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EFFECT OF DIETARY FATTY ACIDS ON BODY ENERGY PARTITIONING THROUGH THE REGULATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS.

DISSERTATION

Presented in Partial Fulfillment of the Requirement for
The Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

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The Ohio State University
2002

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ABSTRACT

Purportedly, dietary fat level and type influence body energy homeostasis. To examine if changes in body weight and body composition were associated with alterations in peroxisome proliferator-activated receptor (PPAR) gene expression, forty-four 3-week-old weanling male Sprague-Dawley rats were fed ad libitum for 5 weeks with either a low fat (15% energy) or high fat (45% energy) diet providing four types of fats, i.e. coconut oil (MCT-SFA), safflower-HOSO oil (MUFA), safflower-HP oil (n-6 PUFA), or flaxseed oil (n-3 PUFA). Our results showed that feeding rats with a high fat diet reduced feed intake regardless of the types of fats. Only in the SFA group did feeding with a high fat diet result in increased weight gain, percent epididymal fat pad, serum and liver TG levels, and liver total lipid concentrations when compared to feeding with low fat (p<0.05). This was accompanied with higher gene expression of epididymal adipose tissue PPARγ and lower expression of liver PPARα (p<0.05). The expression of a downstream gene of PPARγ in adipose tissue, lipoprotein lipase (LPL), was also increased, confirming the enhanced expression of PPARγ. However, expression of liver acyl-CoA oxidase (ACO) was increased on the high fat diet regardless of types of dietary fats. Rats fed high fat SFA, high fat MUFA, and low fat n-3 PUFA partitioned more food energy into body fat than their corresponding fatty acid groups (p<0.05). The consumption of n-3 PUFA suppressed serum cholesterol, liver total lipid and TG concentrations. The fatty acid composition of the liver TG and total lipid reflected dietary lipid manipulation. No difference in serum leptin levels was observed.

This study suggested that during early development when positive energy balance usually occurs, it was the caloric content rather than the amount of food that was under control. Although long-term intake of low MCT diet had the potential for dietary prevention of obesity by suppressing total energy intake, consumption of this readily metabolized fat at a high level may not protect rapidly growing rats against
excess fat accumulation, which may be attributed to an increased adipose tissue LPL gene expression through the activation of PPARγ that enhanced circulating TG removal and hence promoted adipose tissue fat deposition.
DEDICATION

Dedicated with love to my parents.
ACKNOWLEDGMENTS

This dissertation could not be completed without the help of numerous people. First and foremost, I would like to thank Dr. Snook, my advisor. As not every graduate student has an advisor who refines and edits the rough drafts as patiently as Dr. Snook, I would be remiss not to thank her.

The encouragement of Dr. Bray and Dr. Huang of the suicidal idea of this project must be acknowledged. Their tremendous efforts in steering me in the right direction, and in helping me develop the idea out of their busy schedule was outstanding, and will never be forgotten.

I am deeply appreciative of the mental support from friends in Taiwan and in the United States. Their friendship provided me the strength in running this Ph.D. marathon. In order not to miss any one of you, “your name is here.”

Finally, I am grateful to my family. Their unwavering love, encouragement, and patience were no doubt an essential part for the completion of my dissertation.
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PUBLICATIONS

Abstracts


FIELD OF STUDY

Major Field: OSUN Nutrition Program
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<td>AA</td>
<td>arachidonic acid</td>
</tr>
<tr>
<td>ACO</td>
<td>acyl-CoA oxidase</td>
</tr>
<tr>
<td>ADD-1</td>
<td>adipocyte determination and differentiation factor-1</td>
</tr>
<tr>
<td>ALA</td>
<td>α-linolenic acid</td>
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<tr>
<td>BAT</td>
<td>brown adipose tissue</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>CT</td>
<td>resonance tomography</td>
</tr>
<tr>
<td>DHA</td>
<td>docosahexanoic acid</td>
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<tr>
<td>EN</td>
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<td>eicosapentanoic acid</td>
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<td>free fatty acid</td>
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<td>glucose transporter</td>
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<td>hepatocyte nuclear factor-4</td>
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<td>linoleic acid</td>
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<td>long chain fatty acids</td>
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<td>low fat</td>
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<td>LPL</td>
<td>lipoprotein lipase</td>
</tr>
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<td>MCFA</td>
<td>medium chain fatty acids</td>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
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<tr>
<td>MUFA</td>
<td>monounsaturated fatty acid</td>
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<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
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<td>NHLBI</td>
<td>National Heart, Lung, and Blood Institute</td>
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<tr>
<td>NPY</td>
<td>neuropeptide Y</td>
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<tr>
<td>PP</td>
<td>peroxisome proliferator</td>
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<td>peroxisome proliferator-activated receptor</td>
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<td>PPRE</td>
<td>peroxisome proliferator response element</td>
</tr>
<tr>
<td>PUFA</td>
<td>polyunsaturated fatty acid</td>
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<tr>
<td>RT-PCR</td>
<td>reverse transcriptase polymerase chain reaction</td>
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<td>SFA</td>
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<td>SREBP</td>
<td>sterol regulatory element binding protein</td>
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<td>TEF</td>
<td>thermic effect of food</td>
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<td>tumor necrosis factor</td>
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<tr>
<td>UCP</td>
<td>uncoupling protein</td>
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<td>WHO</td>
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<td>week</td>
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CHAPTER I

INTRODUCTION

1.1 Background

Obesity is one of the most important nutritional disorders in industrialized countries. It is defined as an excessive increase in body fat, but not necessarily in body weight. Obese individuals often exhibit a higher risk for chronic diseases, such as hypertension, type II diabetes, and coronary heart disease.

Several studies have related adult obesity to childhood obesity, based on the findings that childhood obesity tends to persist into adulthood. Even though obesity in adulthood is often but not always preceded by childhood obesity, given the difficulty in the treatment of adult obesity, the increasing incidence of overweight in children, and the association between childhood onset obesity and adult morbidity and mortality rate, targeted strategies in decreasing the prevalence of obesity have been directed at preventing obesity at an early age.

In theory, obesity treatment may be more effective in children than in adults because children exhibit marked secular increase in both weight and height and thus may have the opportunity of returning to normal body size if weight is under control. Also, food preferences are learned at an early age; thus eating behavior may be easier to modify in children than in adults. Moreover, while adipose tissue cell size may vary with age; fat cell number is usually determined prior to and during puberty, even though an increased cell size can also occur in adulthood when adipocytes reach a critical size. Once new adipocytes are formed, they do not decline, and only a decrease in size is possible. As a consequence, avoiding an increase in adipose cell number (hyperplasia) earlier may have the potential of preventing and treating obesity later in life.
Obesity is multifactorial and the etiology for the high prevalence of obesity, especially for early-onset obesity, has not been fully elucidated. Possible contributing factors are listed in Table 1.1. Recent studies suggested that obesity occurs as a result of interactions among these factors, rather than any factor alone. On the nutritional basis, it is generally believed that obesity is associated with excessive intake of calories relative to energy expenditure, and high levels of fat intake. However, in contrast to adults, treating childhood obesity by energy or fat restriction needs extreme caution. Childhood represents a critical period in which energy is used not only for weight maintenance but also for growth. Treating childhood obesity by restricting energy intake should be done without impeding normal growth. In children, dietary fats provide essential fatty acids, energy, and enhance the absorption of certain nutrients, such as vitamins A, D, E and K. They are used for the synthesis of hormones, membrane lipids, and biological mediators, and are important for neurological development. Detrimental health problems can result if insufficient fat is consumed.

Literature on the role of dietary fat in obesity remains controversial because of inconsistent results regarding the efficacy of long-term ad libitum low-fat diets in reducing body weight. Since fat is energy dense, and is stored more efficiently than carbohydrate and protein as body fat, a lower fat intake is always recommended in the nutritional treatment of obesity.

Recent emphasis on the influence of dietary fats on obesity has been placed not only on the level but also the type of dietary fats, as well as the molecular mechanisms regulated by fats. However, to date, less effort has been made in examining how these effects influence early stages of adipocyte development.

Studies of the effects of fat type showed that in humans and animals, saturated fats tend to induce a higher rate of fat accumulation than polyunsaturated fats, while the effect of monounsaturated fats is less described. Although the precise mechanisms are not completely understood, several underlying events have been proposed, as summarized in Table 1.2.

Recently, there is evidence showing that dietary fats can also work as mediators of gene expression, and several genes involved in the control of body fat mass have been identified in both humans and animals. Among these are leptin and peroxisome proliferator-activated receptor (PPAR).
Leptin, the obese gene product, is an adipocyte-specific hormone. Its release is proportional to the mass of adipose tissue. It acts as a feedback signal to the hypothalamus, which inhibits food intake and stimulates energy expenditure. Obese subjects usually express hyperleptinemia, suggesting the development of leptin resistance.

PPARs are new members of the steroid/thyroid hormone nuclear receptor superfamily. So far, three isoforms of PPARs, α, β (δ), and γ, have been identified. The expression of PPAR occurs in a tissue-specific manner. PPARα is predominantly expressed in the liver and muscle, tissues with high levels of lipid metabolism. PPARβ is ubiquitously expressed and its function is less well identified. PPARγ is highly expressed in both white and brown adipose tissues, and is important for adipogenesis.

Many proteins involved in lipid metabolism or energy partitioning contain the peroxisome proliferator response element (PPRE) in the promoter region of their genes. For example, acyl-CoA oxidase (ACO), the rate-limiting enzyme in the liver peroxisomal fatty acid β-oxidation, is regulated by PPARα. Lipoprotein lipase (LPL), an important regulator in triacylglycerol metabolism, has been shown to be activated by PPARα in the liver and PPARγ in adipocytes. In addition, the expression of adipocyte leptin has been reported to be under PPARγ regulation.

An array of structurally diverse compounds has been identified as activators for PPAR. These include lipid-lowering drugs, lipid analogs, and naturally occurring lipids. Whether dietary fatty acids act as direct ligands for PPAR in vivo to regulate their own metabolism is not fully investigated. So far, only linoleic acid, linolenic acid and their metabolites have been shown to be specific ligands for PPAR.

Since various dietary fatty acids can differentially modulate body fat metabolism by manipulating the expression of genes involved in lipid metabolism and since many of the genes have been shown to be regulated via the PPAR dependent pathway, the aim of this study is to examine if dietary fatty acid can determine its own metabolic fate by the direct regulation of the expression of PPAR. In addition, because fat accumulation tends to accelerate during a dynamic state, such as during rapid growth, whether fatty acid quality and quantity have a profound impact on body fat deposition by affecting PPAR expression during the early stage of adipogenesis was also examined.
1.2 Hypotheses and objectives

Two hypotheses were proposed in this study, based on the effects of (1) level and (2) type of dietary fats examined, as summarized in Figure 1.1 and Figure 1.2, respectively.

(1) High fat diets induce a higher body fat mass than low fat diets during rapid growth period by shifting body lipid metabolism towards anabolism (adipogenesis and liver lipogenesis) rather than catabolism (liver lipid oxidation).

(2) Polyunsaturated fats, which contain high levels of either 18:2n-6 or 18:3n-3, the potential activators of PPAR, reduce the accumulation of body fat in growing rats by downregulating of adipocyte PPARγ and upregulating of liver PPARα which result in decreased adipocyte differentiation and increased peroxisome β-oxidation, respectively, and also by inhibiting liver lipogenesis.

The specific objectives of this study are:

(1) To investigate whether feeding different levels and types of dietary fats to growing rats could shift body lipid metabolism by measuring:

- Body weight change
- Carcass composition changes
- Liver lipid profile
  - liver total lipid levels
  - liver triacylglycerol levels
  - fatty acid composition of total lipid and triacylglycerol
- Serum lipid profile
  - serum triacylglycerol levels
  - serum cholesterol levels
  - serum leptin levels

(2) To investigate whether feeding dietary fat high in PUFA could down regulate the expression of adipocyte PPARγ and upregulate liver PPARα and thus reduce the accumulation of body fat in growing rats by measuring:
1.3 Significance of study

Dietary fat is widely assumed as an important lifestyle factor responsible for the high prevalence of obesity. Dietary advice for treating and preventing obesity usually focuses on restricting dietary fat intake, with less emphasis on the type of fat. Since a growing body of evidence has indicated that dietary fat composition can influence body energy partitioning, it is thus important to consider fat subtypes when establishing dietary guidelines regarding dietary fat intake and obesity.

Adipose tissue development varies in magnitude depending on age, with rapid growth occurring during infancy and puberty. It is of significance to know whether early adaptation to obesity-inducing fat during the pre-puberty period makes young animals more susceptible to obesity. Appropriate dietary advice can then be given to children, to whom dietary fat is an essential part of the diet, and dietary modification is more likely to succeed.

To date, little information is available regarding the molecular mechanisms underlying lipid homeostatic regulation. One of the molecular targets proposed is PPAR. It is possible that if obesity can be managed by reversing adipocyte differentiation through the regulation of the gene expression of the upstream regulator, PPAR, new approaches in treating and preventing obesity can be achieved by nutritionally modulating dietary fat intake, and pharmacologically introducing PPAR agonists, antagonists, or the gene encoding PPAR. The potential effect of early nutritional intervention in terms of regulating the adaptation of gene expression also makes this study highly significant.
Factors

Environmental risk factors
- decreased infectious disease
- decreased socioeconomic status
- increased economic growth
- increased food supply
- increased fat palatability
- increased prepared food
- increased portion size
- increased technology
- increased sedentary lifestyle
- increased loneliness and social isolation
- increased psychosocial/family problems

Metabolic risk factors
- decreased metabolic rate
- decreased fat oxidation
- decreased insulin sensitivity
- decreased sympathetic nervous activity

Genetic risk factors
(possibly polymorphisms and/or mutations in any of the followings)
- adrenergic receptors
- CRH
- leptin/leptin receptor
- lipoprotein lipase
- NPY/NPY receptor
- PPAR
- TNFα
- UCP

Behavioral risk factors
- decreased activity
- increased caloric intake
- increased fat intake

Table 1.1 Contributing factors for the development of obesity (modified from Kiess et al., 2001). Abbreviations: CRH, corticotrophin releasing hormone; NPY, neuropeptide Y; TNFα, tumor necrosis factorα; UCP, uncoupling protein.
### Table 1.2 Possible mechanisms for the lower body weight or body fatness induced by PUFA compared to SFA.

