher mean 20:5n-3 (eicosapentaenoic acid, EPA) in plasma than those without that advice. In the same trial, all-cause mortality as well as IHD are remarkably lower in patients receiving fish (Burr *et al*, 1989). Experimental study provides evidence that an increase consumption of n-3 PUFAs protects against fatal arrhythmias occurring during myocardial ischaemia and reperfusion (McLennan *et al*, 1990).

Furthermore, fish oil is hyperlipidaemic with documentation dated to 1960s (Goodnight *et al*, 1982; Harris, 1989). It can lower plasma cholesterol and

triglycerides through the inhibition of the synthesis of triglycerides and very-low-density lipoproteins (VLDLs) in the liver, leading to occasional increase in low-density lipoproteins (LDL). Apolipoprotein B production is reduced while high-density lipoprotein (HDL) maintains at a constant level in patients feeding with fish oil than vegetable oils. Also, the effects are more remarkable in the n-3-rich fatty fish oil than in the n-6-rich vegetable oil (Connor, 1997).

1.2.2 n-3 Fatty Acids Improve Hypertension

n-3 PUFAs also protect against hypertension and reduce the risks of CHD. Starting from 1985, various reports indicated that the addition of fatty fish oil or n-3 fatty acids to the diet of patients with mild hypertension could lower blood pressure (Bønaa *et al*, 1990; Radack *et al*, 1991). In the Tromsø Study conducted in 1990, patients with previously untreated stable, mild essential hypertension receiving 6g per day of fish oil are recorded to have the mean systolic and the mean diastolic blood pressures dropped by 4.6mmHg and 3.0mmHg. On the contrary, patients receiving the same amount of corn oil in the same study maintain the blood pressure (Bønaa *et al*, 1990). This improvement in blood pressure may be the result of the endothelium-independent vasorelaxant effect of n-3 fatty acids, especially EPA, through production of prostanoids that activate K^+-ATP channels (Engler *et al*, 2000).

n-3 fatty acids can be a potential treatment for post-transplant hypertension. Background hypertension and cyclosporine-induced nephrotoxicity are common complications in heart transplant recipients. Administration of n-3

fatty acids on these patients results in no observable complications. However, the conditions of the placebo recipients deteriorate such as increase in systolic blood pressure, systemic vascular resistance and plasma creatinine with the decrease in glomerular filtration rate (Holm *et al*, 2001). It suggests that the administration of n-3 fatty acids to the patients can help to maintain the conditions without further deterioration.

1.2.3 n-3 Fatty Acids Protect from Atherosclerosis

The development of an atheromatous plaque in an artery is complicated. Plasma factors such as lipoproteins, together with multiple cellular elements, e.g., platelets and leukocytes interact with the vascular wall (Ross, 1986). The endothelial lining of an artery is normally protected by some anti-thrombotic mechanisms to ensure a smooth blood flow and inhibit clot formation. During injury, infection or inflammation, the procoagulant functions on the artery is upregulated, while the anti-thrombotic properties suppressed, resulting in thrombus formation, leukocyte accumulation and release of mitogenic factors into the arterial wall (Gerlach *et al*, 1990).

Increase in dietary intake of n-3 fatty acids delays and inhibits the formation of experimental atherosclerotic lesion. Possible mechanisms include the anti-inflammatory effects of n-3 PUFAs and its alteration in platelet and leukocyte function, as well as vessel wall metabolism (Kinsella *et al*, 1990).

Fish oil or n-3 PUFAs has been reported to have a variety of modulatory effects on blood components and the vascular endothelium against atherosclerosis.

They decrease platelet adhesion and aggregation to fibrinogen and collagen (Li and Steiner, 1990), platelet thromboxane (TXA_2) synthesis (Goodnight *et al*, 1982), and block the TXA_2 receptor on platelets (Swann *et al*, 1989). The neutrophil chemotaxis (Schmidt *et al*, 1991), inflammation and cytokines in leukocytes are also suppressed. On the endothelium, n-3 fatty acids reduce the macrophage-produced cytokines, various growth factor release and synthesis of platelet-derived growth factor (PDGF) (Endres *et al*, 1989) but elevate endothelium-derived relaxing factor (EDRF), nitric oxide (NO) and prostaglandins PGI_2 and PGI_3 (Li and Steiner, 1990). They also decrease plasma triglycerides, fibrinogen and plasminogen activator inhibitor (PAI) and elevate the concentration of antithrombin III (AT III) and tissue plasminogen activator (tPA) (Weksler, 1992).

The vascular smooth muscle cell (SMC) proliferation is specifically slowed down by n-3 fatty acids. This has not been proved only until recently by Pakala and co-workers in 2000. Mitogens for vascular SMC proliferation, serotonin (5HT) and PDGF can only partially stimulate the process that are preloaded with EPA and DHA when compared to the control cells; and though there are some proliferation found in the presence of n-3 fatty acids, the level of 5HT(2) receptor mRNA remain unchanged. On the contrary, γ-linolenic acid (GLA) and oleic acid (OA) do not block the 5HT and PDGF-induced ^{3}[H]thymidine incorporation. Moreover, the combination of EPA and docosaheptaenoic acid (DHA) same as in fish oil showed a synergistic interaction in inhibiting the vascular SMC proliferation (Pakala *et al*, 2000).

In patients with atherosclerosis, administration of n-3 fatty acids can prolong platelet survival. On the other hand, shortening of platelets survival is observed in the untreated patients due to repeated platelet activation (Levine *et al*, 1989) and thus stimulates blood coagulation (Bevers *et al*, 1987). Multiple-measure of atherosclerosis, namely pulse wave velocity of the aorta, intima-media thickness of the carotid artery and atherosclerotic plaques obtained by ultrasonography, has been recorded to be lower in a Japanese fishing village than in the farming village. The atherosclerotic plaques are 5-8 fold smaller in subjects having high fish intake in their diet. Evaluation of the n-3 fatty acids has shown a negative correlation with the number of plaques while the n-6 fatty acids illustrate a weaker positive correlation (Yamada *et al*, 2000).

Although there are more evidences showing that the n-3 fatty acids are the salvation of CHD and related diseases, similar results can also be obtained with diet enriched with LA (18:2n-6) (Hennig *et al*, 1994).

High consumption of LA or corn oil, in comparison with saturated-fat diets, increases the susceptibility of LDL to oxidative modification, and promotes LDL-induced disruption of endothelial functions in both humans (Reavan *et al*, 1993) and rabbits (Hennig *et al*, 1995). The mechanisms proposed include the LA-related decrease in intracellular ATP levels, local accumulation of the fatty acid within endothelial cells (Hennig and Watkins, 1989), diminishing levels of proteoglycans (Ramasamy *et al*, 1993), enhancement of Ca^{2+}-ATPase (Ramasamy *et al*, 1991) and elastase-like activity (Toborek and Hennig, 1993), and inhibition of gap-junctional intracellular communications (de-Haan *et al*, 1994.) LA can also

act as a potent peroxidant by enhancement of radical adducts in the endothelial cells (Alexander-North *et al*, 1994), depletion of glutathione stores (Toborek *et al*, 1996), and increase in perioxosomal ß-oxidation (Hennig *et al*, 1990). Dietary consumption of n-6 fatty acids led to a decrease in atherosclerotic lesion in LDL-receptor-deficient (LDL-RD) mice by its effects on lipoprotein composition and other potential influences contributed to the anti-atherogenesis (George *et al*, 2000).

1.2.4 PUFAs are Beneficial in Inflammation

Prostaglandins are the metabolites of arachidonic acid (AA, 20:4n-6) and LA (18:2n-6) through the conversion to GLA and dihomo- γ-linoleic acid (DGLA) (Bergström *et al*, 1964). Prostaglandin E (PGE) is known to have a clear role in the regulation of cellular and humoral immune responses since the 1980s. PGE acts as a feedback inhibitor of T cell proliferation, lymphokine production, macrophage and natural killer cytotoxicity in cellular immune response. PGE production is a necessary component in the generation of some type of T suppressor cells. Disturbances in immune function found in several human conditions and diseases have been linked to the changes in PGE-mediated immunoregulation. Either increased production of PGE or increased its sensitivity results in depressed cellular immunity (Roper and Phipps, 1994).

In vitro studies have shown that PGE inhibits both T and B lymphocyte functions. It suggests that the effects of essential fatty acids on immune response are, in part, mediated through eicosanoids. The profile of eicosanoids is found to

vary with the type of stimuli, anatomical sites and the fatty acid composition of tissue lipids, which in turn can be modified by the composition of dietary essential fatty acids (Hwang, 1989).

Administration of n-6 and n-3 fatty acids also shows remarkable effects in improving the condition in patients succumbing to various inflammatory diseases. Combination of fish oil and primrose seed oil supplement with relatively large amount of GLA reduces the cellular and fluid phases of monosodium urate crystal-induced inflammation, thus suppresses the acute and chronic inflammation in rheumatoid arthritis (Tate *et al*, 1988). Dietary deficiency in n-6 essential fatty acids leads to a range of skin inflammation (Wright, 1991). GLA treatments show a dose-related improvement in atopic eczema (Wright and Burton, 1982) while fish oil supplements improve the lesion of half of the psoriasis patients moderately (Maurice *et al*, 1987). Supplementation of fish oil inhibits the production of proinflammatory cytokines, which ameliorates the immune-complex-mediated kidney injury by enhancing the ability of cells to dispose harmful reactive oxygen intermediates in autoimmune lupus-prone mice (Chandrasekar and Fernandes, 1994). Dietary modification of n-3 fatty acids reduces the severity of glomerulonephritis in several autoimmune strains of mice (Robinson *et al*, 1986). Fish oil preparation is also effective in reducing the rate of relapse and maintaining remission in patients with Crohn's disease, an idiopathic inflammatory bowel disease (Belluzzi *et al*, 1996).

1.2.5 n-3 PUFAs Help to Control Tumour Growth

EPA and DHA are found to be beneficial in tumour control. Administration of high amount of n-3 PUFAs leads to a marked suppression (35-46%) of tumour growth in rats over a 12-day period. Both the whole Walker 256 tumour homogenate and the mitochondrial fraction show significant changes in fatty acid composition with elevated levels of EPA and DHA compared to rats fed on chow diet. *In vitro* studies showed a 46% decrease in Walker 256 cell growth in the presence of either EPA or DHA (Colquhoun *et al*, 2001).

Fish oil is certainly not having an even supply to all the populations in the world. Scientists suggest that rapeseed, which is rich in GLA and LNA (18:3n-3), can compensate for the effect of low fish consumption in human. Rapeseed oil administration (50g/day) results in a higher level of 18:3n-3 in triglycerides and cholesterol than administration of sunflower oil and a baseline control. The concentrations of EPA in plasma triglycerides, cholesterol esters and phospholipids are higher in the rapeseed consumers. Phospholipid docosapentaenoic acid (DPA, 22:5n-3) and DHA are also higher in this group. These results resemble the effects of a weekly intake of 50-100g of fish oil (Valsta *et al*, 1996).

n-3 PUFAs are hypolipidemic, antithrombotic and anti-inflammatory which in turn help to prevent and treat heart disease and hypertension. Specific fatty acids of EPA and DHA also have beneficial effects in the treatment of many autoimmune diseases and disorders, e.g., rheumatoid arthritis, lupus

erythematosus, ulcerative colitis and psoriasis and reduction in size and tumours in animal studies (Simopoulos *et al*, 1991).

In the numerous studies conducted by scientists using a variety of measures of atherosclerosis, n-3 fatty acids appear to be the most promising in prevention and treatment of CHD, though n-6 fatty acids are also helpful at times. In populations with limited fish supply, one can use rapeseed oil as a supplement of n-3 fatty acids.

1.3 Obesity and Eating Disorder

1.3.1 Obesity, a Companion of the Modern World

On one hand, famine is a killer in many under-developed countries and people are fighting for their food, while on the other hand, obesity is one of the characteristics in the developed world. It has reached epidemic proportions in the United States, and the prevalence is increasing in most countries, especially the industrialised ones around the world (WHO, 1998). Obesity is usually the result of sedentary lifestyles and high-fat, energy-dense diets. Numerous studies show that obesity, especially abdominal obesity, leads to many life-threatening health burdens such as insulin resistance and non-insulin-dependent diabetes mellitus (NIDDM), hypertension, dyslipidaemia, cardiovascular disease, stroke, premature death, sleep apnea, gall bladder disease, hyperuricaemia and gout, and osteoarthritis.

The participants in the meeting held by World Health Organization (WHO) Consultation on Obesity in Geneva, 1997, agreed on an international standard for

measuring overweight and obesity, the Body Mass Index (BMI, kg/m^2), which is defined as weight (in kg) divided by the square of one's height (in m). For assessing obesity in adult populations, the BMI categories are:

BMI $\geq$ $25kg/m^2$ for overweight (Pre-obese: BMI = 25-29.9 kg/m^2)

BMI $\geq$ $30kg/m^2$ for obesity:

- Class I obese: BMI $\geq$ 30-34.9kg/m^2

- Class II obese: BMI $\geq$ 35-39.9kg/m^2

- Class III obese: BMI $\geq$ $40kg/m^2$.

Recent studies have shown that overweight and obesity affect over half of the adult population in many countries. The prevalence of obesity in adults is 10% to 25% in most countries of Western Europe and 20% to 25 % in some countries in the Americas. This figure increases up to 40% for women in Eastern European and Mediterranean countries, and black women in the USA, even higher in prevalence is observed among American Indians, Hispanic Americans, and Pacific Islanders, with probably the highest rates in the world among Melanesians, Micronesians, and Polynesians. Up to 70% of women and 65% of men on the island of Nauru in Micronesia are Class I obese. Some ethnic groups, especially the Asians, are more susceptible to weight gain and accumulation of abdominal fat (WHO, 1998).

In the United States, the percentage of overweight adults (age 20-74) increased from 43.5% in 1960-62 to 55% in 1988-94. For those who are obese the percentage increased from 12.8% to 22.6%. For the overweight or obese adolescents (age 12-19), the percentage increased from 6.1% in 1971-74 to 10.5%

in 1988-94. Overweight children (age 6-11) increased from 4.2% to 11.4% from 1960s to 1988-94 (Centers for Disease Control and Prevention, 2001). In the European countries, similar increase in overweight is found as well (Seidell *et al*, 1995).

1.3.2 Health Risks Related to Obesity

Overweight is known to be related to diabetes, CHD, osteoarthritis, respiratory disease, gall stones, lipid abnormalities and hypertension. Risks of large bowel and endometrial cancers are also higher in overweight patients (Wilding, 1977).

Studies suggest that a high proportion of fat in the diet is associated with impaired insulin sensitivity and an increased risk of developing diabetes, independent of obesity and body fat localisation (Vessby, 2000).

Chronic arterial hypertension is closely correlated with obesity (Dyer *et al*, 1994). It increases the risk in CHD and stroke. This obesity-induced hypertension is secondary to insulin resistance and hyperinsulinaemia (Reaven, 1998). The kidney and renal sympathetic nerves also play an important role in the development of hypertension (Hall, 1997).

Leptin, besides decreasing appetite and increasing metabolism, has sympathetic, vascular and renal actions that can increase the blood pressure regardless of the reduction in food intake (Allyn *et al*, 1998). The high level of leptin in the leptin-resistance overweight subjects may therefore account for obesity-related hypertension.

Hypertensive effect of obesity has been suggested to carry genetic-neurobiological relationship (Allyn *et al*, 1998). This effect is less prominent in the Pima Indians, Hispanic Americans and African Americans than in the Caucasians (van Itallie, 1985). Moreover, severity of the condition varies from individual to individual. In a research studying the whites on obesity-related intra-arterial pressure, only 5 to 10% of the severely obese patients developed severe hypertension, about 50% has mild to moderate hypertension and the rest with normal arterial pressure (Alexander *et al*, 1962).

Excess weight gain shows a positive correlation with the NIDDM (Colditz *et al*, 1995); on the other hand, physical exercise and the consequent weight loss improve the condition (Manson *et al*, 1991).

Free fatty acids cause insulin resistance, the cause of NIDDM, in muscle and liver and increase hepatic gluconeogenesis and lipoprotein production and perhaps decrease hepatic clearance of insulin. Depressing effect of insulin on the circulating free fatty acid concentration is dependent on the fraction derived from visceral adipocytes, which have a low responsiveness to the anti-lipolytic effect of insulin. Cortisol and/or testosterone induce insulin resistance in muscle; furthermore, cortisol increases hepatic gluconeogenesis. Effects of free fatty acids, cortisol and testosterone can combine to promote insulin resistance, which are active in abdominal visceral obesity (Björntorp, 1991).

Overweight and obesity also show a close and positive correlation to the occurrence of a variety of cancers, e.g., breast, colon, endometrium, prostate, kidney and gall bladder. Bergström and associates (2001) has performed a meta-

analysis and summarised the results of a large number of studies, either cross-sectional or population-based, relating overweight and cancer incidence in the countries of the European Union (Bergström *et al*, 2001). Their summary is shown in Table 1.2:

Table 1.2: Percentage of cancer cases attributable to overweight and obesity in the European Union, by cancer site (Bergström *et al*, 2001).

Cancers	Men		Women	
	Overweight	Obese	Overweight	Obese
Breast			4.1	4.5
Colon	6.9	4.2	5.0	5.7
Endometrium			17.2	22.0
Prostate	2.9	1.6		
Kidney	15.2	10.3	11.1	13.4
Gallbladder	14.7	10.1	10.7	13.0
All cancer sites	2.1	1.3	2.9	3.5

Other cancers like thyroid (Kolonel *et al*, 1990), gastric cardia (Chow *et al*, 1998), and adenocarcinoma of the oesophagus (Lagergren *et al*, 1999) are also found to be positively correlated with overweight.

It is obvious that overweight and obesity are closely related to many life-threatening health problems. Since there is a universal trend of body weight

increase, the burdens exerted onto the public health and medical services might be even more pronounced in the future.

1.3.3 Management of Obesity

Yearly medical costs attributed to obesity directly or indirectly are estimated to approach US$100 billion in the United States (Wolf and Colditz, 1998). Recommendation of weight loss in overweight individuals and maintaining the healthy body weight are agreed among researchers and health associations (Dietary Guidelines for Americans, 1995; National Institutes of Health, 1998).

Together with a suitable restriction in food intake and an increase in physical exercise, dietary modification is the primary approach to decrease cardiovascular risk in obese patients. Diets with progressive restriction of dietary saturated (and *trans*) fatty acids and cholesterol intake, increase in the consumption of foods high in soluble viscous fibres, vegetable proteins, possibly antioxidants such as vitamin E and the isoflavonoids, LNA, consumption of specific plant foods such as green leafy vegetables, nuts and seeds, and dried legumes may be beneficial in the weight loss programme until an ideal body weight is reached (Jenkins, 1995).

It must be noticed that achieving a 5 to 10% weight loss over at least a year and then maintaining the loss through a period of time are equally important in a dieting programme (Goldstein, 1992). Energy balance must be negative by reducing the calorie intake and encouraging physical exercise, but the basic metabolic uses have to be maintained. A reduction of fat to about 30% of total

energy consumption is critical in a healthy diet. Emotional care and urgency to eat without feeling hungry should be relieved and controlled (Thomas, 1998).

In addition to losing the body weight, combating the various diseases related to obesity is necessary. Dietary manipulation can easily be carried out and is as beneficial as reduction of calorie intake.

Different sources of oil provide different profiles of fatty acids in a diet. In a study comparing the effects of fish oil, safflower oil, olive oil and beef tallow with *ad libitum* feeding, olive oil increased the cholesterol and triglycerides in liver and small intestine of rats in comparison to other oils. On the contrary, rats feeding 85% and 68% of food of the *ad libitum* intake result in the opposite fashion. Energy restriction increases triglycerides synthesis rates in intestine of rats fed with fish oil and safflower oil, but not in that of olive oil- and beef tallow-fed animals. The cholesterol synthesis in food-restricted rats treated with olive oil is reduced, contrasting the finding in the *ad libitum* feeding. These results suggest a dietary fat selection to control against hyperlipidemia during consumption of weight-reduction diets (Cha and Jones, 1997).

Insulin resistance and NIDDM are disorders commonly seen in obese patients. Insulin resistance and diseases characterised by it are associated with a specific fatty acid pattern of the serum lipids with increased proportions of PA (16:0) and palmitoleic acids (POA, 16:1 n-7) and reduced levels of LA (18:2n-6). An increased saturation of the membrane fatty acids and a reduced activity of Δ-5 desaturase have been associated with insulin resistance (Vessby, 2000). A previous study with a LA-enriched diet in NIDDM patients shows a significant

improvement in glucose-tolerance test (Houtsmuller *et al*, 1980), causing a less atherogenic lipoprotein profile but not influencing glycaemic control and carbohydrate tolerance (Heine *et al*, 1989). Control of dietary intervention, with less SFAs but more LA, may improve the insulin resistance condition of patients suffering from diabetes.

In patients already succumbing to coronary atherosclerosis, vigorous cholesterol lowering by low fat food and lipid active drugs, control of hypertension, and smoking abstinence stabilise plaque from rupture of lipid rich, less severe coronary artery stenoses. Diet modification can remarkably reduce coronary events and angina pectoris with greater improvement in survival than that reported for elective invasive revascularisation procedures (Gould, 1998).

1.3.4 Care Must be Taken to Prevent the Development of Eating Disorder or Other Psychological Disturbances during Weight Loss Programme

Attention to psychological and behavioural consequences of dieting and weight loss is critical in maintaining the healthy body weight as well as preventing the development of eating disorders. Binge eating disorder (BED), frequent episodes of eating substantially larger amounts of food than do others in similar circumstances accompanied by a feeling of losing control, is the most common eating disorder found in overweight and obese adults (Yanovski, 1999). BED differs from bulimia nervosa by the lack of compensation for the overeating by

purging with vomiting or laxative abuse, fasting, or excessive exercise (American Psychiatric Association, 1994).

Studies on the weight-losing programme on obese patients with moderate energy restriction report no associated binge eating behaviour development (Sherwood *et al*, 1999). On the other hand, it is controversial in the correlation between BED and severe energy restriction with very-low-calorie diets (VLCDs). Participation in binge eating has been reported, especially during the refeeding period that follows the termination of severe energy restriction (Wadden and Bartlett, 1992). This is believed to be compensation to the physiological and the psychological effects of food deprivation. Telch and Agras (1993) reported a pronounced increase in patients having BED after a 12-week VLCDs treatment (Telch and Agras, 1993). Nonetheless, other studies showed no significant changes before and after the treatment of VLCDs on the development of BED (Yanovski *et al*, 1994). Despite the disagreement in the studies, moderate caloric restraint in dieting may be safer than adopting VLCDs.

When attempting to lose weight, 'dieting depression' is usually reported. Patients complain to experience symptoms like weakness, nervousness, irritability, fatigue and nausea (Stunkard and Rush, 1974). On the contrary, more recent studies reported modest improvements in mood, or at least, no worsening in affect (Wing *et al*, 1994). This may be due to the fact that voluntary weight loss becoming more popular in the people having body figure preoccupation. However, psychological problems can be exacerbated by weight loss in those having previous history of such disturbances (O'Neil and Jarrell, 1992; Felitti, 1993).

Regaining of weight can attenuate the improvements in psychological functioning (Brownell and Stunkard, 1981), with mood sometimes rebounding to the initial levels (Wadden *et al*, 1988).

In conclusion, losing weight under a well-planned and well-monitored programme with the help of professionals is critical to reduce problems arisen. Moreover, maintaining the weight at the ideal level is equally important to prevent weight-cycling and another weight loss programme. Expenses on the health care of the overweight population, before, during and after weight loss, can also be reduced to a large extent.

Chapter TWO

WEIGHT CYCLING WITH CHOW DIET

2.1 INTRODUCTION

Weight-cycling (WC) is a common phenomenon of dieting and may not be intentionally practised. It is usually practised by overweighed or obese patients, bulimic patients and people who have body image preoccupation irrespective of their age (Allaz *et al*, 1998; Fallaz *et al*, 1999).

This issue was first brought to wide attention by Brownell's group (1986), suggesting that the process of losing and regaining body weight increases food efficiency and makes subsequent weight loss more difficult (Brownell *et al*, 1986). Since then, a large number of research papers have been published (National Task Force on the Prevention and Treatment of Obesity, 1994). In 1994, this issue was

ranked as a 'special priority' by the Surgeon General's Report on Nutrition and Health (Brownell and Rodin, 1994).

The attempt of repeated weight loss is more popularly known as 'yo-yo dieting'. In fact, 'weight cycling' describes the outcome of the body weight fluctuating with the mode of food intake by 'yo-yo dieting', i.e., intermittent dieting. Even with many other available treatments to obesity, many people will regain the weight they have lost once they lose their patience and give up the therapy. Weight cycling is not only a problem being aware by the scientists or dieticians, but also a problem of the general public (Rovney, 1988; Brody, 1992).

2.1.1 Definition of Weight Cycling

Many independent researches have been carried out in the 1990s. Population studies include male and female, adolescent and adult, obese and normal-weight, sedentary and physically active ones. Variables measured span from weight change, basal metabolic rate (BMR), fat-free mass, waist-to-hip ratio (WHR), blood pressure, serum levels of cholesterol, triglycerides, glucose, insulin and, morbidity and mortality by cardiovascular disease, diabetes mellitus and cancer, as well as the psychological well-being, and so on. However, there is no clear cut definition of weight cycling because of the lack of strong experimental evidence and the limitations due to the small number of samples and methodology (Popkess-Vawter *et al*, 1998). In general, it is widely accepted as an individual who has repeated loss and regain of more than 5 kg yearly over a period of 3-6 years (National Task Force on the Prevention and Treatment of Obesity, 1994).

2.1.2 Incentives Leading to Weight Reduction

Various and diverse populations are available for researches since there are many different motives to drive people to dieting, such as preventing the cardiovascular disease and cancer morbidity, controlling diabetes mellitus, achieving the weight-limits of certain sports like wrestling, and gaining a higher self-esteem by looking like a model.

Pursuing their belief, dieters go under a strict caloric intake period. Undoubtedly, weight is lost in reward to their effort. Satisfaction accompanies the success and brings the withdrawal from the enthusiasm. As a kind of reward, people tend to carve for more food than they require and, usually with high energy-density diets. When the weight lost is regained, feeling of guilt comes to their mind and another energy restriction period returns until they think they have to lose the pounds again. In many mild cases, relapses come and go like tides. However, in worse cases, binge eating and bulimia may result, bringing up even more problems (Ferguson and Spitzer, 1995).

2.1.3 Problems Aroused by Weight Cycling

Controversies are seen among various studies. Nonetheless there are common beliefs that weight cycling makes it harder to lose future weight and is accompanied by increasing total body fat, central adiposity and visceral fat, lowering energy expenditure, increasing fat intake, increasing total cholesterol, triglycerides, fasting plasma insulin and glucose, decreasing high-density

lipoprotein, impairing glucose tolerance and altering fat metabolism (National Task Force on the Prevention and Treatment of Obesity, 1994). Debates on this topic are mainly due to the sampling diversity between different studies, like age and gender variables. However, studies do provide evidence on the lowering rate of the second weight loss (Blackburn *et al*, 1989) and it is obvious that weight cycling has adverse effects on health (Wannamewthree and Shaper, 1990), leading to a higher risk of coronary heart disease and premature death in humans (Lee and Paffenbarger, 1992).

2.1.3.1 Food Preference, Efficiency and Expenditure

Food efficiency is a description of the total energy accumulated per unit total energy intake, i.e. gaining more weight with taking in less calories. Animal and human models are used in researches to prove that there is an increase in food efficiency after repeated weight loss and regain (Reed *et al*, 1988; Desautels and Dulos, 1988; Archambault *et al*, 1989). Moreover, it is also observed that there is a preference of taking in more high energy-density food, especially the high-fat diets, after a fasting-refeeding mode of diets (Drewnowski *et al*, 1992). On the other hand, energy expenditure in resting and exercised conditions is lower in the dieters than the controls but it returns to normal during refeeding (Steen *et al*, 1998; Santos-Pinto *et al*, 2001). All these suggest that a dieter practising yo-yo dieting has a possibility to gain weight much faster than one who is not a weight cycler.

2.1.3.2 Increased Overall and Central Adiposity,

Weight obviously increases after yo-yo dieting and human studies show that the majority of the weight gained is in terms of fat stored inside the body (Manore *et al*, 1991). Specific adiposity in the abdominal region, i.e., central adiposity can be measured by WHR and BMI. Significant positive association has been found in weight cyclers but not in the controls (Ernsberger *et al*, 1996). In the studies on the body composition change after a fasting-refeeding cycle, the weight regained can also be accounted for, to a lesser extent, by the gain in protein and water (Björntorp and Yang, 1982). The surplus energy gained by the increased food efficiency is probably stored in the form of fat, mainly in the central region.

In one detailed study by Pellizzon and associates (2000), six groups of female aging rats were fed a combination of high-fat or low-fat diets, *ad libitum* or food restraint or weight cycling modes with a control base-line group. The weight cycling rats are only fed a high-fat diet; one group started with a weight gain then a weight loss, while the other group had the weight loss first, followed by the weight gain. It has been found that the more the number of weight cycles, the rates of weight gain and feeding efficiencies increased much more significantly but the body fat is not modified permanently from the base-line. The body fat as well as the percentage of internal fat (retroperitoneal and omental) in the *ad libitum* groups with either diet is obviously higher than their food-restricted counterparts. However among the high-fat food restricted subjects, there is no significant difference in body fat between the cyclers and the non-cyclers. These results suggest that repeated weight cycling promotes obesity, and it is more easy

to gain but difficult to lose weight in the previously weight cycled individuals (Pellizzon *et al*, 2000).

2.1.3.3 Increased Morbidity and Mortality of Cardiovascular Diseases

Studies in the last decade on weight cycling mainly focus on the epidemiological field. Positive correlation is found in various studies, e.g., in Western Electric Study, Framingham Study, Japanese American men, on humans and animals between all-cause as well as cardiovascular mortality in different populations (Hamm *et al*, 1989; Lissner *et al*, 1991; Blair, *et al*, 1993; Ernsberger and Kolestsky, 1993; Iribarren *et al*, 1995).

Hyperinsulinaemia and hypertension also cause death in animals with weight cycling (Robert and Williams, 1989). Combined exposure of central-type obesity and weight cycling can raise hypertension (Guagnano *et al*, 1999) though another study suggests this cause-and-effect relation is not essential (Field *et al*, 1999). The selective deposition of fat in the abdomen in the obese population reflects the association with cardiovascular health risks.

In addition to weight cycling, obesity-related health problems such as hyperlipidaemia, hypertension and hyperinsulinaemia can also lead to an increased prevalence of coronary artery disease and NIDDM (National Institutes of Health, 1985; Manson *et al*, 1990). Obese patients are also accompanied by certain types of cancer, degenerative joint disease, sleep apnea, gout and gall bladder disease, decrease in bone mineral density, all these increase the

susceptibility to other health conditions (van Itallie and Lew, 1992; Fogelholm *et al*, 1997).

2.1.3.4 Psychological Impact and Social Consequences

Weight cyclers usually overeat when they have unpleasant feelings or stresses in interpersonal relationships (Popkess-Vawter *et al*, 1998). Repeated failure in attempts to losing the gained weight causes depression and lower self-esteem (Foryet *et al*, 1995). Weight management (physical aspects) is not the only factor, in addition, the psychological and social aspects play a big part in normalising eating and separating actual hunger from emotional hunger (Robison *et al*, 1995).

2.2 *OBJECTIVES*

The objective of the present study was to examine the effects of weight cycling on body composition in rats fed a low-fat chow diet.

2.3 *Materials and Method*

2.3.1 Animal Handling

Twenty male Sprague-Dawley rats (320.5±7.6g) were kept in the Laboratory Animal Service Centre, LASEC, with 2 rats per cage. The room was kept at 23°C with a 12/12 hour light-dark cycle. The body weight and the food intake were recorded every day.

As the experiment was done without replacing the Chow diet (PicoLab® Rodent Diet 20 – Lab Diet, Australia) with any customised one, only one week of stabilisation was introduced. However, in order to make the supply in the food restriction period more accurate the Chow diet was ground into powder. During the stabilisation, the weight of the rats and the food intake (32g/rat/day) were noted so as to help grouping and determining the amount of food to be given in the food restriction period.

After one-week stabilisation, the rats were divided into 2 groups with the initial weight as close as possible between the groups. In each group, there were 10 rats. One of the groups was the Control group (CTL) and the other was the Weight Cycling group (WC). The CTL group rats were allowed to consume food *ad libitum* during the course of experiment while the WC group was subjected to food restriction twice.

The course of experiment was divided into 4 periods. From Day 0 to Day 3 and Day 11 to Day 14, the WC group was allowed to have half of the usual food intake, i.e. 16g/rat/day. From Day 4 to Day 10 and Day 15 to Day 21, both groups were allowed to eat *ad libitum*. The rats were sacrificed on Day 11 and Day 22,

the end of each cycle of food restriction and *ad libitum* period. Five rats were killed for each group each time.

Rats were sacrificed for fatty acid profile determination, proximate analysis and measurement of serum triglycerides and cholesterol levels.

The nutritional value of the Chow diet (PicoLab® Rodent Diet 20 – Lab Diet, Australia) according to the supplier is shown in Tables 2.1 and 2.2. Free access of distilled water was granted during the whole course of experiment.

Table 2.1: Composition of the Chow diet for Weight Cycling Experiment.

Nutrients	Percentage (w/w)
Crude Protein not less than	20.0
Crude Fat (ether extract) not less than	4.5
Crude Fiber not more than	6.0
Nitrogen-Free Extract (by difference)	54.8
Ash not more than	7.0

Table 2.2: Composition of fat of the Chow diet for Weight Cycling Experiment*.

Nutrients	Percentage of Weight of Diet
Linoleic Acid	2.26
Linolenic Acid	0.2
Arachidonic Acid	<0.01
Omega-3 Fatty Acids	0.36
Total Saturated Fatty Acids	0.84
Total Monounsaturated Fatty Acids	1.04
Cholesterol, ppm	149

*Ingredients used by the supplier:
Ground corn, dehulled soybean meal, wheat middlings, ground wheat, fish meal, dried beet pulp, wheat germ, cane molasses, brewers dried yeast, ground oats, dehydrated alfalfa meal, dried whey, soybean oil, calcium carbonate, salt, DL-methionine, choline chloride, vitamin A acetate, cholecalciferol, cyanocobalamin, calcium pantothenate, folic acid, riboflavin, thiamin mononitrate, nicotinic acid, menadione dimethylpyrimidinol bisulphite (source of vitamin K), pyridoxine hydrochloride, DL-alpha tocopheryl acetate, calcium iodate, cobalt carbonate, copper sulphate, manganous oxide, ferrous carbonate, zinc sulphate, zinc oxide.

In each killing, the rats were given nitrogen anaesthesia before exanguination. Tissue towel soaked with diethylether covering the mouth of the rat was used to secure the anaesthesia. Blood was drawn from the abdominal aorta vacuum using Vacutainer (Becton Dickinson) – SST tubes with Gel & Clot Activator and Vacutainer Brand Blood Collection Needles – 18G1 (Becton Dickinson). The chest was massaged to allow a faster flow of blood to the tube. Blood was then kept on ice before centrifugation. Centrifugation of blood to separate serum from other components was done after 30 minutes but no later than 2 hours after the collection. In this period of time, the blood was allowed to clot. The force of centrifugation was maintained at 1000-1300g, and the temperature was kept at 4°C. Once the serum was separated from the blood cells, it was aliquoted into micro-centrifuge tubes and immediately stored at –80°C.

The whole liver, the perirenal and the epidydimal adipose tissues from both sides were excerised. They were washed in chilled 0.9% saline and blotted dry. Then they were weighed separately. They were immediately put into small vials and frozen in liquid nitrogen before being stored at –80°C.

The carcass was also saved for future use. They were wrapped up and put in the –80°C freezer. When they were about to be used, they were allowed to thaw thoroughly overnight at room temperature. Then the whole carcass was ground with a mincer.

The organs and the carcass were all used for fatty acid analysis. The carcass was also used in the proximate analysis. This study was approved by the Animal Research Ethics Committee of the Chinese University of Hong Kong.

2.3.2 LIPID ANALYSIS

Lipid analysis was carried out with Gas Liquid Chromatography. Different fatty acid components can be separated and the ratio can be calculated.

2.3.2.1 Adipose Tissues

Extraction with Chloroform:Methanol (2:1)

One hundred milligrams of the perirenal or the epidydimal adipose tissues were added into a 50ml conical tube. Into each tube, 12mg of tri-heptadecanoin (Sigma) in chloroform:methanol (2:1 v/v) were added as an internal standard for the quantification of fatty acids in adipose tissues. Up to 15ml of $CHCl_3$:MeOH and 3ml of 0.9% NaCl were added to the sample. It was then homogenised with Polytron (Kinematica, Switzerland). The homogenate was centrifuged at 2500rpm and room temperature for 15 minutes. Four millilitres of the bottom organic layer containing the fatty acids were transferred into a clean glass screwed cap tube. The content of the glass tube was allowed to dry with a slow flush of nitrogen in a 30°C water bath.

Methylation

Two millilitres of 14% boron tri-fluoride (Sigma) in methanol and 1ml of toluene were added to the tube in the dark. Flush of N_2 gas was used to expel O_2 from the tube in order to avoid oxidation of the fatty acids in the next step. The content was mixed by vortexing. The tube was screw capped immediately, covered with aluminium foil, and incubated at 95°C for an hour in a dry bath. Then the tube

was allowed to cool down to room temperature before it was uncapped. Three millilitres of hexane and 1ml of water were added and the content was vortexed. The tube was centrifuged at 2500rpm for 15 minutes. The upper hexane layer containing the fatty acyl methyl esters (FAME) was transferred to a clean test tube and allowed to dry in a steady stream of N_2 in a water bath not more than 30°C. The dried FAME was re-dissolved in 1ml of hexane, and transferred to a vial and ready for gas liquid chromatography (GLC).

2.3.2.2 Carcass

Extraction with chloroform:methanol (2:1 v/v)

Six hundred milligrams of the carcass were added into a 50ml conical tube. Into each tube, 8mg of tri-heptadecanoin (Sigma), 1.2mg of L-α-phosphotidyl-choline diheptadecanoyl (Sigma) and 1 mg of heptadecanoic acid (Sigma) in chloroform:methanol with a ratio of 2:1 were added as internal standards for the quantification of triglycerides, phospholipids and free fatty acids, respectively, in the carcass. The steps of extraction of fat were performed as described in section 2.3.2.1 until the first drying by nitrogen.

Separation of Triglycerides, Phospholipids and Free Fatty Acids by Thin Layer Chromatography

A hundred and twenty microlitres of chloroform was used to re-dissolve the fat in the test tube. The re-dissolved fat was applied as a line onto a thin layer chromatography (TLC) plate, DC-Fertigplatten SIL G-25 UV254 (Macherey-

Nagel, Germany). Internal standards were also applied onto the same plate. After the sample was dry, the TLC was run with hexane:diethylether:acetic acid with a ratio of 80:20:1 v/v/v for around an hour until the solvent front reached almost the top of the plate. The plate was taken out from the tank and allowed to air dry in a fume hood. The dried plate was then sprayed with a fluorescent dye, 2',7'-Dichlorofluorescein (Sigma), 0.02% in ethanol. The plate was allowed to dry in a fume hood before being illuminated under an UV lamp at 254nm. The corresponding bands of triglycerides, phospholipids and free fatty acids were identified and marked with a pencil. The bands were scratched out and transferred to the corresponding screw cap tubes separately.

Methylation

The steps of methylation were the same as those in section 2.3.2.1. The dried FAME was re-dissolved in 800µl, 400µl, 200µl and 100µl of hexane, respectively, and transferred to different vials. Glass inserts were used for the tubes containing 200µl and 100µl of FAME and ready for gas liquid chromatography (GLC).

2.3.3 PROXIMATE ANALYSIS

2.3.3.1 Crude Fat

After defrosting, approximately 500mg of carcass were weighed into a 50ml conical tube. Fifteen millilitres of chloroform:methanol (2:1 v/v) and 1 ml of 0.9% NaCl were added in each sample. The samples were homogenised with Polytron (Kinematica, Switzerland). The lower organic layer was transferred

totally to a weighed clean test tube. Then it was dried by a constant flow of nitrogen gas under an evaporator. The fat could be dried in a water bath at around 30°C.

At the same time, the process of homogenisation by adding $CHCl_3$:MeOH and saline was repeated twice. After each repetition, the lower organic layer was transferred to the same corresponding test tube for drying.