<table>
<thead>
<tr>
<th>Factors</th>
<th>PUFA</th>
<th>SFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>lipid oxidation</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>lipogenesis</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>energy expenditure</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>thermic effect of food</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>adipocyte GLUT4 level</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>adipocyte UCP-1 level</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>adipocyte differentiation</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>adipocyte lipolysis</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>adipocyte β-adrenergic receptor sensitivity</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>adipocyte LPL activity</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>muscle LPL activity</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>muscle UCP-2 level</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>hepatic TG secretion</td>
<td>↓</td>
<td>↑</td>
</tr>
</tbody>
</table>

Abbreviations: GLUT4, glucose transporter 4; LPL, lipoprotein lipase; TG, triacylglycerol; UCP, uncoupling protein. References are listed in context (section 2.1.5.3).
Figure 1.1 Proposed effects of fat level on body fat mass regulation. Thick lines and thin lines represent the proposed major (thick lines) and minor (thin lines) sites of the action for high fat intake.
Figure 1.2 Proposed effects of fat type on body fat mass regulation. Thick lines and thin lines represent the proposed major (thick lines) and minor (thin lines) sites of the action for high polyunsaturated fatty acid (PUFA) intake.
CHAPTER 2

LITERATURE REVIEW

2.1 Obesity

2.1.1 Obesity Definition

Obesity, a condition of abnormally elevated body fat content, develops when energy intake exceeds energy output. Because the exact amount of body fat is difficult to measure, a number of techniques are used to estimate body fatness. Widely accepted methods are body mass index (BMI) and skinfold thickness for estimating total body fat, whereas resonance tomography (CT) scan, magnetic resonance imaging (MRI), and waist-to-hip circumference are used for regional fat estimation (Bray, 1994). Among these, BMI is the most widely performed method for practical reasons, even though it only provides an approximation of body fat level. BMI is defined as body weight in kilograms divided by the square of the height in meters (kg/m²). Current guidelines in defining adult overweight and obesity came from the recommendations of the 1997 World Health Organization (WHO) Consultation on obesity (WHO, 1998a), and the 1998 Nutritional Heart, Lung, and Blood Institute (NHLBI) Clinical Guidelines for the Consultation on Obesity (NHLBI, 1998). It is agreed that a BMI equal to or more than 25 and 30 kg/m² in adults is classified as overweight and obesity, respectively, based on Garrow’s definition (Garrow, 1981). A suitable method for assessing childhood obesity is undefined, due to non-linear variation of weight and height for age in many child populations. In 1997, the WHO Internation Obesity Task Force suggested that the cutoffs for adults of 25 or 30 kg/m² and above be used in defining overweight and obesity in children.

2.1.2 Obesity Health Risks and Economic Costs

The association between obesity and disease was first identified by Ayurveda and Samhita as early as 100-200BC (Kahn, 1994). However, it was not until 1985 that the National Institutes of Health (NIH)
consensus conference described it as a chronic disease (NIH, 1985). People who are obese are at higher risk for developing diseases, such as hypertension, type II diabetes, coronary heart disease, lipid disorders, certain cancers, gallstones, respiratory problems, sleep apnea, and osteoarthritis. It has been estimated that obesity is associated with 280,000 to 325,000 deaths a year in USA, making it second only to smoking as a preventable cause of death (Allison et al., 1999).

The total costs of obesity-related disease in the USA approach $100 billion annually (Atkinson, 1998), including $52 billion in direct medical cost (Wolf et al., 1998). In some countries, about 2% to 8% of the total health budget is attributed to obesity (Katsilambros, 2000). It is no doubt that obesity is one of the major burdens in public health economics.

2.1.3 Obesity Prevalence

The prevalence of obesity is high in many developed countries. Evidence has shown that obesity rate is also increasing in the developing world, such as Asia and Latin America. Recent studies indicated that the incidence of obesity can even be higher in lower and middle income countries than in higher income countries (Popkin, 1998). Prevalence and time trends of obesity can vary among countries, even among those with similar economic status.

In the US, based on the third National Health and Nutrition Examination Survey (NHANES III), the prevalence of overweight in adults (BMI≥25) increased from 46% to 54%, while that of obesity increased from 14.5% to 22.5% between 1976 and 1994 (Flegal et al., 1998). According to the first federal obesity clinical guidelines released by NIH in 1998, overweight or obesity affects approximately 97 million or 55% of American adults. This upward trend in obesity led to the prediction that by the year of 2230, all Americans will be obese (Foreyt et al., 1995).

Obesity prevalence in many European countries is also rising. An increase from 10% to 40% in most major countries within the past decade is generally reported. Among European countries, it is estimated that for women and men at ages between 40 and 60 years, respectively, about 10 and 15% in northern Europe, 13 and 16% in western Europe, 18 and 30% in eastern Europe, and 16 and 30% in the
Mediterranean countries are obese (Rissanen et al., 1991). In the UK where obesity is as prevalent as in the US, the 1996 Health Survey suggested that 16% of women and 15% of men were classified as obese (Senior, 1997).

Obesity is also becoming an important public health issue in a number of other nations, where formerly little obesity was described, such as those located in the Middle East, Southeast Asia, Latin America, Australia, Northern Africa, and Caribbean Islands. As the prevalence of obesity continues to increase worldwide, about 300 million people will become obese in 2025 as suggested by WHO (1998b).

2.1.4 Obesity Etiology

Although the ultimate cause of obesity is an increased energy intake or decreased energy expenditure, or both, the precise regulatory mechanisms leading to this positive energy balance are still unclear. Possible contributing factors include those of environmental, metabolic, genetic, and behavioral origins, as listed in Table 1.1. It is increasingly accepted that an excess accumulation of body fat is determined by an interaction among these factors, rather than by one single factor (Grundy, 1998). One of the most important lifestyle factors is dietary fat intake, which still is highly debated.

2.1.5 Dietary fat and Obesity

Studies in both animals and humans have shown that the effects of each macronutrient (protein, carbohydrate and fat) on body energy balance need to be considered separately (Flatt 1988; Flatt 1987). Because protein balance tends to remain relatively constant within a few days, body weight regulation is more closely related to carbohydrate and fat intake.

To date, the relationship between dietary fat and carbohydrate intakes and body weight regulation remains controversial. Most researchers claimed that dietary fat is the major dietary factor determining body weight. On the other hand, it has been noted that “Diets high in fat do not appear to be the primary cause of the high prevalence of excess body fat in society, and reductions in fat will not be a solution.” (Willett, 1998). The concept that the effect of dietary fats on obesity may be overestimated has emerged from the following findings: (1) Some individuals who habitually consume a high fat diet (at least during a certain period) do not necessarily develop obesity (Blundell et al., 1996; Heitmann et al., 1995), (2) Some epidemiological studies showed only modest weight reduction when high fat diets were replaced with low
fat diets (Willett, 2002; Willett, 1998; Lissner et al., 1995a). Judging from the fact that dietary fat represents only one of the several factors involved in obesity development, it is likely that fat per se may not account for the high rate of obesity alone. It is suggested that unless other factors involved in energy balance regulation are clearly considered, the role of dietary fat on obesity will be difficult to identify, and obesity treatment by reducing fat intake will not be as successful as expected.

2.1.5.1 Theoretical Role of Dietary Fat on Obesity Development

In theory, high fat intake is more likely to cause positive energy balance than high carbohydrate intake as a consequence of the induction of both increased energy intake and reduced energy expenditure (Lissner et al., 1995b). Possible mechanisms underlying the high fat: carbohydrate ratio in relation to body weight have been suggested. Because fats offer more diversity, flavor and texture to food, they are more palatable and often stimulate overconsumption. Dietary fats may be overconsumed also because they are energy dense; as a consequence, a larger amount of fat may be consumed before fat-induced hunger suppressing signals become effective (Rolls, 1995; Lawton et al., 1993). Less chewing required for high fat diets compared to carbohydrate rich diets high in fiber and complex carbohydrates may further enhance overconsumption.

Differences in the metabolism of fat compared with other macronutrients may also contribute to obesity. The diet-induced thermic effect is greater for carbohydrate and protein than for fat (Sims et al., 1987). The body’s storage capacity for protein and for carbohydrate as glycogen is limited. With excess intake, both protein and carbohydrate promote their own oxidation. However, for fat, body stores are large, and intake does not cause comparable changes in oxidation (Astrup et al., 1994; Schutz et al., 1989). In addition, the storage cost for fat (4%) is lower than that for carbohydrate (12% for glycogenesis and 23% for de novo lipogenesis) (Lissner et al., 1995b). These results suggest that de novo synthesis of fat from carbohydrate is less favorable and that fat may be utilized and stored as body fat more efficiently.

2.1.5.2 Macronutrient Composition and Obesity

Since dietary fat contains a higher caloric density than carbohydrate, high fat foods are often smaller in portion as compared to isocaloric high carbohydrate foods. As the amount (or weight and volume) of food is an important regulator of food intake, high fat intake is more likely to lead to overeating.
or excess energy intake, and thus promote obesity. This raises the concern about whether the effect of dietary fat on body weight determination, as compared to dietary carbohydrate, is independent of its contribution to total energy intake. Studies aimed at understanding if dietary fat is overconsumed were usually conducted with *ad libitum* food intake. On the other hand, by manipulating the fat to carbohydrate ratio in diets while maintaining energy intake constant, either at, above or below maintenance level, the independent effect of fat content can be examined. The effect of diet composition on food intake and energy balance regulation are discussed below.

Studies with laboratory rats reveal that *ad libitum* high fat feeding produces obesity, which is attributed mostly to hyperphagia (Sclafani *et al.*, 1992), but not always (West *et al.*, 1998). Similar results have been found in humans in which during *ad libitum* intake, dietary fat restriction may not affect body weight change (Knopp *et al.*, 1997) or energy intake (Bell *et al.*, 2001; Rolls *et al.*, 1999). When excess energy is consumed, fat is more efficient than carbohydrate in producing weight gain (Horton *et al.*, 1995).

However, when energy intakes are held constant by manipulating dietary fat and carbohydrate levels, dietary fat *per se* does not affect energy intake in either rats (Ramirez *et al.*, 1989) or humans (Hirsch *et al.*, 1998; Saltzman *et al.*, 1997; Leibel *et al.*, 1992; Stratum *et al.*, 1978). Under conditions of hypocaloric intake, a similar magnitude of weight loss is seen in low fat and high fat groups (Golay *et al.*, 1996; Rumpler *et al.*, 1991).

These data suggest that in most studies in which the individual effect of dietary fat is not separated from the combination effects of dietary fat and total calorie intake, the influence of fat alone on body weight determination may be overestimated. In other words, diets high in carbohydrate and low in fat may not protect against weight gain if total energy is not regulated.

On the other hand, there are researchers who believe that fat is a major determinant of body fat deposition independently of energy intake. For example, Boozer *et al.* (1995) found that when rats were fed different amounts of dietary fat with isocaloric intake, there was a linear relationship between dietary fat content and body fat accumulation. Studies by Romieu *et al.* (1988) in obese women, by Gazzaniga *et
al. (1993) in preadolescent children, and by Tucker et al. (1992) in adult females all showed that, after adjusting for confounding factors, such as physical exercise and energy intake, relative body weight or percent body fat remains highly associated with fat intake.

With *ad libitum* low fat consumption, an association between dietary fat restriction and body weight reduction was observed in humans in both short-term (10 wks by Gatenby et al., 1997; 12 wks by Siggaard et al., 1996; 11 wks by Raben et al., 1995; 12 wks by Schaefer et al., 1995; 11 wks by Kendall et al., 1991; 12 wks by Schaefer et al., 1995; 11 wks by Kendall et al., 1991; 20 wks by Prewitt et al., 1991) and long-term follow-up studies (1 yr by Kasim et al., 1993; 1 and 2 yrs by Sheppard et al., 1991; and 1 year by Thuesen et al., 1986).

During restricted energy intake, Racette et al. (1995) found that a low fat intake resulted in greater weight loss than a high fat diet; however, there was no dietary effect on body composition.

These apparent disagreements on the association between high fat intake and obesity may arise from methodological differences, and interpretive difficulties. For example, different levels of dietary fat have been implicated in obesity studies ranging from as high as 80% to as low as 10% of total energy (Lemonnier, 1972; Mickelson et al., 1955; Fenton et al., 1951). Since humans normally consume a complex food with energy contributed from a combination of carbohydrate, fat and protein, within a realistic range of dietary fat intake, dietary fat may have less of an impact on body fat than those using extreme high intake of dietary fat.