The dried tubes of fat were incubated in a 60°C oven overnight to remove any water in the fat. They were then cooled down to room temperature in a desiccator before weighing. The weight increase in the test tube represents the weight of fat in each sample. The percentage of crude fat was calculated by the weight increase over the weight of sample.

$$\% \text{ Fat} = \frac{\text{(Final weight of test tube with dried fat – Initial weight of clean test tube)}}{\text{Weight of Sample}}$$

2.3.3.2 Crude Protein

The nitrogen determination by Kjeldahl method was adopted in this part of experiment. Approximately 500mg of the thawed carcass were weighed into a 350ml Kjeldahl flask. One piece of Kjeltab (Fisher Scientific) and 12.5ml of 18M sulphuric acid were added into the flask in a fume hood. The digestion process was only carried out in the fume cupboard. The flask was placed in a Digestor (Tecator, Perstorp Analytical, Sweden) and heated to 420°C for 1.5 hours, i.e. about 20 to 30 minutes after the contents of the flask became clear to ensure complete oxidation of the sample. During the process, a conical funnel was put

over the flask in order to condense back the sulphuric acid vapour. Then the Kjeldahl flask was allowed to cool down to room temperature in the fume hood. In the meanwhile, 50ml of 2% solution of boric acid was added into a 250ml conical flask together with a few drops of the screened methyl red indicator. Drop by drop, 0.1M sodium hydroxide was added to the boric acid until the colour just turned green. Then, 0.1M hydrochloric acid was added to the same flask dropwise to just turn the green colour back to pink. By doing so, the solution of boric acid was adjusted to the screened methyl red endpoint.

The cooled Kjeldahl flasks were put in a water bath at room temperature. Then 75ml of double distilled water was added into each tube to dilute the concentrated sulphuric acid. Placing the Kjeldahl flask and the conical flask prepared as above to the Kjeldahl Distilling Unit (Tecator, Perstorp Analytical, Sweden), 50ml of 40% NaOH was mixed with the contents in the Kjeldahl flask. Steam was introduced into the Kjeldahl flask by a steam generator in the distillation set. The distillate was collected in the conical flask with boric acid up to around the 150ml mark on the conical flask.

The original red colour of the boric acid was turned to green by the ammonia from the distillate resulted from the acid digestion. The boric acid was then titrated with 0.1M HCl.

The percentage of nitrogen in the sample can be calculated as followed:

$$\% \text{ Nitrogen} = \frac{(\text{Volume of HCl in ml} \times \text{Exact molarity of HCl} \times 1.4)}{\text{Weight of Sample}}$$

% Protein = % Nitrogen X Factor (for general products: 6.25)

2.3.3.3 Moisture

After thawing, about 1g of carcass was weighed together with a small pre-weighed tray of aluminium foil (initial weight). The samples were then put overnight at 105°C into an air-forced oven (Shel Lab, Sheldon Manufacturing, Inc). Afterwards, they were taken out from the oven and immediately put into a desiccator for cooling down to room temperature. The weight was then measured again with the aluminium foil (final weight).

The difference between the initial and the final weights indicated the moisture in the sample. Then this difference of weights was put over the net initial weight of sample and the percentage of moisture in the sample can be calculated.

$$\% \text{ Moisture} = \frac{\left[\text{Initial weight of MOIST sample with Al tray} - \text{Final weight of DRIED sample with Al tray}\right]}{\text{Weight of Sample}}$$

2.3.3.4 Ash

Around 1g of defrosted carcass were weighed together with a 30ml dried porcelain crucible with lid. Then the crucible with the content was placed in an air-forced oven (Shel Lab, Sheldon Manufacturing Inc) at 105°C overnight to remove the moisture in the carcass and enhance a more complete incineration in a muffle furnace (Vulcan™, NEY). After the crucible was cooled down, it was transferred to the muffle furnace. The samples, inside the crucibles with lids, were allowed to complete oxidation in the furnace at 550°C overnight. Then the crucibles were allowed to cool down in the closed furnace until they could be transferred to a desiccator for further equilibration to room temperature. After

cooling down, the crucibles were weighed again. The increase in weight of the crucibles marked the weight of ash inside.

$$\% \text{ Ash} = \frac{(\text{Weight of crucible with lid and ash} - \text{Weight of empty crucible with lid})}{\text{Weight of Sample}}$$

2.3.4 SERUM ANALYSIS

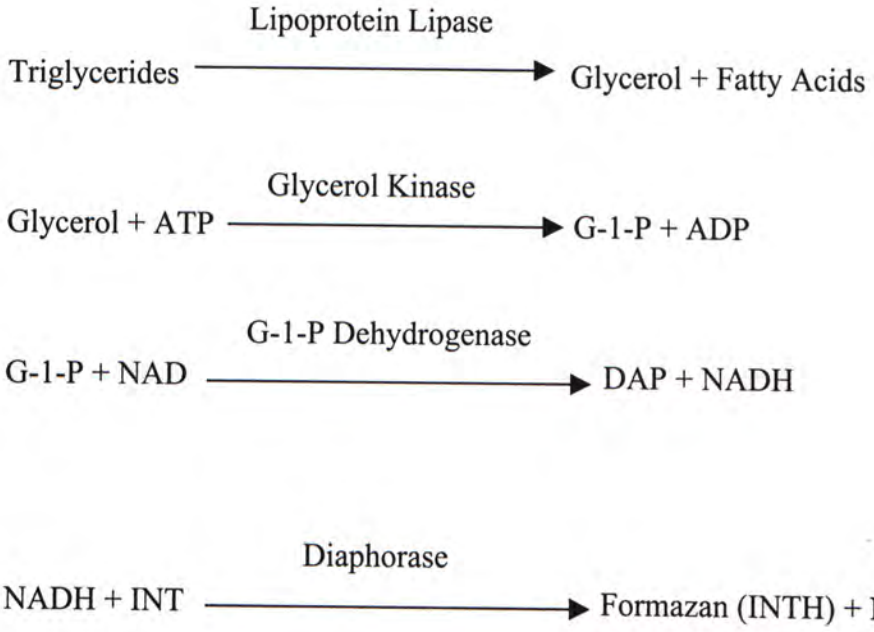
2.3.4.1 Serum Triglycerides

The serum triglyceride level of the non-fasting rats was determined quantitatively by spectrophotometric method with an enzymatic kit (Sigma Diagnostics, Procedure No 336) and triglyceride calibrators (Sigma Diagnostics, Procedure No T 2772).

Ten microlitres of serum sample (or calibrator) were added to 1ml of the reconstituted light-sensitive reagent in a plastic cuvette. After inverted for a few times, it was incubated at room temperature for 18 minutes in the dark. The absorbance of the resultant solution was measured by a spectrophotometer at 500nm against water blank.

$$\text{Triglycerides (mg/ml)} = \frac{A_{TEST} - A_{BLANK}}{A_{CALIBRATOR} - A_{BLANK}} \text{ X [Calibrator]}$$

Principle of the assay:



DAP: dihydroxyacetone phosphate

INT: 2-(p-iodophenyl)-3-p-nitrophenyl-5-phenyltetrazolium chloride

The serum sample was only allowed to be thawed less then 3 times and the serum triglycerides are stable at room temperature for 2 days.

2.3.4.2 Serum Cholesterol

The serum cholesterol level of non-fasting rats was determined quantitatively by spectrophotometric method with an enzymatic kit (Sigma Diagnostics, Procedure No 352) and cholesterol calibrators (Sigma Diagnostics, Procedure No 0284).

Ten microlitres of sample serum (or calibrator) were added to 1ml of the reconstituted light-sensitive cholesterol reagent in a plastic cuvette. After inverted for a few times, it was incubated at room temperature for 10 minutes in the dark.

The absorbance of the resultant solution was measured by a spectrophotometer at 500nm against water blank.

$$\text{Cholesterol (mg/ml)} = \frac{A_{TEST} - A_{BLANK}}{A_{CALIBRATOR} - A_{BLANK}} \text{ X [Calibrator]}$$

Principle of the assay:

$$\text{Cholesterol Esters} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{Fatty Acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2\ H_2O_2 + \text{4-Aminoanipyrine} + \text{p-Hydroxybenzenesulphonate} \xrightarrow{\text{Peroxidase}}$$

$$\text{Quinoneimine Dye} + 4\ H_2O$$

The serum sample was only allowed to be thawed less then 3 times and the serum cholesterol is stable at room temperature for 7 days.

2.3 *RESULTS*

2.4.1 BODY WEIGHT

The body weight was recorded daily (Fig 2.1). From the very beginning of the experiment, the rats weighed 320.5±7.6g before the one-week stabilisation. Before the weight cycling started, the rats weighed 368.0±12.5g and 369.5±7.6g in the CTL group and the WC group respectively.

After the first food restriction for 4-day, the CTL rats weighed 387.0±14.4g while the WC rats weighed 325.5±8.6g. Both groups of the rats gained weight gradually during the 7-day *ad libitum* period, resulting in their body weight gained to 427.5±16.5g (CTL) and 409.0±14.9g (WC). After the second food restriction period, the CTL rats gained weight to 435.0±5.0g while the WC rats lost to 360±14.6g. At the end of the second *ad libitum* period, which is also the end of the experiment, the rats weighed 462±6.7g (CTL) and 431±14.8g (WC). Although the weight of the WC rats could not reach that of the CTL rats, the weight was gained in a faster rate by the WC rats.

2.4.2 FOOD INTAKE

During the stabilisation period, the daily food intake was 32g/rat/day. Thus, the WC rats were given 16g of the powered Chow diet every day. In the *ad libitum* periods, the WC rats took 34.1±3.1g and 34.8±6.2g in the first and the second cycles respectively. In fact, the average food intake of the CTL rats during the

whole experiment was 32.6±4.4g. During free eating, the WC rats showed an increase in food intake at first and gradually returned to the CTL value (Fig 2.2).

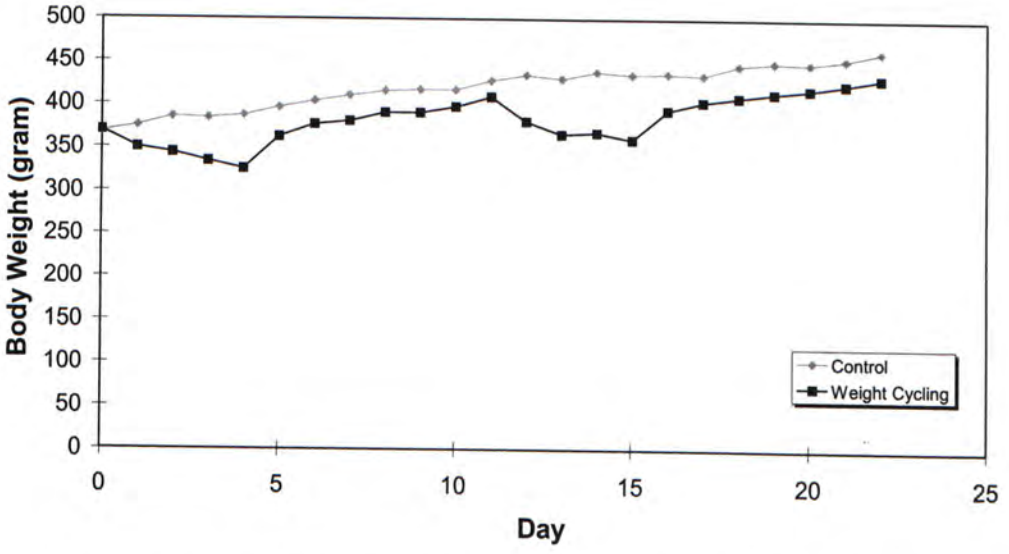


Fig 2.1: Body weight of rats fed a chow diet. (Day 0-Day 11, n=10; Day 12-Day 22, n=5)

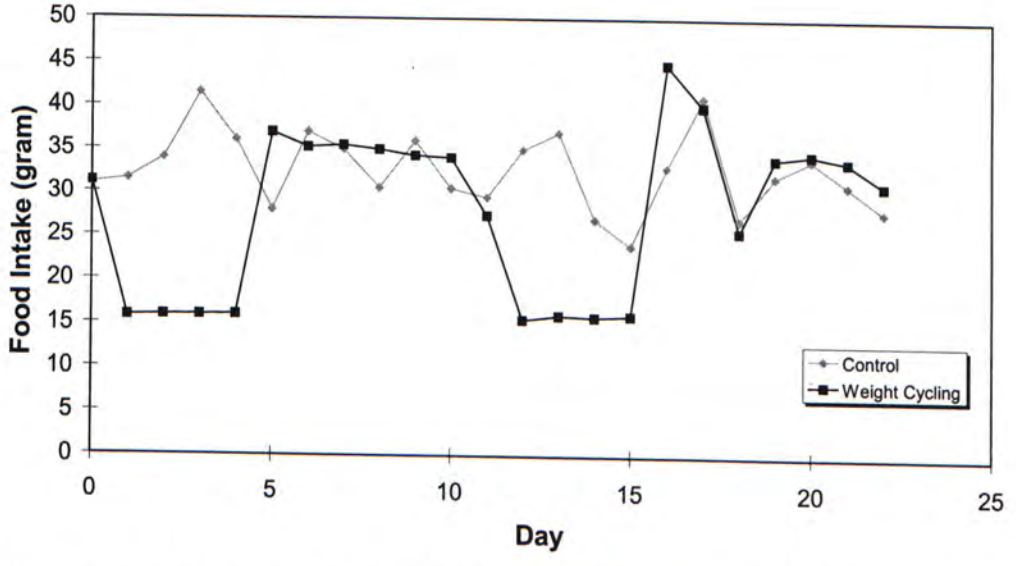


Fig 2.2: Daily diet intake of rats fed a chow diet. (Day 0-Day 11, n=10; Day 12-Day 22, n=5)

2.4.3 ORGAN WEIGHT

Since there was some variation on the body weight of rats being sacrificed even on the same day, the organ weights are also expressed as a ratio to the body weight in order to show a clearer relationship. Differences were compared between the groups killed on the same day by Student's t-test with the notation *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.

2.4.3.1 Liver

After the first cycle, the weights of liver of both groups were similar, 15.7±1.6g (CTL) and 15.5±1.7g (WC). The ratios to the body weight were 0.037±0.002 (CTL) and 0.038±0.003 (WC). After the second cycle, the liver weight gained to 18.8±1.2g and 16.3±1.6g, and the ratios of liver to body weight for the CTL and WC were 0.041±0.003 and 0.038±0.003 for CTL and WC rats, respectively. It appeared that there was a smaller increase in liver weight after weight cycling although no significant difference was noticed (Fig 2.3 and 2.4).

2.4.3.2 Adipose Tissues

The adipose tissues, both the perirenal and the epidydimal fat pads, showed more distinctive changes in their weight throughout the experiment (Fig 2.5 – 2.8).

After Cycle 1, the perirenal adipose tissues weighed 5.04±0.4g (CTL) and 3.48±0.8g (WC). The ratios of the perirenal adipose tissues to body weight for the CTL and WC were 0.012±0.001 and 0.008±0.002 of body weight respectively. After Cycle 2, they weighed 5.68±0.8g for CTL and 4.85±0.7g for WC. The ratios

were 0.012±0.002 for CTL and 0.011±0.002 for WC of body weight respectively (Fig 2.5 and 2.6).

After Cycle 1, the epidydimal adipose tissues weighed 4.00 ±0.40g (CTL) and 3.32±0.1g (WC). The ratios of the epidydimal adipose tissues to body weight for the CTL and WC were 0.009±0.001 and 0.008±0.0001 of body weight correspondingly. After Cycle 2, they weighed 4.31±0.5g for CTL and 3.73±0.4g for WC. The ratios to body weight were 0.009±0.001 of body weight and 0.009±0.001 respectively (Fig 2.7 and 2.8).

Both adipose tissues of the WC rats showed a significant weight decrease compared with those of the CTL rats after the first cycle. However the weights gained were much faster in the WC rats such that at the end of the second cycle the weight was similar to that of the CTL rats.

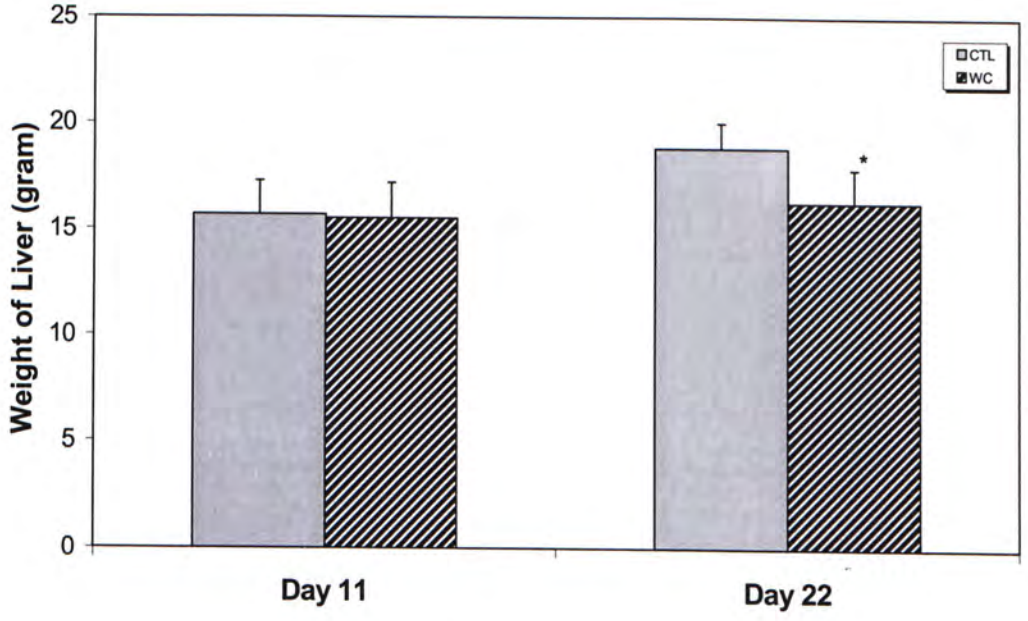


Fig 2.3: Weight of liver of rats fed on chow diet. Grey Bars: Control (CTL); Striped: Weight-Cycled (WC); *: $p<0.05$

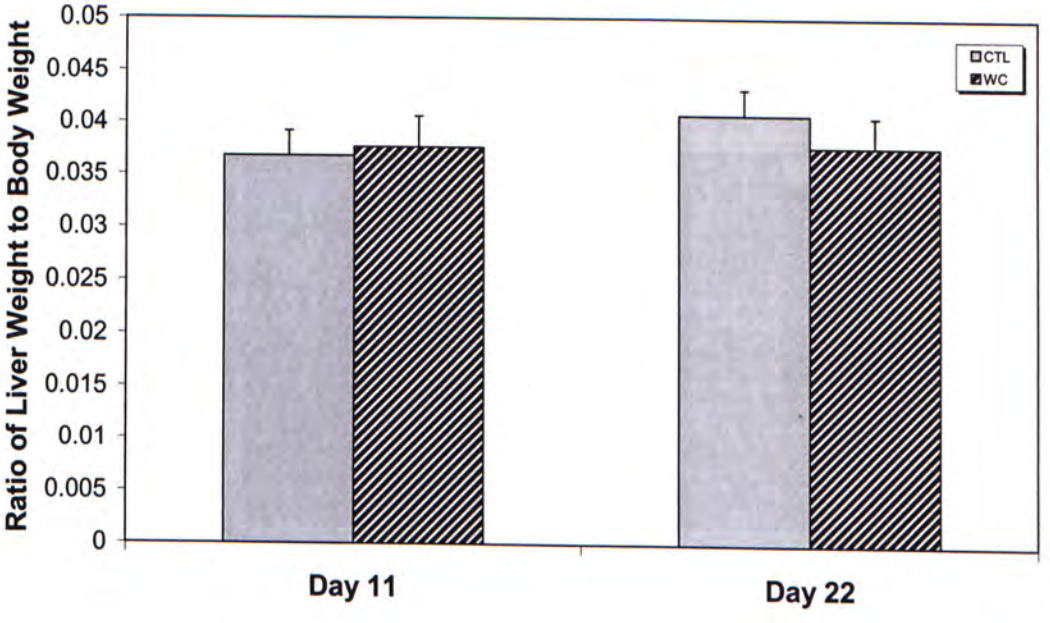


Fig 2.4: Ratio of weight of liver to body weight of rats fed a chow diet. Grey Bars: Control (CTL); Striped: Weight-Cycled (WC)

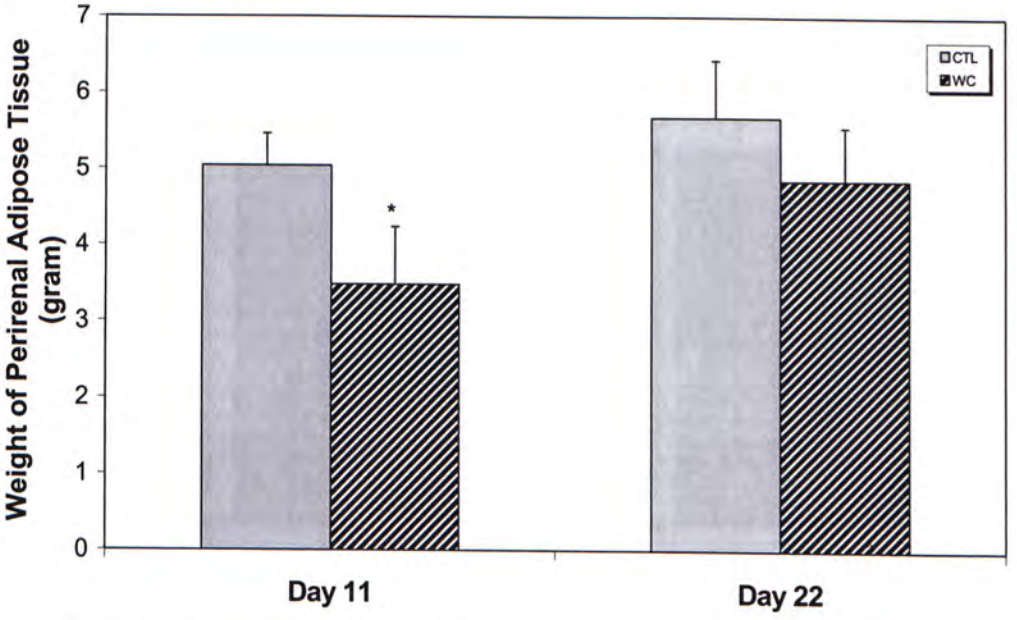


Fig 2.5: Weight of perirenal adipose tissue of rats fed on chow diet. Grey Bars: Control (CTL); Striped: Weight-Cycled (WC); *: $p<0.05$

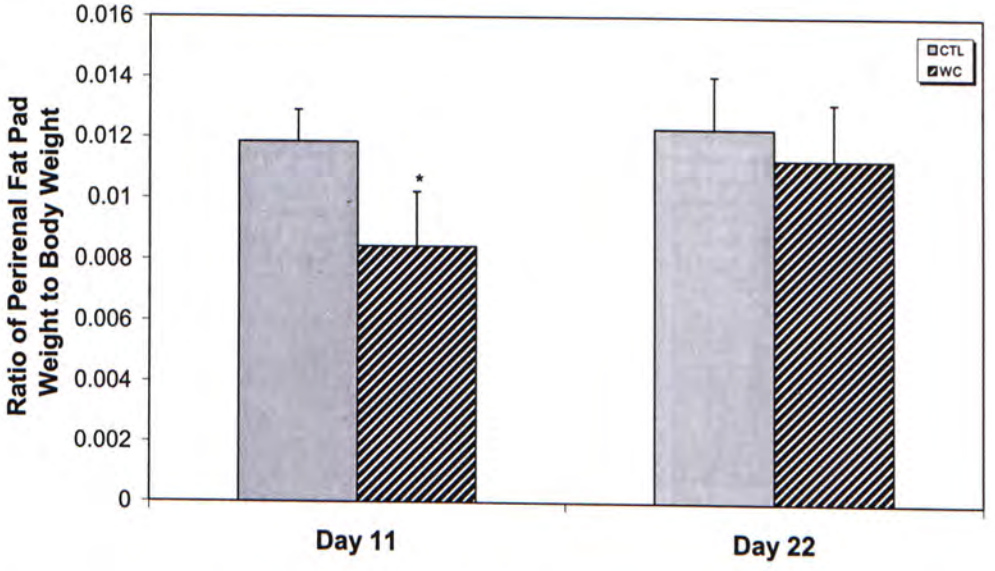


Fig 2.6: Ratio of weight of perirenal adipose tissue to body weight of rats fed a chow diet. Grey Bars: Control (CTL); Striped: Weight-Cycled (WC); *: $p<0.05$

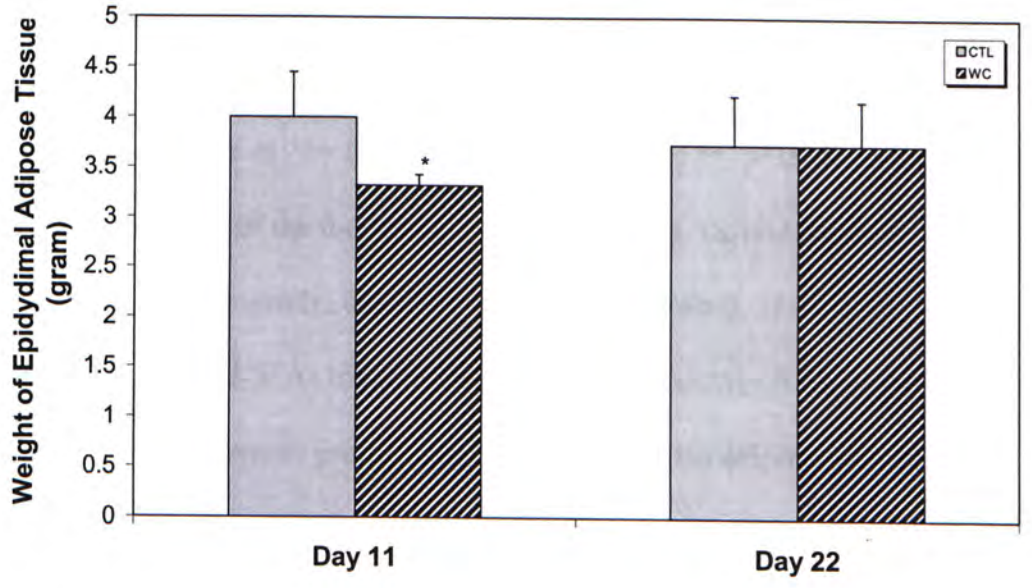


Fig 2.7: Weight of epididymal adipose tissue of rats fed on chow diet. Grey Bars: Control (CTL); Striped: Weight-Cycled (WC); *: $p<0.05$

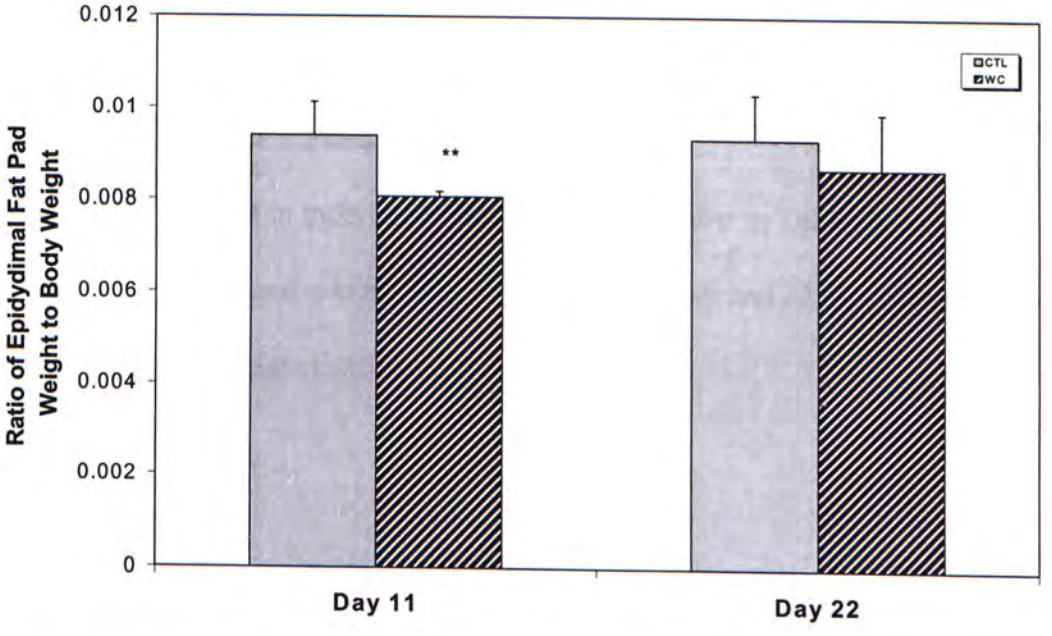


Fig 2.8: Ratio of weight of epididymal adipose tissue to body weight of rats fed a chow diet. Grey Bars: Control (CTL); Striped: Weight-Cycled (WC); **: $p<0.01$

2.4.4 LIPID ANALYSIS

The differences in lipid composition were compared between the two groups killed on the same day by Student's t-test (*: $p<0.05$; **: $p<0.01$; ***: $p<0.005$). Besides the sums of the four categories of fatty acids, individuals were selected and compared separately, they are 16:0, 18:2n-6, 20:4n-6, 18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3. As triglycerides are the major storage form of fatty acids in the fat pads, the overall profiles were determined in the adipose tissues.

2.4.4.1 Adipose Tissues

In general term, the levels of saturated and mono-unsaturated fatty acids (SFAs and MUFAs) increased after cycle 1, and to a larger extent after cycle 2 in the WC group compared with the CTL rats. In contrast, the PUFAs, including n-6 and n-3 PUFAs, decreased after the 2 WC in the WC group compared with those in the CTL rats (Tables 2.3 and 2.4).

The changes in individual fatty acids were shown in Tables 2.3 – 2.4. In general, 16:0 increased while 18:2n-6, 18:3n-3, 20:4n-6 and 22:6n-3 decreased in the WC group compared with the CTL rats.

2.4.4.2 Carcass

The changes in the carcass were similar to those observed in the adipose tissues. Among the saturated fatty acids, 16:0 increased the most in the WC rats after two WC compared with the CTL group. The changes in the n-6 PUFAs were more pronounced in the carcass total lipids, triglycerides and free fatty acids. The

individual n-6 fatty acid which decreased the most was 18:2n-6. Although not all the n-3 fatty acids showed the same general decrease trend, the levels of 18:3n-3 and 22:6n-3 in the WC rats dropped more significantly after the two weight cycling periods (Tables 2.5 – 2.8).

Table 2.3: Fatty acid composition (% of the total) of the perirenal adipose tissues in rats fed on chow diet. Data was expressed as Mean ± SD. The Weight-Cycled (WC) group was significantly different from the Control (CTL) rat at *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.

	Cycle 1 (Day 11)		Cycle 2 (Day 22)	
	CTL	WC	CTL	WC
SFA				
14:0	1.77±0.07	1.77±0.06	1.74±0.09	1.78±0.07
15:0	0.27±0.03	0.27±0.01	0.29±0.03	0.25±0.02
16:0	25.11±1.19	24.80±0.85	25.02±1.37	26.88±1.05**
18:0	4.17±0.32	4.16±0.15	4.45±0.30	4.13±0.24
Sum	31.31±1.01	30.94±0.83	31.50±1.13	33.04±0.94*
MUFA				
16:1n-9	1.40±2.55	0.29±0.07	0.31±0.01	0.21±0.13
16:1n-7	3.60±2.08	4.69±0.23	4.49±0.66	6.23±0.96
18:1n-9	26.34±0.85	28.41±1.21	27.31±1.16	29.84±1.73
18:1n-7	0.97±0.78	1.22±0.95	1.52±0.77	0.86±0.96
20:1n-9	0.45±0.02	0.49±0.03	0.46±0.03	0.45±0.02
20:1n-7	0.24±0.02	0.24±0.03	0.24±0.02	0.25±0.03
Sum	33.01±1.16	35.35±0.54***	34.33±1.33	37.84±1.56*
n-6 PUFA				
18:2n-6	29.66±1.73	28.28±0.72	28.32±2.21	25.36±1.10*
20:2n-6	0.38±0.06	0.35±0.03	0.37±0.03	0.27±0.10
20:3n-6	0.17±0.03	0.15±0.02	0.16±0.03	0.12±0.02
20:4n-6	0.82±0.19	0.60±0.05*	0.79±0.11	0.54±0.07*
22:4n-6	0.18±0.05	0.15±0.03	0.18±0.02	0.12±0.02
Sum	31.21±2.02	29.53±0.69	29.82±2.23	26.40±0.12*
n-3 PUFA				
18:3n-3	2.30±0.13	1.95±0.06***	2.07±0.21	1.82±0.11
20:5n-3	0.28±0.04	0.26±0.03	0.23±0.04	0.27±0.03
22:5n-3	0.48±0.10	0.49±0.05	0.50±0.07	0.45±0.07
22:6n-3	0.97±0.18	0.81±0.08*	0.87±0.22	0.69±0.06
Sum	4.02±0.39	3.50±0.11	3.67±0.38*	3.23±0.21

Table 2.4: Fatty acid composition (% of the total) of the epidydimal adipose tissues in rats fed on chow diet. Data was expressed as Mean ± SD. The Weight-Cycled (WC) group was significantly different from the Control (CTL) rat at *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.

	Cycle 1 (Day 11)		Cycle 2 (Day 22)	
	CTL	WC	CTL	WC
SFA				
14:0	1.55±0.09	1.57±0.04	1.45±0.18	1.01±0.93
15:0	0.33±0.01	0.32±0.01	0.33±0.02	0.28±0.03
16:0	22.95±1.11	23.04±0.51	21.90±1.66	24.69±1.53***
18:0	4.34±0.30	4.26±0.10	4.37±0.22	4.02±0.47
Sum	29.17±0.96	29.18±0.50	28.06±1.68	30.00±1.78**
MUFA				
16:1n-9	0.31±0.05	0.33±0.06	0.37±0.03	0.24±0.16
16:1n-7	4.01±0.86	4.26±0.39	3.55±0.66	5.86±1.63
18:1n-9	26.65±1.16	28.19±1.48	25.87±0.83	29.02±0.98
18:1n-7	0.47±0.68	0.91±0.99	1.92±0.21	0.60±0.87
20:1n-9	0.42±0.02	0.45±0.02	0.44±0.03	0.40±0.05
20:1n-7	0.23±0.02	0.23±0.04	0.23±0.03	0.25±0.05
Sum	32.08±1.27	34.38±0.93**	32.38±1.40	36.40±1.83
n-6 PUFA				
18:2n-6	32.10±1.49	31.36±0.89	31.85±2.00	28.08±2.34***
20:2n-6	0.44±0.05	0.43±0.02	0.50±0.05	0.30±0.09
20:3n-6	0.19±0.02	0.18±0.02	0.23±0.03	0.15±0.03
20:4n-6	0.96±0.17	0.75±0.08*	1.09±0.19	0.63±0.13***
22:4n-6	0.20±0.04	0.18±0.04	0.25±0.06	0.15±0.02
Sum	33.88±1.66	32.90±0.95	33.91±2.23	29.32±2.52***
n-3 PUFA				
18:3n-3	2.49±0.11	2.28±0.10**	2.42±0.16	2.07±0.18***
20:5n-3	0.31±0.09	0.28±0.02	0.35±0.06	0.30±0.04
22:5n-3	0.51±0.12	0.46±0.05	0.64±0.08	0.47±0.15*
22:6n-3	1.18±0.23	0.98±0.07*	1.42±0.32	0.83±0.19**
Sum	4.49±0.49	4.00±0.14	4.83±0.57	3.67±0.38***

Table 2.5: Fatty acid composition (% of the total) of the carcass total lipids in rats fed on chow diet. Data was expressed as Mean ± SD. The Weight-Cycled (WC) group was significantly different from the Control (CTL) rat at *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.

	Cycle 1 (Day 11)		Cycle 2 (Day 22)	
	CTL	WC	CTL	WC
SFA				
14:0	1.76±0.08	1.75±0.05	1.66±0.06	1.81±0.05
15:0	0.32±0.01	0.29±0.00	0.32±0.01	0.28±0.02
16:0	22.41±0.30	22.88±0.66	22.08±0.54	23.73±0.48
18:0	5.69±0.20	5.56±0.23	5.82±0.40	5.333±0.39
Sum	30.19±0.32	30.47±0.49	29.87±0.44	31.15±0.25***
MUFA				
16:1n-9	0.31±0.02	0.31±0.04	0.34±0.02	0.28±0.02**
16:1n-7	3.82±0.41	4.67±0.19	3.71±0.33	5.85±0.69
18:1n-9	24.21±0.47	25.54±0.47	25.09±0.65	26.12±0.62
18:1n-7	2.48±0.06	2.66±0.05	2.61±0.16	2.65±0.08
20:1n-9	0.49±0.02	0.48±0.02	0.50±0.02	0.49±0.02
20:1n-7	0.52±0.08	0.46±0.02	0.47±0.05	0.57±0.13
Sum	31.81±0.57	34.12±0.55***	32.73±0.90	35.96±1.08**
n-6 PUFA				
18:2n-6	30.80±0.67	28.40±0.34***	29.64±0.55	26.42±0.81***
20:2n-6	0.34±0.02	0.32±0.06	0.41±0.03	0.32±0.04
20:3n-6	0.20±0.01	0.20±0.03	0.20±0.01	0.17±0.01
20:4n-6	1.94±0.20	1.86±0.21	1.98±0.23	1.70±0.16
22:4n-6	0.0	0.24±0.02	0.67±0.86	0.27±0.02
22:5n-6	0.17±0.10	0.11±0.02	0.11±0.01	0.09±0.01
Sum	33.44±0.54	31.12±0.62***	33.01±0.89	28.92±0.93***
n-3 PUFA				
18:3n-3	2.03±0.04	1.76±0.03***	1.86±0.05	1.66±0.07***
20:5n-3	0.32±0.03	0.34±0.01	0.31±0.03	0.34±0.03
22:5n-3	0.64±0.13	0.69±0.05	0.66±0.10	0.68±0.09
22:6n-3	1.57±0.15	1.49±0.17	1.57±0.15	1.29±0.15*
Sum	4.56±0.30	4.28±0.20*	4.39±0.25	3.97±0.26

Table 2.6: Fatty acid composition (% of the total) of the carcass triglycerides in rats fed on chow diet. Data was expressed as Mean ± SD. The Weight-Cycled (WC) group was significantly different from the Control (CTL) rat at **: $p<0.01$; ***: $p<0.005$.

	Cycle 1 (Day 11)		Cycle 2 (Day 22)	
	CTL	WC	CTL	WC
SFA				
14:0	1.87±0.09	1.85±0.04	1.78±0.10	1.71±0.10
15:0	0.31±0.00	0.27±0.01	0.31±0.01	0.24±0.02
16:0	21.48±0.31	21.80±1.05	21.71±0.90	21.32±1.01
18:0	4.55±0.29	4.30±0.13	4.84±0.28	3.91±0.32
Sum	28.21±0.51	28.22±1.08	28.64±0.99	27.18±1.19
MUFA				
16:1n-9	0.33±0.02	0.34±0.05	0.37±0.02	0.30±0.03
16:1n-7	4.04±0.53	5.20±0.35	4.01±0.33	5.70±0.67
18:1n-9	25.53±0.41	27.28±0.46	26.60±0.37	24.97±1.21
18:1n-7	2.55±0.08	2.81±0.06	2.85±0.21	2.47±0.12
20:1n-9	0.43±0.01	0.46±0.03	0.44±0.03	0.38±0.02
20:1n-7	0.28±0.01	0.28±0.03	0.26±0.03	0.24±0.03
Sum	33.17±0.68	36.37±0.67***	34.53±0.53	34.06±1.68
n-6 PUFA				
18:2n-6	33.03±0.53	30.49±0.74***	31.52±0.72	25.08±1.02***
20:2n-6	0.36±0.02	0.29±0.03	0.33±0.03	0.21±0.01
20:3n-6	0.15±0.01	0.14±0.01	0.15±0.01	0.11±0.01
20:4n-6	1.02±0.11	0.84±0.07**	1.00±0.05	0.68±0.04***
22:4n-6	0.20±0.03	0.17±0.02	0.22±0.01	0.14±0.01
22:5n-6	0.07±0.01	0.07±0.02	0.08±0.00	0.05±0.01
Sum	34.82±0.53	32.00±0.86***	33.30±0.78	26.26±1.06***
n-3 PUFA				
18:3n-3	2.27±0.06	2.00±0.05***	2.06±0.06	1.66±0.09***
20:5n-3	0.24±0.03	0.27±0.01	0.24±0.02	0.25±0.02
22:5n-3	0.39±0.07	0.40±0.02	0.40±0.03	0.37±0.05
22:6n-3	0.90±0.12	0.74±0.07**	0.82±0.04	0.56±0.04***
Sum	3.81±0.26	3.40±0.12**	3.53±0.15	2.83±0.15***

Table 2.7: Fatty acid composition (% of the total) of the carcass free fatty acids of rats fed on chow diet. Data was expressed as Mean ± SD. The Weight-Cycled (WC) group was significantly different from the Control (CTL) rat at *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.

	Cycle 1 (Day11)		Cycle 2 (Day 22)	
	CTL	WC	CTL	WC
SFA				
14:0	2.01±0.07	2.04±0.05	1.93±0.06	2.02±0.09
15:0	0.40±0.01	0.37±0.01	0.39±0.02	0.35±0.02
16:0	27.10±0.24	28.54±0.63***	27.10±0.77	29.11±0.62***
18:0	6.77±0.20	6.61±0.29	6.83±0.52	6.55±0.40
Sum	36.28±0.27	37.56±0.71**	36.25±1.06	38.03±0.45***
MUFA				
16:1n-9	0.34±0.02	0.36±0.03	0.39±0.02	0.34±0.02
16:1n-7	4.06±0.31	4.61±0.14	3.91±0.33	5.79±0.64
18:1n-9	21.47±0.13	22.39±0.37	21.81±0.60	23.19±0.91
18:1n-7	2.84±0.08	3.08±0.11	3.27±0.24	3.20±0.10
20:1n-9	0.48±0.02	0.50±0.03	0.47±0.02	0.46±0.02
20:1n-7	0.33±0.03	0.34±0.03	0.32±0.04	0.35±0.04
Sum	29.53±0.21	31.29±0.57***	30.16±1.00	33.34±1.40*
n-6 PUFA				
18:2n-6	26.89±0.47	24.64±0.50***	26.37±0.77	22.57±0.99***
20:2n-6	0.38±0.02	0.32±0.04	0.33±0.01	0.25±0.01
20:3n-6	0.24±0.01	0.20±0.02	0.21±0.01	0.19±0.01
20:4n-6	2.17±0.10	1.89±0.16***	2.19±0.35	1.83±0.19
22:4n-6	0.28±0.01	0.26±0.03	0.29±0.02	0.23±0.02
22:5n-6	0.12±0.02	0.11±0.02	0.11±0.01	0.09±0.02
Sum	30.08±0.40	27.40±0.63***	29.49±0.96	25.16±1.21***
n-3 PUFA				
18:3n-3	1.97±0.05	1.69±0.07	1.86±0.11	1.56±0.18
20:5n-3	0.24±0.02	0.28±0.01**	0.26±0.03	0.27±0.04
22:5n-3	0.53±0.04	0.55±0.05	0.57±0.08	0.59±0.10
22:6n-3	1.37±0.04	1.23±0.14*	1.41±0.25	1.04±0.16*
Sum	4.12±0.05	3.75±0.20**	4.10±0.42	3.47±0.44

Table 2.8: Fatty acid composition (% of the total) of the carcass phospholipids of rats fed on chow diet. Data was expressed as Mean ± SD. The Weight-Cycled (WC) group was significantly different from the Control (CTL) rat at *: $p<0.05$.

	Cycle 1 (Day 11)		Cycle 2 (Day 22)	
	CTL	WC	CTL	WC
SFA				
14:0	0.60±0.06	0.58±0.01	0.61±0.08	0.60±0.05
15:0	0.29±0.00	0.29±0.07	0.26±0.01	0.24±0.01
16:0	20.22±0.63	19.69±0.24	19.75±0.29	19.82±0.58
18:0	12.04±0.56	11.47±0.25	11.60±0.86	11.60±0.53
Sum	33.15±1.14	32.03±0.39*	32.22±1.08	32.25±1.00
MUFA				
16:1n-9	0.58±0.63	0.22±0.02	0.22±0.03	0.21±0.02
16:1n-7	1.74±0.20	2.03±0.09	1.77±0.22	2.74±0.33
18:1n-9	16.24±1.13	17.97±0.67	17.22±1.19	17.91±1.16
18:1n-7	2.20±0.07	2.35±0.07	2.31±0.11	2.46±0.20
20:1n-9	0.40±0.01	0.39±0.03	0.41±0.03	0.41±0.01
20:1n-7	0.19±0.01	0.19±0.02	0.23±0.03	0.1954±0.02
Sum	21.36±1.02	23.14±0.71*	22.15±1.44	23.93±1.60
n-6 PUFA				
18:2n-6	24.06±0.67	24.36±0.15	24.14±1.42	23.54±0.57
20:2n-6	0.30±0.04	0.23±0.04	0.39±0.28	0.32±0.34
20:3n-6	0.59±0.13	0.55±0.11	0.51±0.05	0.53±0.02
20:4n-6	8.83±0.50	8.26±0.26*	8.75±0.70	8.51±0.50
22:4n-6	0.81±0.19	0.62±0.03	0.68±0.04	0.64±0.05
22:5n-6	0.33±0.03	0.33±0.03	0.31±0.04	0.31±0.02
Sum	34.91±0.53	34.35±0.30*	34.77±0.69	33.84±0.57*
n-3 PUFA				
18:3n-3	0.69±0.06	0.66±0.04	0.73±0.12	0.65±0.09
20:5n-3	0.78±0.09	0.79±0.05	0.74±0.08	0.75±0.02
22:5n-3	2.23±0.10	2.25±0.03	2.21±0.33	2.42±0.21
22:6n-3	6.89±0.25	6.78±0.14	7.17±0.82	6.17±1.05
Sum	10.59±0.29	10.48±0.12	10.85±0.03	9.99±1.06

2.4.5 PROXIMATE ANALYSIS

In this part, the crude fat, moisture, crude protein and ash were measured in carcass, i.e. whole body excluding blood, liver, perirenal and epidydimal adipose tissue. Differences were compared between the two groups of animal killed on the same day by Student's t-test with the notation *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.

2.4.5.1 Crude Fat

There were no significant differences in the crude body fat between the WC and CTL rats (Fig 2.7) although the former was slightly higher. After cycle 1, the body fat for the WC rats was 10.1%; it increased to 10.5% after cycle 2. The body fat for the CTL group was 9.5% after cycle 1 and increased to 9.7% after cycle 2.

2.4.5.2 Moisture

Moisture in the WC rats was lower compared with the CTL group after cycle 1. However, no difference was observed between the two groups after cycle 2 (Fig 2.8).

2.4.5.3 Crude Protein and Ash

Crude protein in the WC rats was 20.9-21.6% while that of the CTL rats was 21.1% (Fig 2.9). The ash in the CTL rats was 3.2-3.3% while that in the WC rats was 3.1-3.4% (Fig 2.10). However, no significant differences in the crude protein and ash were observed between the CTL and WC rats.

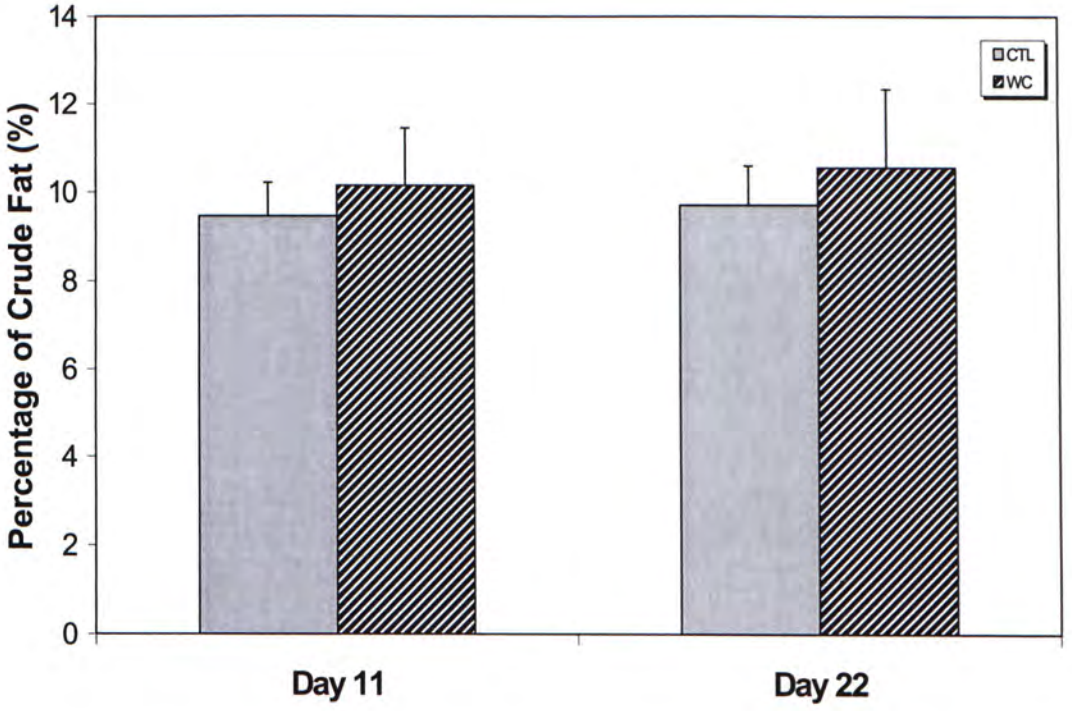


Fig 2.7: Percentage of crude fat of carcass in rats fed a chow diet. Grey Bars: Control (CTL); Striped: Weight-Cycled (WC)

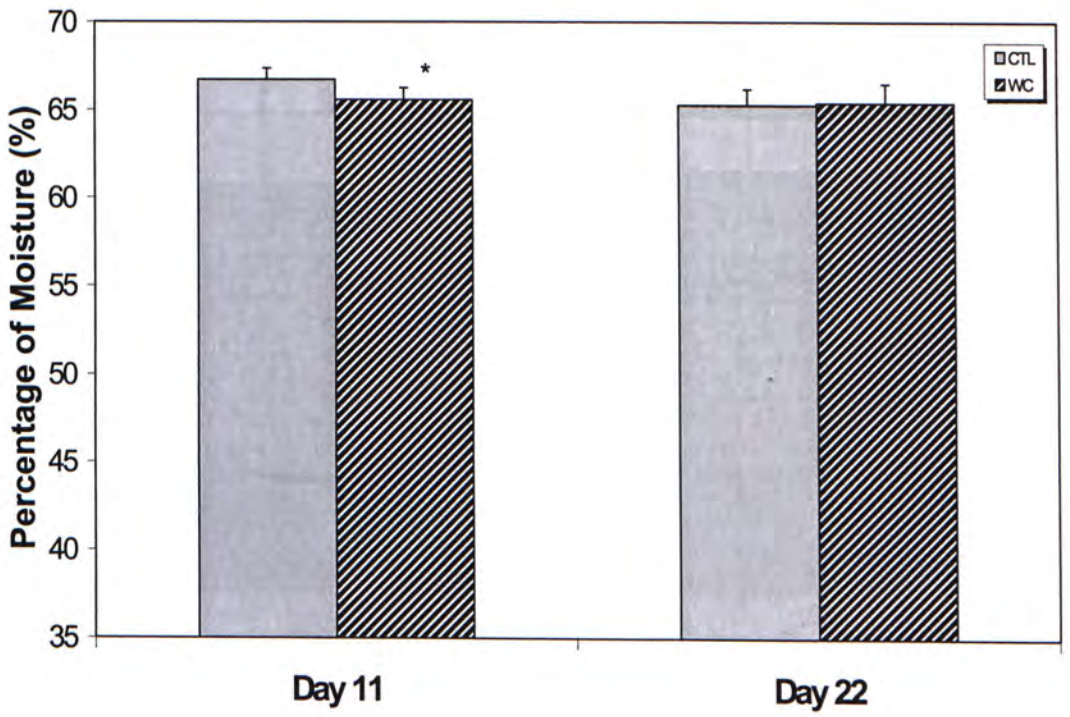


Fig 2.8: Percentage of moisture of carcass in rats fed a chow diet. Grey Bars: Control (CTL); Striped: Weight-Cycled (WC); *: $p<0.05$

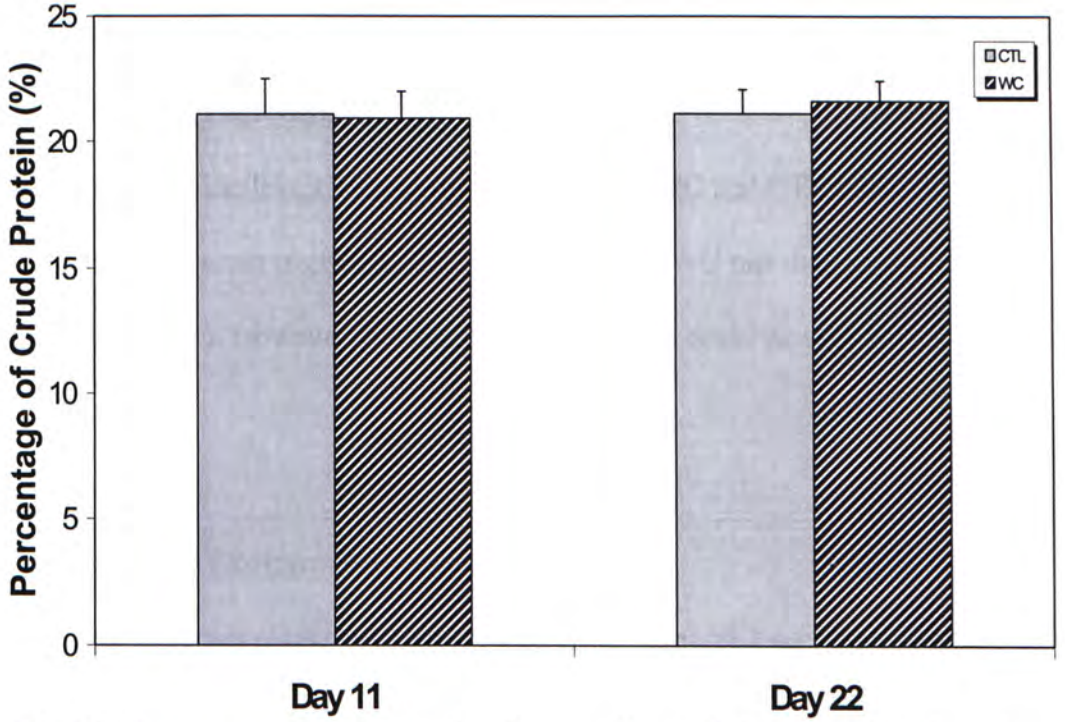


Fig 2.9: Percentage of crude protein of carcass in rats fed a chow diet. Grey Bars: Control (CTL); Striped: Weight-Cycled (WC)

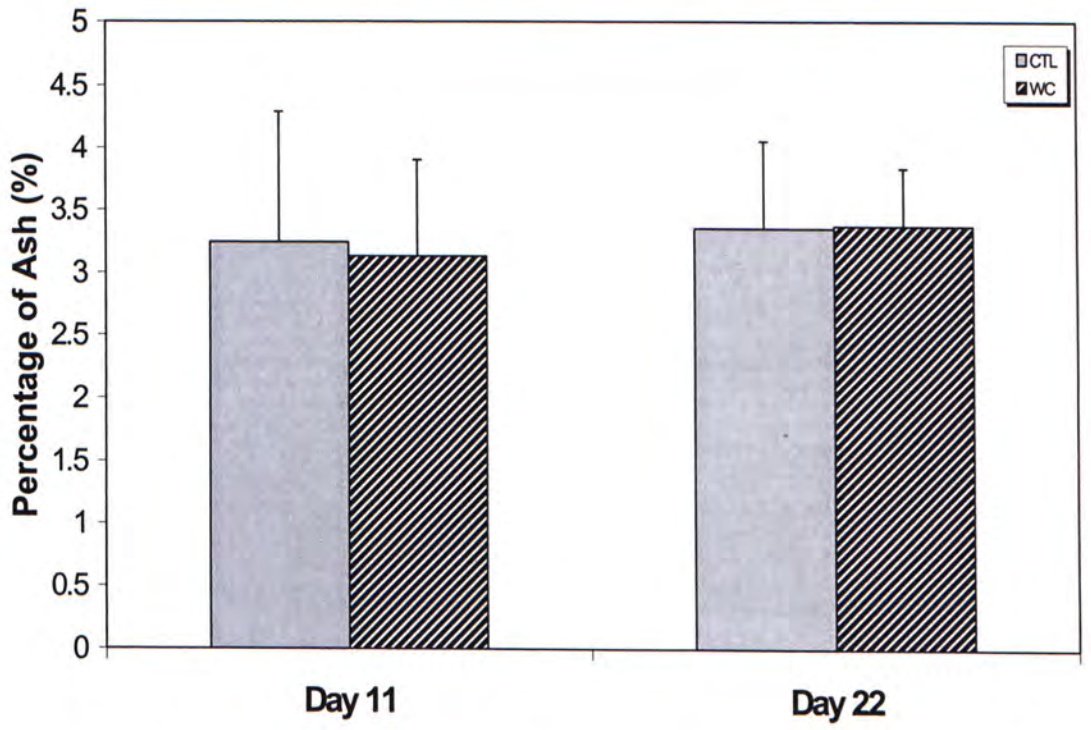


Fig 2.10: Percentage of ash of carcass in rats fed a chow diet. Grey Bars: Control (CTL); Striped: Weight-Cycled (WC)

2.4.6 SERUM ANALYSIS

2.4.6.1 Serum Triglycerides

Serum triglyceride levels were determined in both WC and CTL rats. After cycle 1, the level of serum triglycerides was higher in the WC rats than that in the CTL group (Fig 2.11). However, no significant difference could be observed after cycle 2.

2.4.6.2 Serum Cholesterol

The level of serum cholesterol in the WC rats (81.7mg/dL) was significantly higher than that in the CTL rats (67.4mg/dL) after cycle 1 (Fig 2.12). Similar to the changes in serum triglycerides, the difference in the serum cholesterol levels became insignificant between the two groups after cycle 2.

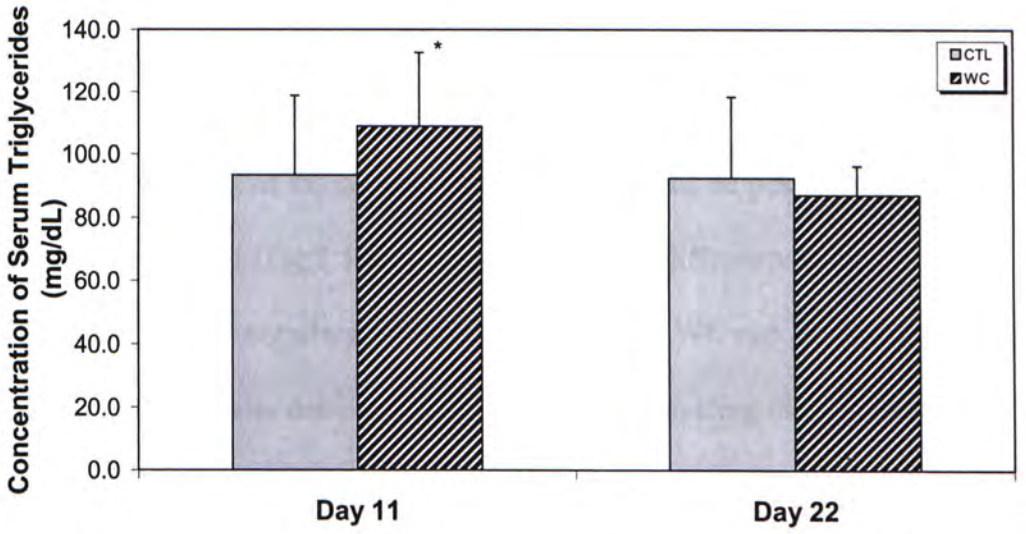


Fig 2.11: Concentration of serum triglycerides in rats fed a chow diet. Grey Bars: Control (CTL); Striped: Weight-Cycled (WC); *: $p<0.05$

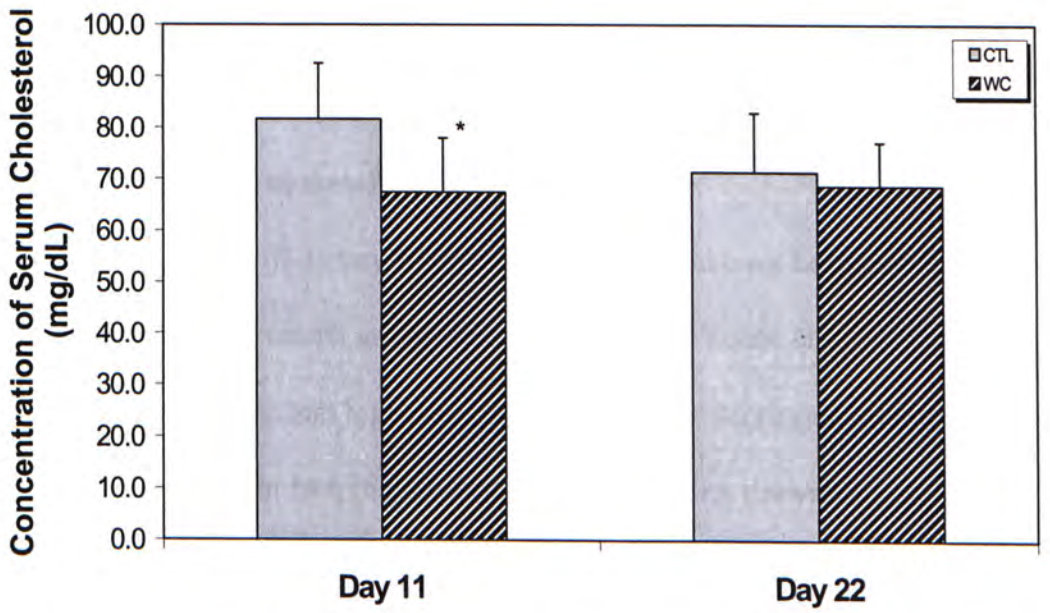


Fig 2.12: Concentration of serum cholesterol in rats fed a chow diet. Grey Bars: Control (CTL); Striped: Weight-Cycled (WC); *: $p<0.05$

2.5 *DISCUSSION*

The weight cycling-induced obesity could not be confirmed in the present study. A commercial chow diet with 5% of energy derived from fat was used during the experiment. Both of the perirenal and the epidydimal adipose tissues decreased only after cycle 1 (Fig 2.5-2.7). After cycle 2, the difference in both adipose tissues became insignificant between the CTL and WC rats. In this regard, the crude body analysis demonstrated that the weight cycling did not change the body fat (Fig 2.9). The present results did not support the idea of 'weight cycling-induced obesity' if the rats were on a low-fat diet. The observation was in agreement with previous studies using also a low-fat diet (Hill *et al*, 1987; 1988; Wheeler *et al*, 1990) or a medium-fat diet (Sea *et al*, 2000). However, weight cycling-induced obesity could occur if a high-fat diet was used (Sea *et al*, 2000). It is concluded that the obesity induced by repeatedly dieting would not be a major problem for a dieter on diet with a low to moderate level of dietary fat if the present data could be transferred to humans.

The levels of dietary fat in relation to body fat have been extensively studied by other research groups (Verboeket-van de Venne *et al*, 1994; Horton *et al*, 1995). A low-fat and high carbohydrate diet is usually associated with a lower level of body fat. In fact, the subjects fed a low-fat diet showed a negative fat-balance, probably due to the net endogenous fat oxidation, leading to the shrinkage of adipose tissues compared with subjects fed with a high-fat diet. It has been shown that high-fat diets promote central adiposity compared with other isocaloric diets (Schutz *et al*, 1992; Boozer, *et al*, 1995). Moreover, Lauer and

associates (1999) recently found no correlation between rats most prone to dietary obesity and those prone to weight gain after weight cycling. They found out that during the weight cycling phase, the weight cycled groups consume less energy and gained less weight than the controls; during the follow-up phase, the weight gain and energy intake did not differ significantly between the control and weight-cycled groups (Lauer *et al*, 1999).

Weight cycling does change the body fatty acid composition although it may not necessarily change the level of body fat. The changes in the fatty acid composition of adipose tissues and carcass were specific during weight-cycling, with selective depletion of n-3 and n-6 PUFAs and preferential accumulation of saturated fatty acids. Among the n-3 and n-6 PUFAs, 18:2n-6, 18:3n-3 and 22:6n-3 decreased the most in response to weight cycling. Among the SFAs, 16:0 increased the most in the adipose tissue and carcass after the two weight cycles. It must be noticed that the same diet was used throughout the experiment, thus, the changes observed in the body fatty acid composition must be a result of weight cycling which causes preferential oxidation of n-3 and n-6 PUFAs and slower oxidation or promoted synthesis of the SFAs.

The changes in the individual fatty acids observed in the present study were in agreement with the previous reports in the literature. In a study by Chen and collegues (1995), the amount of 18:2n-6 and 18:3n-3 in the whole body store is specifically and significantly decreased while the SFAs and MUFAs accumulate simultaneously despite adequate supply of n-6 and n-3 PUFAs in the diet during the *ad libitum* refeeding period. These changes correlate to the number

of weight cycles (Chen *et al*, 1995). In other studies, a 2-fold depletion of 18:2n-6 and 18:3n-3 is observed in WC rats compared with CTL rats (Chen *et al*, 1996; 1997). Although 20:4n-6 and 22:6n-3 are accumulated in the carcass of both of the groups, it has been observed that this accumulation is much lower in the WC rats regardless of the calorie restriction, the pattern of weight cycling, the animal age nor the dietary level of these fatty acids. In humans, selective depletion of the 18:3n-3 in the abdominal and gluteal adipose tissues without any decrease in 22:5n-3 and 22:6n-3 is noticed (Hudgins and Hirsch, 1996).

These differential changes in the body storage of various fatty acids induced by weight cycling may be explained by differential mobilisation and oxidation of specific fatty acid to yield energy during the energy restriction period and accumulation/synthesis during the *ad libitum* refeeding period with sufficient food supply (Leyton *et al*, 1987). Relative mobilisation of fatty acid is defined as '% in FFA / % in TG' by Raclot and Groscolas (1993). Under *in vitro* and *in vivo* conditions of stimulated lipolysis from the white adipocytes, there is a correlation between the preference of mobilisation and the molecular structure of a fatty acid. For a given chain length, the relative mobilisation increases exponentially with unsaturation. Amongst the fatty acids with 18 to 22 carbon atoms, shorter-chain fatty acids have higher values of relative mobilisation. On the other hand, for a given unsaturation, the relative mobilisation decreases with increasing chain length (Raclot and Groscolas, 1993; 1995).

The different oxidative rate of fatty acids may be one of the factors contributing to the weight cycling-induced changes in body fatty acid composition

observed in the present study. In a study using rat liver mitochondria, it has been found that 18:2n-6 is oxidised at a rate 1.2 times higher than that of 16:0 (Reid and Husbands, 1985). In a more detailed study, the oxidation of 18:2n-6 and 18:3n-3, as percentage of dietary amount in rats, has been found to increase 66% and 78% to 82% and 88%, respectively, after a 4-weight cycle treatment (Chen *et al*, 1996).

Preferential biosynthesis and storage of SFAs and MUFAs after weight cycling would be another important factor. It has been shown that the amount of radioactivelly labelled acetate incorporated into 16:0, 18:0, 16:1n-7, 18:1n-9 are 2, 3, 8 and 24-fold higher, correspondingly, after refeeding a fat-free diet for a day in rats previously fasted for two days (Allman *et al*, 1965).

In conclusion, starvation-refeeding promotes the biosynthesis of SFAs and MUFAs. The PUFAs, especially 18:2n-6 and 18:3n-3, are preferably mobilised for oxidation during the energy restriction period. Higher rate of oxidation of PUFAs in the mitochondria further triggers the mobilisation of PUFAs from the adipose tissues. As a result, the percentage of SFAs and MUFAs increases while the reserve for the PUFAs decreases in the adipose tissue and carcass triglycerides.

Moreover, the present study showed that the change in the fatty acid profile was more pronounced in the epidydimal adipose tissue than the perirenal adipose tissue after the second WC (Fig 2.3-2.4). It suggested that the epidydimal adipose tissue was more important to supply the energy with PUFAs and the selective storage of SFAs during the food restriction period. In fact, the original reserve of SFAs was observed to be smaller in the epidydimal adipose tissue than

in the perirenal adipose tissue; while the reserves of both of the n-6 and n-3 PUFAs were larger in the epidydimal fat pad. Therefore, the mobilisation of PUFAs from the epidydimal fat pad was more preferred. However, the difference in the fatty acid metabolism in the two fat pads needs to be proven.

For carcass phospholipids, the changes in most of the individual fatty acids after the 2 WC were not significant. It may be because phospholipids function mostly in the cell membrane structure. Even though there was an introduction of energy restriction, the structure of the cell membrane had to be maintained (Fig 2.8).

The effect of weight cycling on serum lipoprotein profile remains inconclusive. The present study demonstrated that serum triglyceride and cholesterol levels in the WC rats were significantly different from those of the CTL only at the end of cycle 1 but not at the end of cycle 2. The observation was in agreement with previous findings, which in general showed no significant increase in the total cholesterol and triglycerides levels in the weight-cycled humans (Lissner *et al*, 1990; Melby *et al*, 1991; Jeffery *et al*, 1992). In contrast, Olson and associates (2000) has demonstrated that the level of HDL cholesterol in the weight cyclers is lower than that in the non-cyclers, which is obviously believed to have correlation to the risk in cardiac events (Olson *et al*, 2000). On the other hand, in a study with the starvation-refeeding lasted for 8 cycles, there is an approximately 2-fold decrease in postprandial serum triglyceride level (Kochan *et al*, 2001).

Chapter THREE

DEGREES OF FOOD RESTRICTION ON BODY FATTY ACID COMPOSITION

3.1 INTRODUCTION

Dieting, or food restraint is so easily to be practised amongst the general public, in different genders, age groups and figures. Dieters adopt different methods in their dieting programme and most of them cut the energy intake in the first place. They may skip a meal, reduce the intake in every meal, or have small but frequent meals during a day.

3.1.1 SKIPPING BREAKFASTS

Skipping breakfast is a commonly used method to cut caloric intake during a day. However, eating breakfast is associated with improved strength and endurance, better attitude toward school or work, maintenance of a constant blood glucose

level, and prevention of hunger and subsequent overeating later in the day (Zabik, 1987; Schlundt *et al*, 1992). Many studies have found out that not having breakfast is closely related to depression (Allgower, 2001). Breakfast skippers may in turn develop night eating syndrome (NES: morning anorexia, evening hyperphagia and insomnia). Night eaters have higher depression, lower self-esteem, less hunger, and a trend for more fullness before the daytime test meal than the others. The NES is a syndrome with distinct psychopathology and increased food intake later in the day, both of which may contribute to poorer weight loss outcome (Gluck *et al*, 2001).

The previous studies suggest that skipping breakfast should not be practised. A morning fast results in a diet that tends to have a lower percentage of energy from carbohydrate (Taylor and Garrow, 2001). On the contrary, regular cereal breakfast can lead to reduced intake of total and saturated fatty acids of the daily diet and consequently to reduction in serum cholesterol level (Kleemola *et al*, 1999). The subjects who consume cereal breakfast have a more positive mood at the start of the test sessions, perform better on a spatial memory task, and feel calmer at the end of the work (Smith *et al*, 1999).

3.1.2 Nibbling, Grazing vs Gorging

Nibbling, spreading energy intake over small meals, is known to control blood glucose, serum lipids and body fat accretion but the mechanisms are still poorly understood. Men exhibit more of a gorging eating pattern, taking large but less frequent meals, than women and are also more prone to the metabolic

complications of obesity (Kral *et al*, 2001). In the short term, meal frequency and duration of fasting have no major impact on energy intake or expenditure but the energy is spent more during the day with a higher meal frequency compared with a lower meal frequency. This might be attributed to the thermogenic effect of food continuing into the night when a later, larger meal is given (Taylor and Garrow, 2001).

In a study feeding rabbits with a high-fat diet, Juhel and his colleagues (2000) have found out that postprandial lipid responses and plasma accumulation of dietary lipids are significantly higher in the gorging group than in the nibbling group, despite higher post-heparin plasma lipase activities. Atheroma deposition is significantly increased by gorging the HF diet and has been found to correlate with levels of most postprandial lipid variables (Juhel *et al*, 2000).

Nibbling diet reduces fasting serum concentrations of total cholesterol, low-density lipoprotein cholesterol and apolipoprotein B. Although the mean blood glucose level and serum concentrations of free fatty acids, 3-hydroxybutyrate, and triglyceride are similar during both diets, with the nibbling diet the mean serum insulin level decreased (Jenkins *et al*, 1989). In smokers, grazing, i.e., snacking between meals, has shown to have a correlation with a reduced risk of developing symptomatic peripheral atherosclerosis which, in turn leads to cardiovascular disease (Powell *et al*, 1999).

Moreover, Kral and associates (2001) showed that an increased eating rates in severely obese men and women promote central fat distribution, fatty liver and elevate serum lipids (Kral *et al*, 2001).

3.1.3 Reducing Food Intake in Meals

It has long been known that having moderate food restrain can promote longevity and reduce cardiovascular risks. Scientists are still gathering information and evidence to confirm this hypothesis.

3.1.3.1 Anti-Aging Action

The median length of life and age of the tenth percentile survivors are similar for rats receiving 60% of the *ad libitum* intake in a single meal or split into two meals and much greater than those having free-access of food. Both modes of dietary restriction influence age-associated disease processes in a similar fashion. Thus, although the temporal pattern of food intake influences circadian rhythms of food-restricted rats, it does not significantly affect the anti-aging action (Masoro *et al*, 1995). Numerous studies from other groups of scientists also provide similar evidence on the association of moderate food restriction and prolongation of life span (Yu *et al*, 1985; Hubert *et al*, 2000).

Food restriction to 50-70% in rodents is found to be beneficial with a noticeable increase in longevity, retardation of age-associated physiological deterioration, delay and, in some cases, prevention in age-associated diseases. These actions are due to the anti-aging action of caloric restriction. There are three proposed mechanisms of this anti-aging action, namely attenuation of oxidative damage, modulation of glycemia and insulinemia and, hormesis (Masoro, 2000).

3.1.3.2 Effects on Other Health Issues

In a two-year long study on male SD rats performed by Kemi and colleagues (2000), it has been found that both groups of rats fed *ad libitum*, with diets of 4.1% or 15.7% of fat, have more severe cardiomyopathy at the end of the experiment. Moderately restricted food intake to 65% of the *ad libitum* consumption in rats with either of the diets show an increase in survival and decrease in risks on obesity and heart disease. Only slight improvements in the severity and progression of spontaneous cardiomyopathy are seen by modification of the protein, fibre, fat, and energy content of the diet if fed *ad libitum*. The effects on prevention and control of the progression of cardiomyopathy are more pronounced by moderate fasting than by changing the *ad libitum* diet composition (Kemi *et al*, 2000).

Moderate dietary restriction shows a remarkably decrease in the incidence and delay on the onset of leukaemia in Fischer 344 rats. They are found to be related to the total cumulative energy intake of the rat expressed in age multiplied by mean daily energy intake. The duration, rather than the age of initiation, of dietary restrain shows a closer correlation to the prevention of leukaemia (Simokawa *et al*, 1993; Higami *et al*, 1994). Implanted tumour growth is also found to be suppressed in 60% food-restricted mice with the plasma concentrations of interferon-gamma and tumour necrosis factor-alpha greater than the control ones (Matsuzaki *et al*, 2000). Mild food restriction increases

ubiquinone contents but decreases the incidence of hepatcellular carcinoma in male Winstar rats (Wang *et al*, 2000).

Significant increase in water intake per gram body weight is found in both young and old caloric-restricted mice when compared to their free-eating counterparts. This may contribute to the prevention of autoimmune disease with age and differences in longevity by increasing the renal expression, in both protein and mRNA levels, of AQP1 and AQP2, members of the aquaporin family of cell membrane water channel transport proteins which have been implicated in the regulation of renal water excretion (Mittal *et al*, 2000).

Caloric restraint helps to delay the age-related changes of physiological system in rats, e.g., serum triglycerides, free fatty acids, phospholipids and cholesterol levels, and hence prolong life-span (Leipa *et al*, 1980). Decrease in serum triglycerides, cholesterol, HDL and LDL are observed in healthy volunteers living in Biosphere 2 with two-year food restriction while in some participants, there is an increase in the HDL2 subfraction. This study suggests that energy restriction substantially reduces risk for atherosclerosis and consequent coronary artery and cerebrovascular disease (Verdery and Walford, 1998).

Food restriction to around 60% also proves to delay or prevent severe renal lesions, interstitial cell tumours of the testes, bile duct hyperplasia, myocardial fibrosis, myocardial degeneration, and gastrocnemius muscle mass decline (Yu *et al*, 1982), renal membrane lipid deposition (Eiam-Ong and Sabatini, 1999), and pituitary adenomas (Hubert *et al*, 2000). Bone growth and maturation are slowed down, but the rats having only 60% of usual food intake do not

experience senile bone loss or marked terminal increase in circulating parathyroid hormone observed in the free-eating group (Kalu *et al*, 1984). Energy restriction can also enhance insulin sensitivity (Vessby, 2000), decrease hyperglycaemia (Cameron-Smith *et al*, 1994), reverse hepatic insulin resistance (Brazilai *et al*, 1998), and increase insulin-stimulated glucose transport in skeletal muscle (Gazdag *et al*, 1999) as well as in adipocytes (Craig *et al*, 1987). This improvement in insulin sensitivity has been suggested to be further related to decreased visceral fat (Barzilai *et al*, 1998).

3.1.3.3 Energy Expenditure

Energy expenditure is generally reduced during the fasting period until food supply returns. Resting energy expenditure (REE) falls during the weight loss period while the patients decrease their weight, the body fat and the fat-free mass (Wadden *et al*, 1996). In animals subjected to mild food restriction (10 to 20%), there is a sustained decrease in oxygen consumption that lasts until refeeding of the animals. It suggests that the energy conservation mechanism is active from little food restriction to higher levels of restriction, in a proportional manner, and the decreased energy expenditure is maintained during the whole food restriction period (Santos-Pinto *et al*, 2001).

3.2 OBJECTIVES

The objective of the present study was to investigate the effects of different levels of food restriction on body fatty acid composition in rats fed on MF diet.

3.3 MATERIALS AND METHOD

3.3.1 ANIMAL HANDLING

Forty male Sprague-Dawley rats (462.0±28g) were kept in the Laboratory Animal Service Centre, of the Chinese University of Hong Kong with 2 rats per cage. The room was kept at 23°C with a 12/12 hour light-dark cycle. The body weight and the food intake were recorded every day.

Four weeks of stabilisation period was introduced by replacing the Chow diet on a semi-synthetic diet, which provided 20% of the energy derived from canola oil. The nutritional value of the diet is shown in Table 3.1. The diet was put into a small can, which was placed in a larger one, for preventing food spillage. The cans were then fixed by screwing onto the base of the cage. Food restriction lasted for 2 more weeks with the same customised diet. During the course of experiment, the animals were allowed to have free access to tap water.

During the 4-week stabilisation period, the rats were allowed to take in food *ad libitum*. The average daily food intake was 28g/rat/day. Then the rats were randomly divided into 7 groups and assigned to varying levels of diet restriction: Control (Day 0), 100%, 90%, 80%, 70%, 60% and 50% of the usual food intake. The averaged body weight of rats was similar prior to the food restriction.

After the 4-week stabilisation, the rats weighed 504±35g. Four rats were sacrificed on Day 0, the last day of the stabilisation period. The other groups (n=6 each) were given the diet for 2 more weeks as assigned. Group 100% rats were fed *ad libitum* (28g/rat/day) while groups 90%, 80%, 70%, 60% and 50% were

given 25.2, 22.4, 19.6, 16.8 and 14g/rat/day respectively. At the end of the two-week food restriction (Day 14), all the rats were killed.

Rats were sacrificed for fatty acid profile determination, proximate analysis and measurement of serum triglyceride and cholesterol levels. For detailed material and methods, please refer to sections 2.3.2 to 2.3.4.

Table 3.1: Composition of the semi-synthetic diet for the different degrees of food restriction experiment.

Ingredients	Percentage (w/w)
Corn Starch	**33.8**
Canola Oil	**10.0**
Casein	23.5
Sucrose	**24.3**
Mineral Mixture	3.5
Cellulose	3.2
Vitamin Mixture	1.0
Choline Bitartrate	0.4
D/L-Methionine	0.3
Total	100.0

3.4 RESULTS

3.4.1 BODY WEIGHT

The body weight of the rats was recorded daily (Fig 3.1). As all the rats went through the stabilisation period with the same diet, the body weights of rats among the groups could be maintained to be the same, 501.6±34.9g, at the beginning of the 2-week food restriction period. Throughout the food restriction period, the rats with 100% and 90% food supply had almost gained the similar body weight; the 80% group of rats gained weight gradually; the 70% and the 60% groups of rats maintained their weight and the 50% group of rats lost weight (Fig 3.1). At the end of the experiment, the body weights of the rats for the 100%, 90%, 80%, 70%, 60% and 50% groups were 551.7±53.9g, 549.2±27.1g, 528.3±35.7g, 508.3±40.6g, 501.7±30.8g and 463.3±18.4g respectively.