### 2.1.5.3 Dietary Fat Composition and Obesity

Increasing evidence has shown that the influence of high fat diets on body weight may depend on the dietary fat subtypes. Several studies suggested that polyunsaturated fats are anti-obesogenic, and thus are a preferred dietary fat source to saturated fats in body weight manipulation (Cha et al., 1996; Shimomura et al., 1990). The effect of monounsaturated fats, however, is less documented. Dietary fats consisting mostly of triacylglycerols, differ from each other in many aspects: the chain length of the constituent fatty acids (*Table 2.1*), the steric configuration (cis or trans) of double bonds, the number and position of double bonds, and the distribution of the fatty acids on the glycerol structure (*Table 2.2*). Hypothetically, each fatty acid has its unique biological pathway which can contribute to different levels of

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<table>
<thead>
<tr>
<th>Fat or oil</th>
<th>4-11:0</th>
<th>12:0</th>
<th>14:0</th>
<th>16:0</th>
<th>16:1</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>other</th>
</tr>
</thead>
<tbody>
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<td>Beef fat*</td>
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<td>0.1</td>
<td>3.3</td>
<td>25.5</td>
<td>3.4</td>
<td>21.6</td>
<td>38.7</td>
<td>2.2</td>
<td>0.6</td>
<td>4.5</td>
</tr>
<tr>
<td>Butter*</td>
<td>9.3</td>
<td>3.1</td>
<td>11.7</td>
<td>26.2</td>
<td>1.9</td>
<td>12.5</td>
<td>28.2</td>
<td>2.9</td>
<td>0.5</td>
<td>3.7</td>
</tr>
<tr>
<td>Chicken fat*</td>
<td>0.2</td>
<td>1.3</td>
<td>23.2</td>
<td>6.5</td>
<td>6.4</td>
<td>41.6</td>
<td>18.9</td>
<td>1.3</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Cocoa butter</td>
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<td>25.8</td>
<td>0.3</td>
<td>34.5</td>
<td>35.3</td>
<td>2.9</td>
<td></td>
<td></td>
<td></td>
<td>1.1</td>
</tr>
<tr>
<td>Coconut oil b</td>
<td>14.9</td>
<td>48.5</td>
<td>17.6</td>
<td>8.4</td>
<td>2.8</td>
<td>5.8</td>
<td>1.8</td>
<td></td>
<td></td>
<td>0.1</td>
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<td>0.1</td>
<td>2.2</td>
<td>27.5</td>
<td>57.0</td>
<td>0.9</td>
<td>0.1</td>
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<td>Flaxseed oil b</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.8</td>
</tr>
<tr>
<td>HOSO b</td>
<td>4.3</td>
<td>1.9</td>
<td>74.6</td>
<td>14.4</td>
<td>4.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.8</td>
</tr>
<tr>
<td>HPSO b</td>
<td>4.3</td>
<td>1.9</td>
<td>14.4</td>
<td>74.6</td>
<td>4.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.8</td>
</tr>
<tr>
<td>Lard*</td>
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<td>0.1</td>
<td>1.5</td>
<td>24.8</td>
<td>3.1</td>
<td>12.3</td>
<td>45.1</td>
<td>9.9</td>
<td>1.1</td>
<td>3.0</td>
</tr>
<tr>
<td>Olive oil*</td>
<td>13.7</td>
<td>1.2</td>
<td>2.5</td>
<td>25.1</td>
<td>71.1</td>
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<td>0.6</td>
<td>0.9</td>
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<td>0.3</td>
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<tr>
<td>Palm oil a</td>
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<td>45.1</td>
<td>0.1</td>
<td>4.7</td>
<td>38.8</td>
<td>9.4</td>
<td>0.3</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Rapeseed oil a</td>
<td>0.1</td>
<td>2.8</td>
<td>0.2</td>
<td>1.3</td>
<td>23.8</td>
<td>14.6</td>
<td>7.3</td>
<td>49.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.1** Fatty acid compositions of common oils and fats. (%)

* From White, 1992


Abbreviations: HOSO, high oleic safflower oil; HPSO, high polyunsaturated safflower oil.

<table>
<thead>
<tr>
<th>Fat or oil</th>
<th>Major triacylglycerols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef tallow</td>
<td>POO POO POS</td>
</tr>
<tr>
<td>Butter</td>
<td>PPB PPC POP</td>
</tr>
<tr>
<td>Cocoa butter</td>
<td>POS SOS POP</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>DDD CDD CDM</td>
</tr>
<tr>
<td>Corn oil</td>
<td>LLL LOL LLP</td>
</tr>
<tr>
<td>Cottonseed oil</td>
<td>PLL POL LLL</td>
</tr>
<tr>
<td>HOSU</td>
<td>OOO POO SOO</td>
</tr>
<tr>
<td>Lard</td>
<td>SPO OPL OPO</td>
</tr>
<tr>
<td>Olive oil</td>
<td>OOO OOP OLO</td>
</tr>
<tr>
<td>Palm oil</td>
<td>POP POO POL</td>
</tr>
<tr>
<td>Palm kernel oil</td>
<td>DDD MOD ODO</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>LLL OLL LOO</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>LLL LLO LLP</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>LLL LLO LLP</td>
</tr>
</tbody>
</table>

**Table 2.2** Major triacylglycerols of some natural fats and oils. (From Small, 1991). Abbreviations: B, butyric; C, Capric; D, lauric; M, myristic; P, palmitic; S, stearic; O, Oleic; L, linoleic acid; HOSU, high oleic sunflower oil.
lipid accumulation. The proposed mechanisms accounting for the enhanced body fat deposition or weight gain by saturated fatty acids as compared to polyunsaturated fatty acids are discussed as follows, and are summarized in Table 1.2.

2.1.5.3.1 Fatty Acid Digestion and Absorption

Lipid digestion begins in the upper intestinal tract where lingual or gastric lipase hydrolyzes part of the fatty acids at the sn-3 position (Figure 2.1). Once they have entered the duodenum, the enzyme pancreatic lipase and its colipase act predominantly at the sn-1 position and generate 2-monoacylglycerol, 2,3-diacylglycerol, and free fatty acid. 2,3-Diacylglycerol is then attacked by carboxylesterase or pancreatic lipase and produce another fatty acid and 2-monoacylglycerol. Thus, fatty acids in the sn-2 position are preferentially absorbed into the enterocyte as a 2-monoacylglycerol (Small, 1991), with about 75% of the fatty acids in the sn-2 position being conserved in the triacylglycerol in lymph (Mattson, et al., 1964). Fatty acids in the sn-1,3 position are released in the small intestine and partly excreted in the feces through the formation of insoluble calcium soaps (Mattson et al., 1973; Widdowson, 1965). After diffusing across the brush border membrane, 2-monoacylglycerols are reesterified with fatty acids at the sn-1,3 position to reform triacylglycerol.

Figure 2.1 Lipid digestion1,2 (From Bracco, 1994). Abbreviations: R1, R2, and R3, fatty acids; b.s.s.l., bile-salt-stimulated lipase; DGs, diglycerides; MG, monoglycerides.

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The absorption of fatty acids can be divided into two pathways: long chain fatty acids having chain length greater than 12 are less water soluble and are incorporated into chylomicrons as triacylglycerol and secreted into lymph, initially bypassing the liver. Fatty acids with chain length less than or equal to 12, so called short chain or medium chain triacylglycerol (MCT), have a pK_a of ≈4.5 and are soluble in water when protonated and ionized. They are absorbed directly into the liver as free fatty acids via the portal vein and then rapidly oxidized in the liver after carnitine-independent transport into the mitochondria (Figure 2.1) (Small, 1991; Bach et al., 1982).

The different routes of fatty acid absorption, i.e., via the portal-venous or chylomicron triacylglycerol transport, may contribute in part to the various adipogenic effects of a particular fatty acid.

2.1.5.3.2 Fat Oxidation

The rate of oxidation varies among fatty acids depending on the chain length and degree of saturation. Overall, short-chain fatty acids (Leyton et al., 1987), MUFA (Bottino et al., 1965; Coots et al., 1964), and PUFA (Birt et al., 1990; Jones et al., 1988; Cenedella et al., 1969; Dupont et al., 1969) are more preferentially oxidized than long-chain SFA (DeLany et al., 2000) in both animal and human studies. One exception is n-3 rich lipids, particularly fish oil, which contains the very long chain fatty acids, DHA and EPA. Reduced fat mass and body weight have been found in both animals (Fickova et al., 1998; Cha et al., 1996; Belzung et al., 1993; Hill et al., 1993; Su et al., 1993) and humans (Mori et al., 1999) fed fish oil compared to other oils. It is suggested that increased mitochondrial carnitine palmitoyltransferase activity by n-3 fatty acid may facilitate fatty acid β-oxidation and lead to a decreased body fat storage (Gavino et al., 1991).

These results suggest that body fat accumulation is higher when a diet containing predominantly SFA is consumed.

2.1.5.3.3 Thermic Effect of Food

After a meal, metabolic rate increases by an average of 10% above that of the resting level (Strubbe, 1994). This is known as the thermic effect of food (TEF) or the diet induced thermogenesis (DIT). The TEF differs among macronutrients, with amino acids, glucose, and lipid representing 23%, 9%,
and 4% of TEF, respectively, in humans (Wahlqvist et al., 1999). Studies have shown that TEF also varies among fatty acids, with PUFA tending to induce a greater TEF than SFA. The increased TEF may explain the lower body fatness with PUFA consumption.

2.1.5.3 Other Mechanisms

Other metabolic events involved in the lowering effect of PUFA on body fat deposition as compared to other types of dietary fats, especially SFA, have been proposed. Those include first, increased lipoprotein lipase activity in the heart and skeletal muscle, and decreased LPL synthesis and activity in adipose tissue which lead to enhanced fat oxidation and decreased serum triacylglycerol level (Shimomura et al., 1990). Second, decreased hepatic lipogenesis (Ikeda et al., 1998; Surette et al., 1992) due to reduced lipogenic enzyme gene expression (Iritani et al., 1998; Cheema et al., 1996; Iritani et al., 1995). Third, decreased liver triacylglycerol secretion (Ikeda et al., 2001) and subsequent hypotriglyceridemia (Roche et al., 2000; Mori et al., 1999). Fourth, decreased adipose tissue GLUT4 level. Fifth, increased β-adrenergic receptor affinity (Matsuo et al., 1997), probably due to alterations in fatty acid composition of membrane structural lipids. Sixth, increased adipose tissue lipolysis, and thus less PUFA stored in adipose tissue under lipolytic stimuli (Raclot et al., 1997). Seventh, increased total body energy expenditure, partly due to increased UCP levels in both adipose tissue (UCP-1) and muscle (UCP-2) which enhance energy dissipation as heat. Finally, with the finding that fatty acids can act as gene modulators, the effect of PUFA is further identified as down-regulation of genes involved in lipid synthesis and adipocyte differentiation (Iritani et al., 1998; Clarke et al., 1997).

2.1.5.4 Factors Influencing the Effect of Dietary Fat on Obesity

Because the effect of dietary fat on body fat may depend on energy intake, other factors participating in energy intake regulation may influence the causal effect of dietary fat on energy intake and thus obesity development. As a consequence, these factors need to be considered before the role of dietary fat on obesity is determined. Possible confounding variables include the dietary factors (dietary components other than fat), environmental factors (physical activity), genetic factors, age of animals and subjects, initial adiposity of animals and subjects, gender, duration of the study, and accuracy of self-reported dietary intake in human studies.
2.1.5.4.1 Genetic factors

Obesity can be under genetic control (Pérusse et al., 2000; Ravussin et al., 1992; Bouchard, 1991). Epidemiological studies have suggested that between one-fourth and one-half of the differences in body weight in humans can be attributed to genetic variations (Bluchard et al., 1993). Differences in obesity susceptibility among rodents and among general populations in humans have been observed.

In rodents, both single-gene and polygenic models of obesity have been identified. The former includes agouti, ob, db, fat, and tub genes in mice and the fatty gene in rats. Polygenic models include New Zealand obese mice, KK mice and Wellesley mice. Different responses to HF diets among strains within a species have also been described (Wang et al., 1998; West et al., 1995; West et al., 1992). For instance, Schemmel et al. (1970) found that the S5B/P1 rat was more resistant to a HF diet than the other 6 strains of rats examined.

For humans, it has been reported recently that >100 genes or marker loci have the potential of affecting obesity (Chagnon et al., 1998). The role of genetic factors in obesity induction is evidenced by the findings that not all people eating a high fat diet are obese (Blundell et al., 1996; Heitmann et al., 1995), and that high fat consumption promoted weight gain (Heitmann et al., 1993) and reduced fat oxidation (Astrup et al., 1994; Astrup, 1993) only in predisposed women (those who are overweight with one or both overweight parents). Also, ethnic differences in fat deposition have been identified, such that the incidence of obesity is higher in Pima Indians (Story et al., 1999; Broussard et al., 1991; Knowler et al., 1991) and African Americans (Weyer et al., 1999; Allison et al., 1997) than in whites. Studies in twins further provide evidence that the individual response to dietary fat is heritable (Heitmann et al., 1997; Saltzman et al., 1997; Stunkard et al., 1986a; Stunkard et al., 1986b). These results suggest that genetic differences may explain variable adipogenic responses to high fat diets among studies and the weak association between dietary fat and obesity development found in some epidemiological studies.

Several pathways involved in energy balance regulation have been identified which are under genetic regulation, as summarized in Table 2.3 (Lissner et al., 1995).
Genetically determined energy regulation

- β-adrenergic receptor activity
- Capacities for fat and carbohydrate oxidation
- Diet induced thermogenesis
- Insulin sensitivity
- Lipolysis
- Lipoprotein lipase activity
- Metabolic rate
- Physical activity
- Protein deposition
- Respiratory quotient level
- Smoking
- Taste preference
- Type and number of muscle fibers

Table 2.3 Genetic modification of energy balance (Lissner et al., 1995).

2.1.5.4.2 Age

In rats, the age at which an obesity-inducing diet is initiated may influence the extent of body fat deposition (West et al., 1998). In general, the earlier an obesity promoting diet is introduced, during which weight gain is more rapid, the greater the extent of body fat gain. In humans, body fat content changes with age in two patterns, either with a steady increase throughout life, or shows a curvilinear pattern, i.e., body fat increases through middle age (about 60 to 70 years old) even when body weight remains unchanged (Roberts et al., 1994), then decreases (Mott et al., 1999; Going et al., 1995; Silver et al., 1993). The increased percent body fat with age may be due to increased body fat mass (Melanson et al., 1997; Flynn et al., 1989), decreased muscle mass (Forbes, 1999), decreased body fat oxidation (Melanson et al., 1997; Calles-Escandon et al., 1995), and impaired regulation of food intake (Moriguti et al., 2000) and energy expenditure (Das et al., 2001). Studies have shown that a child who is obese has a higher chance of being obese in adulthood (Rolland-Cachera et al., 1987; Fisch et al., 1975). These results suggest that age-related variations in energy balance do exist, prevention of obesity early in life may decrease its prevalence in adulthood, and that each age group should be considered individually.
2.1.5.4.3 Regional Fat Distribution

Mammalian adipose tissue can be divided into omental (in the abdominal cavity), subcutaneous (under the skin) and inter- or intramuscular (within the musculature) depots according to their anatomical locations (Pond, 1992). Studies have shown that different depots can vary in their ability to differentiate (Soret et al., 1999; Kirkland, 1996; Hauner et al., 1991), and in their ability to induce obesity-related metabolic disorders. This can be explained by differences in blood supply and innervation (Portillo et al., 1999; West et al., 1989). In general, central obesity (an increased accumulation of intraabdominal adipose fat, or visceral fat, in the upper body) appears to be a stronger risk factor than subcutaneous obesity. A plausible explanation is that intraabdominal adipose tissue has a higher rate of triacylglycerol turnover and release of fatty acids into the portal circulation in response to lipolytic stimuli. Increased fatty acid concentration in the liver may enhance liver gluconeogenesis and LDL secretion while inhibiting hepatic insulin clearance, resulting in hyperinsulinemia and insulin resistance. Consequently, the role of individual fat depots as opposed to total body fat needs to be acknowledged when comparing effects of dietary fat on body composition among studies.