3.4.2 FOOD INTAKE

The average daily food intake of the rats during the stabilisation period was recorded as 28g/rat/day. Therefore, the amount of food given to the rats with 100%, 90%, 80%, 70%, 60% and 50% food supply were 28.0g, 25.2g, 22.4g, 19.6g, 18.6g and 14.0g correspondingly. As there was always some spillage of the powered food, the actual amount of food taken was recorded to be 27.9±2.1g, 23.7±0.7g, 22.2±0.2g, 19.6±0.1g, 16.3±0.1g and 13.7±0.4g respectively (Fig 3.2).

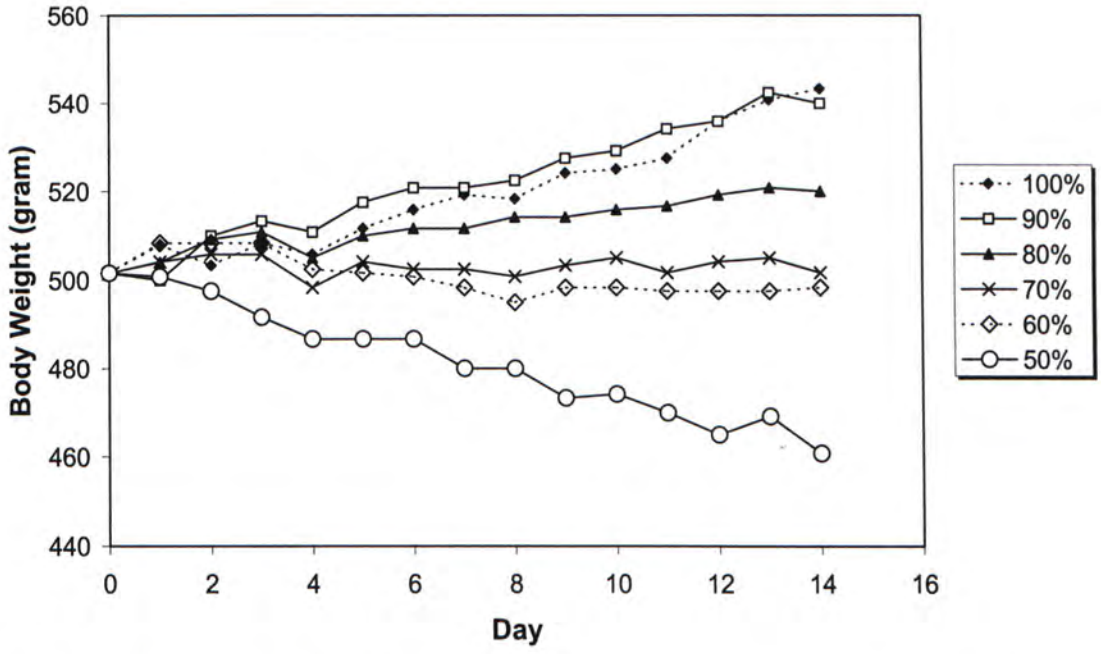


Fig 3.1: Changes in the body weight of rats undergoing varying levels of food restriction with medium fat diet.

3.4.3 ORGAN WEIGHT

Since there was some variation on the body weight of the rats being sacrificed even on the same day, the organ weights are presented against the corresponding body weights in order to show a clearer relationship. Differences were compared with those of the 100% group by Student's t-test with the notation *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.

3.4.3.1 Liver

Weights of liver decreased after food restriction both when measured as actual weight and relative to the body weight. The less the food given to the rats, the smaller the resulting liver weight.

Before the fasting period, the liver weighed 20.7±4.2g with a ratio to the body weight being 0.041±0.01. After the 14-day treatment, the liver weighed from 20.9 to 12.6g with the ratio to the body weight from 0.038 to 0.027 in the rats fed 100% to 50% of food respectively. When expressed as a ratio of body weight, no significant difference was noticed (Fig 3.2 and 3.3).

3.4.3.2 Adipose Tissues

Both the weights of the perirenal and the epidyidmal adipose tissues decreased when there was a shortage of food supply. In general, the less the food given, the smaller the resulting adipose tissue weights.

Right after the stabilisation stage, perirenal fat pads weighed 14.65±4.73g with a ratio to the body weight being 0.029±0.01. By the end of the 14-day

treatment, the liver weighed between 19.1 and 11.4g with a ratio to the body weight between 0.035 and 0.025 (Fig 3.4 and 3.5). At the beginning of the experiment, epidydimal fat pads weighed 9.85±2.70g with a ratio to the body weight being 0.020±0.01. After the 14-day treatment, the fat pads weighed between 11.9 and 7.3g with the ratio to the body weight ranged from 0.022 to 0.016 (Fig 3.6 and 3.7).

In both of the adipose tissues, there was a slight increase in the weights in rats having 90% and 80% of food supply. However, the weights decreased gradually when the food supply was more limited. No significant difference and trend were noticed for these mild changes of adipose tissue weights.

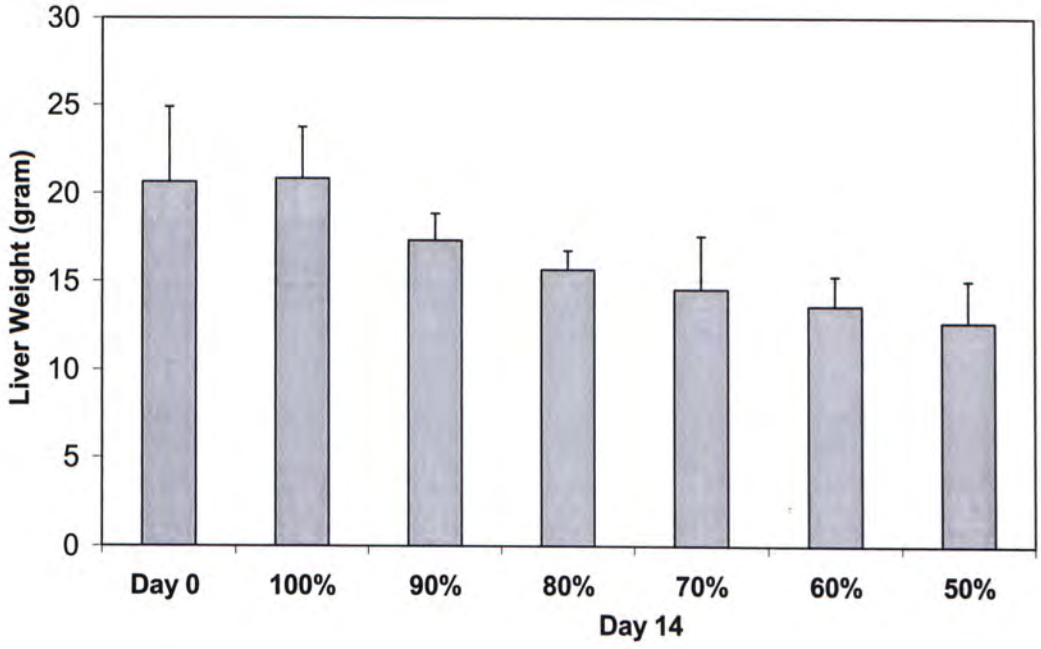


Fig 3.2: Weight of liver of rats undergoing different levels of food restriction with medium fat diet.

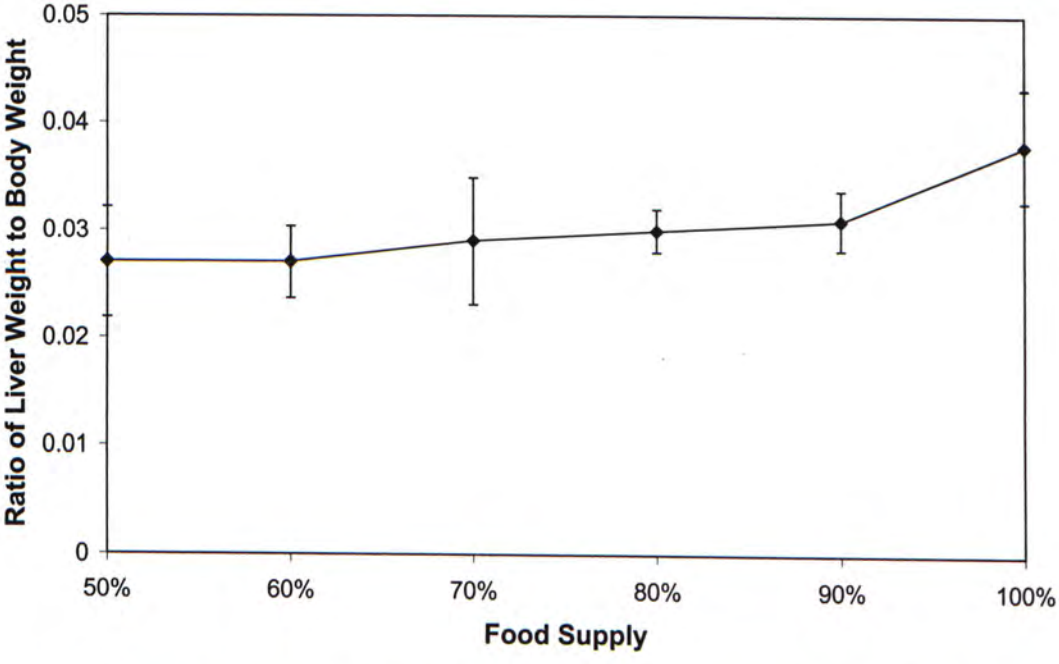


Fig 3.3: Ratio of weight of liver to body weight of rats undergoing different levels of food restriction with medium fat diet.

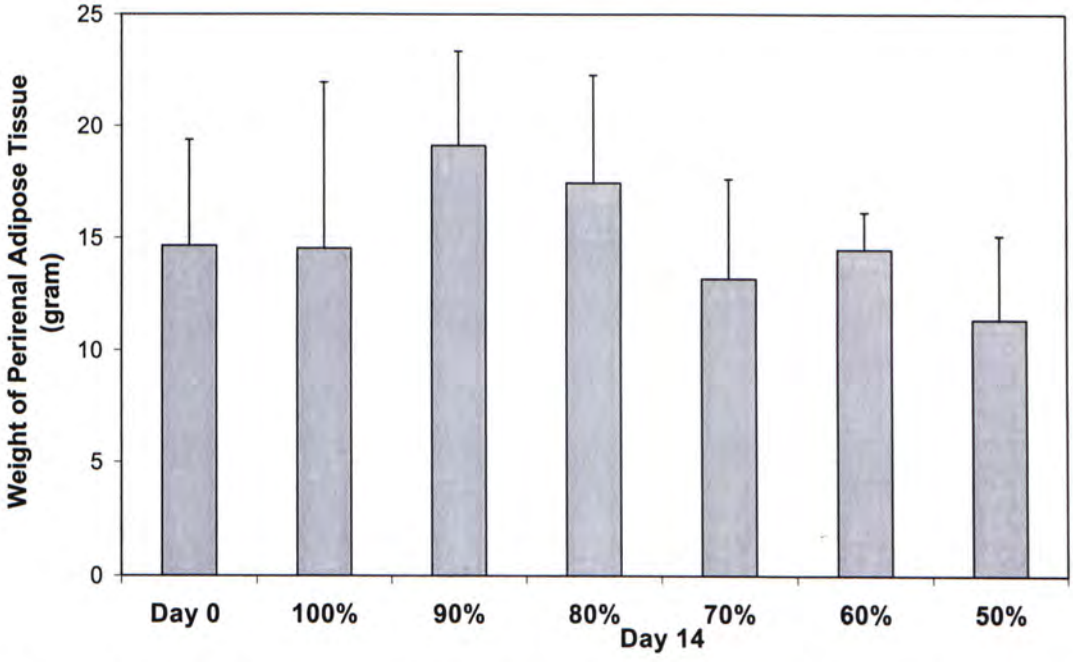


Fig 3.4: Weight of perirenal adipose tissues of rats undergoing different levels of food restriction with medium fat diet.

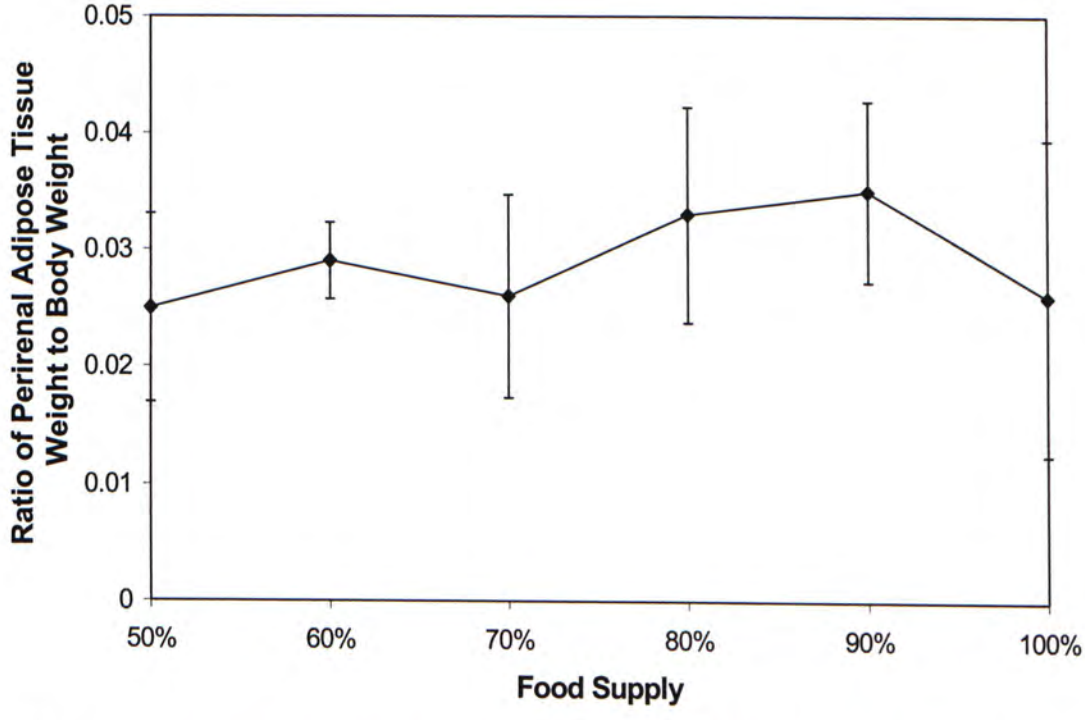


Fig 3.5: Ratio of weight of perirenal adipose tissues to body weight of rats undergoing different levels of food restriction with medium fat diet.

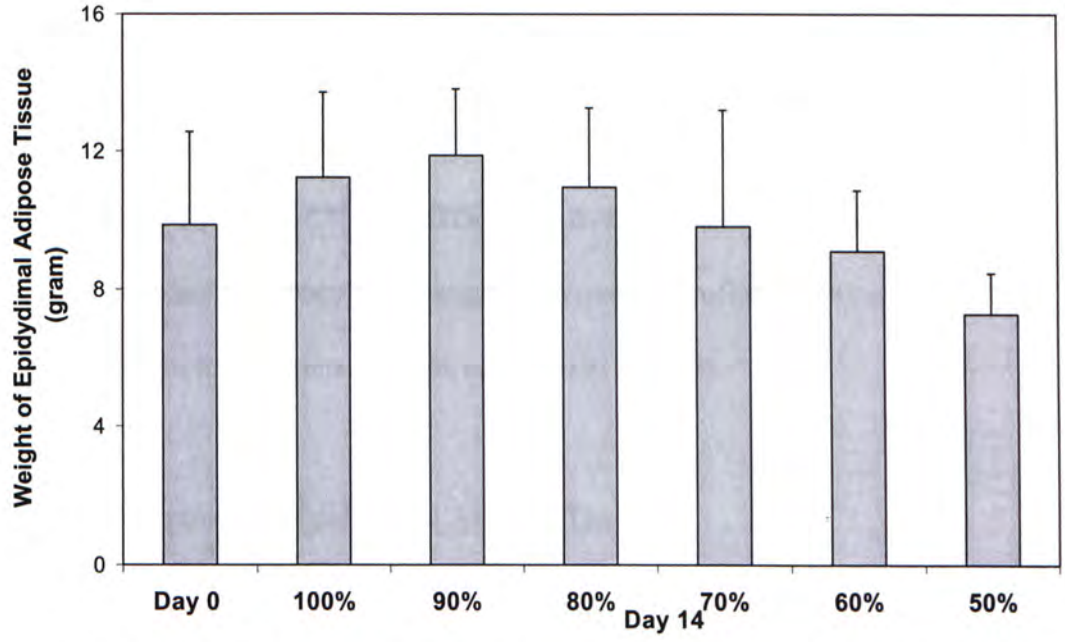


Fig 3.6: Weight of epidydimal adipose tissues of rats undergoing different levels of food restriction with medium fat diet.

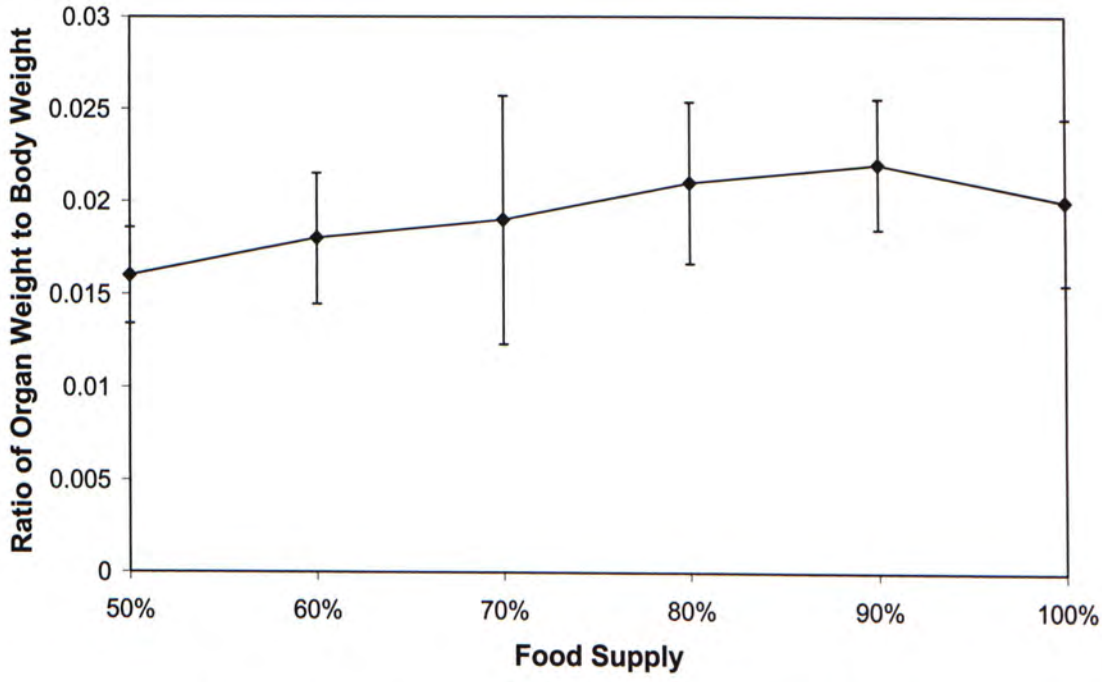


Fig 3.7: Ratio of weight of epidydimal adipose tissues to body weight of rats undergoing different levels of food restriction with medium fat diet.

3.4.4 Lipid Analysis

Fatty acid composition was measured by gas-liquid chromatography. Differences were compared with those of the 100% group by Student's t-test with the notation *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$. Since over 90% of the fatty acids are stored as triglycerides in adipocytes in fat pads, an overall profile of fatty acids were determined in the perirenal and the epidydimal fat pads.

3.4.4.1 Perirenal and Epidydimal Adipose Tissues

In general term, all the 4 categories of fatty acids, namely SFAs, MUFAs, n-6 and n-3 n-6 PUFAs showed little differences between the groups on Day 14 (Tables 3.2 –3.3). The levels of SFAs and n-6 PUFAs rather fluctuated among different groups. On the other hand, MUFAs showed a mild increase with increase in energy restriction. A more distinctive difference can be observed in n-3 PUFAs, in the 50% group n-3 PUFAs was significantly lower than that in the control 100% group.

The fatty acids 16:0 and 18:2n-6 remained almost the same. However, 20:4n-6 dropped significantly in perirenal adipose tissues. In accordance with the general change observed in n-3 PUFAs, most of the individual fatty acids also decreased by Day 14.

3.4.4.2 Carcass

The overall changes of the SFAs, MUFAs and n-6 PUFAs in the carcass total lipids, triglycerides and free fatty acids showed some trends but not as

pronounced as among the groups after the caloric restriction. On the contrary, the changes in the n-3 PUFAs and also those of the 4 categories of fatty acids in the carcass phospholipids illustrated more significant trends. The 18:3n-3 fatty acid showed a general decrease in all of the carcass lipids while the other individual n-3 fatty acids fluctuated among the groups (Tables 3.4 – 3.7).

Table 3.2: Fatty acid composition (% of the total) of the perirenal adipose tissues in rats undergoing different levels of food restriction with medium fat diet. Data was expressed as Mean ± SD. The food restricted groups were significantly different from the 100% food intake rats at *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.

	Day 0	Day 14		
		100%	90%	80%
SFA				
14:0	1.23±0.12	1.05±0.10	1.19±0.14	1.14±0.10
15:0	0.16±0.02	0.16±0.02	0.14±0.02	0.14±0.02
16:0	20.60±2.56	18.49±1.58	20.24±1.33*	19.58±1.47
18:0	2.85±0.12	2.69±0.18	3.23±0.86	2.88±0.15
Sum	24.84±2.75	22.39±1.71	24.79±2.11	23.73±1.65
MUFA				
16:1n-9	0.43±0.04	0.49±0.02	0.40±0.04	0.43±0.04
16:1n-7	4.36±1.09	3.19±0.54	4.00±0.89	3.54±0.74
18:1n-9	46.18±0.43	49.09±1.00	47.31±1.63	48.77±1.40
18:1n-7	3.59±0.22	3.59±0.29	3.69±0.18	3.48±0.13
20:1n-9	0.35±0.02	0.33±0.02	0.34±0.03	0.34±0.02
20:1n-7	0.32±0.03	0.27±0.02	0.29±0.03	0.25±0.04
22:1n-7	0.72±0.06	0.73±0.04	0.72±0.05	0.74±0.05
Sum	55.94±0.68	57.69±0.59	56.75±0.83*	57.56±0.82
n-6 PUFA				
18:2n-6	15.17±3.02	15.82±1.11	14.42±1.14	14.86±1.03
20:2n-6	0.12±0.03	0.13±0.02	0.13±0.01	0.12±0.02
20:3n-6	0.07±0.01	0.06±0.01	0.06±0.01	0.05±0.01
20:4n-6	0.27±0.03	0.25±0.07	0.24±0.03	0.21±0.03
22:4n-6	0.07±0.02	0.05±0.01	0.03±0.03	0.05±0.03
Sum	15.71±3.04	16.30±1.20	14.88±1.16	15.30±1.01
n-3 PUFA				
18:3n-3	3.19±0.36	3.35±0.23	3.29±0.24	3.20±0.23
20:5n-3	0.02±0.01	0.01±0.02	0.01±0.01	0.00±0.00
22:5n-3	0.12±0.03	0.11±0.02	0.13±0.04	0.10±0.04
22:6n-3	0.18±0.04	0.15±0.01	0.14±0.03	0.12±0.03
Sum	3.51±0.38	3.62±0.25	3.58±0.26	3.42±0.26

Table 3.2: (Con't)

	Day 14		
	70%	60%	50%
SFA			
14:0	1.19±0.13	1.11±0.07	1.09±0.08
15:0	0.14±0.03	0.14±0.02	0.14±0.03
16:0	19.38±2.34	19.30±1.28	18.70±0.98
18:0	2.96±0.17	3.00±0.20	3.04±0.14
Sum	23.67±2.56	23.55±1.42	22.99±1.12
MUFA			
16:1n-9	0.46±0.05	0.44±0.05	0.49±0.05
16:1n-7	3.64±0.92	3.43±0.59	3.03±0.56
18:1n-9	48.34±1.51	49.07±1.01	49.50±1.21
18:1n-7	3.44±0.15	3.55±0.35	3.43±0.41
20:1n-9	0.35±0.04	0.32±0.02	0.31±0.02
20:1n-7	0.23±0.02	0.21±0.01	0.15±0.01
22:1n-7	0.73±0.07	0.75±0.03	0.80±0.08
Sum	57.18±0.96	57.78±0.34	57.71±0.89
n-6 PUFA			
18:2n-6	15.32±1.87	15.07±1.06	16.04±0.96
20:2n-6	0.12±0.02	0.11±0.02	0.12±0.02
20:3n-6	0.06±0.01	0.06±0.01	0.04±0.03
20:4n-6	0.21±0.04	0.19±0.04	0.17±0.04*
22:4n-6	0.05±0.04	0.02±0.03	0.02±0.03
Sum	15.77±1.83	15.45±1.00	16.39±1.03
n-3 PUFA			
18:3n-3	3.15±0.22	2.99±0.20*	2.72±0.18***
20:5n-3	0.01±0.02	0.02±0.03	0.03±0.04
22:5n-3	0.10±0.02	0.10±0.02	0.07±0.02**
22:6n-3	0.12±0.03	0.12±0.02*	0.08±0.02***
Sum	3.38±0.22	3.22±0.22*	2.91±0.19***

Table 3.3: Fatty acid composition (% of the total) of the epidydimal adipose tissues of rats undergoing different levels of food restriction with medium fat diet. Data was expressed as Mean ± SD. The food restricted groups were significantly different from the 100% food intake rats at *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.

	Day 0	Day 14		
		100%	90%	80%
SFA				
14:0	1.11±0.14	0.98±0.11	1.14±0.15	1.01±0.13
15:0	0.19±0.02	0.17±0.04	0.17±0.03	0.17±0.02
16:0	19.23±2.23	17.90±1.98	19.11±1.42	18.66±1.93
18:0	2.78±0.12	2.76±0.11	2.71±0.22	2.69±0.09
Sum	23.31±2.26	21.80±2.14	23.13±1.63	22.59±2.03
MUFA				
16:1n-9	0.45±0.06	0.48±0.04	0.45±0.03	0.47±0.03
16:1n-7	3.98±1.01	3.11±0.51	4.24±0.84	3.65±0.90
18:1n-9	44.82±1.23	47.53±1.04	45.37±1.12	47.17±1.53
18:1n-7	3.64±0.31	3.60±0.26	3.62±0.29	3.48±0.10
20:1n-9	0.35±0.01	0.34±0.02	0.34±0.02	0.35±0.02
20:1n-7	0.34±0.01	0.30±0.02	0.32±0.03	0.29±0.05
22:1n-7	0.66±0.04	0.69±0.05	0.67±0.04	0.69±0.04
Sum	54.24±1.96	56.05±0.93	55.00±0.65**	56.10±0.87
n-6 PUFA				
18:2n-6	17.95±3.26	17.56±1.92	17.06±1.83	16.92±1.34
20:2n-6	0.15±0.03	0.14±0.05	0.17±0.05	0.14±0.02
20:3n-6	0.06±0.04	0.08±0.03	0.07±0.02	0.06±0.03
20:4n-6	0.30±0.15	0.32±0.17	0.36±0.13	0.29±0.09
22:4n-6	0.07±0.01	0.08±0.04	0.08±0.02	0.07±0.03
Sum	18.53±3.44	18.18±2.13	17.75±1.96	17.48±1.35
n-3 PUFA				
18:3n-3	3.42±0.28	3.53±0.27	3.59±0.21	3.47±0.33
20:5n-3	0.08±0.06	0.06±0.07	0.07±0.04	0.04±0.02
22:5n-3	0.15±0.06	0.14±0.05	0.18±0.06	0.13±0.05
22:6n-3	0.27±0.12	0.23±0.20	0.29±0.17***	0.19±0.08
Sum	3.92±0.48	3.97±0.55	4.12±0.34	3.83±0.44

Table 3.3: (Con't)

	Day 14		
	70%	60%	50%
SFA			
14:0	1.11±0.14	1.10±0.05	1.03±0.11
15:0	0.17±0.03	0.17±0.02	0.20±0.03
16:0	18.32±1.86	18.58±1.16	17.45±0.68
18:0	2.72±0.17	2.71±0.19	2.78±0.26
Sum	22.32±1.86	22.55±1.17	21.47±0.92
MUFA			
16:1n-9	0.49±0.05	0.48±0.03	0.54±0.04
16:1n-7	3.52±0.89	3.73±0.67	2.99±0.67
18:1n-9	46.61±0.49	46.53±1.16	46.23±1.76
18:1n-7	3.52±0.20	3.50±0.47	3.58±0.35
20:1n-9	0.35±0.02	0.33±0.01	0.32±0.02
20:1n-7	0.28±0.03	0.25±0.03	0.23±0.05
22:1n-7	0.68±0.04	0.69±0.03	0.65±0.24
Sum	55.46±1.11	55.511.36	54.54±1.91*
n-6 PUFA			
18:2n-6	17.74±2.84	17.57±1.74	19.83±0.87*
20:2n-6	0.15±0.03	0.14±0.03	0.18±0.03
20:3n-6	0.08±0.01	0.07±0.01	0.08±0.01
20:4n-6	0.29±0.05	0.29±0.11	0.31±0.08
22:4n-6	0.15±0.21	0.20±0.33	0.09±0.03
Sum	18.41±2.77	18.28±2.12	20.48±0.90*
n-3 PUFA			
18:3n-3	3.44±0.15	3.27±0.25	3.18±0.18*
20:5n-3	0.03±0.01	0.03±0.03	0.04±0.03
22:5n-3	0.15±0.04	0.14±0.06	0.11±0.06
22:6n-3	0.19±0.04*	0.21±0.11	0.19±0.06
Sum	3.82±0.16	3.63±0.42	3.52±0.25

Table 3.4: Fatty acid composition (% of the total) of the carcass total lipids in rats undergoing different levels of food restriction with medium fat diet. Data was expressed as Mean ± SD. The food restricted groups were significantly different from the 100% food intake rats at *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.

	Day 0	Day 14		
		100%	90%	80%
SFA				
14:0	1.30±0.06	1.16±0.10	1.35±0.12	1.21±0.11
15:0	0.17±0.01	0.16±0.01	0.15±0.02	0.16±0.02
16:0	19.42±1.01	18.11±1.20	19.39±0.90	18.44±1.21
18:0	3.21±0.22	3.25±0.19	2.94±0.26	3.02±0.32
Sum	24.11±0.89	22.67±1.23	23.83±0.76	22.82±1.04
MUFA				
16:1n-9	0.43±0.04	0.49±0.03	0.40±0.03	0.44±0.02
16:1n-7	4.51±0.66	3.66±0.59	4.92±0.98	4.51±1.19
18:1n-9	45.07±0.48	47.29±0.54	46.70±1.08	46.99±0.59
18:1n-7	3.05±0.14	3.09±0.29	2.30±1.11	2.91±0.21
20:1n-9	0.68±0.04	0.72±0.03	0.67±0.03	0.69±0.04
20:1n-7	0.31±0.03	0.31±0.05	0.30±0.04	0.30±0.02
22:1n-7	0.00	0.00	0.00	0.00
Sum	54.06±0.94	55.58±0.36	55.29±0.38	55.83±0.47
n-6 PUFA				
18:2n-6	16.59±1.32	16.17±0.94	15.70±0.78	16.03±0.93
20:2n-6	0.17±0.02	0.25±0.07	0.19±0.01	0.22±0.02
20:3n-6	0.09±0.01	0.09±0.01	0.07±0.01	0.08±0.01
20:4n-6	0.96±0.11	1.04±0.12	0.80±0.11	0.94±0.15
22:4n-6	0.11±0.02	0.12±0.02	0.09±0.01	0.10±0.01
Sum	17.92±1.46	17.67±1.08	16.85±0.89	17.36±1.11
n-3 PUFA				
18:3n-3	3.06±0.13	3.12±0.15	3.25±0.12	3.11±0.19
20:5n-3	0.06±0.01	0.06±0.02	0.06±0.01	0.04±0.02
22:5n-3	0.25±0.05	0.29±0.05	0.23±0.02	0.25±0.03
22:6n-3	0.5±0.05	0.61±0.10	0.50±0.09	0.58±0.10
Sum	3.97±0.21	4.08±0.20	4.03±0.16	3.99±0.29

Table 3.4: (Con't)

	Day 14		
	70%	60%	50%
SFA			
14:0	1.23±0.07	1.23±0.06	1.12±0.05
15:0	0.16±0.01	0.16±0.01	0.17±0.02
16:0	18.13±1.07	18.20±0.63	17.40±0.57
18:0	3.12±0.28	3.14±0.30	3.48±0.25
Sum	22.65±0.90	22.74±0.52	22.17±0.38
MUFA			
16:1n-9	0.47±0.04	0.46±0.03	0.51±0.02
16:1n-7	4.11±0.73	4.25±0.67	3.38±0.54
18:1n-9	46.97±0.52	47.14±0.59	47.17±0.43
18:1n-7	3.02±0.25	3.14±0.25	3.38±0.23
20:1n-9	0.72±0.04	0.73±0.02	0.81±0.04
20:1n-7	0.33±0.03	0.32±0.02	0.38±0.05
22:1n-7	0.00	0.00	0.00
Sum	55.62±0.77	56.04±0.74	55.63±0.52
n-6 PUFA			
18:2n-6	16.47±1.06	16.26±0.81	17.19±0.58
20:2n-6	0.24±0.06	0.18±0.03	0.19±0.02
20:3n-6	0.08±0.01	0.08±0.00	0.09±0.00
20:4n-6	0.96±0.13	0.95±0.10	1.13±0.13
22:4n-6	0.10±0.01	0.11±0.01	0.13±0.02
Sum	17.86±1.22	17.59±0.85	18.73±0.73
n-3 PUFA			
18:3n-3	2.97±0.08*	2.75±0.10***	2.4±0.09***
20:5n-3	0.06±0.01	0.06±0.01	0.07±0.01
22:5n-3	0.27±0.05	0.26±0.02	0.29±0.04
22:6n-3	0.54±0.07	0.56±0.08	0.67±0.09
Sum	3.84±0.15*	3.63±0.18**	3.50±0.11***

Table 3.5: Fatty acid composition (% of the total) of the carcass triglycerides in rats undergoing different levels of food restriction with medium fat diet. Data was expressed as Mean ± SD. The food restricted groups were significantly different from the 100% food intake rats at *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.

	Day 0	Day 14		
		100%	90%	80%
SFA				
14:0	1.23±0.05	1.15±0.12	1.27±0.14	1.16±0.13
15:0	0.17±0.02	0.16±0.01	0.15±0.02	0.15±0.01
16:0	19.17±0.86	17.91±1.61	19.09±1.11	17.91±1.48
18:0	2.75±0.16	2.74±0.12	2.59±0.22	2.48±0.22
Sum	23.32±0.73	21.96±1.72	23.10±1.06	21.69±1.45
MUFA				
16:1n-9	0.44±0.04	0.51±0.02	0.41±0.03	0.44±0.03
16:1n-7	4.60±0.61	3.75±0.57	4.99±1.01	4.72±1.22
18:1n-9	49.96±1.09	50.51±2.41	49.55±2.04	50.59±1.36
18:1n-7	0.24±0.33	2.23±1.77	1.31±1.37	1.47±1.55
20:1n-9	0.75±0.04	0.80±0.03	0.71±0.04	0.72±0.04
20:1n-7	0.20±0.01	0.55±0.79	0.17±0.02	0.17±0.01
22:1n-7	0.00	0.00	0.00	0.00
Sum	56.18±1.32	58.35±0.25	57.13±0.37***	58.11±0.22
n-6 PUFA				
18:2n-6	16.51±1.66	15.76±1.36	15.64±0.95	16.12±1.16
20:2n-6	0.14±0.02	0.13±0.01	0.13±0.01	0.12±0.01
20:3n-6	0.07±0.01	0.05±0.01	0.05±0.01	0.06±0.01
20:4n-6	0.40±0.03	0.38±0.04	0.35±0.04	0.36±0.04
22:4n-6	0.07±0.01	0.07±0.01	0.06±0.01	0.06±0.01
Sum	17.18±1.72	16.39±1.40	16.23±1.00	16.72±1.18
n-3 PUFA				
18:3n-3	2.93±0.20	2.93±0.21	3.19±0.13*	3.12±0.24
20:5n-3	0.03±0.00	0.02±0.01	0.03±0.01	0.05±0.06
22:5n-3	0.12±0.02	0.13±0.03	0.12±0.02	0.11±0.02
22:6n-3	0.24±0.04	0.21±0.02	0.20±0.03	0.19±0.04
Sum	3.32±0.25	3.29±0.24	3.53±0.15*	3.48±0.29

Table 3.5: (Con't)

	Day 14		
	70%	60%	50%
SFA			
14:0	1.21±0.08	1.20±0.08	1.06±0.08
15:0	0.16±0.01	0.15±0.01	0.16±0.02
16:0	17.62±1.26	17.49±0.90	15.90±0.99*
18:0	2.60±0.15	2.62±0.21	2.73±0.16
Sum	21.59±1.18	21.46±0.82	19.86±0.98
MUFA			
16:1n-9	0.48±0.04	0.46±0.02	0.51±0.02
16:1n-7	4.25±0.76	4.43±0.72	3.60±0.66
18:1n-9	49.75±1.07	49.86±0.55	49.96±0.36
18:1n-7	2.39±1.16	2.86±0.19	4.02±0.36
20:1n-9	0.76±0.03	0.77±0.03	0.86±0.03
20:1n-7	0.21±0.05	0.20±0.03	0.25±0.02
22:1n-7	0.00	0.00	0.00
Sum	57.83±0.68	58.60±0.50	59.21±1.18
n-6 PUFA			
18:2n-6	16.62±1.19	16.29±0.98	18.02±0.78*
20:2n-6	0.14±0.02	0.14±0.01	0.15±0.01
20:3n-6	0.06±0.01	0.06±0.01	0.03±0.04
20:4n-6	0.36±0.04	0.32±0.03*	0.32±0.05*
22:4n-6	0.06±0.01	0.07±0.01	0.03±0.05
Sum	17.24±1.16	16.88±1.00	18.56±0.81*
n-3 PUFA			
18:3n-3	3.01±0.16	2.74±0.14	2.68±0.11*
20:5n-3	0.03±0.01	0.04±0.01*	0.00±0.00***
22:5n-3	0.13±0.03	0.11±0.02**	0.11±0.02
22:6n-3	0.17±0.04*	0.18±0.02*	0.13±0.03***
Sum	3.34±0.15	3.06±0.14*	2.92±0.13**

Table 3.6: Fatty acid composition (% of the total) of the carcass free fatty acids in rats undergoing different levels of food restriction with medium fat diet. Data was expressed as Mean ± SD. The food restricted groups were significantly different from the 100% food intake rats at *: $p<0.05$; **: $p<0.01$.

	Day 0	Day 14		
		100%	90%	80%
SFA				
14:0	1.08±0.05	1.01±0.12	1.10±0.23	1.02±0.12
15:0	0.19±0.02	0.19±0.01	0.17±0.02	0.19±0.02
16:0	22.88±0.99	21.78±1.22	22.90±0.94	23.09±1.09
18:0	4.87±0.77	5.01±0.59	4.27±0.38	4.86±0.56
Sum	29.02±1.03	27.99±1.41	28.44±0.88	29.15±0.91
MUFA				
16:1n-9	0.42±0.03	0.49±0.03	0.40±0.03	0.44±0.02
16:1n-7	4.41±0.67	3.55±0.64	4.78±0.97	3.99±1.61
18:1n-9	39.77±1.45	40.53±1.09	40.82±0.92	39.25±1.99
18:1n-7	4.72±0.29	4.51±0.18	4.33±0.10	4.08±0.20
20:1n-9	0.79±0.03	0.80±0.05	0.78±0.05	0.74±0.09
20:1n-7	0.26±0.02	0.27±0.02	0.21±0.02	0.21±0.04
22:1n-7	0.00	0.00	0.00	0.00
Sum	50.37±2.23	50.14±1.44	51.31±0.80	48.72±1.12
n-6 PUFA				
18:2n-6	14.24±0.89	14.20±0.90	13.78±0.56	14.19±0.49
20:2n-6	0.18±0.02	0.18±0.04	0.17±0.01	0.17±0.02
20:3n-6	0.15±0.02	0.14±0.02	0.11±0.01	0.13±0.03
20:4n-6	1.94±0.53	2.35±0.48	1.68±0.23**	2.29±0.42
22:4n-6	0.16±0.03	0.20±0.03	0.14±0.03	0.65±1.17
Sum	16.67±1.47	17.07±1.10	15.88±0.76	17.44±1.05
n-3 PUFA				
18:3n-3	2.79±0.10	3.02±0.32	3.18±0.04	3.02±0.24
20:5n-3	0.05±0.04	0.09±0.04	0.05±0.01*	0.07±0.02
22:5n-3	0.30±0.07	0.41±0.16	0.30±0.02	0.39±0.12
22:6n-3	0.79±0.17	1.28±0.56	0.84±0.13*	1.22±0.46
Sum	3.93±0.31	4.80±0.59	4.37±0.14	4.70±0.45

Table 3.6: (Con't)

	Day 14		
	70%	60%	50%
SFA			
14:0	1.16±0.11	1.26±0.12	1.20±0.10
15:0	0.19±0.01	0.20±0.02	0.21±0.02
16:0	22.80±1.30	23.51±0.86*	22.97±0.95
18:0	4.68±0.50	4.30±0.36	4.61±0.53
Sum	28.82±0.94	29.28±0.88*	28.99±0.77
MUFA			
16:1n-9	0.50±0.05	0.53±0.04	0.63±0.03
16:1n-7	3.97±0.71	4.24±0.58	3.43±0.68
18:1n-9	40.03±1.14	39.29±1.60	40.98±1.27
18:1n-7	4.43±0.25	4.24±0.30	4.01±0.23
20:1n-9	0.81±0.06	0.75±0.11	0.83±0.09
20:1n-7	0.25±0.05	0.22±0.02	0.30±0.05
22:1n-7	0.00	0.00	0.00
Sum	49.99±1.29	49.27±1.62	50.18±1.20
n-6 PUFA			
18:2n-6	14.29±1.02	14.91±0.65	15.71±0.36
20:2n-6	0.18±0.02	0.17±0.02	0.17±0.02
20:3n-6	0.12±0.01	0.10±0.01	0.11±0.01
20:4n-6	2.15±0.50	1.91±0.22	1.71±0.39**
22:4n-6	0.16±0.02	0.15±0.02	0.13±0.03
Sum	16.91±1.52	17.24±0.80	17.83±0.50
n-3 PUFA			
18:3n-3	2.92±0.17	2.95±0.24	1.99±1.01*
20:5n-3	010±0.07	0.06±0.01	0.05±0.03*
22:5n-3	0.44±0.19	0.31±0.04	0.25±0.07*
22:6n-3	0.81±0.31	0.89±0.15	0.70±0.13*
Sum	4.27±0.31	4.21±0.37	2.99±1.61**

Table 3.7: Fatty acid composition (% of the total) of the carcass phospholipids in rats undergoing different levels of food restriction with medium fat diet. Data was expressed as Mean ± SD. The food restricted groups were significantly different from the 100% food intake rats at *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.

	Day 0	Day 14		
		100%	90%	80%
SFA				
14:0	0.16±0.04	0.16±0.09	0.21±0.10	0.13±0.03
15:0	0.95±0.18	1.25±0.22	0.90±0.27	0.84±0.13
16:0	17.28±1.45	18.66±0.64	17.34±1.47	16.03±0.51***
18:0	11.08±1.00	11.33±0.78	9.27±0.82	10.04±1.37
Sum	29.46±2.60	31.40±1.31	27.71±2.27*	27.03±1.25*
MUFA				
16:1n-9	0.23±0.05	0.22±0.11	0.21±0.03	0.18±0.01
16:1n-7	1.72±0.25	1.48±0.23	2.16±0.57	1.47±0.34
18:1n-9	29.58±3.21	27.57±1.54	32.58±2.87	30.79±3.22
18:1n-7	3.05±0.23	3.25±0.14	2.98±0.23	4.52±3.93
20:1n-9	0.59±0.07	0.53±0.26	0.57±0.05	0.53±0.06
20:1n-7	0.20±0.02	0.19±0.09	0.19±0.01	0.20±0.03
22:1n-7	0.00	0.00	0.00	0.00
Sum	35.36±3.11	33.23±1.66	38.68±3.03*	37.70±3.57
n-6 PUFA				
18:2n-6	14.35±0.75	13.00±0.66	14.34±0.40***	13.94±1.68
20:2n-6	0.26±0.04	0.20±0.10	0.21±0.03	0.22±0.04
20:3n-6	0.54±0.11	0.39±0.19	0.39±0.05	0.39±0.06
20:4n-6	8.13±0.45	9.23±0.85	7.04±0.97*	8.20±1.10*
22:4n-6	0.62±0.07	0.49±0.24	0.48±0.06	0.57±0.06
Sum	23.90±0.72	23.30±0.54	22.47±1.22	23.33±1.94
n-3 PUFA				
18:3n-3	1.17±0.14	1.12±0.11	1.58±0.17***	1.19±0.20
20:5n-3	0.47±0.09	0.44±0.25	0.37±0.03	0.43±0.08
22:5n-3	2.25±0.31	2.43±0.22	2.04±0.13	2.19±0.37
22:6n-3	7.41±0.84	8.08±0.84	7.16±0.97*	8.14±1.08
Sum	11.29±0.86	12.07±0.62	11.15±0.75***	11.95±1.30

Table 3.7: (Con't)

	Day 14		
	70%	60%	50%
SFA			
14:0	0.17±0.04	0.18±0.05	0.39±0.35
15:0	0.95±0.10	1.11±0.10	0.84±0.37
16:0	16.16±0.32***	16.07±0.51***	15.94±2.82*
18:0	9.94±1.71	9.11±0.86	6.73±1.49
Sum	27.22±1.74***	26.48±1.24***	23.89±2.72***
MUFA			
16:1n-9	0.19±0.01	0.20±0.02	0.25±0.16
16:1n-7	1.68±0.50	1.70±0.23	2.01±0.60
18:1n-9	32.26±3.95	32.88±2.01	38.72±3.31
18:1n-7	2.77±0.33	2.62±0.18	2.50±0.41
20:1n-9	0.58±0.11	0.58±0.03	0.67±0.13
20:1n-7	0.23±0.05	0.22±0.02	0.22±0.03
22:1n-7	0.00	0.00	0.00
Sum	37.71±4.06*	38.21±2.04***	44.35±3.81***
n-6 PUFA			
18:2n-6	14.34±0.57**	14.76±1.09**	17.31±1.34***
20:2n-6	0.20±0.05	0.19±0.02	0.15±0.02
20:3n-6	0.38±0.06	0.36±0.06	0.24±0.06
20:4n-6	8.02±1.65	7.96±0.81*	5.61±1.98***
22:4n-6	0.54±0.07	0.50±0.23	0.41±0.13
Sum	23.47±1.92	23.77±0.87	23.73±2.16
n-3 PUFA			
18:3n-3	1.20±0.21	1.15±0.14	1.47±0.51*
20:5n-3	0.42±0.06	0.47±0.15	0.29±0.12
22:5n-3	2.24±0.63	2.16±0.44	1.36±0.59***
22:6n-3	7.15±0.87	7.38±0.86	4.91±2.04
Sum	11.01±1.29*	11.15±1.22	8.03±2.19***

3.4.5 PROXIMATE ANALYSIS

In this part of tests, crude fat, moisture, crude protein and ash were measured in carcass, i.e. whole body after the removal of blood, liver, perirenal and epidydimal adipose tissue. Differences were compared with those of the 100% group by Student's t-test with the notation *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.

3.4.5.1 Crude Fat

Crude fat from the carcass, in general, was decreasing when the food supply was limited (Fig 3.8). Before food restriction, crude fat contributed to 18.5±3.0% by weight of the carcass. After the 2-week food restraint, it was recorded between 14.3% and 21.3%. A slight increase of fat can be observed in the rats with 90% food provided in the decreasing trend although there was no significant difference with the rats without undergoing any food restriction. From that onwards, less fat was stored in the body of the rats and when the rats were only given half of their required food, a significant difference was noticed compared to the 100% group.

3.4.5.2 Moisture

On the contrary, the change of moisture in the carcass showed an opposite trend from that of crude fat (Fig 3.9). A slight increase was seen when the rats had less food. At the beginning of the experiment, moisture was measured as 57.9±1.1%. At the end, the percentage was recorded from 56.6% to 63.2%. Two significant differences were observed against the control group. Rats with 60% and 50% food supply showed differences of $p<0.05$ and $p<0.005$ correspondingly.

3.4.5.3 Crude Protein and Ash

Before any food restriction, crude protein was recorded as 20.1±1.2%. After that, the percentage fluctuated between 20.0% and 30.4%. The highest and the lowest percentages of crude protein were measured in the groups of 90% and 50%. The latter showed a significant difference with $p<0.05$ compared with the 100% group (Fig 3.10).

Percentage of ash showed no constant trend among the groups (Fig 3.11). Before energy restriction, percentages of ash were 2.9±0.5%. At the end of the 2-week food restriction, the percentage measured between 2.3% and 3.1%. Difference was noticed in the groups of 90% and 80% compared with the control group.

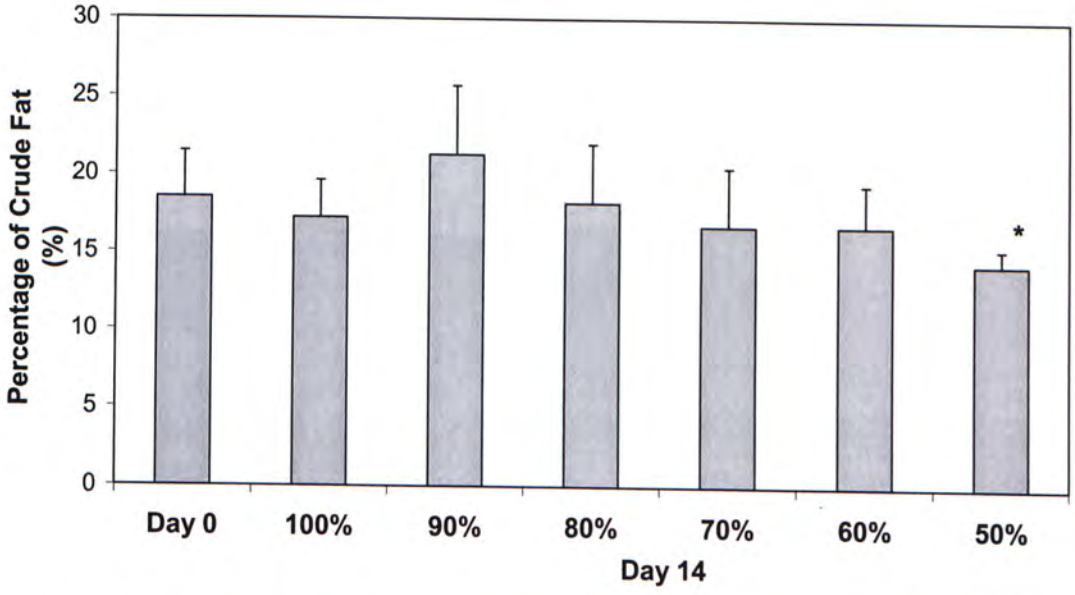


Fig 3.8: Percentage of crude fat of carcass of rats undergoing different levels of food restriction with medium fat diet. *: $p<0.05$

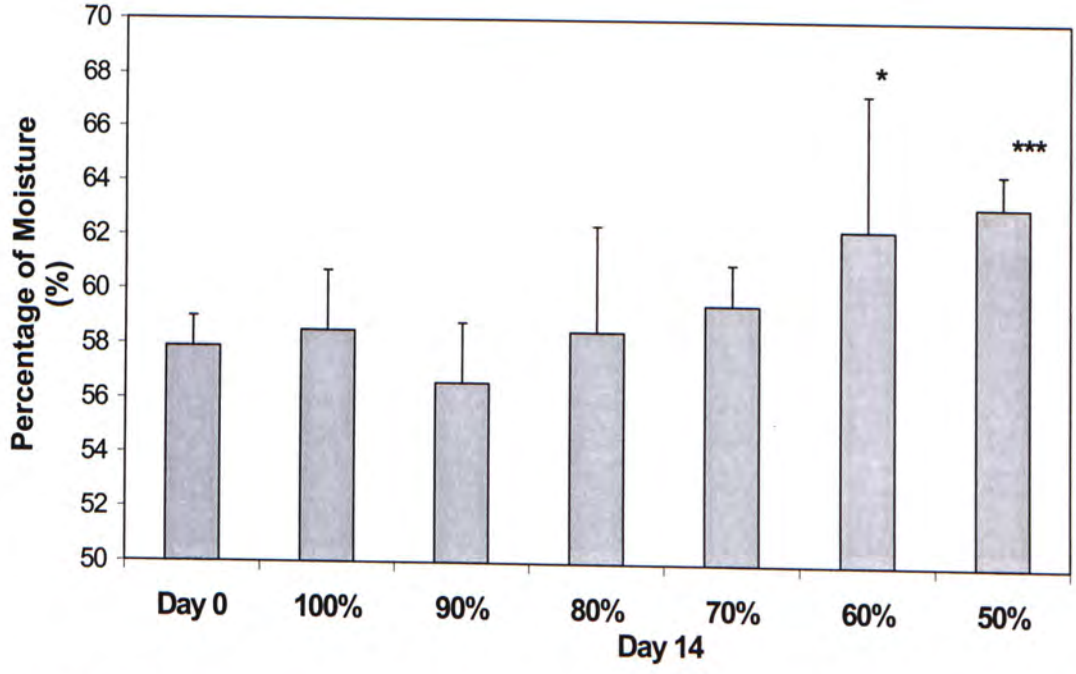


Fig 3.9: Percentage of moisture of carcass of rats undergoing different levels of food restriction with medium fat diet. *: $p<0.05$; ***: $p<0.005$

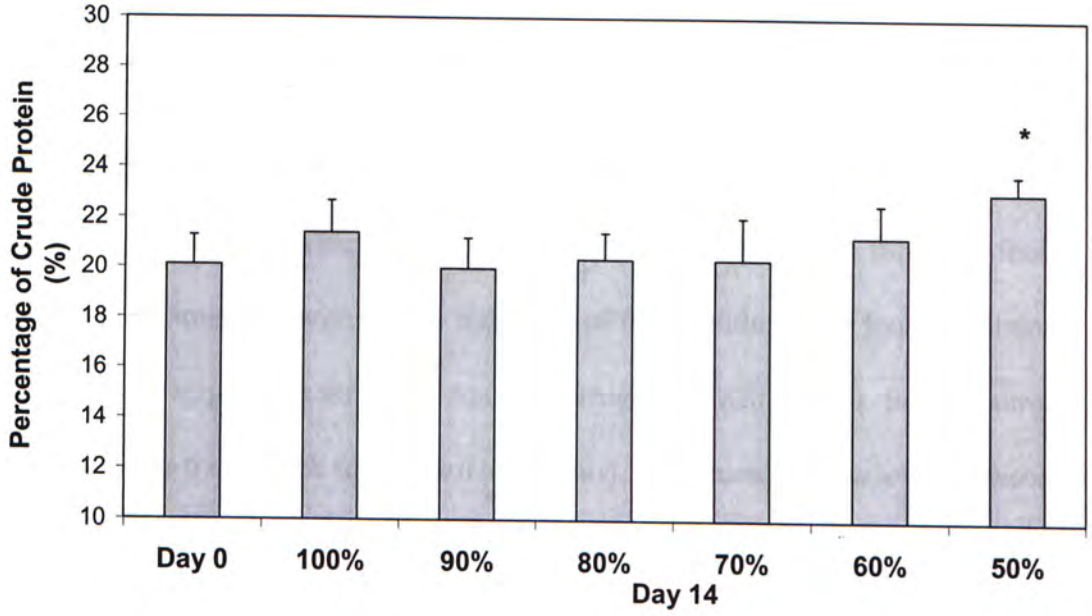


Fig 3.10: Percentage of crude protein of carcass of rats undergoing different levels of food restriction with medium fat diet. *: $p<0.05$

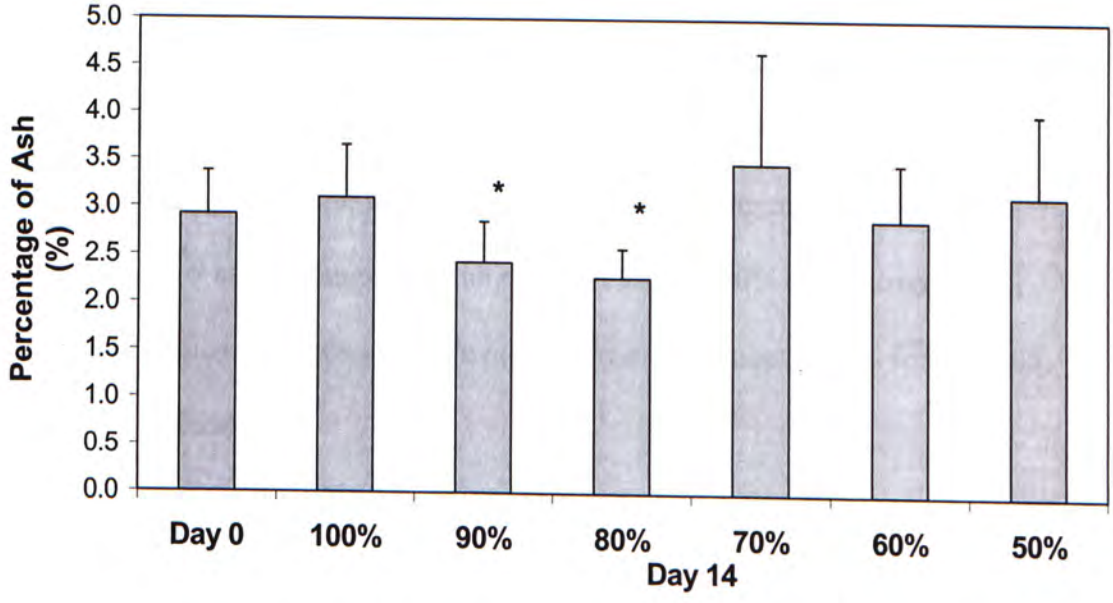


Fig 3.11: Percentage of crude fat of carcass of rats undergoing different levels of food restriction with medium fat diet. *: $p<0.05$

3.4.6 SERUM ANALYSIS

3.4.6.1 Serum Triglycerides

Concentration of serum triglycerides was decreasing with decreasing food supply to the rats (Fig 3.12). This decrease was sharp from 100% to 80% supply of food while it became mild with further reduction of food. Without any food restriction, the serum triglycerides increased from 193.1mg/dL to 220.2mg/dL in the control group. After the 2-week food restriction period, it decreased to the level between 201.9mg/dL to72.9mg/dL in the rats with 90% to 50% of the usual food intake. Differences were noticed from the groups of rats feeding with 80% or less food.

3.4.6.2 Serum Cholesterol

Concentration of serum cholesterol also dropped with the decreasing food supply (Fig 3.13). This decrease was milder among the different groups. Without any food restriction, the serum cholesterol level increased from 116.7mg/dL to 128.1mg/dL in the control group. Cholesterol level decreased to the values between 116.9 and 92.6mg/dL in the rats with 90% to 50% of the usual food intake. Significant differences were noticed from the groups of rats feeding with 80% or less food.

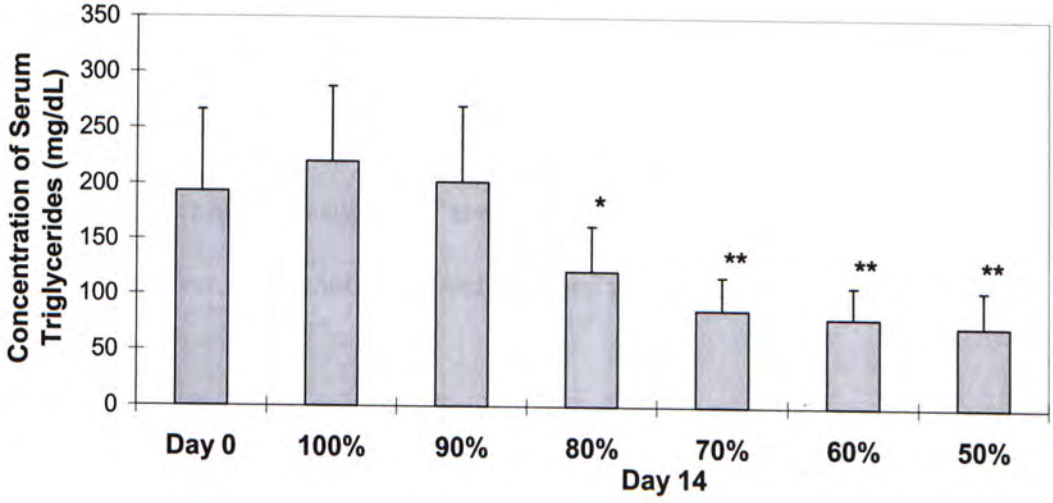


Fig 3.12: Concentration of serum triglycerides of rats undergoing different levels of food restriction with medium fat diet. *: $p<0.05$; **: $p<0.01$

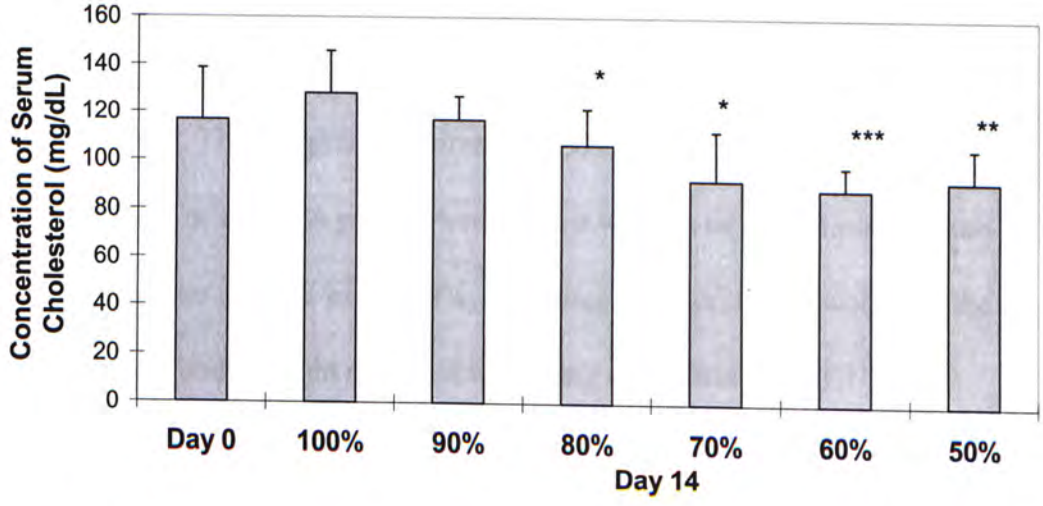


Fig 3.13: Concentration of serum cholesterol of rats undergoing different levels of food restriction with medium fat diet. *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$

3.5 *DISCUSSION*

In the present study, an MF diet with 25% of energy coming from fat was used. Weights of liver decreased with the decreased energy supply during the food restriction stage. Obviously, it was due to the depletion of energy reserve in the liver when the exogenous supply of energy is limited. Glycogen, as storage of energy in the liver, were mobilised and oxidised to yield energy for daily metabolism.

Fat in the form of triglycerides, which is the major form of energy reserve inside the body. As compared in a gram-by-gram basis, fat yielded 2-fold as much energy as the carbohydrates. A similar trend should be expected in the decrease in weight of the adipose tissues as that observed for the liver weight. In general, the fat reserve in the adipose tissues shrank when food supply was limited. However, the rats with 10% food restriction (the 90% group), not the rats with free access of food (100%), showed the heaviest adipose tissues for both the perirenal and the epidydimal ones. The weights of perirenal adipose tissue of the 100% group resembled those of the 80% group whereas their weights of epidydimal adipose tissues was similar to those of the 70%, no matter whether the net weight or the organ weight to body weight ratio that was being considered (Fig 3.4-3.7).

This finding suggested that mild (10 to 20%) food restriction promotes lipogenesis and lipid storage in the adipose tissues. The oxidation of the liver energy store became the major energy release when the food supply was not very scarce. A portion of exogenous energy supplies was preferentially stored up for the future use rather than use immediately.

In the present study, the SFAs, MUFAs and n-6 PUFAs in both of the adipose tissues and the carcass fluctuated among the groups receiving different amount of food supply. A selective depletion of the percentage of n-3 class of PUFAs was observed. This decrease in the reserve of n-3 PUFAs was mainly compensated by the increase in the MUFAs. It must be noticed that the same diet was used throughout the experiment.

The depletion of the n-3 PUFAs can be explained by the studies reported by Raclot and Groscolas (1993, 1995). In both of their *in vitro* and *in vivo* studies, energy depletion results in a higher mobilisation of fatty acids with shorter chain length than longer ones for a given unsaturation, with higher unsaturation than the saturated ones for a given chain length, and with the double-bonds nearer to the methyl end of the chain (Raclot and Groscolas, 1993; 1995). This difference in the mobilisation increased the chances of usage of the n-3 PUFAs as an energy source during caloric restriction. Evidence also shows a higher potential to oxidise unsaturated fatty acids than the saturated ones in rat liver mitochondria (Reid and Husbands, 1985). For details, please refer to section 2.5.2.

The PUFAs, especially the n-3 PUFAs, are preferably mobilised for oxidation during the energy restriction period. A higher rate of oxidation in the mitochondria further consumes the mobilised n-3 PUFAs. As a result, in the triglyceride storage in the adipose tissue and carcass an increasing percentage of SFAs and MUFAs and a selective depletion of the n-3 PUFAs were observed.

For the percentage of n-3 PUFAs to the total fatty acids of both of the adipose tissues, there was a similar decreasing trend (Tables 3.2-3.3). However, it

was interesting to notice that the changes of the individual n-3 fatty acids of 20:5n-3 and 22:6n-3 in the epidydimal fat pad and 20:5n-3 in the perirenal fat pad levelled off or even showed a rebounce in their percentage of the total fatty acids. It might suggest that there is a reservation of specific n-3 fatty acids while 18:3n-3 was more preferred to be metabolised for energy compared to other n-3 PUFAs when the supply of n-3 fatty acid was limited. This feature was observed when the food supply was restricted to 70% or less.

In carcass, decrease in the reservation of the sum of the n-3 PUFAs was observed in all the fractions, namely triglycerides, free fatty acids and phospholipids. However, this decreasing trend was not observed in every n-3 component. Regarding the 20:5n-3, 22:5n-3 and 22:6n-3, highest free fatty acid levels were observed when the rats received 70% of the *ad libitum* food consumption (Tables 3.4-3.7). These two features suggested that the change in the fatty acid reserve and the possible adverse effect are not proportional to the food supply during caloric restriction. They may also give an idea that dieting with 70% to 50% of the usual food intake can be less harmful in the cardiovascular burden. Nevertheless, the individual potencies of 20:5n-3 and 22:6n-3 in the prevention and treatment of CHD have to be further determined before concluding this observation can be beneficial in the dieters or not.

The serum triglycerides and total cholesterol were measured without giving rats any fasting. When food supply was limited, the triglyceride level dropped noticeably (Fig 3.12 and 3.13). A clear and sharp drop was detected between the 90% and the 70% groups.

Serum cholesterol dropped slightly and smoothly among the groups receiving different amount of food even though the food is cholesterol-free by using canola oil as the fat source. These drops agreed with previous researches showing decreases in the serum triglycerides and cholesterol during food restriction, which suggest a longer life span and lower risks on cardiovascular disease and atherosclerosis (Leipa *et al*, 1980; Verdery and Walford, 1998).

The fat in the carcass generally decreased when there was a caloric restriction, the rats with 50% food restriction differed significantly from the *ad libitum* group. Similar to the phenomenon seen in the weights of the adipose tissues, the proportion of fat in the carcass of the 90% group rats was the highest. Similarly, the percentage of fat in carcass of the control group fell between those of the 80% and 70% groups. This suggested an overall fattening of the body under mild energy restriction.

Chapter FOUR

FOOD RESTRICTION WITH DIETS CONTAINING VARIOUS AMOUNT OF FAT

4.1 INTRODUCTION

Fat, for its high energy density, is more or less like a poison in dieters' eyes. Many low-fat or fat-free food and beverages are easily available in the supermarkets. Low fat recipes are published in popular magazines and newspapers. The energy supplied by fat is replaced with that provided by carbohydrates which have a lower energy density but lead to a higher satiety. Steaming, hard-boiling, roasting and barbecuing are recommended methods to cook food instead of frying, no matter it is deep or shallow. American fast food is obviously regarded as the culprit of obesity, especially in children. On the contrary, dieters look for fatty food at times in their dieting course.

It may be due to the special flavour provided by the fat and, the texture, the orosensory and post-ingestive effects of food after deep or shallow frying that

other macronutrients cannot replace, so some of the dieters are still addicted to fat. Genetic factors may explain the fat and carbohydrate preference in different individuals (Reed *et al*, 1997; Rha *et al*, 1997), but a larger portion of the variation in total energy and macronutrient intake can be accounted for by environmental effects (Perusse *et al*, 1988). Consumption of high-fat diets is associated with socio-economic, demographic and lifestyle characteristics, e.g., habit of smoking, low level of leisure time and physical activity as well as low consumption of alcohol (Mattisson *et al*, 2001).

The appetite control in habitual high and low fat consumers is different. Habitual high-fat consumers eat a constant weight of food whereas low-fat ones have a more constant level of energy intake as they are believed to be more fat sensitive (Cooling and Blundell, 1998). After all, fat and oil are still the love and hate to the dieters.

4.1.1 Adverse Effects of High-Fat Diets

High fat diets have been known for many years to be related to the increase in the risk of cardiovascular disease and cancer (Berström *et al*, 2001). High dietary fat intake has been proven to have correlation to impair glucose tolerance and decrease insulin sensitivity (Lichtenstein and Schwab, 2000). It decreases insulin suppressibility of hepatic glucose production (Oakes *et al*, 1997) and whole body glucose disposal rate (Oakes *et al*, 1997; Holness and Sugden, 1999), which in turn, impairs pancreatic ß-cell function (Kaiyala *et al*, 1999) and reduces insulin-stimulated skeletal muscle glucose transport (Kim *et al*, 1996; Han *et al*, 1997).

Saturated fat intake is found to have a correlation to insulin resistance, but the adverse effects caused by high-fat diets on insulin sensitivity can be improved by substitution of n-3 fatty acids (Lichtenstein and Schwab, 2000; Vessby, 2000). Dietary fat content also modifies whole body glucose oxidation and intra-abdominal adipocyte glucose uptake during weight loss (Cha *et al*, 2001).

In LDL-receptor negative mice, high-fat and high-cholesterol diets can increase the incidence of infection-independent gastritis by more than 10 fold. In these gastritis mice, an elevation of incidence of atherosclerosis has also been reported (Laurila *et al*, 2001).

The weight gained by consuming high-fat diets is related to inflammation as BMI and waist circumference are found to be positively related to C-reactive protein (CRP), an inflammatory-response protein, a marker for low-grade systemic inflammation that is a strong, independent predictor of cardiovascular mortality. It suggests that weight loss by very-low-fat, energy-restricted diets (5700 kJ, 15% fat) can proportionally lower the level of CRP (Heilbronn *et al*, 2001).

4.1.2 Adverse Effects of Low-Fat Diets

Low-fat diets may not be as good as it seems. Volunteers in Hudgins' research (1996) were provided with a non-restricted low-fat diet with 10% calories as fat. By Day 10, their VLDL triglyceride was markedly enriched in 16:0 and deficient in 18:2n-6 in all subjects on the low-fat diet but no such observation is found in the subjects with diet of 40% fat by energy. Since over 40% of the fatty acids in

the VLDL triglycerides are newly synthesised in the low-fat diet subjects, they concluded that having low fat in diet caused an increase in the biosynthesis of 16:0, diluting the other PUFAs. Depletion of PUFAs by low-fat diets could have adverse effects on the cardiovascular system (Hudgins *et al*, 1996; Mittendorfer and Sidossis, 2001).

Comparing diets with 25% and 45% calories as fat, after a 2-week consumption period, the volunteers taking the lower fat diet were recorded with higher fasting plasma triglycerides, remnant lipoprotein (RLP) cholesterol, RLP triglyceride, and lower HDL cholesterol concentrations, without any change in LDL cholesterol concentration. These changes in lipoprotein metabolism elevated the atherogenic potential in the body (Abbasi *et al*, 2000; McLaughlin *et al*, 2000). Other study also supports that very low-fat (10%), high-carbohydrate diets increased the fraction of newly synthesised fatty acids, together with the plasma triglycerides. Furthermore, the concentration of the saturated fatty acid (e.g. 16:0) increases and the concentration of the essential polyunsaturated fatty acid (18:2n-6) decreases in triglycerides and VLDL triglyceride. However, the mechanism of these effects by low-fat and high-carbohydrate effects is still uncertain (Hudgins, 2000).

Food restriction with low-fat diet allows the dieters to lose more weight than consuming low-fat diet *ad libitum* or restricting with a higher-fat diet. This reduction in weight is mainly due to the loss in body fat; on the contrary, the loss in lean body mass and resting metabolic rate remain similar in these dieting programmes (Borne *et al*, 1996).

Decreases in LDL cholesterol during active weight loss by caloric restriction do not solely depend on the amount of fat intake, but also on the amount of saturated fat. On the other hand, changes in HDL cholesterol between diets appear to be dependent on both the fat content of the diet and the duration of energy restriction (Heilbronn *et al*, 1999; Noakes and Clifton, 2000).

4.2 OBJECTIVES

The present study was to investigate the effect of amount of fat used in diet on the whole body fatty acid composition of rats given 50% food restriction for 2 weeks.

4.3 MATERIALS AND METHOD

4.3.1 ANIMAL HANDLING

Fifty male Sprague-Dawley rats (316±14g) were kept in the Laboratory Animal Service Centre, LASEC, with 2 rats per cage. The room was kept at 23°C with a 12/12 hour light-dark cycle. The body weight and the food intake were recorded every day.

The rats were grouped randomly making sure that the average weight of rats in each group remained almost the same. In each group, there were 10 rats. Right after grouping, the chow diet was replaced with the powdered form of a set of customised diets with varying fat content. The rats were given diets with 0%, 5%, 15%, 30% and 45% of the total energy provided by canola oil and they were named accordingly.

The customised diets used for all the rats in this part of the experiment are shown as in Table 4.1. Only the amounts of oil, starch and sucrose were altered in the different diet. The rats were allowed to have free access to fresh water.

Starting from the replacement of the diet, a 4-week stabilisation period was given during which the rats ate *ad libitum*. In this period of time, the weight of the rats and the food intake were recorded so as to determine the amount of food to be given to the rats later over the food restriction period. The initial weights of the rats in groups 0%, 5%, 15%, 30% and 45% were 447.5±28g, 458±32g, 464.5±27, 477.5±23g and 461±38g while the daily food intakes were 33.4g, 32.6g, 30.2g, 30.2g and 26.6g per rat respectively.

After the 4-week stabilisation period, at Day 0, 5 rats in each group were sacrificed as the CTL group. The experiment lasted for 2 more weeks with the same customised diet with 1 rat in each cage. In this 2-week time, the rats were given half of the amount of food they took in *ad libitum*, i.e. having 50% food restriction. Therefore the amount of food given to the groups 0%, 5%, 15%, 30% and 45% were 16.7, 16.3, 15.1, 15.1 and 13.3g/rat/day respectively. After the 2-week food restriction treatment, the remaining rats, 5 per group, were sacrificed on Day 14.

The analyses of carcass and adipose tissue fatty acid, serum triglyceride and cholesterol levels and carcass proximate composition have been described in section 2.3.2 to 2.3.4.

Table 4.1: Composition of five customised diets.

Ingredients	**Percentage (w/w)**				
FAT (e/e)	0%	5%	15%	30%	45%
Starch	**61.9**	**59.6**	**55.5**	**48.3**	**39.2**
Canola Oil	**0.0**	**2.5**	**7.0**	**15.0**	**25.0**
Casein			23.5		
Sucrose	**6.2**	**6.0**	**5.6**	**4.8**	**3.9**
Mineral Mix			3.5		
Cellulose			3.2		
Vitamin Mix			1.0		
Choline Bitartrate			0.4		
D/L-Methionine			0.3		
Total	100	100	100	100	100

4.4 RESULTS

4.4.1 BODY WEIGHT

From the very beginning of the experiment, before stabilisation, the rats weighed 313.0±13.6g, 316.5±13.8g, 312.5±14.0g, 309.0±13.1g, and 310.0±13.9g in the groups of the rats having 0%, 5%, 15%, 30% and 45% fat in diet. However, as the rats underwent the stabilisation with different diets containing varying percentage of fat as energy, the weights of rat differed after the 4-week pre-treatment. Before any food restriction, the rats having 0%, 5%, 15%, 30% and 45% fat in diet weighed 447.5±27.5g, 458.0±32.2g, 464.5±27.0g, 477.5±22.9g and 461.0±38.1g respectively.

After the 2-week 50%-food restriction period, they weighed 403±9.1g (0%), 421±24.3g (5%), 431±21.0g (15%), 451±17.1g (30%) and 417±24.7g (45%) (Fig 4.1). The differences of the body weight were 44.5g, 37g, 33.5g, 26.5g and 44.0g in each group before and after the 50% food restriction period.

4.4.2 FOOD INTAKE

From the stabilisation period, the daily food intakes in each group were recorded as 33.4g (0%), 32.6g (5%), 30.2g (15%), 30.2g (30%) and 26.6g (45%). Therefore, foods given to rats every day were 16.7g (0%), 16.3g (5%), 15.1 (15%), 15.1 (30%) and 13.3g (45%) during the 50% food restriction period. However, spillage was often found, so the actual amounts of food consumed were 16.4±0.13g, 15.48±0.17g, 14.65±0.10g, 14.81±0.19g and 13.22±0.12g correspondingly (Fig 4.2).

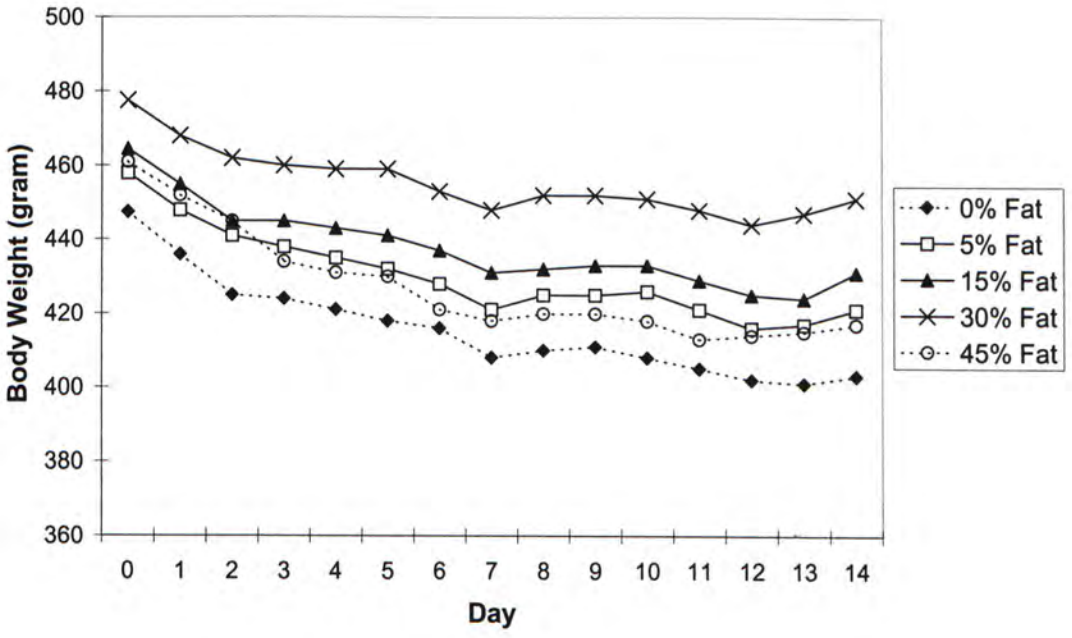


Fig 4.1: Body weight of rats undergoing food restriction with different amount of fat in diets.

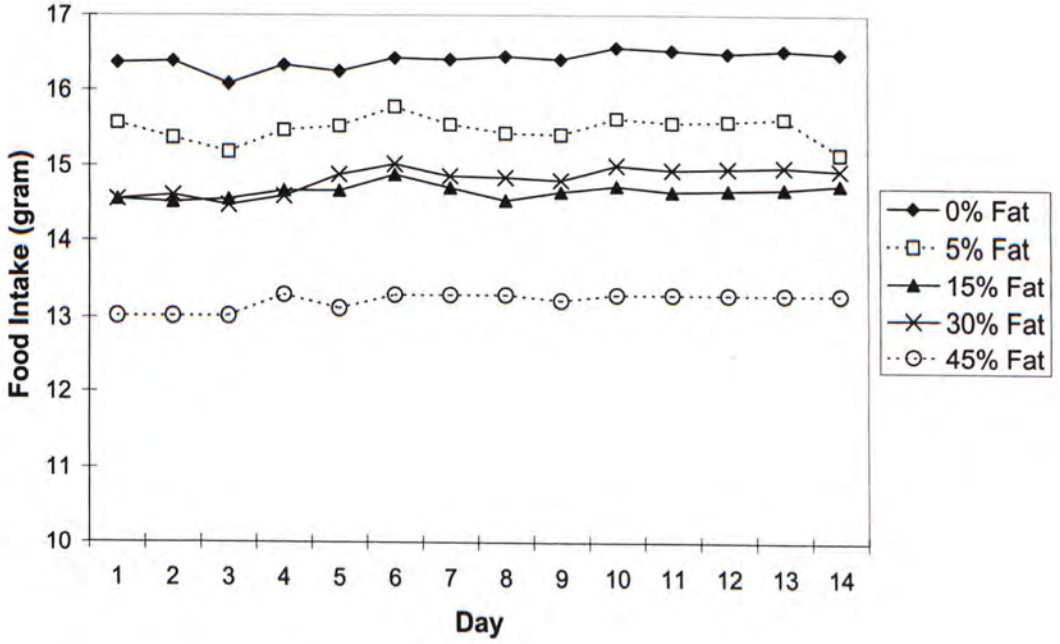


Fig 4.2: Daily food intake of rats undergoing food restriction with different amount of fat in diets.