2.1.5.4.4 Other Dietary Components

Dietary factors other than fat may be of significance for weight control through direct regulation of energy intake (Figure 2.2) (McCrory et al., 2000). Combined results from the literature suggest that increased prevalence of obesity is related to increased dietary variety (McCrory et al., 1999; Rolls, 1985), increased dietary viscosity (Bennett et al., 1999), increased portion size (Edelman et al., 1986; Nisbett, 1968), increased energy density of food (Rolls, 2000; Rolls et al., 1999a; Prentice, 1989), increased high glycemic index food intake (Ludwig, 2000; Kabir et al., 1998), increased palatability of diet (McCrory et al., 2000), increased water incorporation in food (Rolls et al., 1999b), increased level of food preparation, and decreased dietary fiber intake (Burton-Freeman, 2000; Subar et al., 1994).

2.1.5.4.5 Animal vs. Human Studies

In general, animal studies tend to give conclusive results, in that high fat may induce an increase in body fat content that is dependent on the amount of fat consumed and the duration of the feeding. However, the applicability of animal studies to the human situation is always questioned. For example,
animal studies usually use a single diet or forced feeding regimen, while in human studies, free selection of a variety of foods which is more comparable to normal lifestyle is applicable. It is found that self-selection of food can influence obesity development. In rats, fat preference is associated with either higher energy intake and weight gain (Shor-Posner et al., 1991), or higher body fat and no change in energy intake (Smith et al., 1998), and increased adipose tissue lipoprotein lipase activity (Cook et al., 1995). Studies in adult male subjects with spontaneous high fat or high carbohydrate consumption also showed that obesity was almost absent in those with high preference for high carbohydrate intake (Blundell et al., 1996). These findings indicate that dietary preferences and habits as well as nutrient intake need to be considered when evaluating the effects of food intake on obesity, and that defects in psychological mechanisms involved in food intake regulation may be responsible for obesity development.

Human studies usually provide suggestive but not definite results with either no correlation or a positive correlation found between dietary fat intake and body weight gain. This discrepancy can be explained by underreporting of dietary intake, especially in obese individuals (Goris et al., 2000) and
individuals with high energy intake, such as trained athletes. In addition, the difficulties in measuring habitual intake of free-living subjects consuming self-selected foods can further interfere with the conclusions.

2.1.5.4.6 Other Factors

Inconsistent results among studies can also be attributed to other factors. These include the population studied, such as obese vs. lean, men vs. women, and children vs. adults; environmental factors, such as level of physical activity, consumption of alcohol and smoking; experimental diet: liquid or solid, formula or modified, ad libitum or hypo- or hyper-caloric intake; duration of study: short-term or long-term; and experimental design, such as the implication of ecological, cross-sectional, longitudinal or interventional studies in epidemiological research.

2.1.6 Adult and Childhood Obesity

The prevalence and severity of obesity and overweight in children are increasing worldwide, even in countries where the prevalence used to be rare, such as in Thailand (Mo-Sowan et al., 1996). Different rates of childhood obesity have been observed among countries (Flodmark, 1998). In the US where overweight is viewed as the most prevalent nutritional disease among children, approximately one-quarter of children between ages 6 and 17 are identified as overweight (Davison et al., 2001). Based on the American National Health and Nutrition Examination Survey (NHANES), the prevalence of overweight in children was more than doubled between NHANES II and III (Steinbeck, 2001).

Childhood obesity is associated with several health risk factors, as listed in Table 2.4. These adverse effects can be detected either immediately (during childhood or adolescence) or in later life (during adulthood). Because many of the adverse consequences may begin in childhood, and the probability of successfully treating adult obesity and its complications is usually low, preventing childhood obesity has become one of the utmost strategies for preventing and treating obesity.

There are a number of theoretical reasons why early prevention of obesity may give better results in obesity management. First, children are known to have longitudinal changes in both height and weight. If body weight is kept constant, percent body fat can gradually decline as height increases with age. Thus, obese children may benefit from the rapid height increase when controlled for body weight. Second, food
preference in children is easier to modify than that in adults. Developing healthy eating habits may help to
decrease the prevalence of childhood obesity. Third, obesity in childhood is characterized by an increase in
fat cell size (hypertrophy), and especially, an increase in the total number of adipocytes (hyperplasia)
(Bonnet, 1981a). Although fat cell hyperplasia can occur during adulthood (Klyde et al., 1979; Faust et al.,
1978; Lemonnier, 1972), under normal conditions, the specific number of fat cells is usually determined by
puberty (Hausman et al., 2001). Since hyperplasia is irreversible, preventing the hyperplastic activity of
adipocytes which occurs more likely in juvenile onset obesity may lead to the achievement of ideal body
weight.

Consequences

<table>
<thead>
<tr>
<th>Early consequences</th>
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<tbody>
<tr>
<td>Physical appearance</td>
</tr>
<tr>
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</tr>
<tr>
<td>Orthopaedic</td>
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<tr>
<td>Metabolic disturbances</td>
</tr>
<tr>
<td>Nightly hypoventilation and sleep apnoea syndrome</td>
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<td>Immune system and infections</td>
</tr>
<tr>
<td>Skin alterations</td>
</tr>
<tr>
<td>Physical handicap</td>
</tr>
<tr>
<td>Increased blood pressure and hypertension</td>
</tr>
<tr>
<td>Liver steatosis cholecystolithiasis</td>
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<table>
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<tr>
<th>Late consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistence of early co-morbidities</td>
</tr>
<tr>
<td>Obese adults</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>Cancer</td>
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</tbody>
</table>

Table 2.4 Consequences of childhood obesity (Wabitsch, 2000).
2.2 Molecular Targets for the Treatment of Obesity

Considerable progress in recent obesity research has been made by the discovery that fatty acids can act as signaling molecules in the regulation of gene expression by modulating the amount and the transactivating capacity of involved transcription factors. These target genes code for proteins involved in fatty acid transport or metabolism, and thus may play an important role in energy metabolism. It is suggested that these transcription factors may serve as new targets for rational pharmaceutical or nutritional treatments of obesity, as well as advancing our knowledge of obesity at the molecular level. The major selected fatty acid-responsive transcription factors are those involved in liver lipid metabolism: sterol regulatory element binding protein (SREBP), nuclear factor-Y/CCAAT-binding factor (NF-Y/CBF), and hepatocyte nuclear factor-4 (HNF-4), and those involved in the control of adipogenesis: CCAAT/enhancer binding protein (C/EBP), peroxisome proliferator-activated receptor (PPAR), and adipocyte determination and differentiation factor-1 (ADD-1)/SREBP-1c. It is possible that other transcriptional factors will be identified in the future.

2.2.1 Hepatocyte Nuclear Factor-4 (HNF-4)

HNF-4 is also a member of the nuclear receptor superfamily. This nuclear receptor is involved in the regulation of genes related to amino acid and cholesterol metabolism, and gluconeogenesis. Three isoforms of HNF-4 have been identified, namely α1, α2, and α3 (Wolfrum et al., 2000). The expression of HNF-4 in human adults is found in a limited number of tissues, with higher levels found in the liver, intestine and kidney. Like PPAR, HNF-4 also binds to a direct repeat-1 motif; however, the putative ligands of HNF-4 are fatty acyl-CoA esters (Hertz et al., 1998). It is transcriptionally active as a homodimer. Binding of HNF-4 to fatty acyl-CoA esters suppresses its promoter enhancing activity of target genes. The role of HNF-4 in hepatic lipid metabolism is suggested from the findings that the HNF-4 response element resides in the PUFA-response region of L-pyruvate kinase (L-PK) (Liimatta et al., 1994), and in the glucose response region of fatty acid synthase (Jump et al., 1999). Other genes that contain HNF-4 recognition sites in their promoters include L-PK, apoprotein III, tyrosine aminotransferase, and...
phosphoenol-pyruvate carboxykinase (Jump et al., 1999). It remains to be determined how acyl-CoA regulation of HNF-4 influences gene transcription, as well as whether acyl-CoA or fatty acid is the foremost regulator in gene expression, through the activation of HNF-4 or PPAR.

2.2.2 Sterol Regulatory Element Binding Protein (SREBP)

SREBPs are unique transcription factors in that they are synthesized as 125 kDa precursor proteins which, upon proteolysis, release a 68 kDa amino-terminal segment from endoplasmic reticulum membranes for subsequent nuclear translocation (Brown et al., 1997). Three isoforms of SREBP have been found, designated as SREBP-1a and SREBP-2 in humans and rodents, and SREBP-1c in humans or ADD-1 in rats (Shimomura et al., 1997). All three isoforms are capable of activating the same genes but to various degrees, and they appear to act independently. SREBP-1a has a longer amino-terminal domain than SREBP-1c, and it is much more active in inducing transcription (Shimano et al., 1997). However, in adult animals, SREBP-1c is present more abundantly than 1a in most organs, including liver and adipose tissue. SREBP-1a functions predominately in both fatty acid and cholesterol synthesis, whereas SREBP-1c, the major form of SREBP-1, is found to regulate adipocyte differentiation, and liver and adipose tissue lipogenesis. SREBP-2 is a determinant of cholesterol biosynthesis (Osborne, 2000; Horton et al., 1998).

The mature form of SREBP activates transcription of genes encoding enzymes for cholesterol synthesis; these include 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) synthase, HMG-CoA reductase, farnesyl diphosphate synthase and squalene synthase. It also activates genes involved in fatty acid synthesis, such as acetyl CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase-1 (Brown et al., 1997).

Several studies have shown that PUFA exerts its inhibitory effect on lipogenic gene expression by reducing hepatic SREBP-1 content. However, not all glycolytic and lipogenic genes that are suppressed by PUFA contain the SREBP-1 response element (Clarke, 2000). This and the finding that SREBP-1 is a weak trans-activator suggested that SREBP-1 alone is not sufficient to account for the PUFA-mediated suppression of lipogenesis, and other mechanisms secondary to the regulation of SREBP-1 may exist. One

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proposed mechanism is SREBP-1, by binding to its recognition site, may induce the transcriptional activity of other transcription factors, that in turn control lipogenic genes. NF-Y has been suggested to be one of the co-regulatory nuclear proteins.

2.2.3 Nuclear Factor-Y (NF-Y)

NF-Y, also referred to as the CCAAT-binding factor (CBF), is highly conserved among species, and is ubiquitously expressed. NF-Y binds to an inverted CCAAT box present in the promoter region of many genes and stimulates their transcriptional activities. Functional NF-Y is a heterotrimeric protein, consists of three different subunits, A, B, and C (Mantovani, 1999). NF-Y has been shown to function as an accessory DNA-binding protein, acting synergistically with SREBP in mediating gene expression. NF-Y binding sites have been identified in the promoter of genes involved in fatty acid and cholesterol synthesis, such as stearoyl-CoA desaturase, fatty acid synthase, and HMG-CoA synthase (Zhang et al., 2001).

2.2.4 CCAAT/enhancer Binding Protein (C/EBP)

C/EBPs are transcriptional regulators belong to the basic leucine zipper family. To date, six isoforms have been characterized, α, β, γ, δ, ε, and ζ (Roesler, 2001). All C/EBPs recognize a common C/EBP consensus sequence and can form both homodimers or heterodimers with each other (Cao et al., 1991). Even though all C/EBPs show structural similarities, and their tissue distribution often overlaps, they vary from each other in their functional properties and downstream regulated genes. Among the C/EBPs, α, β, and δ are expressed in adipose tissues, and are important in adipocyte differentiation (Tanaka et al., 1997).

The expression of C/EBPα, β, and δ during adipocyte differentiation shows a temporal pattern (Morrison et al., 2000; Rangwala et al., 2000). C/EBPβ and δ are induced early in the process, whereas C/EBPα is expressed in the later stage. The elevated levels of C/EBPβ and δ act synergistically in stimulating the expression of C/EBPα and PPARγ, both of which contain the C/EBP enhancer sequence. Once activated, C/EBPα and PPARγ co-regulate each other to maintain adipocyte differentiation, despite the decreasing levels of C/EBPβ and δ.
Another member of C/EBPs, C/EBPζ, has also been found in adipose tissue. C/EBPζ functions as a negative inhibitor of C/EBPs by avidly preventing the heterodimer formed between C/EBPζ and other C/EBPs from binding to DNA (MacDougald et al., 1995). The role of C/EBPζ in adipogenesis is not well documented. However, the finding that enforced expression of C/EBPζ in 3T3-L1 cells caused a suppression of adipogenesis suggested its inhibitory potential (Batchvarova et al., 1995).

A number of adipocyte-related genes have been identified to be activated by C/EBPs. Those include stearoyl-CoA desaturase-1 (SCD-1), glucose transporter-4 (GLUT-4), phosphoenolpyruvate carboxykinase (PEPCK), fatty acid binding protein (aP2), and uncoupling protein (UCP) (Roesler, 2001).