4.4.3 ORGAN WEIGHT

Differences were compared within the same group of rats before and after the food restriction and they were determined by Student's t-test with the notation *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.

4.4.3.1 Liver

The weights of liver in all the groups significantly decreased after the 2-week 50% food restraint period (Fig 4.3 and 4.4). They decreased from 18.3g to 11.3g (0%), 17.3g to 11.6g (5%), 18.7g to 12.0g (15%), 18.6g to 12.3g (30%) and 18.9g to 12.6g (45%). This trend can also be found in their ratio to body weight. They decreased from 0.041 to 0.028 (0%), 0.038 to 0.027 (5%), 0.040 to 0.028 (15%), 0.039 to 0.0272 (30%) and 0.041 to 0.030 (45%).

4.4.3.2 Adipose Tissues

The shrinkage of the adipose tissue was not as significant as that in the liver throughout the food restriction period. Before the energy restriction period, the weights of the perirenal adipose tissues ranged between 11.1g and 11.8g in the rats. The ratios ranged from 0.024 to 0.028, with little variation between the groups. After food restraint, they were decreased to values between 6.6g and 10.7g while the ratio to the body weight were recorded from 0.016 to 0.024 respectively (Fig 4.5 and 4.6).

The weights of the epidydimal adipose tissues before the energy restraint were measured between 7.0g and 7.6g and the ratios to the body weight ranged from 0.015 to 0.016 with little variation among the groups. At the end of the 50% food restriction, they decreased to the range of 4.2g to 7.8g and the ratios were observed to be from 0.012 to 0.017, respectively (Fig 4.7 and 4.8).

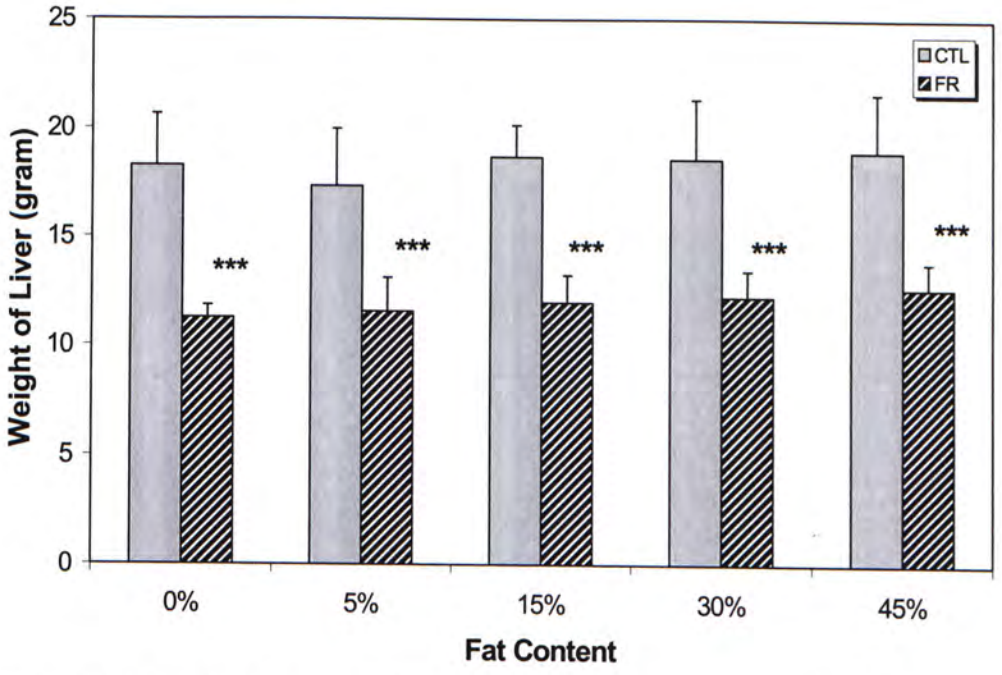


Fig 4.3: Weight of liver of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); ***: $p<0.005$

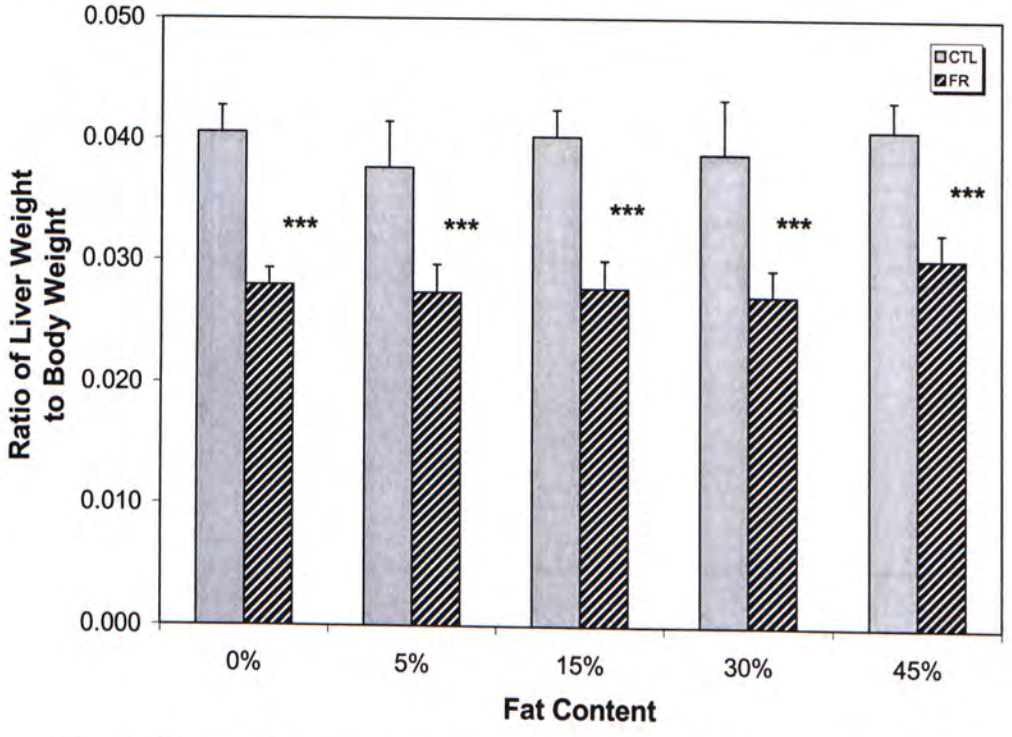


Fig 4.4: Ratio of weight of liver to body weight of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); ***: $p<0.005$

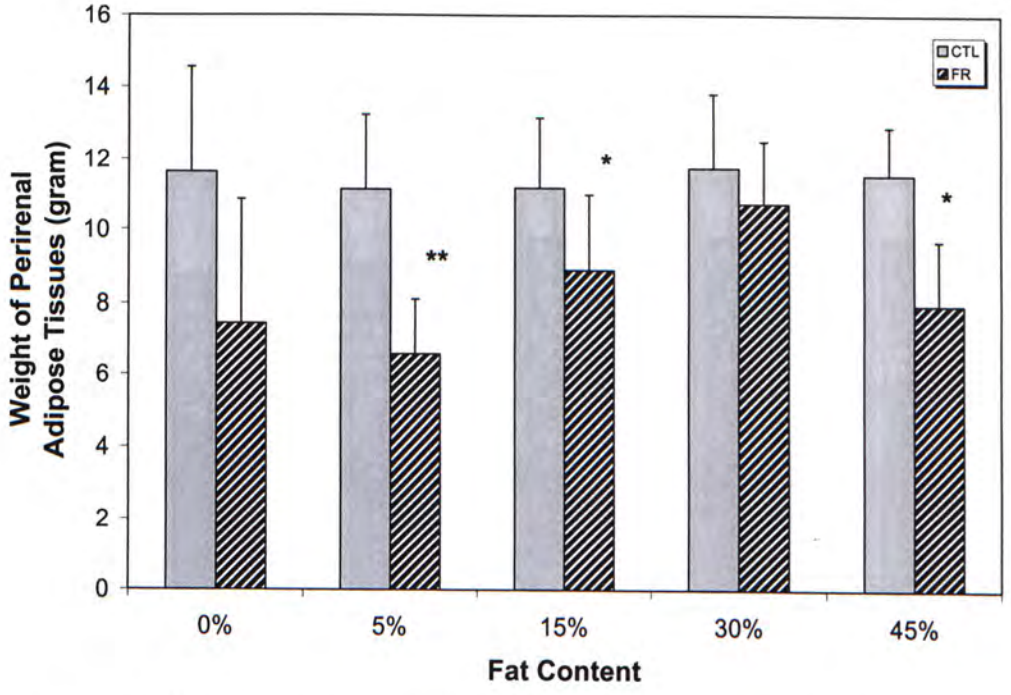


Fig 4.5: Weight of perirenal adipose tissues of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; **: $p<0.01$

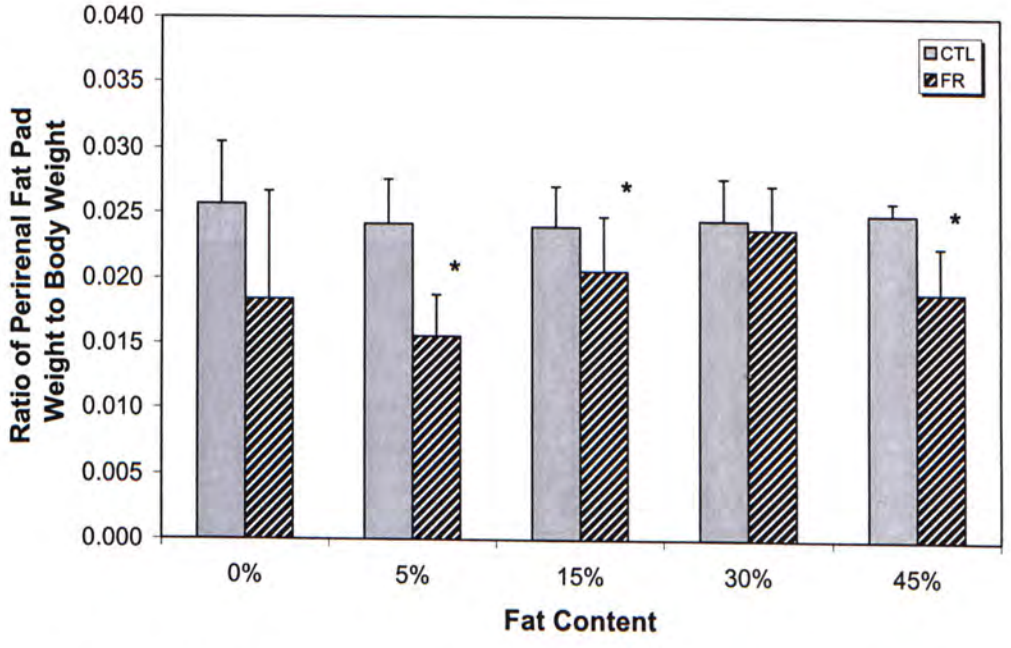


Fig 4.6: Ratio of weight of perirenal adipose tissue to body weight of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$

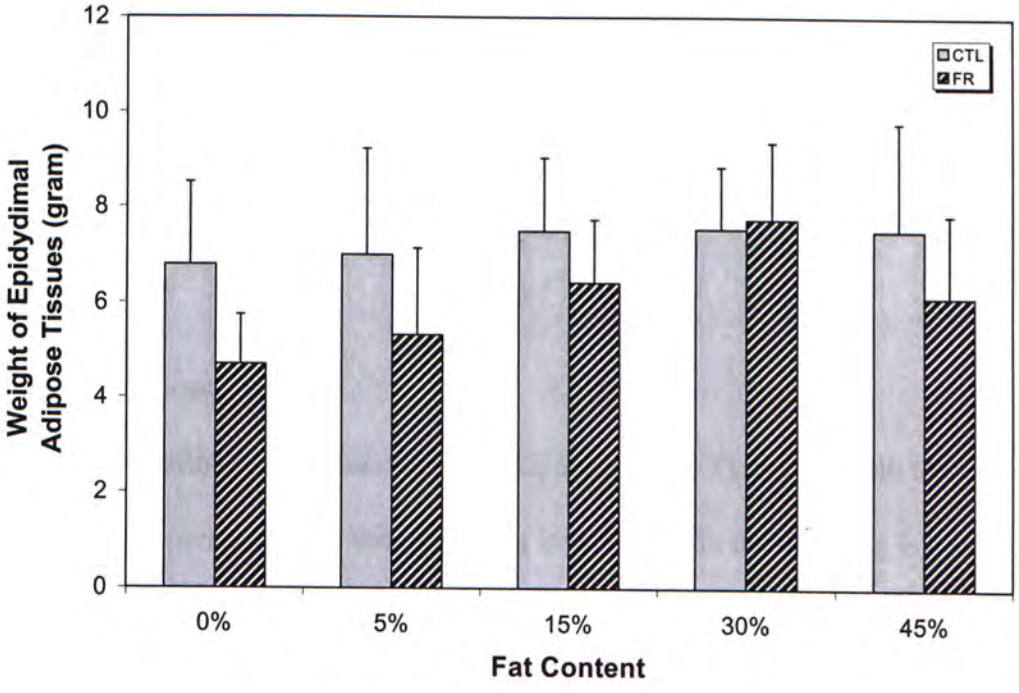


Fig 4.7: Weight of epidydimal adipose tissues of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR)

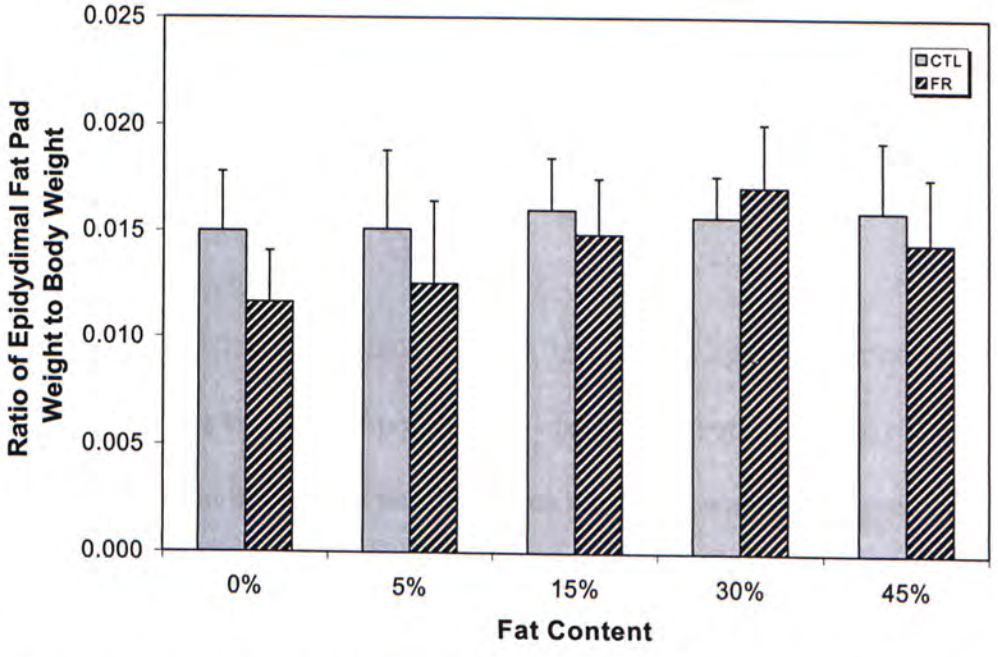


Fig 4.8: Ratio of weight of epidydimal adipose tissue to body weight of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR)

4.4.4 Lipid Analysis

Differences were compared within the same group of rats before and after the food restriction and they were determined by Student's t-test with the notation *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.

4.4.4.1 Adipose Tissues

After the stabilisation period, the MUFAs, n-6 and n-3 PUFAs in both the CTL and FR rats increased with the increasing levels of fat in the diet (Fig 4.9 and 4.12). The reversal was observed for SFAs, which decreased gradually with the increasing levels of dietary fat.

When the food-restricted rats were compared with the CTL, it was found that the energy restriction elevated the total MUFAs but it lowered the total n-3 PUFAs. The individual n-3 fatty acids that were decreased with the food restriction included 18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3 (Fig 4.11 to 4.14).

4.4.4.2 Carcass

The four major categories of fatty acids in the different lipids of carcass showed the same changes as in the adipose tissues observed in both CTL and FR groups except MUFAs in the carcass phospholipids which remained unchanged or decreased slightly with the increasing levels of fat in diet (Fig 4.23). Differences within the same group through the fasting period became more significant in this general view.

The individual component of 20:4n-6 fluctuated among the groups with significant decreases within the same group after the treatment. The 16:0 percentages decreased with time but increased when the fat content in the diet increased. On the other hand, the 18:2n-6 showed the opposite change similar to the general trends observed in n-3 PUFAs, the various individual components dropped distinctively within the same group while the less the fat content in the diet, the less the n-3 PUFAs in the carcass lipids (Fig 4.15 to 4.23).

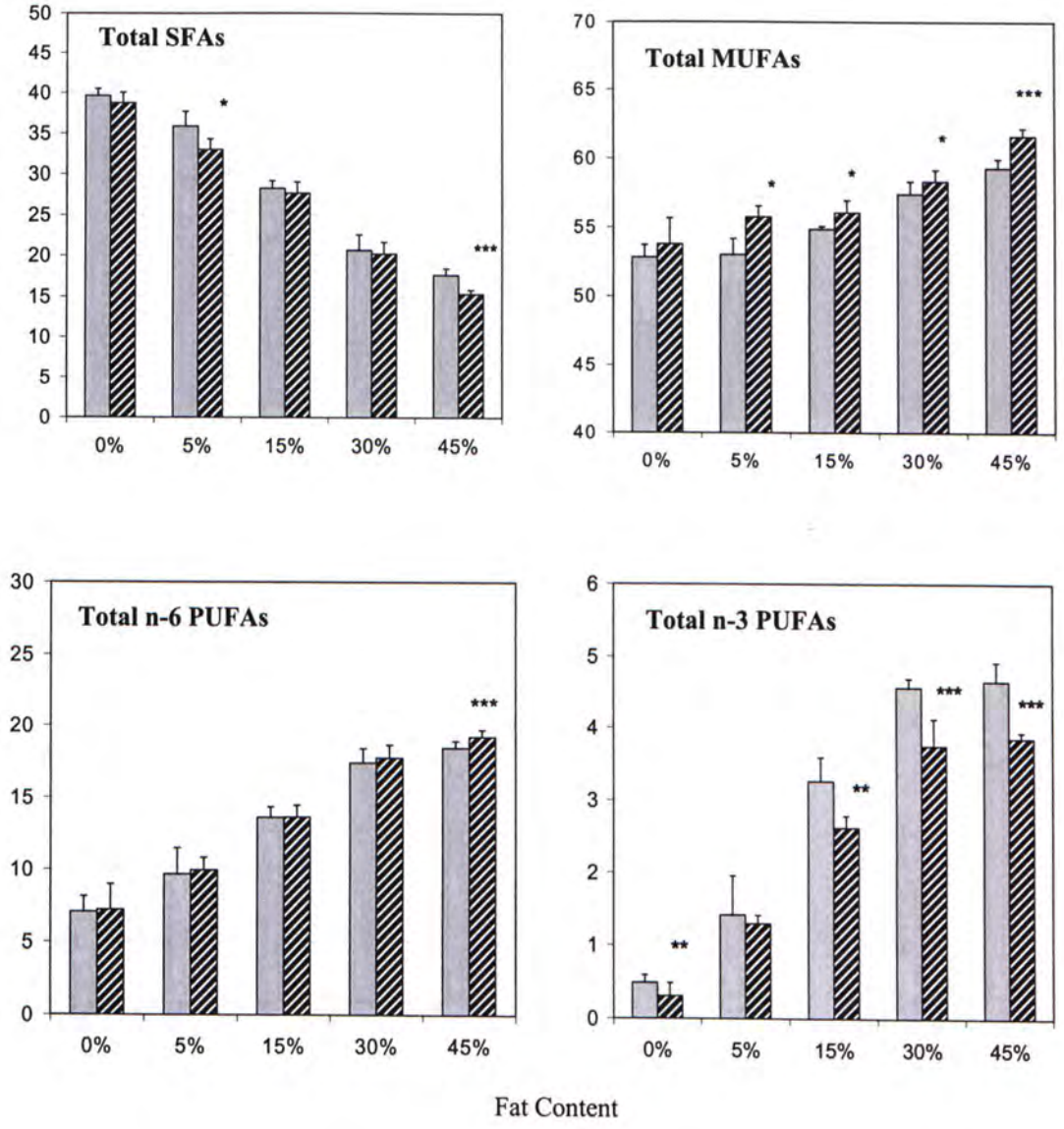


Fig 4.9: Percentage of different categories of fatty acids in perirenal adipose tissues of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$

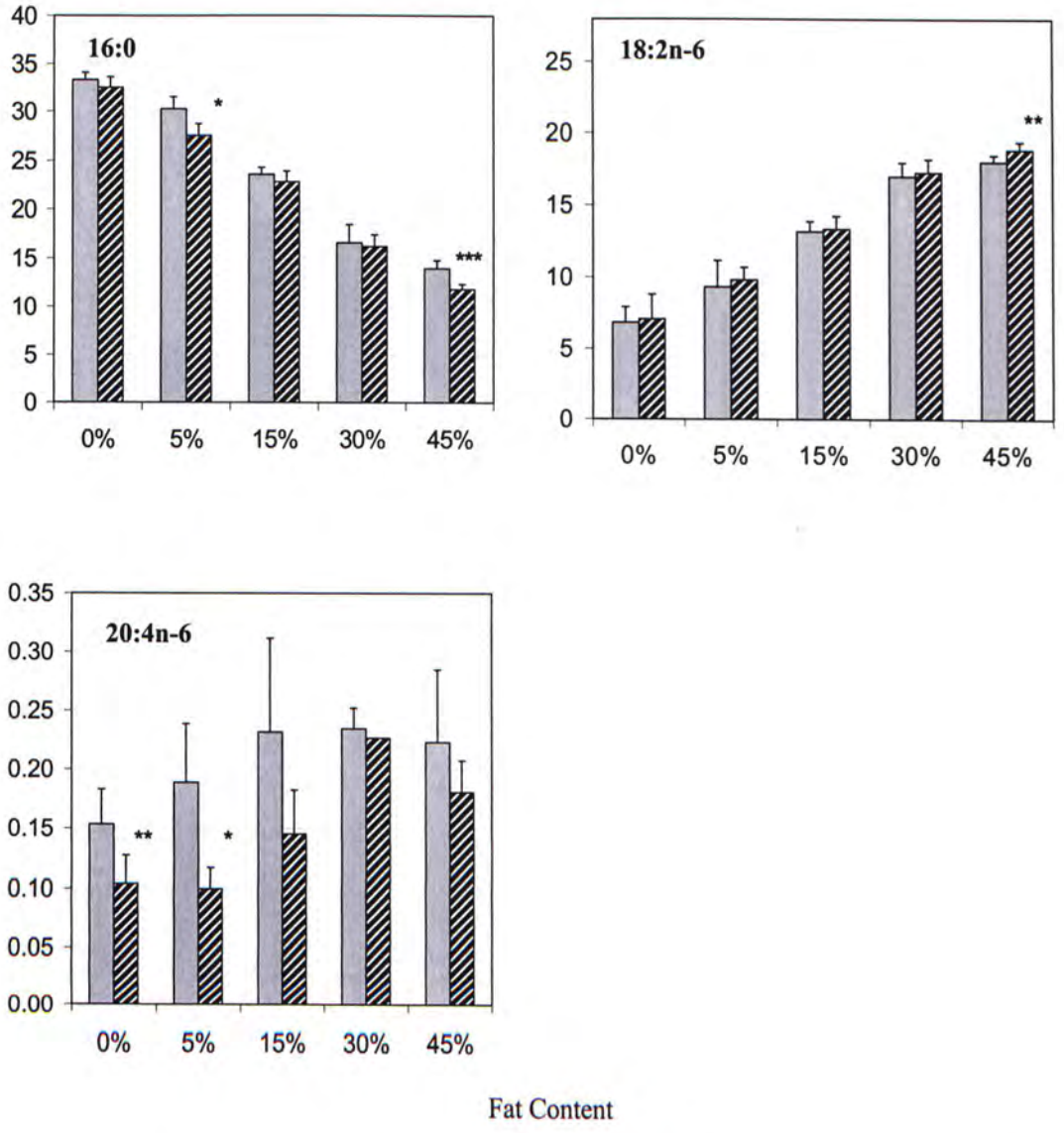


Fig 4.10: Percentage of selected SFA and n-6 PUFAs in perirenal adipose tissues of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$

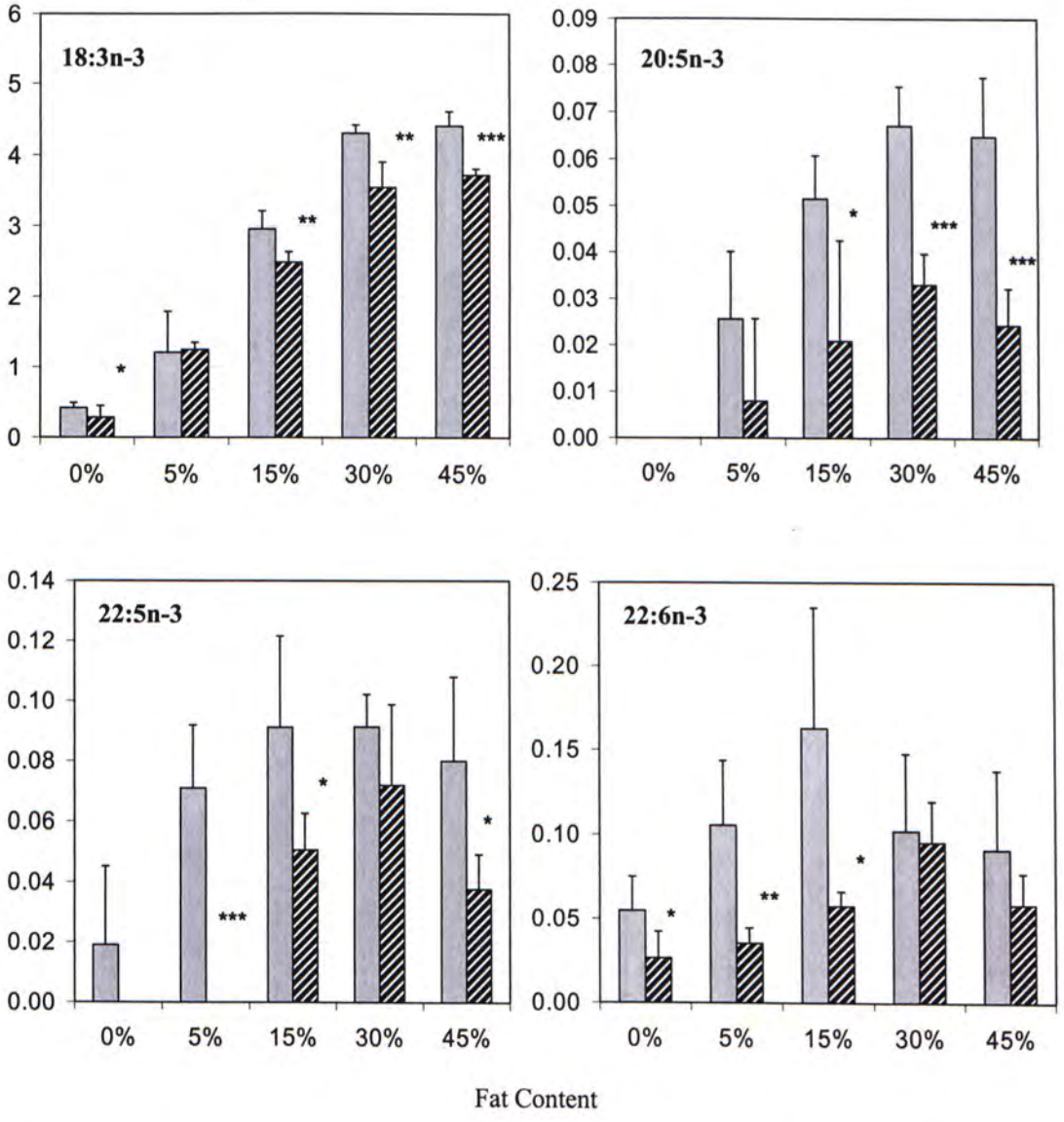


Fig 4.11: Percentage of selected n-3 PUFAs in perirenal adipose tissues of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$

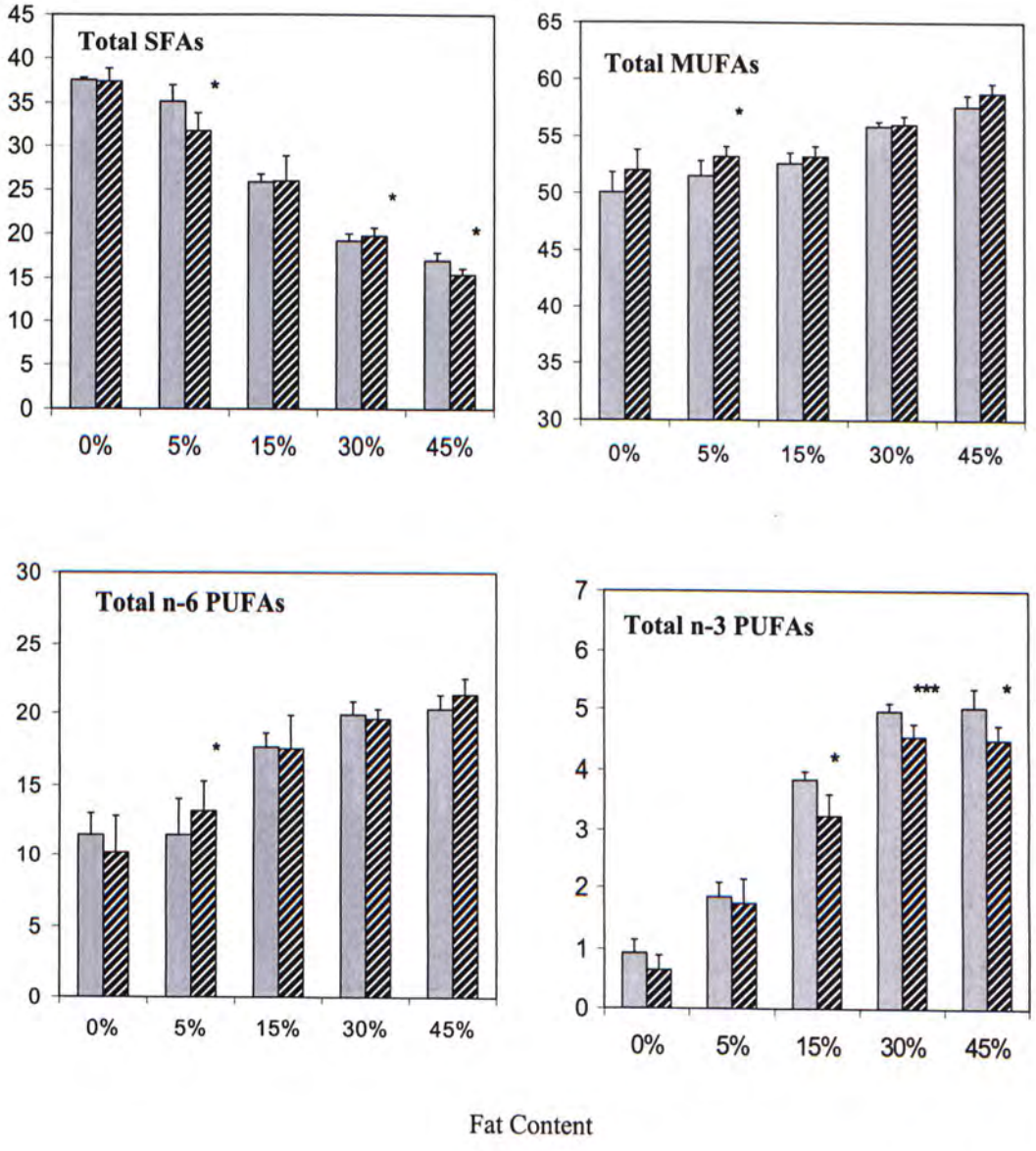


Fig 4.12: Percentage of different categories of fatty acids in epidydimal adipose tissues of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; ***: $p<0.005$

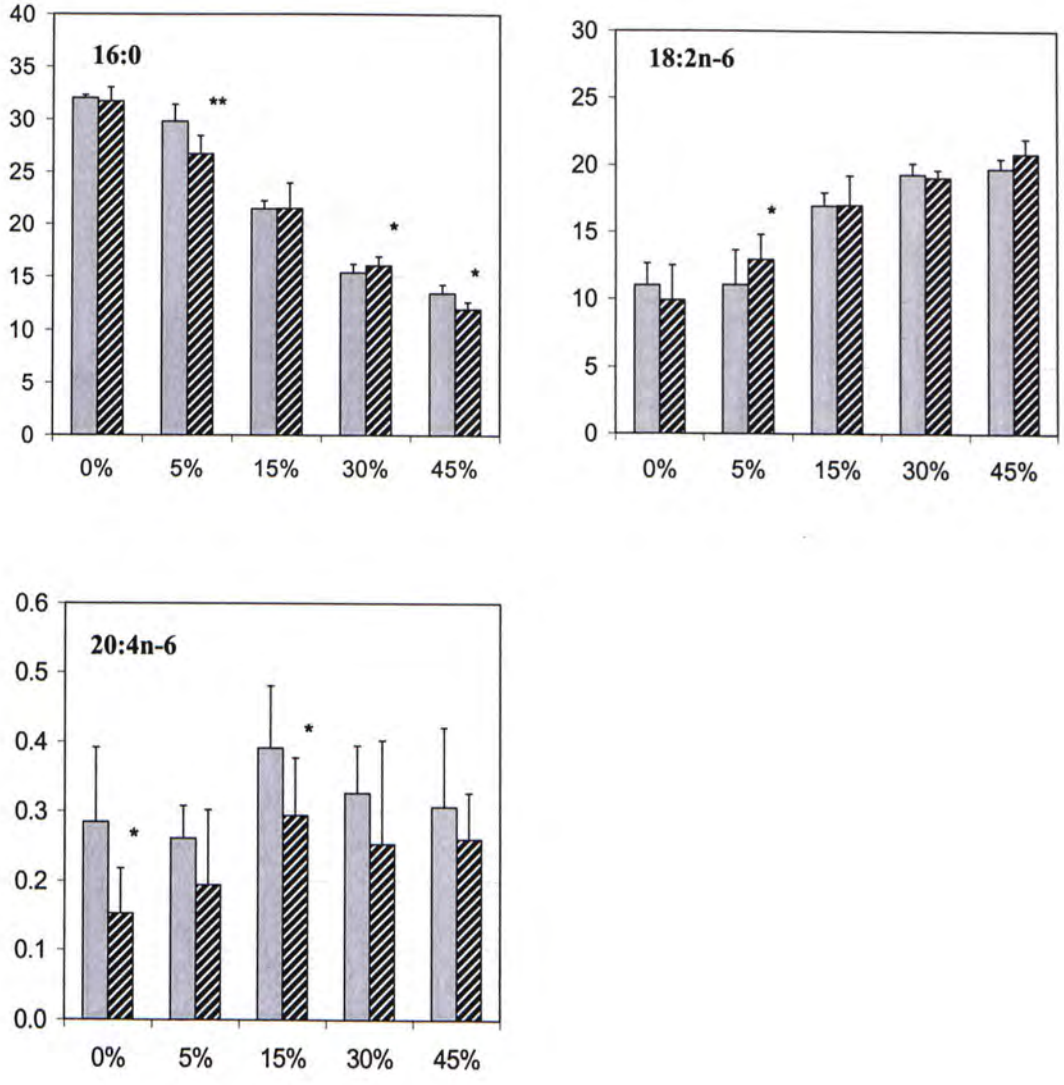


Fig 4.13: Percentage of selected SFA and n-6 PUFAs in epidydimal adipose tissues of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; **: $p<0.01$

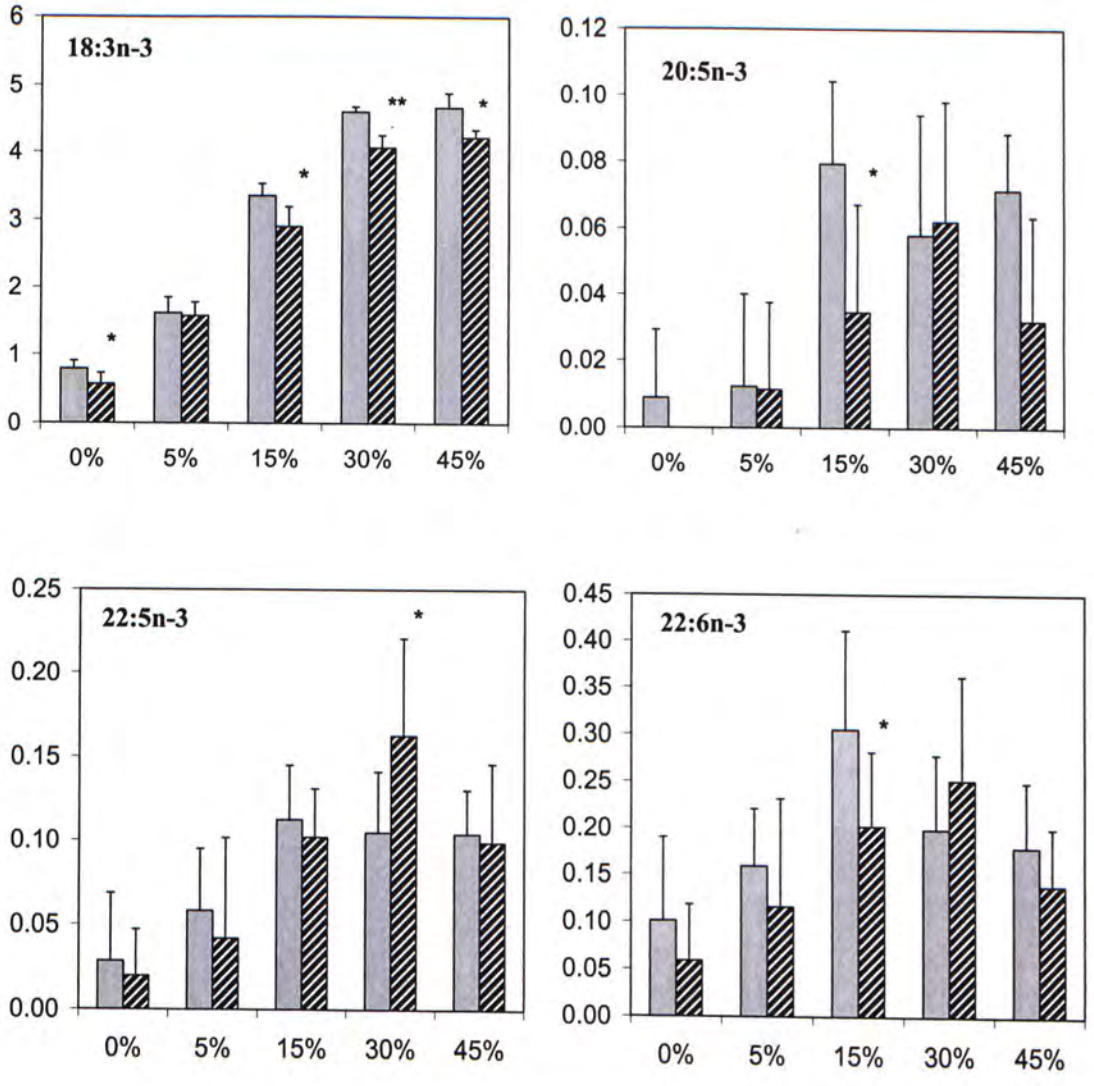


Fig 4.14: Percentage of selected n-3 PUFAs in epidydimal adipose tissues of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; **: $p<0.01$

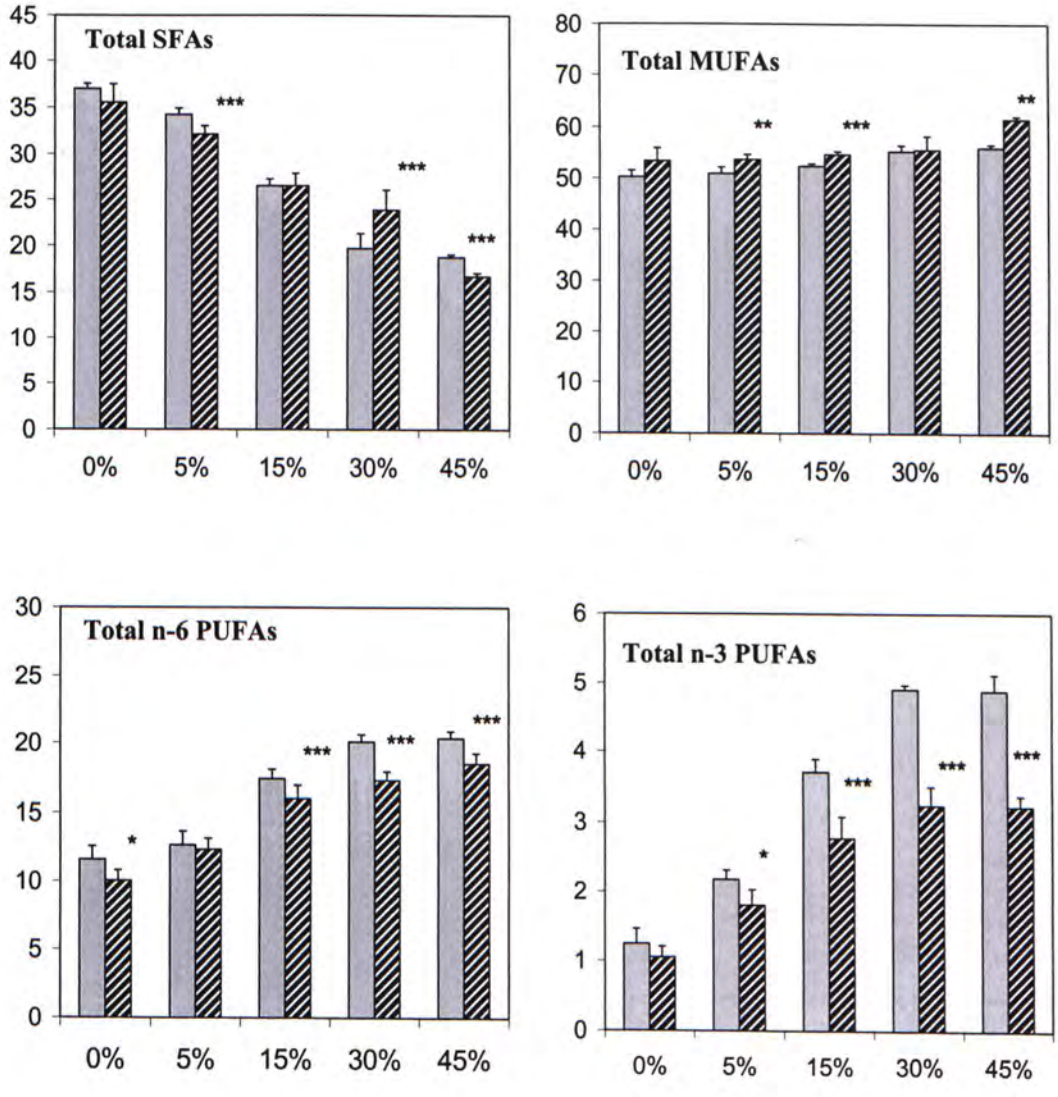


Fig 4.15: Percentage of different categories of fatty acids in carcass total lipids of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$

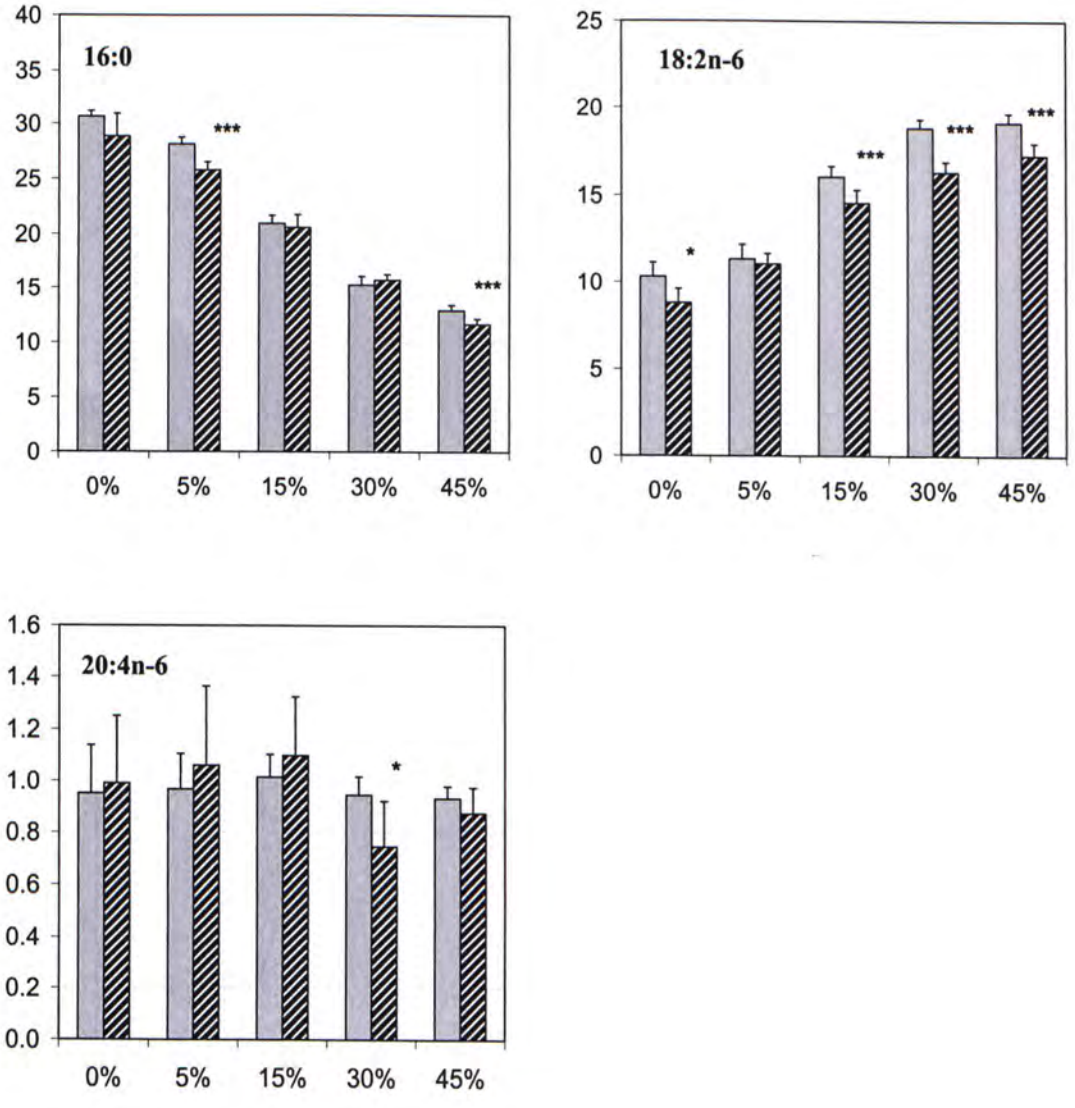


Fig 4.16: Percentage of selected SFA and n-6 PUFAs in carcass total lipids of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; ***: $p<0.005$

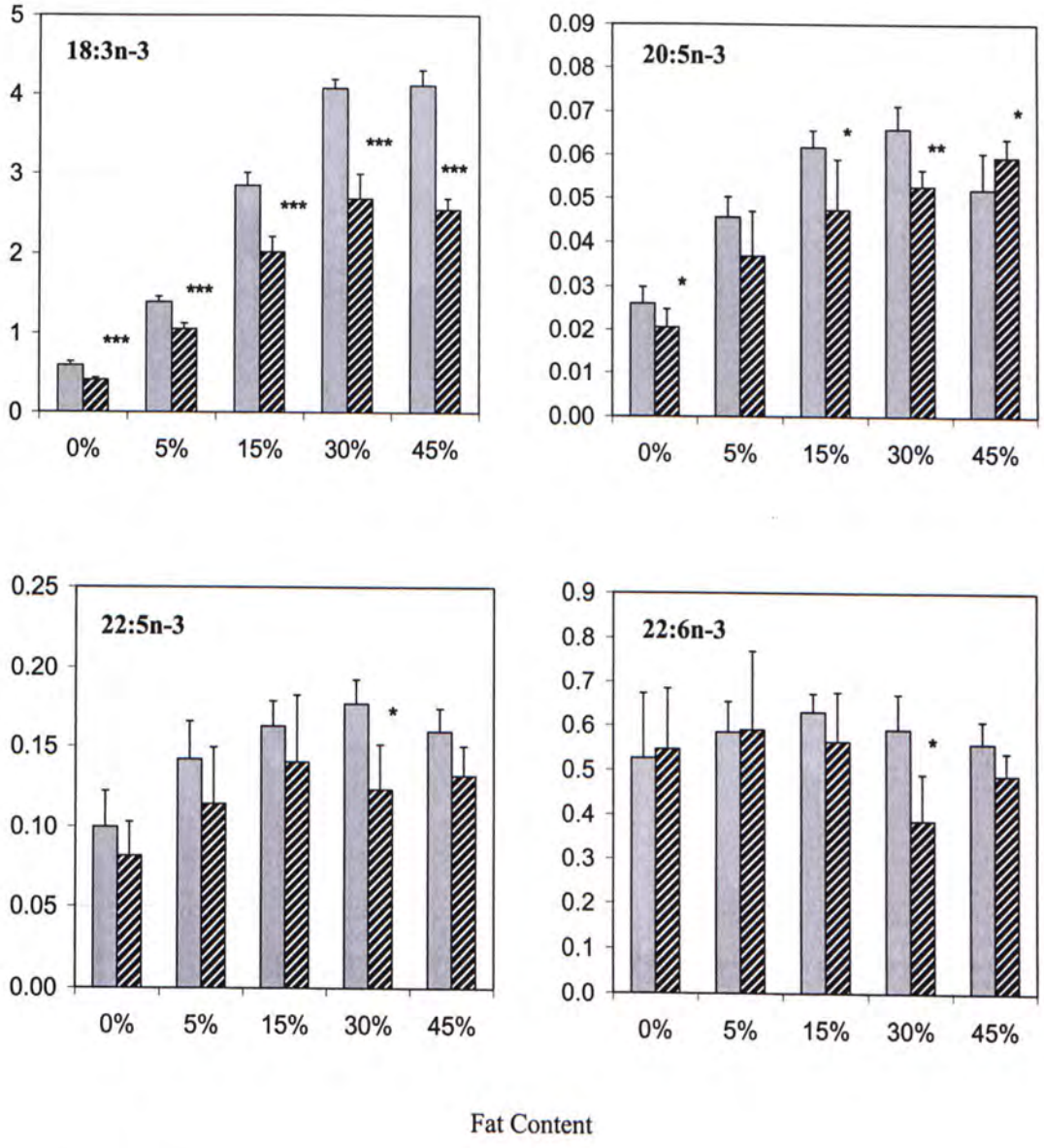


Fig 4.17: Percentage of selected n-3 PUFAs in carcass total lipids of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$

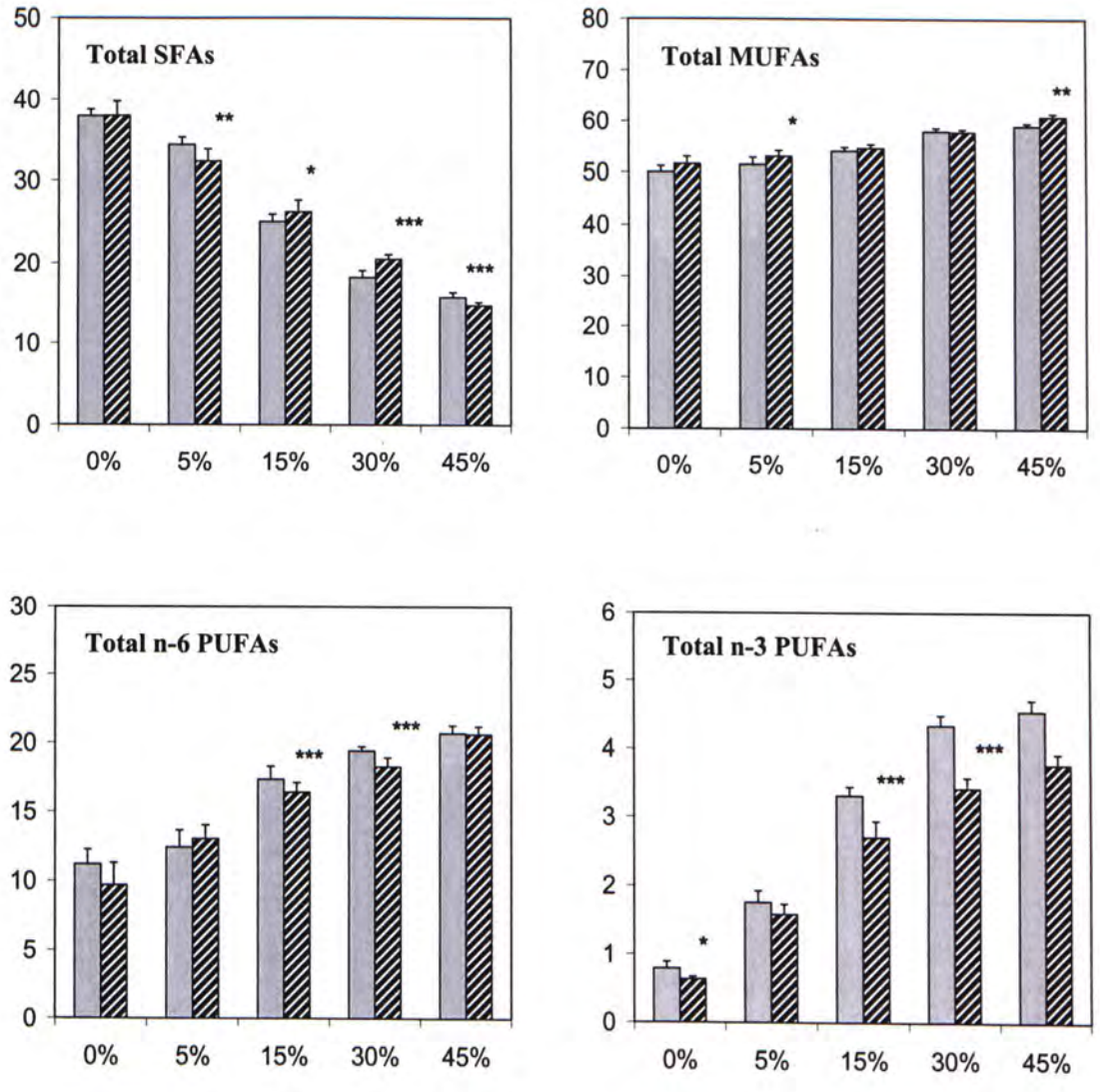


Fig 4.18: Percentage of different categories of fatty acids in carcass triglycerides of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$

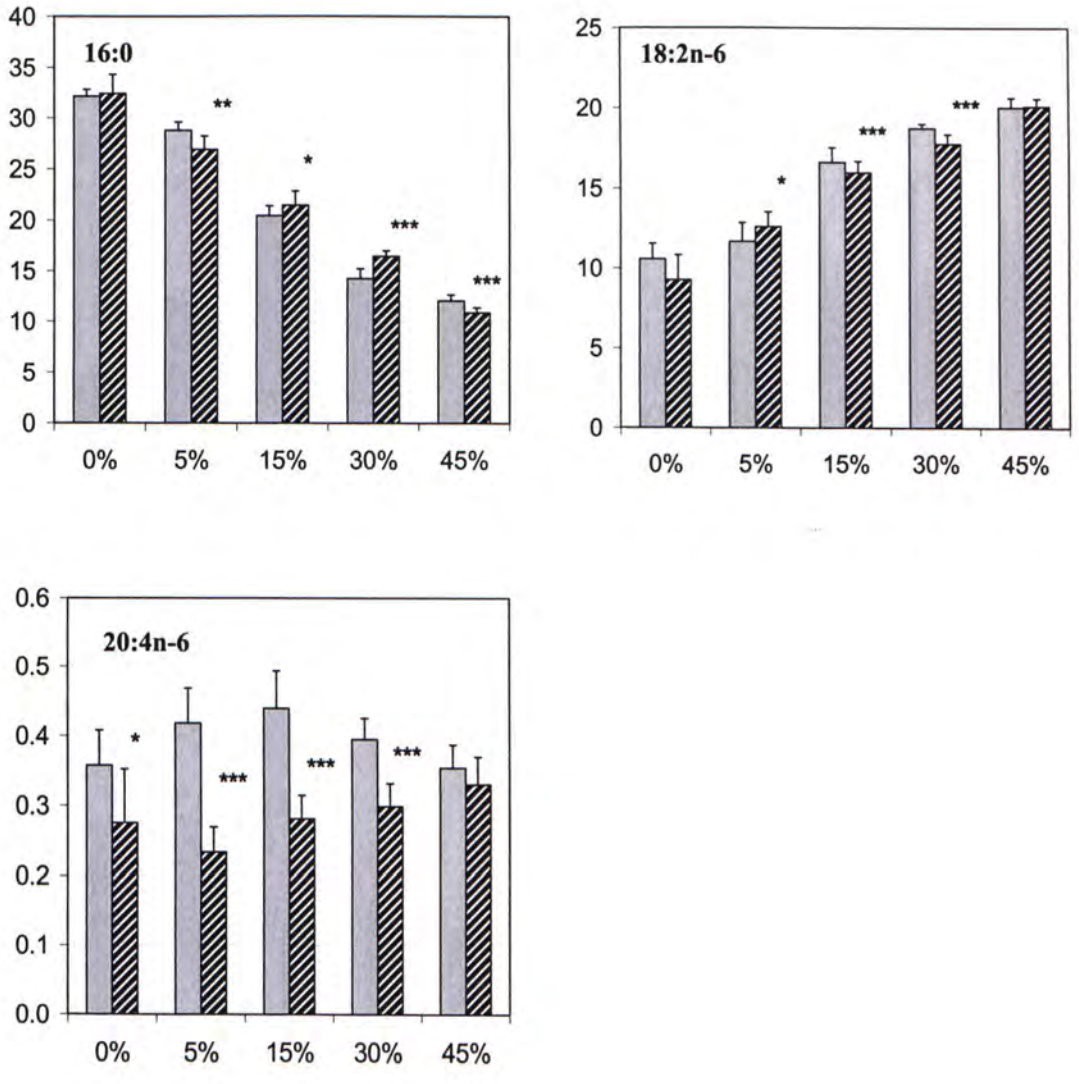


Fig 4.19: Percentage of selected SFA and n-6 PUFAs in carcass triglycerides of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$

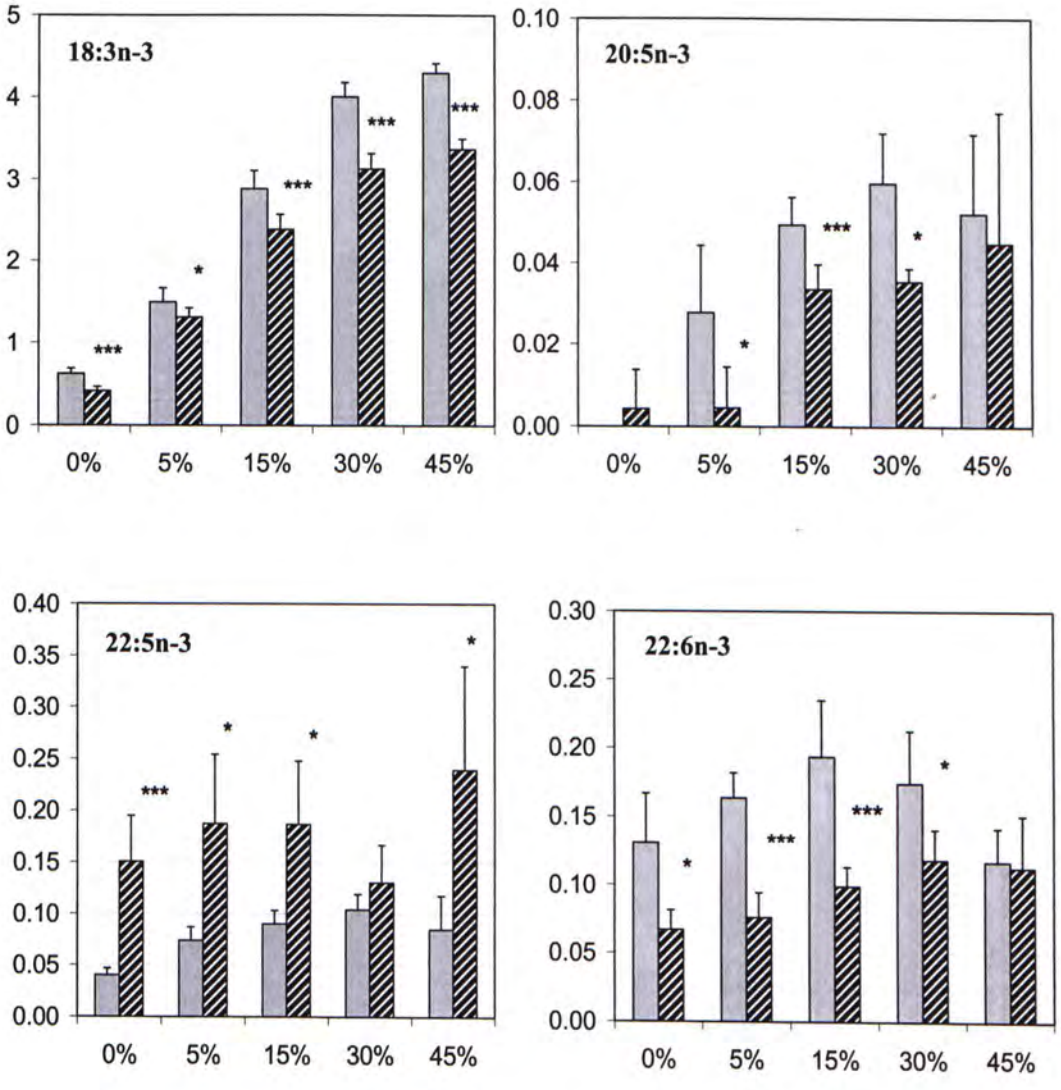


Fig 4.20: Percentage of selected n-3 PUFAs in carcass triglycerides of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; ***: $p<0.005$

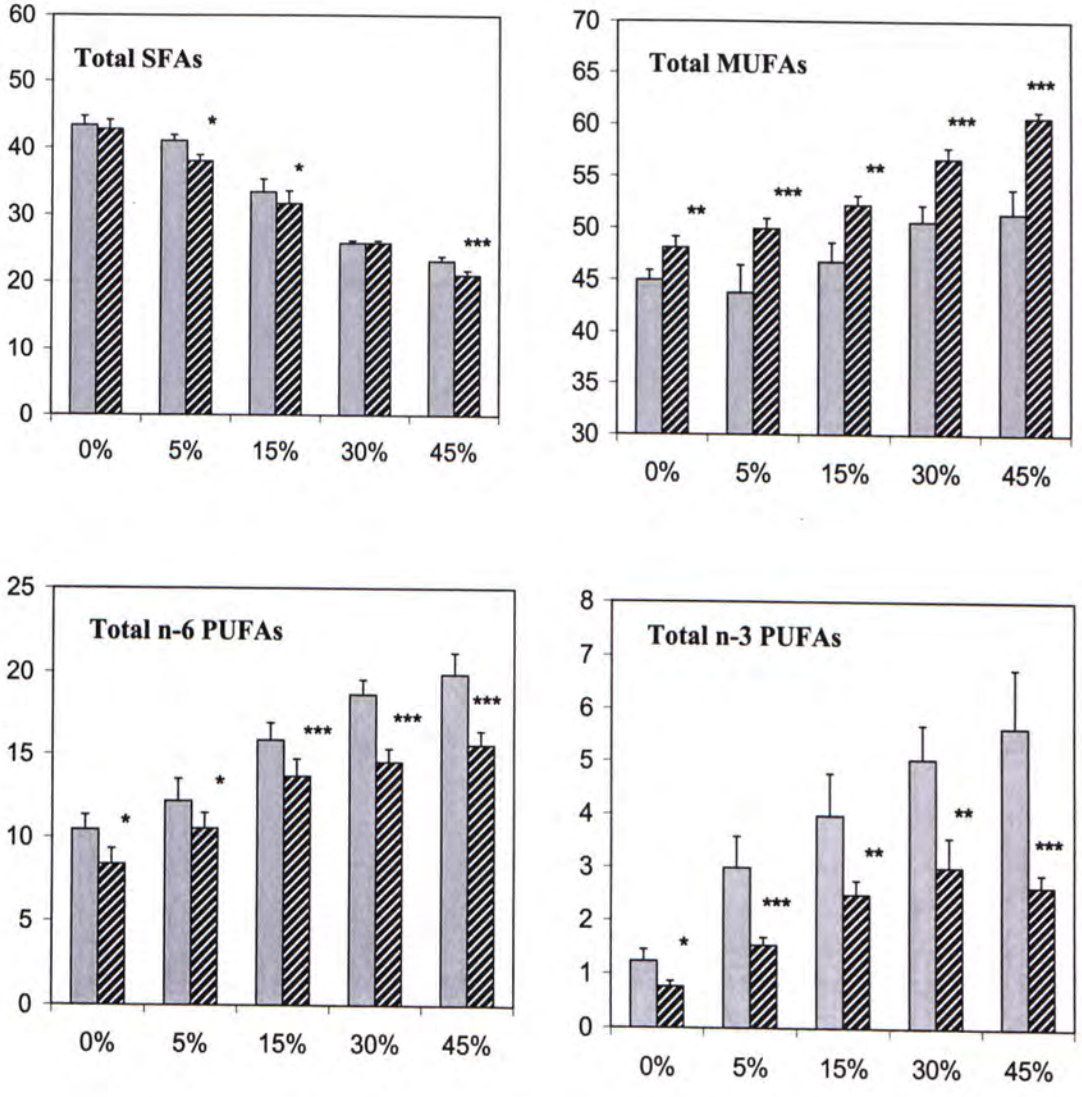


Fig 4.20: Percentage of different categories of fatty acids in carcass free fatty acids of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$

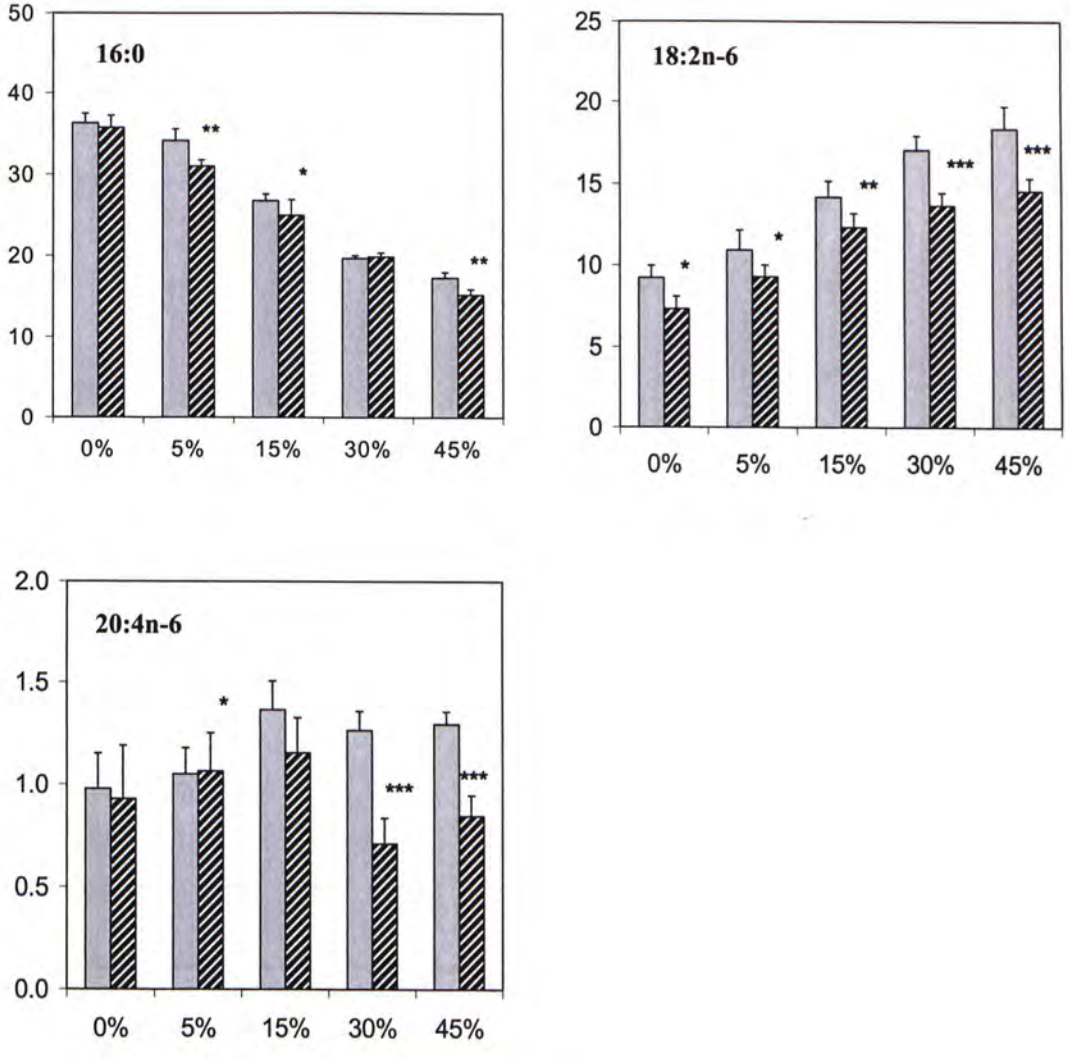


Fig 4.21: Percentage of selected SFA and n-6 PUFAs in carcass free fatty acids of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$

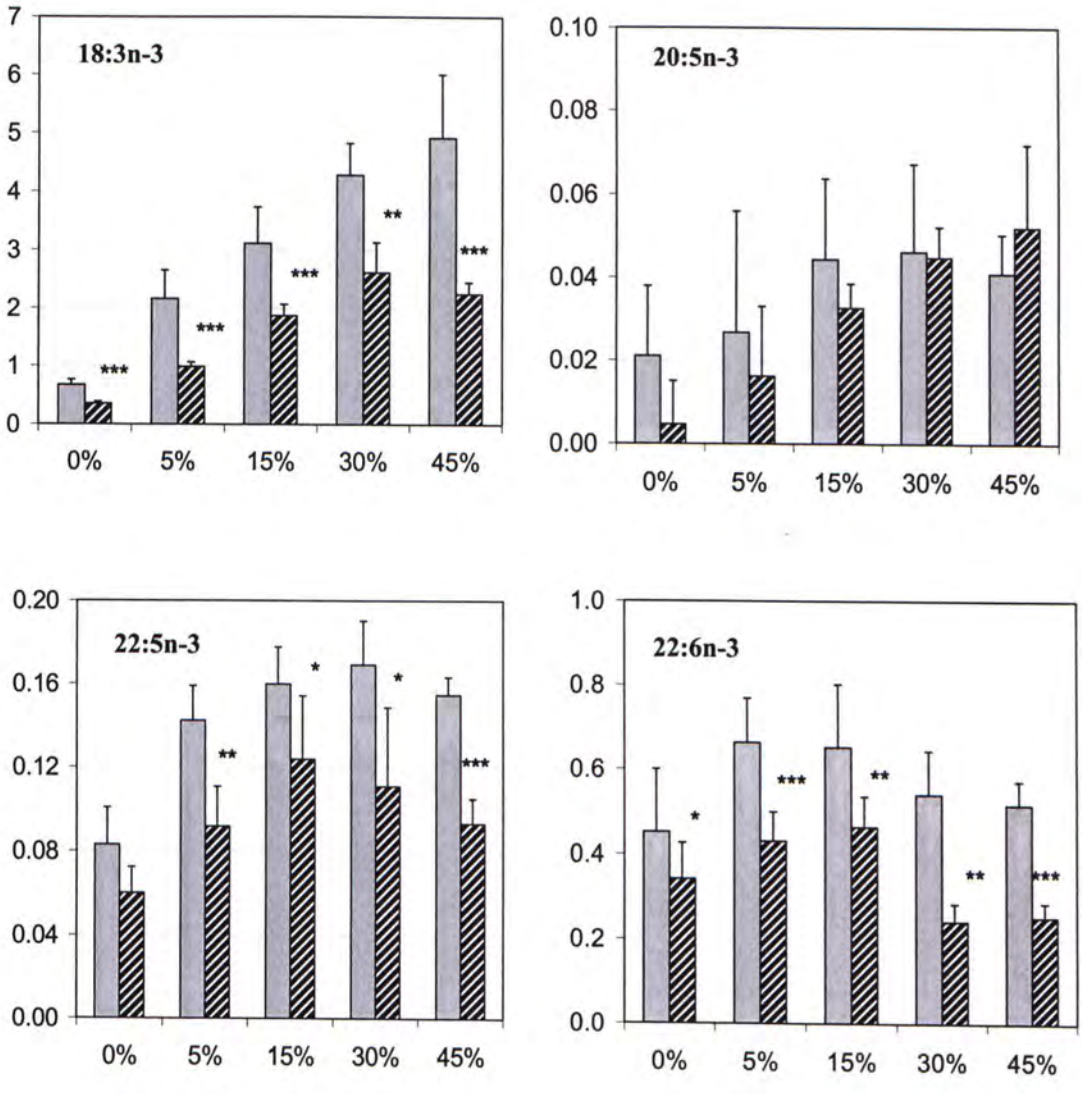


Fig 4.22: Percentage of selected n-3 PUFAs in carcass free fatty acids of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$

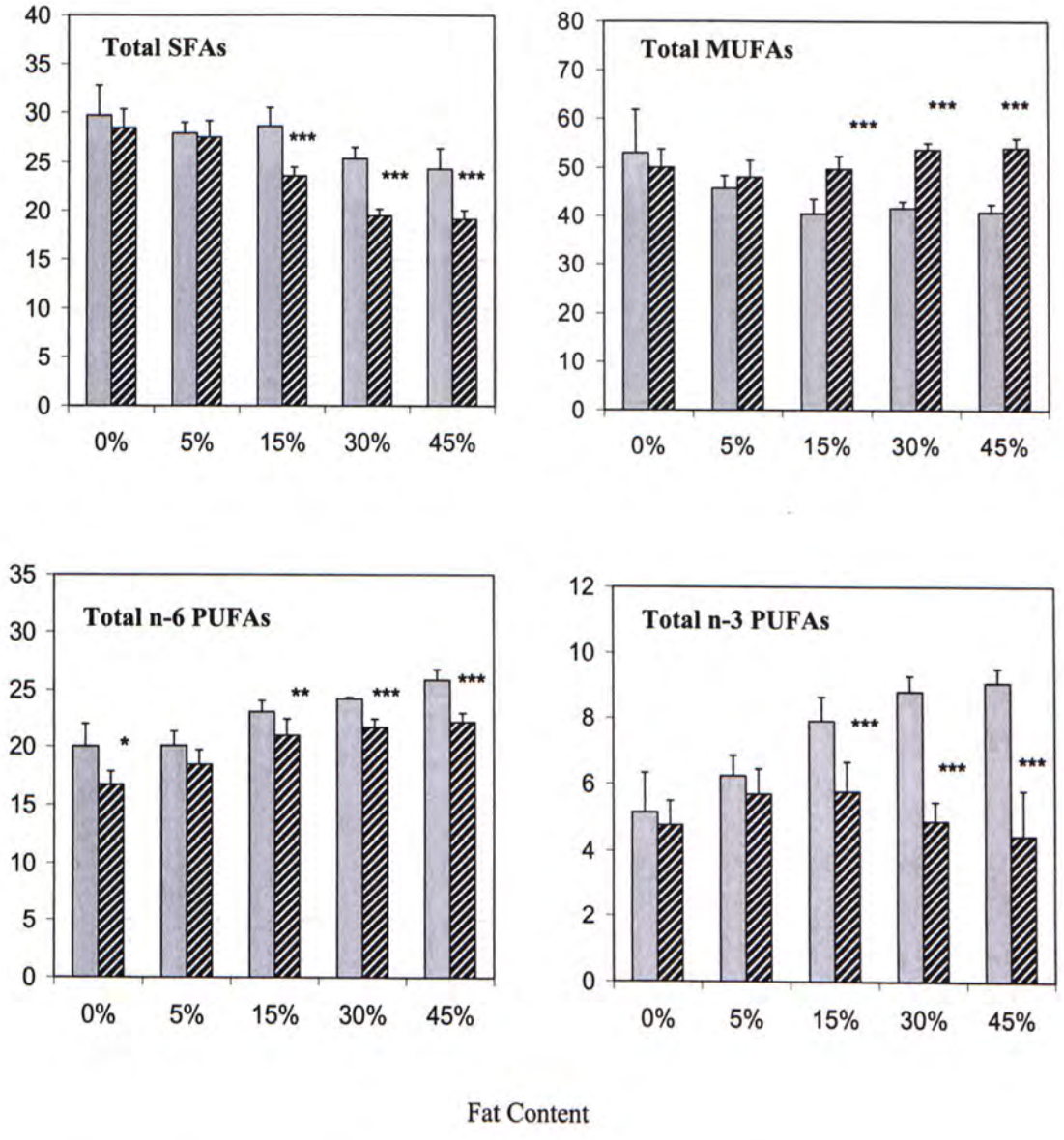


Fig 4.23: Percentage of different categories of fatty acids in carcass phospholipids of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; ***: $p<0.005$

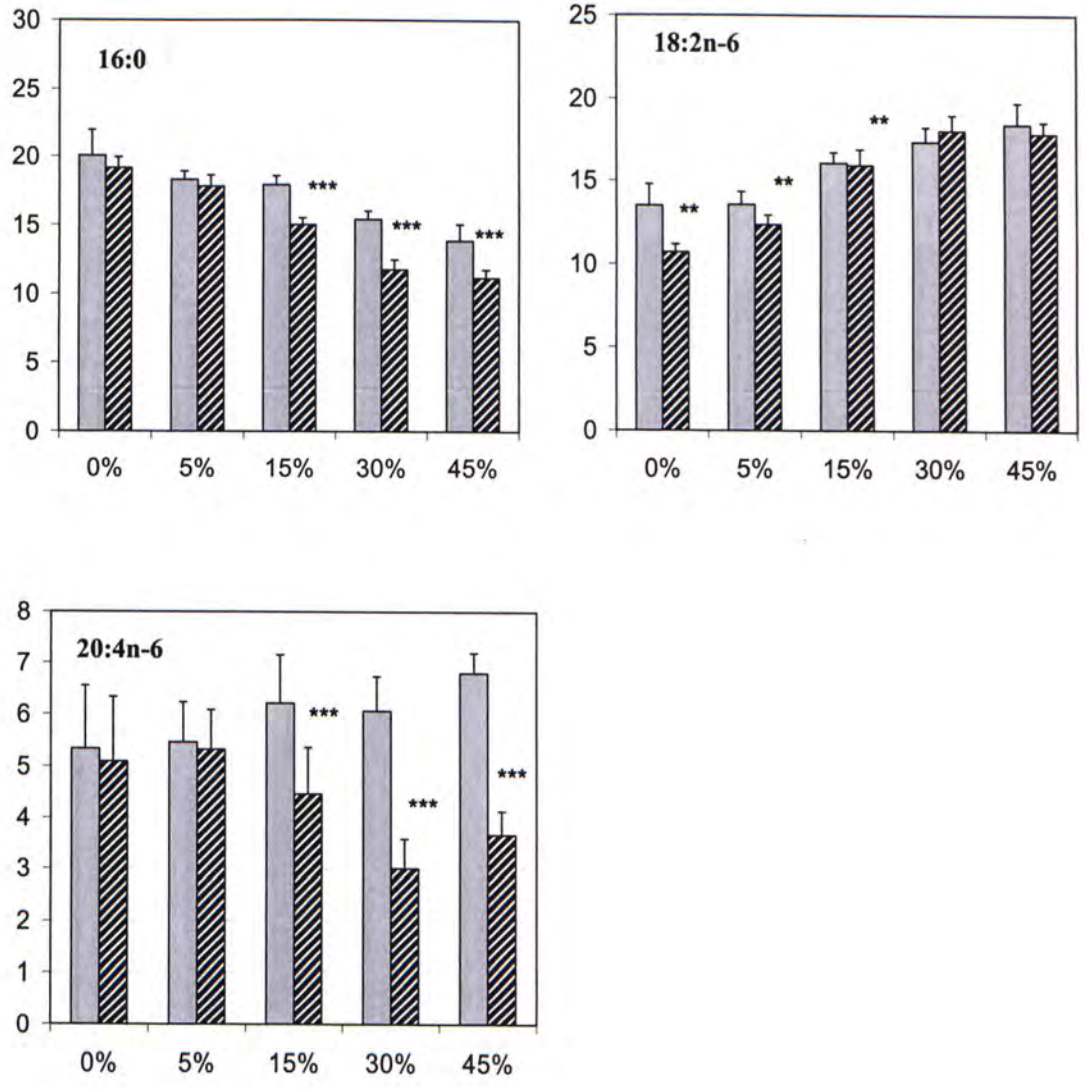


Fig 4.24: Percentage of selected SFA and n-6 PUFAs in carcass phospholipids of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); **: $p<0.01$; ***: $p<0.005$

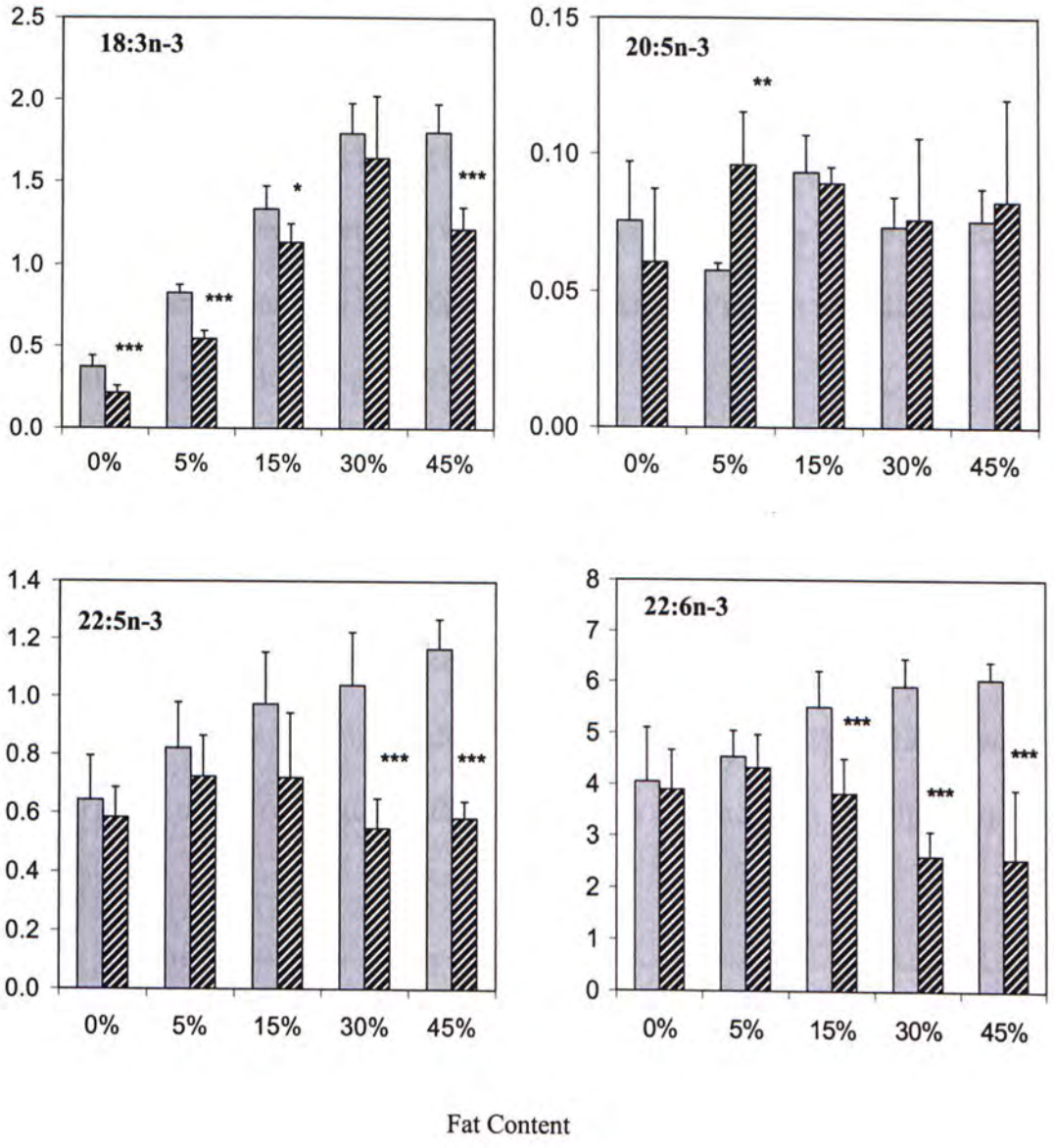


Fig 4.25: Percentage of selected n-3 PUFAs in carcass phospholipids of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$

4.4.5 PROXIMATE ANALYSIS

In this part of tests, crude fat, moisture, crude protein and ash were measured in carcass, i.e. whole body, excluding blood, liver, perirenal and epidydimal adipose tissue. Differences were compared within the same group of rats before and after the food restriction and they were determined by Student's t-test with the notation *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.