2.2.5 Peroxisome Proliferator-activated Receptor (PPAR)

2.2.5.1 Peroxisome β-oxidation

Fatty acid oxidation takes place in three organelles, the coexistence of β-oxidation in mitochondria and peroxisomes, and ω-oxidation in smooth endoplasmic reticulum. In yeast, fatty acid β-oxidation occurs almost exclusively in peroxisomes (Kunau et al., 1988). However, in mammals, peroxisome β-oxidation accounts mostly for very long chain fatty acids (VLCFA, >C_{20}) due to lack of very long chain acyl-CoA synthetase in mitochondria. Peroxisomes also participate in the metabolism of long chain fatty acids (LCFA, C_{12}-C_{20}), which are oxidized predominantly in mitochondria, as well as prostaglandins, bile acid precursors, and some xenobiotic compounds. It was estimated that in rat liver, up to 50% of fatty acids with chain length longer than 16 were metabolized in peroxisomes (Piot et al., 1998). The energy produced by fatty acid oxidation is used preferentially in the skeletal and cardiac muscle under nonfasting conditions.

The concentration of plasma free fatty acids released from adipose tissues roughly reflects the rate of fatty acid oxidation with higher plasma fatty acid concentration accompanying higher rates of oxidation (Gibbons et al., 2000). Even though both mitochondria and peroxisomes conduct β-oxidation, they are functionally different but complementary (Table 2.5), and are regulated by unique enzymes encoded by different genes (Figure 2.3).

In mitochondria, fatty acids are first converted to their acyl-CoA thioesters by long chain acyl-CoA synthetase located on the mitochondrial outer membrane, and then transported into the matrix by
Figure 2.3. Fatty acid β-oxidation (Reddy et al., 2001).
<table>
<thead>
<tr>
<th>Determinant</th>
<th>Mitochondria</th>
<th>Peroxisomes</th>
</tr>
</thead>
</table>
| Substrates | long chain fatty acids  
medium chain fatty acids | very long chain fatty acids  
long chain fatty acids  
long chain dicarboxylic acids  
branched chain fatty acids  
bile acid precursors  
xenobiotics |
| Component | inner membrane and matrix  
associated | enzymes having different fatty acid  
specificities and enzymes having  
overlapping carbon chain length specificities |
| Regulation | long term  
carnitine, malonyl-CoA  
some enzymes inducible | none known  
enzymes of classical set inducible |
| short term | hypoketotic hypoglycemia  
organic aciduria  
cardiac dysfunction  
hepatocellular dysfunction  
marked lipid accumulation  
attack, sudden death | cerebral dysgenesis  
demyelination  
severe hypotonia  
progressive  
accumulation of very long chain fatty acids |
| Clinical symptoms | hypoketotic hypoglycemia  
organic aciduria  
cardiac dysfunction  
hepatocellular dysfunction  
marked lipid accumulation  
attack, sudden death | cerebral dysgenesis  
demyelination  
severe hypotonia  
progressive  
accumulation of very long chain fatty acids |
| Main roles | ATP synthesis | detoxification |

Table 2.5 Comparisons of the two fatty acid β-oxidation systems (Reddy et al., 2001; Hashimoto, 1996).
carnitine acyltransferase/carnitine translocase. In peroxisomes, at least two acyl-CoA synthetases exist: long chain and very long chain acyl-CoA synthetase, and carnitine is not required for the entry of fatty acids.

The first step of peroxisomal β-oxidation differs from that of mitochondria in that the rate limiting enzyme acyl-CoA oxidase catalyzes flavine adenine dinucleotide (FAD)-dependent dehydrogenation by transferring electrons from acyl-CoA to O₂ to form H₂O₂. This is in contrast to mitochondrial β-oxidation in which the first reaction is directly coupled to the electron transfer system for ATP synthesis. The H₂O₂ generated is cleaved to O₂ and H₂O by the enzyme catalase. Since the reduced acyl-CoA oxidase is directly reoxidized by O₂, peroxisome β-oxidation can proceed regardless of the energy status. This makes the peroxisome the ideal organelle for detoxification. Peroxisomes contain two different acyl-CoA oxidases: one for straight chain and one for 2-methyl-branched chain fatty acids. The enzymes involved in the first pathway can be induced by PPARα activators. Due to the fact that peroxisome acyl-CoA oxidase is almost inactive toward fatty acyl-CoAs having 8 or fewer carbon atoms, no complete degradation of fatty acid to acetyl-CoA is found. Those chain-shortened fatty acids are transported to mitochondria for further oxidation.

The hydration of enoyl-CoA to 3-hydroxyacyl-CoA and the subsequent NAD⁺-required dehydrogenation are catalyzed by a bifunctional enzyme, which is distinguished from the trifunctional enzyme in mitochondria. The last reaction of peroxisomal β-oxidation, the cleavage of the thio group of 3-ketoacyl CoA, is activated by 3-ketoacyl-CoA thiolase.

### 2.2.5.2 Peroxisome Proliferation

Peroxisome proliferation is a pleiotropic response marked by an increase in the number and size of peroxisomes, and induction of certain associated enzymes. This phenomenon appears to be species specific since humans (Blaauboer et al., 1990; Holden et al., 1999), non-human primates, hamsters, guinea pigs and rabbits (Chinje et al., 1994; Foxworthy et al., 1990; Makowska et al., 1992; Pacot et al., 1996) do not display the same degree of PP-induced responses. A comparison of the PP-induced responses between rodents and humans is listed in Table 2.6 (Gonzalez et al., 1998). Several naturally and biochemically occurring compounds, as well as synthetic chemicals have been identified as PP, i.e., agents capable of...
inducing peroxisome proliferation. These PP share little or no structure similarity (Figure 2.4). One most common property for some, but not all PP, is their potential to transform into carboxylic acid derivatives. This structural requirement may be the key element for the induction of peroxisome proliferation (Reddy et al., 2001).

Synthetic PP include certain hypolipidemic drugs (clofibrate, fenofibrate, bezafibrate, ciprofibrate, beclofibrate, etofibrate, gemfibrozil, BR931, and Wy14,643), and xenobiotics (phthalate and adipate ester plasticizers, herbicides, leukotriene antagonists, acetylsalicylic acid, thio-substituted fatty acids, and food flavors).

Natural regulators of peroxisome proliferation include high fat diets, phytanic acid, eicosanoids (from arachidonate via both lipoxygenase and cyclooxygenase pathways), and adrenal steroid dehydroepiandrosterone. Diets high in VLCFAs and PUFA have a more profound effect on peroxisome proliferation than diets high in other types of fatty acid (Flatmark et al., 1993; Forman et al., 1998; Osmundsen et al., 1991). Several lines of evidence suggest hormonal regulation of peroxisome proliferation: (1) male rats display greater response to PP than females (Harbhajan et al., 1994), (2) dehydroepiandrosterone treatment in rodents induces peroxisome proliferation (Frenkel et al., 1990), (3) peroxisomal enzymes are increased by glucocorticoid administration (Sorensen et al., 1993) and

Table 2.6. Differences between mice and humans in response to peroxisome proliferators (Gonzalez et al., 1998). +/10: humans have less than one tenth the level of hepatic PPARα than mice and rats.

<table>
<thead>
<tr>
<th>Response to Peroxisome proliferators</th>
<th>mice and rats</th>
<th>PPARα-null mice</th>
<th>humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARα expression</td>
<td>+</td>
<td>-</td>
<td>+/10</td>
</tr>
<tr>
<td>Increase in peroxisomes</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Enzyme induction</td>
<td>+</td>
<td>-</td>
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<td>Cell proliferation</td>
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<td>Apoptosis inhibition</td>
<td>+</td>
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<tr>
<td>Anti-inflammatory effects</td>
<td>+</td>
<td>-</td>
<td>?</td>
</tr>
<tr>
<td>Increased risk of cancer</td>
<td>+</td>
<td>-</td>
<td>-</td>
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Figure 2.4 Structures of known ligands and activators of PPAR (Escher et al., 2000).
hyperthyroidism (Kerckaert et al., 1989). Various physiological and nutritional conditions have also been shown to affect peroxisome proliferation. Enhanced proliferation is observed in response to fasting (Kersten et al., 1999) and during uncontrolled diabetes (Thomas et al., 1989).

The biological significance of PP is not only restricted to peroxisomes but also other cellular compartments (Table 2.7). This suggests that PP may participate in multiple physiological pathways (Latruffe et al., 1997).

2.2.5.3 Peroxisome Proliferator-activated Receptor (PPAR)

The ability of peroxisome proliferators (PP) to control gene transcription led to the discovery of a new nuclear hormone receptor, accordingly called peroxisome proliferator-activated receptor PPAR). However, it was not identified until a decade after the first report of PP in 1965 (Hess et al., 1965). The first PPAR cDNA was isolated from mouse liver (Issemann et al., 1990) and was named mPPARα (m for mouse, and α for the first mPPAR identified).

PPAR belongs to the steroid hormone receptor superfamily, which includes receptors for steroids, thyroid hormone, glucocorticoid, progesterone, mineralocorticoids, oestrogen, androgen, vitamin D, and retinoic acid. Two types of receptor-mediated signaling mechanisms exist in organisms. Hydrophilic signaling molecules such as peptide hormones, growth factors and neurotransmitters bind to cell surface receptors and activate a cascade of second messengers, which regulate gene transcription. For lipophilic ligands such as PP, steroids, certain hormones and vitamins that can enter the cell by simple or facilitated diffusion, direct binding to the nuclear receptor present in the cytoplasm or nucleus leads to the activation of these receptors. Activated nuclear receptors act as transcription factors that regulate target genes directly at the transcription level. This group of nuclear receptor represents a diverse and ancient component in the biological system, as is found in nearly all species, including worms, insects, and vertebrates, and is highly conserved throughout evolution. By 1988, all members of the nuclear receptor superfamily had been identified (Giguere et al.) and now comprise over 30 distinct mammalian genes (Mangelsdorf et al., 1995).
### Effects on peroxisomes

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>Fractional volume of peroxisomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>++</td>
<td>Peroxisomal β-oxidation of</td>
</tr>
<tr>
<td></td>
<td>-monocarboxylyl-fatty acyl-CoA</td>
</tr>
<tr>
<td></td>
<td>-omega-hydroxymono carboxylyl-CoA</td>
</tr>
<tr>
<td></td>
<td>-dicarboxylyl-CoA</td>
</tr>
<tr>
<td></td>
<td>-prostaglandyl-CoA</td>
</tr>
<tr>
<td></td>
<td>-tri hydroxy coprostanoyl-CoA and other branched chain acyl-CoAs (pristanoyl-CoA and 2-methylpalmitoyl-CoA)</td>
</tr>
<tr>
<td></td>
<td>Carnitine acyltransferase (COT, CAT)</td>
</tr>
<tr>
<td></td>
<td>Hydrogen peroxide-generating oxidizing activity on:</td>
</tr>
<tr>
<td></td>
<td>-glycolate</td>
</tr>
<tr>
<td></td>
<td>-urate</td>
</tr>
<tr>
<td></td>
<td>-D-amino acids</td>
</tr>
<tr>
<td></td>
<td>Catalase</td>
</tr>
<tr>
<td></td>
<td>Dihydroxyacetone phosphate acyltransferase</td>
</tr>
<tr>
<td></td>
<td>Acyl-CoA hydrolase</td>
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### Effects on mitochondria

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>Fractional volume of mitochondria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to +</td>
<td>Mitochondrial β-oxidation of straight-chain substrates</td>
</tr>
<tr>
<td></td>
<td>-monocarboxylic fatty acids</td>
</tr>
<tr>
<td></td>
<td>-omega-hydroxymono carboxylic acids</td>
</tr>
<tr>
<td></td>
<td>Mitochondrial β-oxidation of branched-chain substrates</td>
</tr>
<tr>
<td></td>
<td>-isovalerate</td>
</tr>
<tr>
<td></td>
<td>-isobutyrate</td>
</tr>
<tr>
<td></td>
<td>-2-methylbutyrate</td>
</tr>
<tr>
<td></td>
<td>Carnitine acyltransferases (CPT, CAT)</td>
</tr>
<tr>
<td></td>
<td>Enoyl-CoA reductase</td>
</tr>
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</table>

### Effects on microsomes

<table>
<thead>
<tr>
<th>Stimulation</th>
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</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Cytochromes P450s</td>
</tr>
<tr>
<td></td>
<td>-in general</td>
</tr>
<tr>
<td></td>
<td>-P452 (laurate omega-hydroxylase) [Cyt P450 1V A6]</td>
</tr>
<tr>
<td></td>
<td>Epoxide hydrolase</td>
</tr>
<tr>
<td></td>
<td>Glycerol-3-phosphate acyltransferase</td>
</tr>
</tbody>
</table>

### Effects on other components or observed on total liver homogenate

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>Fatty acid binding protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ to ++</td>
<td>Acyl-CoA hydrolases</td>
</tr>
<tr>
<td>++</td>
<td>Total liver content of CoA</td>
</tr>
<tr>
<td>+</td>
<td>Total liver content of carnitine</td>
</tr>
<tr>
<td>+</td>
<td>Total liver content in polyamines</td>
</tr>
<tr>
<td>+</td>
<td>17β-hydroxysteroid dehydrogenase [153]</td>
</tr>
</tbody>
</table>

Table 2.7 Effects induced by peroxisome proliferators (Latruffe et al., 1997). 0, little or no change; +, less than 2-fold increase; ++, 2- to 10-fold increase; ++++, stimulation factor superior to 10; -, decreased activity; CAT, carnitine acetyltransferase; COT, carnitine octanoyltransferase; CPT, carnitine palmitoyltransferase.
2.2.5.4 Subtypes

To date, three types of PPAR have been identified in different species and different tissues, namely PPARα, β (also known as PPARδ or fatty acid activated receptor (FAAR) in rodents, and NUC1 in humans), and γ (Table 2.8). These subtypes are encoded by separate genes located in different chromosomes in different species but display high sequence homology (Jump et al., 1999; Lemberger et al., 1996c; Seldin et al., 1994; Zhu et al., 1993). PPARγ exists as three isoforms, γ1, γ2 and γ3, which differ only in their 3’ end. The γ3 isoform is found only in humans so far, and it encodes for the same protein as PPARγ1 mRNA (Auwerx, 1999; Fajas et al., 1998). All isoforms are derived from the same gene by alternative splicing and promoter usage (Fajas et al., 1997; Zhu et al., 1995).

2.2.5.5 Tissue Distribution and Function

The expression of PPAR varies widely among tissues and may therefore reflect their specific function (Table 2.8) (Auboeuf et al., 1997; Kliewer et al., 1994).

PPARα is predominately expressed in tissues with a high level of fatty acid catabolism and high peroxisomal activities, such as liver, heart, muscle, brown adipose tissue, adrenal gland, retina, renal proximal tubule cells and enterocytes. The distribution pattern is similar between rodents and humans; however, expression is lower in humans than in rodents (Holden et al., 1999; Palmer et al., 1998; Wolfrum et al., 2000). A wide variety of structurally diverse compounds are known to activate PPARα, including the fibrate class of hypolipidemic drugs, fatty acids, plasticizers and herbicides (Forman et al., 1997; Issemann et al., 1993; Murakami et al., 1999). Among the fatty acids, long chain fatty acids and their metabolites, such as linoleic acid, arachidonate and leukotriene B4 appear to be more potent than saturated and medium/short chain fatty acids in activating PPARα (Dussault et al., 2000; Green, 1995). In the liver, PPARα is involved in the oxidation of fatty acids and detoxification of xenobiotic compounds. Since a great number of genes is shown to be regulated by liver PPARα (Table 2.9), it is generally believed that PPARα may also participate in inflammation (via eicosanoids synthesis), energy homeostasis (via triacylglycerol-rich lipoprotein synthesis), and cholesterol homeostasis (via cholesterol-7-hydroxylase
<table>
<thead>
<tr>
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<th>Tissue distribution</th>
<th>Ligand/activators</th>
<th>Major physiologic function</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>liver</td>
<td>fatty acids</td>
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</tr>
<tr>
<td></td>
<td>heart</td>
<td>fibrates</td>
<td>peroxisome proliferation</td>
</tr>
<tr>
<td></td>
<td>kidney</td>
<td>LT-B4</td>
<td>inflammation</td>
</tr>
<tr>
<td></td>
<td>muscle</td>
<td>8S-HETE</td>
<td>energy/cholesterol homeostasis</td>
</tr>
<tr>
<td></td>
<td>BAT</td>
<td>Wy-14643</td>
<td></td>
</tr>
<tr>
<td></td>
<td>retina</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>enterocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>adrenal gland</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β(δ)</td>
<td>ubiquitous</td>
<td>fatty acid</td>
<td>increases HDL-cholesterol</td>
</tr>
<tr>
<td>NUC-1</td>
<td>carba-prostacyclin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAAR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ</td>
<td>WAT</td>
<td>15-deoxy-PGJ2</td>
<td>adipocyte differentiation</td>
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<tr>
<td></td>
<td>BAT</td>
<td>thiazolidinediones</td>
<td>insulin sensitization</td>
</tr>
<tr>
<td></td>
<td>Macrophage</td>
<td>BRL 49653</td>
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</tr>
<tr>
<td></td>
<td>Retina</td>
<td>9-HODE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13-HODE</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.8 Peroxisome proliferator-activated receptors. Abbreviations: WAT, white adipose tissue; BAT, brown adipose tissue; HODE, hydroxyl-octadecadienoic acid; LT-B4, leukotriene B4; PG, prostaglandin.
### PPAR activation

**Stimulated**
- Liver peroxisomal acyl-CoA synthetase
- Liver cytosolic fatty acid binding protein
- Liver peroxisomal β-ketothiolase
- Liver microsomal cytochrome P450 IV A1
- Liver peroxisomal bifunctional enzyme (enoyl-CoA hydratase/β-hydroxyacyl-CoA dehydrogenase)
- Liver cytosolic malic enzyme
- Liver mitochondrial acyl-CoA dehydrogenase
- Liver mitochondrial HMG-CoA synthase
- Liver and adipocyte cytosolic PEPCK
- Activating adipocyte protein (aP2)
- Stearoyl-CoA Δ⁹ desaturase (mouse)

**Suppressed**
- Liver fatty acid synthase
- Liver apolipoprotein A1
- Liver apolipoprotein CIII
- Liver S14 protein gene

---

**Table 2.9 Rodent genes whose expression is controlled upon PPAR activation** (from Latruffe et al., 1997). Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; PEPCK, phosphoenolpyruvate carboxykinase.

activation) (Tobin et al., 2000), and rodent hepatocarcinogenesis (via hydrogen peroxide production) (Gonzalez et al., 1998; Holden et al., 1999).

PPARβ displays an ubiquitous pattern of expression. It is particularly present in the entire nervous system (hippocampus, retina, and cerebellum) and epidermis (in hair follicles and sebaceous glands, but not in the keratinocytes). PPARβ is also expressed in the entire digestive tract, but not in the smooth muscle surrounding it; in the liver, pancreas, nephron, lymphocyte proliferation centers of the immune systems (spleen and ileal Peyer’s patches), and the genital system. PPARβ, like PPARα, binds to a diverse spectrum of compounds, including PUFAs and carbaprostacyclin, a synthetic prostanoid (Forman et al., 1997). The function of PPARβ remains largely inconclusive but it has been proposed to be also involved in lipid metabolism, especially in inducing HDL cholesterol levels, as well as oligodendrocyte maturation and membrane sheet formation. A disruption of the PPARβ gene in mice is associated with alterations in myelination and epidermal proliferation (Peters et al., 2000).
Very high levels of PPARγ have been found in both white and brown adipose tissue. Several non-adipose tissues, such as mucosa of duodenum, retina, and lymphocyte proliferation centers of the immune system, also express PPARγ, but to a lesser extent. The three subtypes of PPARγ show a slightly different expression pattern: PPARγ1 is ubiquitously expressed, PPARγ2 is present in adipose tissue and colon epithelium, and PPARγ3 is expressed in macrophage, adipose tissue and colon epithelium.

PPARγ is a potent stimulator of adipocyte differentiation (Brun et al., 1997a, 1997b; Spiegelman et al., 1997; Tontonoz et al., 1995) and its expression is markedly increased early in this event (Chawla et al., 1994). Naturally occurring compounds, such as ω-3 polyunsaturated fatty acids, α-linoleic acid, eicosapentaenoic acid (EPA), and docosahexanoic acid (DHA) were shown to be active ligands for PPARγ (Krey et al., 1997; Kliewer et al., 1997; Forman et al., 1997). Among these, the arachidonic acid metabolite, 15-deoxy-A12, 14-prostaglandin J2 displays the highest affinity to PPARγ, and is found to be the most potent ligand (Forman et al., 1995; Kliewer et al., 1995). Recently a class of antidiabetic drug, the thiazolidinediones, has been identified as a synthetic PPARγ ligand (Auwerx et al., 1996). This suggests that PPARγ may also participate in the regulation of insulin resistance. Studies are undergoing to investigate the therapeutic implication of PPARγ agonists on non-insulin-dependent diabetes mellitus (Murakami et al., 1998; Shibata et al., 1999).

Several pieces of evidence point to the effects of PPARγ on inflammation, including macrophage differentiation and foam cell formation: (1) macrophage-derived foam cells of both early and advanced atherosclerotic lesions usually contain highly expressed PPARγ, (2) PPARγ ligands, 9-hydroxyoctadecadienoic acid (HODE) and 13-HODE are present in foam cells and oxidized low-density lipoproteins, known contributors to atherogenic plaques (Nagy et al., 1998; Tontonoz et al., 1998), (3) certain non-steroidal anti-inflammatory drugs (NSAID) act as high affinity ligands for PPARγ, and (4) treatment of monocytes with PPARγ agonists inhibits activation of macrophages and production of cytokines (TNFα, IL-1β, and IL-6) (Auwerx, 1999; Jiang et al., 1998; Ricote et al., 1998; Marx et al., 1998). PPARγ is believed to regulate the last process by limiting the production of transcription factors involved in cytokine expression, i.e., nuclear factor (NF)-κB, activating protein (AP)-1, and signal
transducers and activators of transcription (STAT). Potential roles of PPARγ in the cell cycle, apoptosis and carcinogenesis have been addressed. PPARγ is found to inhibit growth and induce apoptosis in breast (Elsner et al., 1998; Mueller et al., 1998) and prostate (Kubota et al., 1998; Mueller et al., 2000) cell line studies. However, additional studies are needed to evaluate the therapeutic potential of PPARγ in humans and in other types of malignancies.

2.2.5.6 Structure

PPAR, like other members of the nuclear hormone receptor superfamily, is characterized by five to six functional domains (Figure 2.5) (Bagchi, 1998; Desvergne et al., 1998; Escher et al., 2000; Schoonjans et al., 1996).

The N-terminal A/B domain is poorly conserved, and is composed of a constitutive ligand-independent activation function region (AF-1). The DNA binding domain (DBD) or C domain is the most conserved region. The DBD contains two zinc fingers that bind to a specific DNA sequence, called peroxisome proliferator response element (PPRE). Each zinc finger contains a zinc atom situated by 4 cysteine residues. One region on the C terminus of the first zinc finger, called P-box, determines specific contacts between the receptor and the DNA, while the other, D-box, which represents the region between the first two cysteine residues of the second zinc finger, is responsible for protein-protein interactions, such as receptor dimerization, and recognition of the spacing between half-sites. A non-conserved C-terminal extension of the DBD domain, D domain, is involved in protein bending or conformational change. The multifunctional ligand binding domain (LBD), E domain, is highly conserved and contains a ligand-dependent transactivation region (AF-2). The LBD is relatively large, which may explain the possibility of PPAR being activated by a spectrum of diverse compounds, and the various degree of regulation by the same ligand among the three isotypes. Besides ligand binding, the LBD is also known to be used for dimerization, nuclear localization signaling, intermolecular silencing, intramolecular repression, and heat-shock protein (HSP) association. The F domain is relatively small and is thought to be where the nuclear receptors interact with coactivators and corepressors (Qi et al., 2000).
2.2.5.7 Peroxisome Proliferator Response Element (PPRE)

Despite the great similarities in structure and function, nuclear hormone receptors can be divided into four groups based on their specific DNA binding and dimerization properties (Schoonjans et al., 1996).

Class I receptors contain the steroid hormone receptors which are located in the cytoplasm in association with HSP and translocate to the nucleus upon ligand binding. They bind as homodimers with high affinity to two DNA half-sites arranged as inverted repeats or palindromes. This group includes glucocorticoid, androgen, progesterone, mineralocorticoid and estrogen receptors. Class II receptors form heterodimers with retinoid X receptor (RXR) and bind to direct repeats (DR) separated by various numbers of bases or spacer nucleotides. Ligand activation is not required for nuclear translocation. Thyroid receptor, retinoic acid (RAR) receptor, vitamin D receptor and PPAR belong to this group. The class III subgroup interacts with DR as homodimers. This subfamily contains 9-cis retinoic acid receptor, HNF-4 and COUP/ARP. Class IV binds as monomers to a single hexameric core recognition motif, which contains flanking sequence in the 5' end. Examples of this receptor are Rev-erbα (EAR-1), Rev-erbβ (RVR), and SF-1. Most nuclear receptors belong to class I, II, and III, or generally categorized as the thyroid/retinoid receptor.
The specific DNA recognition sequence for the PPAR/RXR heterodimer is located in the promoter region of target genes, and is referred to as PPRE. PPRE contains DR of conserved AGGTCA hexamers separated by one nucleotide (thus named DR-1) (Tugwood et al., 1992; Umesono et al., 1991). The AGGTCA motif is recognized by the CEGCKG primary sequence of the P-box in PPAR. Studies have shown that the 5' flanking region of DR-1, the polarity of PPAR/RXR onto the DR, and the spacing nucleotide between the two hexamers are important for PPAR binding: (1) The PPAR/RXR binds to PPRE in such a manner that RXR occupies the 3' hexamer of PPRE while PPAR binds to the 5' extended half-site, (2) adenine is the preferred nucleotide spacing between the two half-sites (Desvergne et al., 1998; Ijpenberg et al., 1997; Palmer et al., 1995).

2.2.5.8 PPAR Regulation

Once PPAR is synthesized, its activity is subject to various ways of regulation (Figure 2.6), including (1) post-transcriptional modification, including phosphorylation and ligand binding, with the later discussed in the previous section; (2) protein-protein interaction, including interaction either with the same or a different family of transcription factors to form a DNA-binding dimer, or with cofactors (Calkhoven et al., 1996).

![Figure 2.6 Mechanisms of transactivation (Escher et al., 2000).](image-url)
CHAPTER 3

EFFECTS OF FEEDING DIFFERENT LEVEL AND TYPE OF DIETARY FATS ON BODY FAT DEPOSITION IN GROWING RATS.

3.1 Introduction

Obesity is rapidly emerging as an important global health problem, and has been recognized as a chronic disease. The first attempt in treating most chronic diseases, including obesity, is usually through dietary modification. However, preventing or curing obesity via dietary treatment is not always successful. Current dietary approaches to obesity prevention and treatment often have focused on both the amount and the type of dietary fat consumed.

Excess fat intake has been widely assumed to be a strong predictor of body weight change. Because dietary fat is energy dense, there is a tendency toward greater energy intake with high fat foods, when consuming diets with similar weight. The question of whether it is the percentage of energy from fat or the total energy intake that contributes to obesity remains controversial. Since some studies showed a lack of effect of macronutrient composition (especially the fat to carbohydrate ratio) on energy intake when food energy density was kept constant (Rolls et al., 1999a; Saltzman et al., 1997), it is possible that when accurate caloric compensation is achieved, as has been reported in lean or obesity-resistant animals and subjects, who are able to accurately adjust food self selection (Loh et al., 1998; Levin et al., 1989), dietary fat per se may not predict obesity.

The effect of fatty acid composition on obesity is of considerable interest but has not been fully understood. It is suggested that the unique composition and structure of each fatty acid can influence its own metabolism, including digestion, absorption, transport, tissue uptake, storage, and mobilization, and thus lead to different levels of fat oxidation and deposition.
It is well known that the earlier the adipose tissue development is induced, the more significant an effect is on body fat accumulation (Bonnet et al., 1981b). Therefore, if various types and levels of dietary fat induce body fat storage differentially, an early and sustained nutritional intervention involving manipulating level and type of fat consumed is likely to produce a profound difference in the degree of obesity. Since early-onset obesity has been reported to be associated with higher rates of morbidity and mortality in adulthood (Must et al., 1999), it is necessary to identify if dietary fat intake (level and type) influences obesity during early adipose tissue development, when both adipocyte size and number can be expanded rapidly.