4.4.5.1 Crude Fat

Food restriction decreased the percentage of fat in the carcass, except in the group of 30% fat. They decreased from the range of 15.6-17.7% to 10.2-12.8%. However, an increase of body fat after food restriction, from 17.2% to 18.3% was noticed in the rats having a diet with 30% of energy derived from fat (Fig 4.26).

4.4.5.2 Moisture

The changes in moisture opposed that of the crude fat. The percentages increased from the range of 59.4-60.9% to 62.2-65.7%. An exception was observed in the rats having 30% fat in the diet during their food restriction. In this group of rats, the percentage of moisture decreased from 59.7% to 58.9%. Besides group 0%, the other groups showed significant changes (Fig 4.27).

4.4.5.3 Crude Protein and Ash

The percentages of crude protein fluctuated within a narrow range, with a general increase (Fig 4.28). They ranged from 20.2% to 21.5% before food restriction and 20.4% to 22% after food restriction.

Percentages of ash also fluctuated among different groups (Fig 4.29). No special trend or difference can be concluded. Before food restriction, the ash content was between 2.7% to 3.6% while after food restriction, it was 3.3% to 3.8%. No significant difference was observed within the same group before and after the caloric restriction.

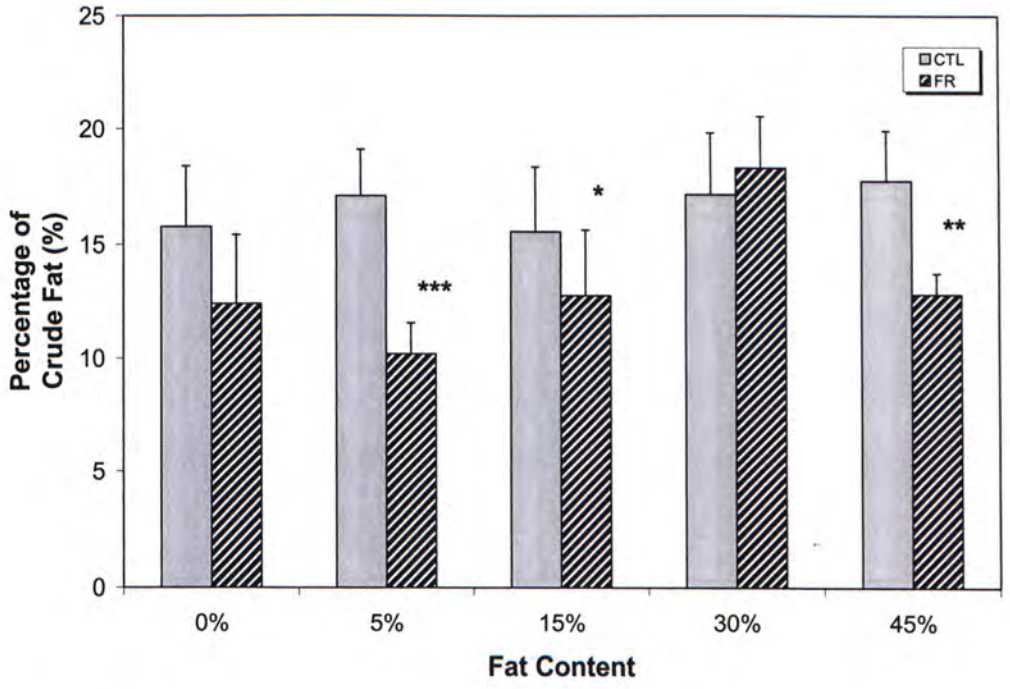


Fig 4.26: Percentage of crude fat of carcass of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$

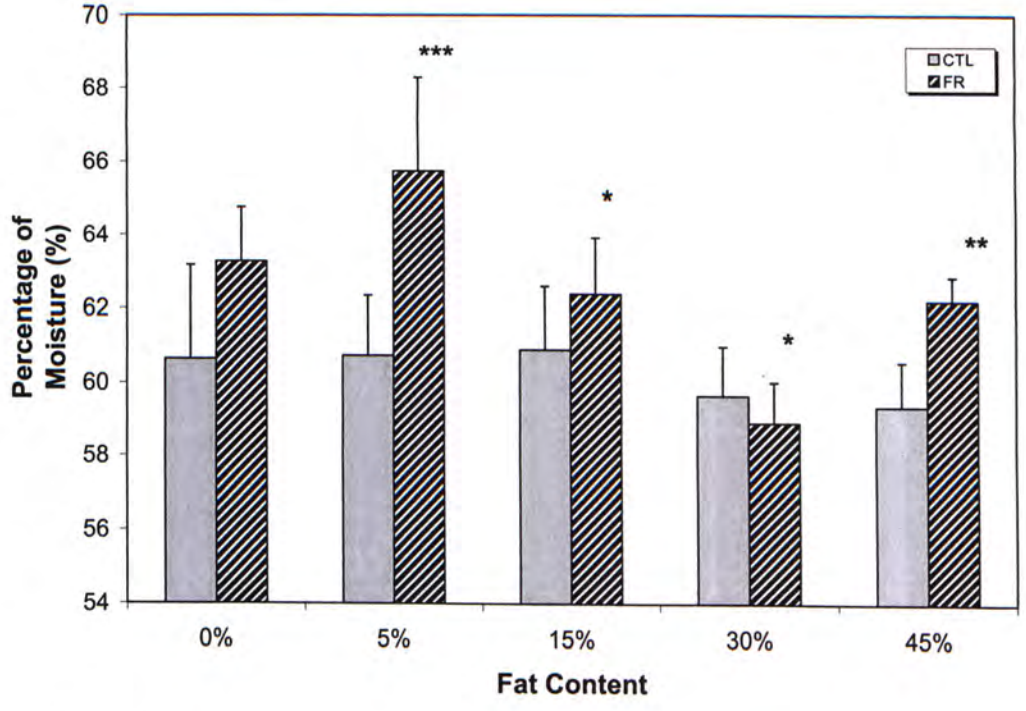


Fig 4.27: Percentage of moisture of carcass of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$

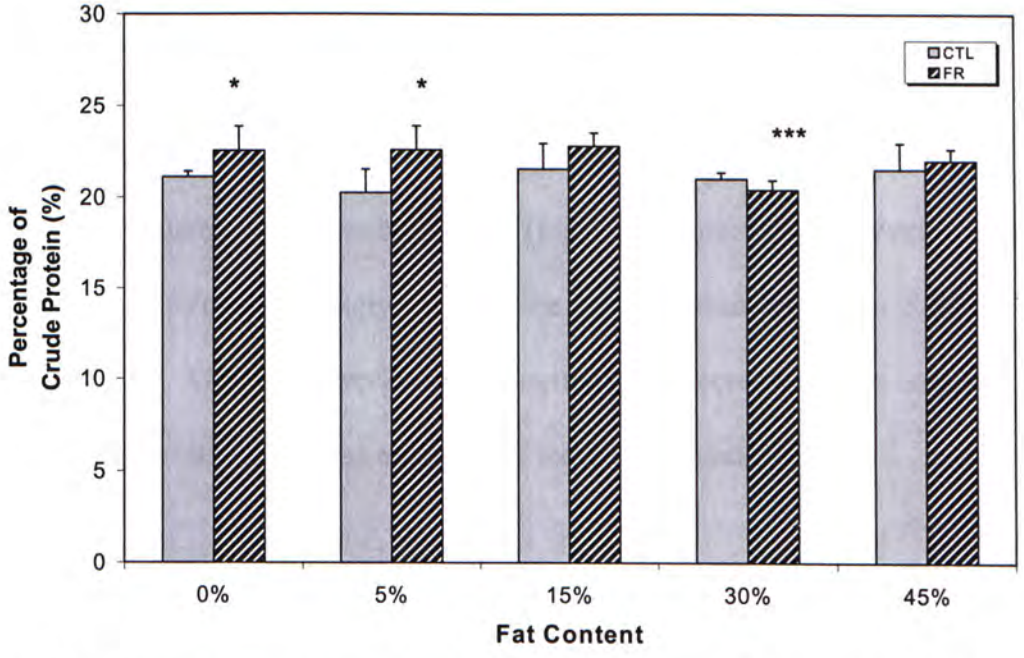


Fig 4.28: Percentage of crude protein of carcass of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; ***: $p<0.005$

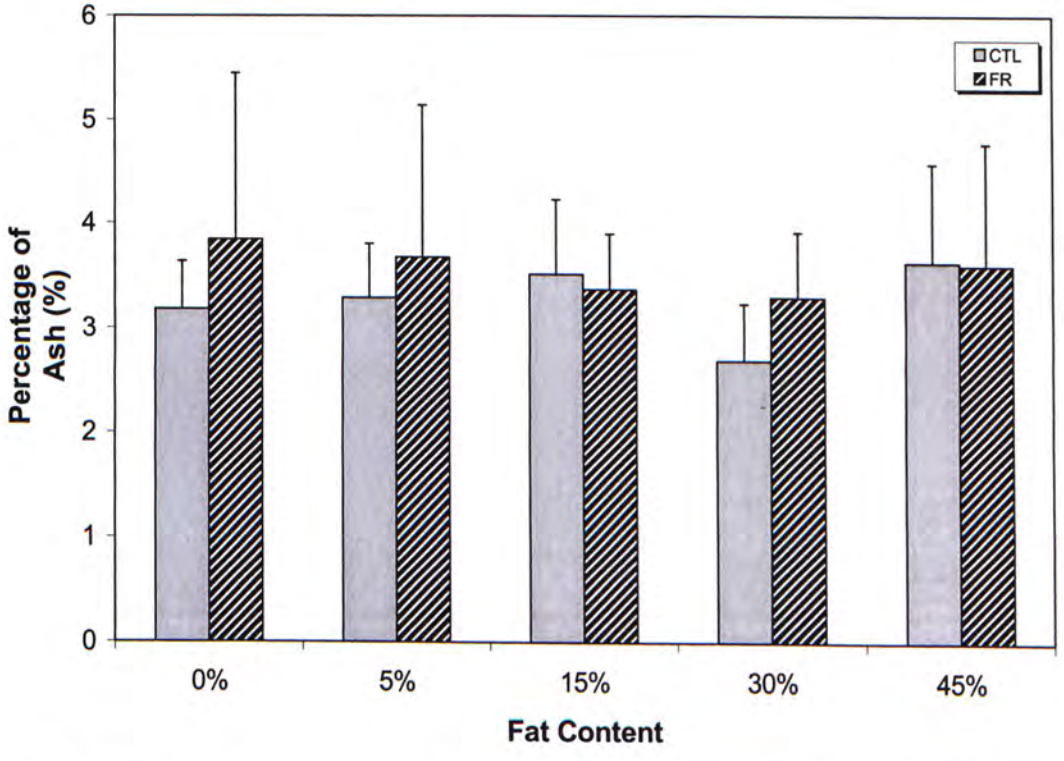


Fig 4.29: Percentage of ash of carcass of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR)

4.4.6 SERUM ANALYSIS

4.4.6.1 Serum Triglycerides

The serum triglycerides significantly decreased by 60% in the food restricted groups compared with those in the CTL (Fig 4.30). Before energy restriction, the concentrations of serum triglycerides in the rats were measured from 206.9 to 140.4mg/dL. After the 2-week food restriction, they decreased to the range of 59.4-49.4mg/dL, which was one-third of the control concentration.

4.4.6.2 Serum Cholesterol

The serum cholesterol also decreased in all the food restricted groups, but to a lesser extent compared with that observed for serum triglycerides (Fig 4.31). Before the food restriction, the concentrations (mg/dL) ranged from 99.1 to 117.4mg/dL. After the 2-week food restriction, they decreased to the range of 71.1-85.1mg/dL.

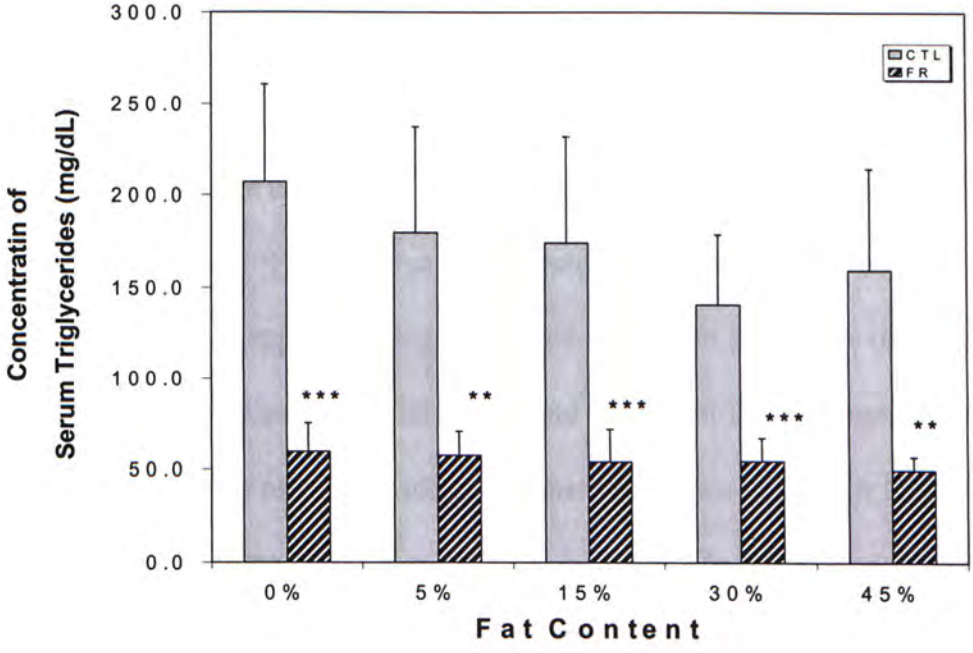


Fig 4.30: Concentration of serum triglycerides of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); ***: $p<0.005$

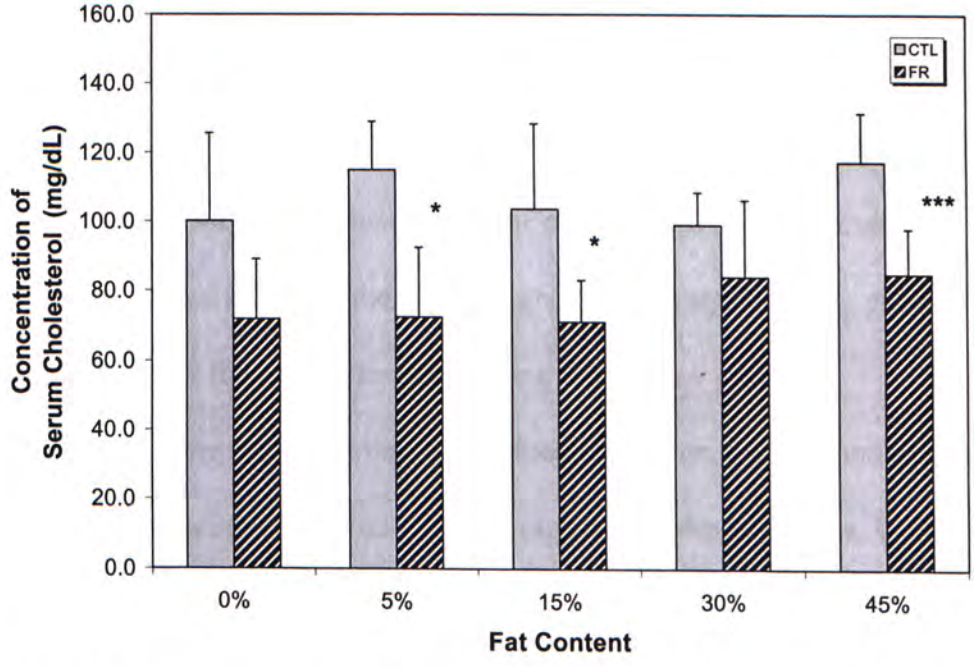


Fig 4.31: Concentration of serum cholesterol of rats undergoing food restriction with different amount of fat in diets. Grey Bars: Control (CTL); Striped: Food Restricted (FR); *: $p<0.05$; ***: $p<0.005$

4.5 *DISCUSSION*

In the present study, a series of diet with 0%, 5%, 15%, 30% and 45% of energy provided from fat were used. Weights of liver decreased significantly after food restriction. Obviously, it may be due to the depletion of energy reserve in the liver when the exogenous supply of energy is limited. Glycogen, as storage of energy in the liver, was mobilised and oxidised to yield energy for the daily metabolism.

Fat in the form of triglycerides is another but, in fact, the major form of energy reserve inside the body, as gram-by-gram, fat yields energy as double as the carbohydrates do. A similar difference should be expected in the decrease in weight of the adipose tissues as it did to the liver weight. However, regarding the weight of adipose tissues to the body weight, there were little changes observed in perirenal ones and this change did not show any correlation with the amount of fat provided. In the case of the epidydimal adipose tissues, there were no noticeable changes within the same group before and after the food restriction. Although not significant, there was a slight increase in the ratio of both of the perirenal and the epidydimal adipose tissues to the body weight in the group accessing diet with 30% fat after 50% food restriction comparing to the other groups.

This finding suggested that during food restriction, a consumption of more fat might promote lipogenesis and lipid storage in the adipose tissues. In all these groups, the oxidation of the liver energy storage became the major energy release when the energy supply was scarce. A portion of energy supplied exogenously was preferred to be stored for future use rather than immediate use.

In the present study, the changes in SFAs, MUFAs n-6 and n-3 PUFAs in both of the adipose tissues and the carcass fluctuated in a similar manner among the groups receiving different amount of fat in their diet. The rats having a fat-free diet showed a strong preference in reserving SFAs. It can be explained by the high stability, the low mobility and rate of oxidation of SFAs during energy-limited period. Energy supplied by the dietary carbohydrates was transformed into SFAs and MUFAs.

The percentage of MUFAs and n-6 PUFAs increased together with the increase of fat supplied to the rats, showing a consequent slight elevation of fatty acids after food restriction. Comparing with the other groups, the depletion of the percentage of n-3 class of PUFAs was much more remarkable.

The depletion of the n-3 PUFAs can be explained by the studies done by Raclot and Groscolas (1993; 1995). In both of their *in vitro* and *in vivo* studies, energy depletion results in a higher mobilisation of fatty acids with shorter chain length a given unsaturation, with higher unsaturation for a given chain length, and the double-bonds nearer to the methyl end of the chain (Raclot and Groscolas, 1993; 1995). This difference in the mobilisation leads to a more convenient usage of the n-3 PUFAs as an energy source during caloric restriction. Evidence showed a higher potential to oxidise unsaturated fatty acids in rat liver mitochondria than the saturated ones (Reid and Husbands, 1985). For details, please refer to section 2.5.2.

In a human study using non-restricted low-fat diet, with 10% of energy supplied as fat, a specific enrichment in 16:0 in the VLDL triglyceride and

deficiency in 18:2n-6 are found in all subjects after 10 days of treatment. More than 40% of the VLDL fatty acids are newly synthesised under such condition, causing accumulation of triglyceride 16:0 and "dilution" of 18:2n-6 (Hudgins *et al*, 1996).

Low-fat diet consumption without food restraint is associated with noticeably greater total n-3 PUFAs, 20:5n-3 and 22:6n-3 levels in plasma phospholipids and cholesterol esters, resembling the effects of feeding n-3 long-chain fatty acids, while high-fat diets elevate the total PUFAs and 18:2n-6. This change is possibly related to decreased competition for the enzymes of elongation and desaturation. Reduction of total intake of 18:2n-6 favours elongation and desaturation of available n-3 fatty acids (Raatz *et al*, 2001).

The PUFAs, especially on the n-3 PUFAs and 18:3n-3, are preferably mobilised for oxidation in the energy restricted period. Higher rate for oxidation in the mitochondria further consumes the mobilised n-3 PUFAs. As a result, in the triglyceride storage in the adipose tissue and carcass, there were are observed with an increasing percentage of SFAs and MUFAs while the reserves are selectively depleting in the n-3 PUFAs.

Furthermore, similar trends of increasing n-3 PUFAs were observed in the adipose tissues and carcass from the groups of rats receiving 0%, 5%, 15%, 30% to 45% of energy derived from fat. The n-3 PUFAs decreased after 2-week food restriction in all the groups. This decrease was noticed to be more distinct in the groups receiving more fat in their diet (Fig 4.9, 4.12, 4.15, 4.18, 4.20 and 4.23). Rats receiving fat-free food had a large depletion of n-3 PUFAs during the *ad*

libitum stabilisation period as the dietary supply of the fatty acids was scarce. A 50% food restriction decreased the n-3 reserve in the whole body. This decrease was less significant when the fat supply was less. It suggested that the body tends to have a lower mobilisation and utilisation of n-3 fatty acids or to reserve the n-3 fatty acids when dietary fat was greatly limited. This drop in the n-3 reserve after food restriction became very significant when there was 30% or more fat in a diet. It gave an idea that when there was a sufficient amount of dietary fat in the diet, the body tends to mobilise n-3 PUFAs for energy generation. This feature was also observed in 18:3n-3 in the whole body, 20:5n-3 in the perirenal fat pad, 22:5n-3 and n-6 PUFAs in carcass phospholipids, 22:6n-3 and 20:4n-6 in the carcass free fatty acids and phospholipids as well as18:2n-6 in the carcass total lipid and free fatty acids (Fig 4.11, 4.14, 4.16, 4.17, 4.20, 4.22 and 4.25). These may illustrate that discriminated mobilisation selectively occurs in n-6 and n-3 PUFAs after food restriction, leading to a higher cardiovascular risk.

On the contrary, this difference was the least in the rats undergoing 50% food restriction with a diet containing 5% of fat as energy. In the perirenal and the epidydimal adipose tissues and carcass triglycerides, the differences of the n-3 PUFAs and 18:3n-3 before and after food restraint were not significant in the 5% fat group, while the others showed significant changes (Fig 4.11, 4.14 and 4.20). Similar observation was obtained in n-6 PUFAs and 18:2n-6 in carcass (Fig 4.15 and 4.16) while an increase in carcass triglycerides (Fig 4.18 and 4.19) despite the other groups having decreases after food restriction. This observation may suggest that a 50% food restriction with a diet containing 5% of fat as energy does not

trigger a significant change in the fatty acid metabolism, especially the depletion of the PUFAs. Hence if these findings in rat model can be applied to human, dieting with this condition may be less harmful regarding the cardiovascular risk brought about by the imbalance of body PUFAs. Dieting with higher fat meals may likely expose the dieters to a higher risk to CHD and related diseases due to the depletion of PUFAs.

The serum triglycerides and total cholesterol were measured without giving rats any fasting treatment. As food supply was limited, the triglyceride level dropped drastically in all of the groups comparing with those having the same diet but with free access or under food restriction. They dropped to a level below half of their free-eating counterparts.

Serum cholesterol also decreased, although less drastically, amongst the groups receiving different amount of fat. Borne and co-workers (1996) provided evidence on the decrease in the serum cholesterol level and the mean arterial blood pressure in dogs receiving a high-fibre, low-fat diet (Borne *et al*, 1996). Food restriction can cause a drop in the serum cholesterol level even though the food is cholesterol-free by using canola oil as the fat source.

The fat in the carcass generally decreased when there was a caloric restriction. In rats without any food restriction, there was a slight increase of body fat content amongst the groups receiving different amount of fat in their diet as energy source. However, the group receiving 30% fat in their diet had the highest percentage of body fat, even higher than the corresponding control. Similar to the phenomenon seen in the weights of the adipose tissues, the proportion of fat in the

carcass of the 30% fat group rats was the highest. This suggests an overall fattening of the body in this group. Comparing the differences between the fat content of rats in 5%, 15% and 45% fat groups, it agreed with the previous finding by Portillo and co-workers (1999) that 25% food-restricted obese rats receiving 10% and 50% calories as fat do not have any significant difference in their body composition and lean body mass (Portillo *et al*, 1999).

Chapter Five

FUTURE PROSPECTS

The fatty acid compositions, the serum triglycerides and cholesterol measurement and the proximate analysis in present study showed that there is a vigorous change in the body condition during the course of dieting, despite the modes taken. However, it is important to note that they are the consequences of many biochemical changes inside the body. Therefore, to have a clearer picture of how the processes going on deep inside, more tests can be done in the midways at hormonal, enzymatic and molecular levels.

5.1 LEPTIN

Leptin is a hormone. It is cloned and identified as the *ob* gene product (Zhang *et al*, 1994). The hormone is absent in obese mice with *ob/ob* (Boston *et al*, 1997).

In the white adipose tissues of obese human subjects, there is always an overexpression of leptin in the blood stream (Lönnqvist *et al*, 1995; Hamilton, *et al*, 1995). This serum concentration of leptin showed a positive correlation with the body mass index both in human and rodents (Maffei *et al*, 1995).

Leptin is mapped to mouse Chromosome 6 and the human homologue on the position of chromosome 7q31. It is a secretory protein with 167-amino acid open reading frame and a 21-amino acid signal sequence, encoded by a 4.5kb mRNA (Zhang *et al*, 1994).

The leptin receptor belongs to class I cytokine receptor (Tartaglia *et al*, 1995). There are at least 5 different isoforms of leptin receptors, depending on the alternative splicing on a single leptin receptor gene (Lee *et al*, 1996). They are the long receptor, OB-Rl, short receptors, OB-Rs and a soluble form OB-Re.

Leptin acts as an 'adipostat' to sense and regulate the body energy source and the homeorhetic regulator of energy homeostasis (Houseknecht and Portocarrero, 1998). This hormone regulates the appetite and the energy expenditure favouring the usage of energy stored in the body. Leptin applied exogenously reduces food intake in both obese and normal mice (Halaas *et al*, 1995). It decreases body weight and improves metabolic control. It acts on both the hypothalamus and various peripheral tissues like lung, kidney, liver, adipose tissue and pancreatic ß-cells (Lollmann *et al*, 1997; Ghilardi *et al*, 1996).

The long form of leptin receptor is mostly found in the hypothalamus, where the key regulatory centre for appetite control is located ventral-medially, but it is expressed in a much lower level in the periphery (Tartaglia *et al*, 1995;

Ghilardi *et al*, 1996). The short isoforms are found in almost all the peripheral tissues and exerts a rather direct effect *in vitro* (Muoio *et al*, 1997).

The actions of leptin on the periphery includes inhibition of insulin secretion (Emilsson *et al*, 1997), timing of puberty (Foster and Nagatani, 1999), maintenance of the normal growth hormone and thyroid stimulating hormone secretion (Clement *et al*, 1998), as well as regulation of growth in lungs, bone mass and intestinal cells (Morton *et al*, 1998). Studies also show a correlation of the high leptin level in obese people and the promotion of human platelet aggregation. In the hypothalamus, the situation is complicated. First the hormone has to be transported through the blood-brain barrier via a saturable transport system (Golden *et al*, 1997). The mediator neuropeptide Y (NPY), a potent stimulator of food intake, in the arcuate nucleus is then inhibited by leptin (Baskin *et al*, 1999). Therefore the appetite is reduced, thermogenesis is stimulated and the plasma insulin and the glucocorticoid concentration are reduced.

Leptin can also inhibit intracellular lipid concentrations by reducing fatty acid and triglyceride synthesis and then increase lipid oxidation (Shimabukuro *et al*, 1997). The activity of acetyl-CoA carboxylase (ACC), the rate-determining enzyme in fatty acid synthesis, can be inhibited by leptin. The consequential mitochondria fatty acid uptake and oxidation result in lower intracellular fatty acid and triglyceride concentrations (Bai *et al*, 1996).

On the other hand, leptin increases glucose utilisation of brown adipose tissue, lipolysis in white adipose tissue and expression of malic enzyme (ME) and lipoprotein lipase (LPL) in brown adipocytes in culture (Fruhbeck *et al*, 1997).

Although the leptin levels regulate food intake and energy expenditure and are positively correlated to the adipose tissue mass and fat-cell size in fully fed animals and humans, obese people may exhibit insensitivity to the hormone (Houseknecht and Portocarrero 1998). These patients usually have high serum leptin level but normal to low leptin level in the cerebro-spinal fluid (Mantzoros *et al*, 1997). The resistance may suggest the presence of receptor mutation followed by absence or inappropriate signalling or the defect in transporting leptin across the blood-brain barrier.

It is clear that there are close relations between leptin and the food intake, the usage of energy and the synthesis of fatty acids and triglyceride in adipose tissues. Monitoring the serum leptin levels may be useful in gaining a better concept of the long-term effect of satiety and food supply to the body in various tissues on the hormonal level. However, there are no studies to date that have examined the effects of weight cycling and levels of food restrictions on expression of leptin and its receptors.

5.2 ENZYMES

Fatty acids are the components of triglycerides, phospholipids and glycolipids, which act mainly as the energy source for the body and the building block of cell membranes and some other functions.

Their turnover depends on their synthesis and usage upon oxidation. These metabolisms are all enzyme-dependent processes. The activities of the enzymes

involved will determine the resulting storage of fatty acids in different parts of the body, mainly in adipose tissues.

Saturated fatty acids are synthesised in cytosol. Acetyl-CoA and CO_2 form a malonyl-ACP molecule with the help of ACC. It further condenses with an acetyl bound to the Cys-SH, yielding acetoacetyl-ACP and releasing CO_2. With NADPH as an electron donor, it is then reduced to a D-ß-hydroxy derivative, dehydrated to the *trans*-Δ^2-unsaturated acyl-ACP and reduced again to butyryl-ACP. Palmitoyl-ACP is yielded by the elongation of 6 more malonyl-ACP adding to the carboxyl end of the fatty acid chain, catalysed by fatty acid synthase (FAS) which consist of an acyl carrier protein (ACP) and a 6-enzyme complex. This fatty acid synthase contains –SH groups to act as carriers of the fatty acid intermediates. Upon hydrolysis, free palmitic acid (16:0) is released.

The acetyl-CoA required in this fatty acid biosynthesis is formed from pyruvate, a product of glycolysis from glucose in mitochondria. Pyruvate is transported into cytosol through the pyruvate/malate cycle. Pyruvate kinase (PK) is a key enzyme for the production of pyruvate from phosphoenolpyruvate. The pyruvate/malate cycle generates NADPH needed for fatty acid synthesis, by the oxidative decarboxylation of malate with ME.

Palmitic acid can be further elongated to stearic acid (18:0). These two fatty acids can be desaturated by the action of mixed-function oxidases to POA (16:1n-9) and OA (18:1n-9). LA(18:2n-6) cannot be synthesised in human, therefore, it must be obtained from diets. Other n-6 PUFAs can be converted from the exogenous LA source.

Triglycerides are formed by two fatty acyl –CoA with glycerol-3-phosphate to form phosphatidate, followed by dephosphorylation to diacylglycerol. A third fatty acyl-CoA is acylated to the diacylglycerol to form triglycerides. Endogenous and exogenous triglycerides are carried in the blood stream by chylomicrons and VLDL. However, whatever is the source, LPL is needed for the hydrolysis before the transportation of triglycerides into the adipose tissues.

Utilisation of fatty acids takes place in the mitochondrial matrix in a process known as ß-oxidation. A cascade of enzymatic reactions, including dehydrogenation, hydration, a second dehydrogenation and a further cleavage, remove a single acetyl-CoA unit in each cycle. The acetyl-CoA molecules are then oxidised into CO_2 via citric acid cycle. Energy is yielded by the production of NADH and $FADH_2$ through the removal and subsequent oxidation of an acetyl-CoA. ATPs can then be produced by the re-oxidation through oxidative phosphorylation, yielding energy need for body activities.

Studies showed that LPL activity is directly correlated with the uptake of fatty acids from the serum triglycerides into adipocytes (Garfinkel *et al*, 1967). The activity of LPL is also found to vary with the nutritional and physiological states of fasting, diabetes and genetic factor, etc (Ong and Kern, 1989).

Previous studies illustrate LPL, FAS, ME and ACC were suppressed during energy restriction but induced during refeeding (Sea *et al*, 2000). Since many steps determine the rate of synthesis and the storage of fatty acids inside the body, a study on the change in activities of these enzymes may shed light on the

changes in the proportion of different fatty acids in storage and the enlargement of adipose tissues under various styles of food restriction, and whether there is any qualitative change associated with the change in levels of restriction and/or fat content.

Furthermore, the activities of the enzymes depend very much on the amount of enzyme present as well as the activity and the affinity of the enzymes to the substrates. Therefore, the corresponding mRNA levels can give an idea of the potency of each enzyme under different stress conditions induced by different modes of energy restriction.

Chapter Six

CONCLUSION

Overweight and obesity have been the topic of numerous studies for many decades. They are not the immediate causes of diseases, but they are found to be positively related to many fatal health disturbances (National Task Force on the Prevention and Treatment of Obesity, 2000; Bergström *et al*, 2001). The mechanism lying underneath on how obesity is related to the diseases is still not yet fully understood. Although it is still controversial on the correlation and some negative findings are reported at times, experts generally agree that losing weight is critical in preventing or improving the conditions (WHO, 1998).

In the present study, we have used 3 different styles of dieting that humans usually practise. The results demonstrated the modification of the fatty acid profiles in the perirenal and the epidydimal adipose tissues and the carcass of

normal, not genetically obese rats, resembling the majority of the people carrying out weight loss programme by cutting down the calorie intake.

On the fatty acid profile basis, a depletion of n-3 fatty acids, especially in 18:3n-3 and 22:6n-3, and less pronounced depletion of n-6 fatty acids, 18:2n-6 and 20:4n-6, were observed in fasting state and after refeeding during weight cycling. This depletion varies with the severity of food restriction, fat intake restraint, and the cycles of yo-yo dieting. Similar but opposite variation of SFAs was observed. In the serum, the triglycerides and cholesterol levels decreased after food restriction but not after refeeding. This decrease varied with the level of food restriction but not the fat restraint. On the body composition basis, fat content varied obviously with the restricted calorie intake, but food restriction caused no further compositional changes.

SFA proportion increases specifically during energy restriction due to the elevation of lipogenesis and storage in the adipose tissues during the refeeding. PUFA mobilisation for energy release increases with the limited calorie supply. It resulted in the disruption in the normal fatty acid profile in rats and exposure to health risks related to the functions of the PUFAs, especially the n-3 fatty acids.

Specifically speaking, consuming around 70% of a usual medium-fat food intake can help moderate the fatty acid profile remodellation and effectively decrease the serum triglyceride level. Moreover, adopting a 50% caloric restriction with a diet containing 5% of energy derived from fat can relieve the PUFA depletion caused by dieting with higher fat intake. Both of the conditions

were believed to be beneficial in reducing the cardiovascular risks and prevention of a range of cancers.

If this animal study represents the real situation happens inside a human body undergoing similar dieting, it suggests an underlying health risk in those individuals attempting to lose weight. In fact, the dieting population only sees the changes in appearance. They try everything they can in order to achieve their goal without seeking professional advice or realising the problems inside.

Nevertheless, weight loss is almost compulsory for an obese individual so as to reduce the risks of CHD, atherosclerosis, hypertension, and a range of cancers, caused by overweight. Care must be taken during a weight loss programme by health and medical practitioners, monitoring the up-coming but similar risks due to dieting.

Beliefs and proposals of alternative 'undieting' or 'non-dieting' approaches of weight control have gathered attentions since the last decade. Most of them deals with the will power of the participants, such as increasing the awareness of the ill effects of dieting, correcting the belief of 'thinner is better', accepting ones appearance and, enhancing ones self-esteem by inner beauty (Polivy and Herman, 1992; Ciliska, 1998; Goodrick *et al*, 1998). If dieting is chosen for the weight loss, a carefully designed programme must be followed. In this regard, severe energy restriction is not appropriate because it remarkably reduces whole body n-3 PUFA reserve based on the present results. It is wise to choose a dieting programme with moderate energy restriction because less effect on the body fatty acid composition is expected. Dieters are encouraged to

abandon dieting with very strict caloric limitation and total avoidance of fat intake. In addition, exercise is necessary for the promotion of energy output, a healthier body and a better figure.

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