The purpose of the present study was therefore to examine the prolonged effects of early exposure to different amounts and types of dietary fats on body energy regulation and obesity development in rapidly growing rats.

3.2 Methods

3.2.1 Experimental Considerations

The epididymal fat pad was used not only as an indirect indicator of body fat status, but also for experimental analysis. This site of adipose tissue has been widely used for studies of the adipocyte metabolism in animals, and its development is well documented. Several researchers found that in male rats, epididymal fat cell size and number continue to increase until about 3 months of age (Björntrop et al., 1982; Lemonnier, 1981). Thus, in the present study, weanling young male rats when fed the experimental diets for 5 weeks were in their 4th to 8th week of life, when both fat cell number and size were not yet completely fixed. This time frame provided us the opportunity to investigate the influence of dietary fats on adipose tissue mass, due to either hyperplasia (increased cell number) and/or hypertrophy (increased cell size) during a highly developing stage.

Human obesity studies conducted in free-living subjects are likely to be biased by the obesity-promoting environment, i.e., increased availability and variety of food supply, as well as socioeconomic factors. As a consequence, laboratory rats were chosen as the experimental model to control for the exogenous causes of weight gain.
3.2.2 Animals and Diets

The study was approved by the Ohio State University Animal Care and Use Committee, including the protocol for sacrificing animals. Studies using animals permit analysis of the whole liver and adipose tissue, which aid in the understanding of metabolic events occurring during development. After 5 weeks of \textit{ad libitum} feeding, the rats were killed, and epididymal fat pads and liver were excised, weighed, immediately frozen in liquid nitrogen, and stored at -70°C until analysis.

Forty-four 21-day-old male Sprague-Dawley rats obtained from Harlan (Indianapolis, IN) were housed individually under a 12-12 h light-dark cycle, lights on at 1am. Animals were acclimated to their new environment with an \textit{ad libitum}-fed rat Chow diet for two to five days (Figure 3.1) before random assignment to one of the eight diets: low-fat diet: SFA (n=5), MUFA (n=6), n-6 (n=5), n-3 (n=6), and high-fat diet: SFA (n=6), MUFA (n=5), n-6 (n=6), n-3 (n=5). All diets provided the same level per unit food energy of protein, vitamins, minerals, and fiber (Table 3.1).

The high fat diets were formulated by replacing carbohydrate with experimental lipids. The food products providing the experimental fats in both diets were as follows: high SFA, coconut oil (Dyets, Bethlehem, PA); high MUFA, high oleic, safflower oil (Hein Food Group, Uniondale, NY); high n-6, safflower oil (Dyets, Bethlehem, PA), and high n-3, flaxseed oil (Spectrum Essentials, Petaluma, CA). Briefly, the diet contained 17% energy as protein, 68% (LF) or 43% (HF) as carbohydrate, and 15% (LF) or 40% (HF) as fat. The ratio of simple to complex carbohydrate was kept constant and was based on the rodent AIN-93G diet to avoid effects of taste preference and rate of carbohydrate metabolism on energy intake (corn starch: Dyetrose: sucrose= 3.97: 1.32: 1, g/kcal). Each diet contained 6 g linoleic acid/kg diet as 11.3 g soybean oil (Dyets, Bethlehem, PA) per kg diet to provide essential fatty acid. \textit{t}-Butylhydroquinone (TBHQ) was added at 0.02 g/100 g lipid to prevent lipid oxidation. Food intake and body weight were monitored daily throughout the experiment.

3.2.3 General Methods

3.2.3.1 Body Composition Analysis

Epididymal fat pads were weighed and used as an indicator of adiposity. Carcasses [whole body- (liver + epididymal fat pads + gut + stomach + blood)] were kept at -20°C until use. They were autoclaved.
for 15 minutes in large beakers covered with aluminum foil, blended with equal amount of water in a
Waring blender until homogenous, and weighed. Homogenized samples (20g) were dried to a constant
weight to measure sample water content. Carcass body fat was determined by Soxhlet ether extraction
(Appendix A).

3.2.3.2 Lipid Extraction and Fatty Acid Analysis

Total lipids were extracted from liver according to Folch et al. (1957). Aliquots of lipids were
converted to fatty acid methyl esters (FAME) using 14% boron trifluoride (BF$_3$)-methanol. TG fractions
obtained by thin-layer chromatography (TLC) separation of total lipids were scraped and subjected to
transesterification directly on the silica. FAME were separated and quantitated by gas-liquid
chromatography (GC) using triheptadecanoin (C17:0) as the internal standard (Appendix B).

3.2.3.3 Serum TG and Cholesterol Analysis

Serum TG and cholesterol were measured enzymatically by Sigma Infinity Triglyceride Reagent
and Infinity Cholesterol Reagent, respectively (St. Louise, MO) on frozen samples (Appendix C, D).

3.2.3.4 Serum Leptin Analysis

Blood samples were taken by cardiac puncture right after animals were sacrificed with CO$_2$. Serum samples were separated by centrifugation at 500×g for 20 minutes and kept at -70°C until analyzed
by a murine leptin ELISA kit (Diagnostic System Laboratories, Webster, TX) (Appendix E).

3.2.4 Statistical Analysis

Data were analyzed using the Statistical Analysis System (SAS)-PC for WINDOWS (version 8.0;
SAS Institute Inc., Cary, NC). Values were expressed as mean±SD. Two-way ANOVA was used to assess
effects of type and amount of fat on all variables. If no interactions were observed, comparison of group
mean was then performed by t-test to investigate the influence of level of fat, and one-way ANOVA
followed by Tukey's multiple range test to analyze the effect of type of fat. The level of significant
difference was set at p<0.05.
Figure 3.1 Experimental protocol timeline. Rats arrived on day 0. Semi-purified powder diets were fed for 35 days, starting from day 2 (SFA), day 3 (MUFA), day 4 (n-6 PUFA), and day 5 (n-3 PUFA). Rat Chow diet was fed before the experimental diets. Rats were killed and samples were taken on day 37 (SFA), day 38 (MUFA), day 39 (n-6 PUFA), and day 40 (n-3 PUFA).
<table>
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</tbody>
</table>

Table 3.1 Composition of experimental diets. The four experimental oils used were coconut oil high in SFA, safflower oil rich in MUFA or n-6 PUFA, and flaxseed oil rich in n-3 PUFA. Antioxidant was added at 0.02g/100g lipid.
3.3 RESULTS

3.3.1 Food intake

Figure 3.2 shows the changes in intake in grams and kcal/day of rats fed a low (LF) and high (HF) amount of different types of dietary fats. When the food intake is expressed in grams, both the main effect of type (p=0.02) and level (p<0.0001) was significant. However, there was no type-by-level interaction (p=0.41) for food intake in this group (Panel A). All rats fed LF diets exhibited higher food consumption (p<0.05) than HF, and coconut oil-fed rats ate significantly less food than flaxseed oil-fed rats when both fats were fed at 15% energy level (p<0.05). However, when food intake was expressed as kcal/d (panel B), only the effect of the type of dietary fat was significant (p=0.02). The significant difference in daily food energy intake was seen only in the LF group, in which the caloric intake was significantly lowered in the SFA diet than in the n-3 diet (p<0.05).

3.3.2 Body weight gain and feed efficiency ratio

Body weight gain was measured daily and is depicted in Figure 3.3. There was no main effect of type (p=0.52) and level (p=0.06), and the type-by-level interaction (p=0.06) for net body weight gain (g) did not quite reach significance (Figure 3.4). However, body weight gain was significantly attenuated by LF-SFA when compared to LF-MUFA, PUFAs, and HF-SFA (p<0.05).

After adjusting daily body weight gain for daily energy intake by calculating the feed efficiency ratio (FER), only the main effect of level was observed (p=0.05) (Figure 3.5). The significant difference in FER was found only in the SFA groups, in which HF feeding resulted in a higher value (p<0.05).

3.3.3 Body composition

Epididymal fat was used as an indirect evidence of body fat deposition and accumulation. For epididymal fat wet weight per final body weight (%), the main effect of type (p=0.002) and the type-by-level interaction (p=0.02) were both significant. For percent carcass body fat (carcass fat vs. final body weight), no main effect of type (p=0.25) or level (p=0.67) was observed, but the interactive effect was significant (p<0.05).

Effects of amount and type of dietary fat on percent epididymal fat and body composition are shown in Figure 3.6 and Figure 3.7, respectively. Both dietary fat type and level were important

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determinants of body composition. When dietary fat was consumed at 15% daily energy level, SFA, MUFA and PUFAs are equally effective in partitioning food energy into epididymal fat and carcass total body fat, regardless of body size.

However, when fat intake was increased to 40% energy, more dietary energy was partitioned into epididymal fat by SFA (p<0.05), and more into carcass lipid by both SFA and MUFA (p<0.05) under similar calorie intake and body weight gain.

HF-SFA caused an increase in percent epididymal fat and percent carcass body fat as compared to LF-SFA (p<0.05). A significantly higher proportion of carcass lipid was also observed in rats fed HF-MUFA than LF-MUFA (p<0.05), and in rats fed LF-n-3 than HF-n-3 (p<0.05).

3.3.4 Liver lipid profile

3.3.4.1 Liver total lipid levels

The total lipid content of liver is given in Figure 3.8. Two-way ANOVA showed that the liver total lipid levels were significantly influenced by fat level and type (p<0.05), and the effect of type was highly dependent on the level of fat, as indicated by the significant interaction (p<0.05).

In LF-fed animals, both MUFA and n-6 PUFA induced a greater increase in liver lipid content (6.25±0.30, and 6.75±0.64, respectively) than SFA (5.09±0.60) and n-3 PUFA (4.71±0.26) (p<0.05). As dietary fat level increased, all HF diets were able to significantly enhance liver total lipid concentration (p<0.05). The highest levels were observed in rats fed MUFA (24.57±2.37) and n-6 (28.52±3.29), in comparison to SFA (19.96±2.50) and n-3 (12.57±0.82). This increase was considerably lower in the n-3 than in the SFA group, resulting in a 0.6-fold lower liver lipid content when compared to SFA (p<0.05).

3.3.4.2 Fatty acid composition of liver total lipid

Table 3.2 shows the detailed fatty acid composition (%) of the lipids extracted from rat liver fed a low (LF) and high (HF) amount of different types of dietary fats. It is observed that the relative proportions of particular fatty acids (12:0, 18:1n-9, 18:2n-6, 18:3n-3) reflected the dietary intake of that particular type of fat. The relative content of most major fatty acids (12:0, 16:0, 18:1n-9, 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3, 22:6n-3) were affected by dietary fat type, level and the interactive effect of type and level (p<0.05).
Data summarized by grouping fatty acids into saturates, monounsaturates, and n-6 and n-3 polyunsaturates are listed in Figure 3.9. The main effects of type, level and the type-by-level interaction were all significant (p<0.05), indicating that the effect of type was highly dependent on the level of fat, and vice versa. In the rat liver total lipid, trace amounts of n-3 PUFA were found in coconut, high-oleic and high-linoleic safflower oil-fed animals, suggesting the existence of de novo synthesis from the desaturation and elongation pathways. The highest n-3 PUFA level was found in rats fed flaxseed oil, mostly in the 18:3n-3 and EPA fractions. Of rats fed safflower oil rich in linoleic acid, there were increased 18:2n-6, arachidonic acid, and total n-6 PUFA, and decreased EPA and DHA (p<0.05). With high-oleic safflower oil feeding, the proportion of 18:1n-9 in liver total lipid was significantly increased (p<0.05).

HF intake resulted in an increased proportion of a particular fatty acid based on the type of experimental diet consumed. As a consequence, there was a 4.8-fold increase of 12:0 in the SFA group (p<0.05), a 1.7-fold increase of 18:1n-9 in the MUFA group (p<0.05), a 1.8-fold increase of 18:2n-6 in the n-6 PUFA group (p<0.05), and a 2.6-fold increase of 18:3n-3 in the n-3 PUFA group (p<0.05), compared to their LF groups individually.

3.3.4.3 Liver TG levels

Changes in liver TG in relation to fat level were significantly different among the fatty acids, as indicated by the significant type×level interaction (p<0.05). The main effect of fat level was significant (p<0.0001). The effect of fat type on liver TG was observed in the HF groups, but not in the LF groups (Figure 3.8). PUFA rats had significantly lower liver TG levels (7.64±2.55 for n-6; 6.42±0.66 for n-3) than MUFA rats (10.93±0.99), whose levels were significantly lower than that of SFA rats (14.22±0.98) (p<0.05).

The main effect of fat level on liver TG was also significant (p<0.0001), but was not the same for all the fatty acids. HF intake led to significantly increased liver TG only in the SFA, MUFA and n-3 (p<0.05) but not in the n-6 (p=0.06) group, as compared to LF.

3.3.4.4 Fatty acid composition of liver TG

The fatty acid composition of liver TG, analyzed by GC of rats fed a low (LF) and high (HF) amount of different types of dietary fats is shown in Table 3.3. The proportions of total saturates,
monounsaturates, and n-6 and n-3 polyunsaturates for each group are given in Figure 3.10. As was observed in liver total lipid, the composition of fatty acid in the TG fraction also reflected the composition of dietary oils. Thus, the proportion of SFA, monoenes, n-6 and n-3 PUFA was substantially higher in rats fed coconut oil, high-oleic safflower oil, high PUFA safflower oil and flaxseed oil, respectively.

3.3.5 Serum lipid profile

3.3.5.1 Serum TG levels

Two-way ANOVA showed that no main effect of type (p=0.21) or type-by-level interaction (p=0.27) for serum TG was observed. For LF groups, serum TG levels were 67.1±19.5, 65.0±23.3, 63.8±15.3, and 67.8±20.6 mg/dl for SFA, MUFA, n-6 and n-3, respectively; and for HF groups, 120.8±28.1, 89.8±34.0, 80.6±17.7, 83.0±32.5 mg/dl. A significantly higher serum TG was associated with HF-SFA, as compared to LF-SFA (p<0.05) (Figure 3.11).

3.3.5.2 Serum cholesterol levels

The effects of dietary fat level and type on serum cholesterol levels (mg/dl) in rats fed a low (LF) and high (HF) amount of different types of dietary fats are given in Figure 3.12. There was no significant effect of type-by-level interaction (p=0.71). When each dietary group was modeled separately, there was significant difference among and within dietary groups (type effect, p<0.0001; level effect, p=0.0067). Rats fed n-3 had significantly lower serum cholesterol concentrations than those fed SFA, after both LF and HF consumptions. The decreased serum cholesterol by n-3 as compared to MUFA and n-6 was considerable only in the HF feeding groups (p<0.05).

3.3.5.3 Serum leptin levels

Figure 3.13 depicts dietary treatment (amount and type of fat) for serum leptin concentrations. Two-way ANOVA showed that there was no significant interactive effect of type and level (p=0.57), nor was there a significant effect of type (p=0.34) and level (p=0.06) on serum leptin levels. Thus, no difference in serum leptin was observed throughout groups.

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Figure 3.2 Average daily food intakes in grams (A) and kcal (B) of rats fed a low (LF) and high (HF) amount of different types of dietary fats. Data were subjected to two-way ANOVA for significance of effects due to level and type of dietary fat. Mean values with different superscripts are significantly different among fat types \((p<0.05)\). Mean values with * are significantly different within fat levels \((p<0.05)\). Error bars are SD.
Figure 3.3 Daily weight gains of rats fed low fat diets (panel A) or high fat diets (panel B) on different types of dietary fats. Weight gain was measured daily during the 35-day study period.
Figure 3.4 Final body weight gains of rats fed a low (LF) and high (HF) amount of different types of dietary fats. Data were subjected to two-way ANOVA for significance of effects due to level and type of dietary fat. Mean values with different superscripts are significantly different among fat types (p<0.05). Mean values with * are significantly different within fat levels (p<0.05). Error bars are SD.
Figure 3.5 Feed efficiency ratio (FER) in rats fed a low (LF) and high (HF) amount of different types of dietary fats. FER was defined as average daily weight gain (g) / average daily food intake (kcal) × 10^2. Data are subjected to two-way ANOVA for significance of effects due to level and type of dietary fat. Mean values with * are significantly different within fat types (p<0.05). Error bars are SD.
Figure 3.6 Percent epididymal fat pad of rats fed a low (LF) and high (HF) amount of different types of dietary fats. Epididymal fat pad wet weight (g) / final body weight (g) × 100. Data were subjected to two-way ANOVA for significance of effects due to level and type of dietary fat. Mean values with different superscripts are significantly different among fat types (p<0.05). Mean values with * are significantly different within fat levels (p<0.05). Error bars are SD.
Figure 3.7 Carcass body composition (A) and carcass lean body mass (B) in rats fed low (LF) or high (HF) amount of different types of dietary fats. % Carcass body fat = carcass fat mass (g) / final body weight (g) × 100. % Carcass lean body mass = [total carcass body weight (g) - carcass fat mass (g)] × 100. Data were subjected to two-way ANOVA for significance of effects due to level and type of dietary fat. Mean values with different superscripts are significantly different among fat types (p<0.05). Mean values with * are significantly different within fat levels (p<0.05).
Figure 3.8 Liver total lipid (TL) and TG levels of rats fed a low (LF) or high (HF) amount of different types of dietary fats. Data were subjected to two-way ANOVA for significance of effects due to level and type of dietary fat. Mean values with different superscripts are significantly different among fat types (p<0.05). Mean values with * are significantly different within fat levels (p<0.05).
Figure 3.9 Fatty acid composition (percentage) of liver total lipids in rats fed a low (LF) or high (HF) amount of different types of dietary fats. Data were subjected to two-way ANOVA for significance of effects due to level and type of dietary fat. Mean values with different superscripts are significantly different among fat types (p<0.05). Mean values with * are significantly different within fat levels (p<0.05).
Figure 3.10 Fatty acid composition (percentage) of liver TG in rats fed a low (LF) or high (HF) amount of different types of dietary fats. Data were subjected to two-way ANOVA for significance of effects due to level and type of dietary fat. Mean values with different superscripts are significantly different among fat types (p<0.05). Mean values with * are significantly different within fat levels (p<0.05).
Figure 3.11 Serum TG levels in rats fed a low (LF) and high (HF) amount of different types of dietary fats. Data were subjected to two-way ANOVA for significance of effects due to level and type of dietary fat. Mean values with * are significantly different within fat types (p<0.05). Error bars are SD.
Figure 3.12 Serum cholesterol levels in rats fed a low (LF) and high (HF) amount of different types of dietary fats. Data were subjected to two-way ANOVA for significance of effects due to level and type of dietary fat. Mean values with different superscripts are significantly different among fat types (p<0.05). Error bars are SD.
Figure 3.13 Serum leptin levels in rats fed a low (LF) and high (HF) amount of different types of dietary fats. Data were subjected to two-way ANOVA for significance of effects due to level and type of dietary fat. Error bars are SD.
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**Table 3.2** Fatty acid composition of liver total lipid in rats fed a low (LF) and high (HF) amount of different types of dietary fats. Represented as percent of total fatty acids (%). Values are mean±SD.
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<td>22:5n-3</td>
<td>0.1±0.2a</td>
<td>0.0±0.1a</td>
<td>0.1±0.1a</td>
<td>3.0±1.2b</td>
<td>0.2±0.0a</td>
<td>0*</td>
<td>0.0±0.0*</td>
<td>2.8±0.5b</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>0.6±0.2*</td>
<td>0.2±0.1a</td>
<td>0*</td>
<td>1.5±0.8b</td>
<td>0.7±0.1a</td>
<td>0*</td>
<td>0.1±0.0b</td>
<td>1.4±0.4b</td>
</tr>
</tbody>
</table>

Table 3.3 Fatty acid composition of liver TG in rats fed a low (LF) and high (HF) amount of different types of dietary fats. Represented as percent of total fatty acids (%). Values are mean±SD.
3.4 Discussion

3.4.1 Food Intake

In the present study, weanling rats fed the calorie dense high fat diets for 45 days decreased their food intake, and thus did not have a higher energy intake than rats fed the low fat diets in any of the fatty acid subgroups. This result suggests that during early development, rats can adjust food intake to avoid any alterations in total energy intake, which is essential for normal growth. Likewise, no difference in body weight gain was observed between low fat and high fat groups except for rats fed the SFA-containing diets, in which a significant increase in body weight was seen with high fat feeding. Although researchers have shown that animals consuming a high fat diet increase body weight or adiposity or both, either dependent (Levin et al., 1993; Ramirez et al., 1989; Naim et al., 1985) or independent (Schemmel et al., 1969; Mickelson et al., 1955) of energy intake, this is not universally the case. The inconsistent results may arise from several factors, including the strain and age of the animals used, the duration of the study, and the diet composition.

Depending on the strain of the animals, high fat-induced increase in energy intake can vary from 5% to over 25% (Kanarek et al., 1988). When fed an energy dense and palatable cafeteria diet, body weight gain and body fat were reported to be greater in Sprague-Dawley and Alderly Park rats than in Lister rats (Rothwell et al., 1982). This suggests that the rat model (Sprague-Dawley) used in the present study is more susceptible to dietary manipulations, and that genetic influences need to be considered when comparing studies.

How animals respond to the high fat diets is also affected by age. Various studies have suggested that young animals are less likely to develop obesity than older animals when fed a high fat diet, and early exposure to high fat feeding in non-obese rats does not always predispose animals to obesity later in life. Only when high fat feeding is sustained for several months does obesity occur (Brooks et al., 1999; Iglesias et al., 1986; Rothwell et al., 1982a). The accompanying biological events responsible for the different responses to high fat diets as a function of age in growing rats are not fully documented. Both morphologic alterations in adipose tissue and metabolic changes in most tissues have been identified to be associated with age.
In growing rats, early exposure to a cafeteria diet results in an increased brown adipose tissue mass, due to both hypertrophy and hyperplasia (Rothwell et al., 1982a; Rothwell et al., 1982b). This greater thermogenic capacity may explain the higher energy expenditure, and thus the resistance to obesity at a younger age.

Metabolic patterns may also be influenced by age. Studies in rodents have shown that when exposed to high fat diets, young animals displayed an increased liver fatty acid synthesis even though dietary fat supply is high (Gaiva et al., 2001; Brooks et al., 1999; Volpe, 1978). Increased muscle glucose oxidation and liver glycolysis have also been reported in rats (Brooks et al., 1996). In addition, it is estimated that young animals had a 50% higher metabolic rate than older animals (Brody, 1945). Taken together, there is a greater increase in body energy storage in young animals than in adult animals. These observations suggest that young animals have an increased need for fat and energy during development to compensate for the higher metabolic rate. Therefore, young animals, at least in rodents, may not develop obesity as readily as adult animals when fed an obesity-producing diet.

In this study, a high intake of the SFA produced a significantly higher weight gain than the low fat SFA diet. This result is mostly accounted for by an increased body fat mass, as indicated by a significant increase in both epididymal fat pad and percent carcass total fat. Since similar calorie intake was observed, the excess body fat storage can not be explained by a loss of control of appetite and satiety, as have been assumed for the high-fat induced obesity in some rodent studies (Sclafani et al., 1992). The most likely mechanism is an alteration in feed efficiency and energy expenditure, which may be attributed to the high MCT content of coconut oil, the major SFA source of the experimental diet.

It is known that MCT are absorbed and oxidized more readily than LCT, and have the potential of enhancing energy expenditure and TEF (Dulloo et al., 1996; Hill et al., 1989). Controversy remains whether a diet high in MCT relative to LCT protects against obesity, and whether this property can be explained by a modulation of food intake. Previous studies on the effect of MCT as opposed to LCT consumption on energy intake showed inconsistent results. Both no change and decreased food intake have been reported, as summarized by Bach et al. (1996).
The mechanisms responsible for the regulation of dietary intake by MCT have not been elucidated. It is suggested that first, MCT possess less energy density than LCT (Livesey et al., 1988). Second, MCT are less palatable than LCT. Although MCT are clear, light, and odorless, MCFA exhibit a goat smell (thus the name caproic, caprylic and capric acids). Since lingual lipase is very active on MCT (Hamosh, 1984), the special taste and smell generated during chewing when consuming a MCT diet may affect food intake as the taste of food has a big impact on satiety and eating. Third, MCT may decrease food intake by sparing carbohydrate. According to the Randle cycle, increased fat oxidation by MCT may spare carbohydrate oxidation and thus delay the onset of hunger thereby reducing food intake (Wymelbeke et al., 2001; Flatt et al., 1985). Fourth, differential secretion of postabsorptive substances between MCT and LCT may modulate satiety differently. MCT, which is more likely to increase insulin secretion, induce mild hypoglycemia, reduce hepatic glucose output, and increase hepatic ketone bodies (Nakamura et al., 1994; Yeh et al., 1976), may prolong the feeling of satiety (Carpenter et al., 1983). Finally, MCT may reduce food intake by producing gastrointestinal discomfort (MacDonald et al., 1985).

Our data showed that the 15% coconut oil diet produced a lower energy intake than 15% safflower and flaxseed oils. This result suggests that even though the natural source of MCT, coconut oil, is clear, light, colorless, and contains a light sweet coconut taste and some natural aroma, it may be as effective as MCT oil in influencing appetite (Edans et al., 1984). However, it is not known if differences in food perception among experimental oils is secondary to body metabolic changes, or it is the food preference that influences feeding, and the subsequent metabolic consequences. On the other hand, the rats fed the diet containing 40% coconut oil did not alter energy intake as compared to 40% safflower and flaxseed oils, but gained significantly more body fat. Therefore, the results from the low fat group agreed with most studies that replacement of LCT with MCT reduced energy intake, but data from the high fat group showed no effect. It is likely that the age of the animals and the composition of the diet may contribute to the differences as Kaunitz et al. (1958) also found no effect of 20 or 30% energy MCT or lard on energy intake in rapidly growing rats.

The underlying mechanisms for the lack of an effect of high intake of MCT on food intake are not clear. Because previous studies showing that MCT inhibited food intake were mostly observed in the short-
term, and only a few in the long-term studies, it is likely that the disappearance of dietary intake regulation was due to an adaptation of the rats to the MCT diet. If this is the case, the reasons for why the adaptation occurs only with high intake of MCT need to be investigated.

3.4.2 Body Weight and Body Composition

Previous studies investigating the effect of dietary fat composition on the development of obesity have not reached a conclusion. High intake of SFA as compared to PUFA has been shown to produce a higher (Shimada et al., 1995; Birt et al., 1990), lower (Gaiva et al., 2001; Pan et al., 1993), or similar (Degrace et al., 1998; Cha et al., 1996; Gaiva et al., 1996; Hill et al., 1993; Su et al., 1993; Awad et al., 1990; Shimomura et al., 1990; Bourgeois et al., 1983; Lemmonier et al., 1973) body weight change. Body fat mass or distribution has been examined in some studies; results indicated either an increase (Shillabeer et al., 1994; Belzung et al., 1993; Hainault et al., 1993; Hill et al., 1993; Bourgeois et al., 1983), a decrease (Toyomizu et al., 1992; Shimomura et al., 1990) or no change (Gaiva et al., 2001; Cha et al., 1996; Hill et al., 1993; Su et al., 1993; Lemmonier et al., 1973) in body fat level with high SFA than with high PUFA consumption. Inconsistent results may arise from a number of factors, such as the chain length and the number of double bonds present in the experimental oils.

The effect of fatty acid chain length on body weight control has long been investigated since the 1950's (Kaunitz et al., 1958). However, no conclusion has been reached to date. As summarized by Bach et al. (1996), about two-thirds of the literature reported reduced body weight while about one-third of the studies found no change in body weight with MCT consumption as compared to LCT. Again, these discrepant results can stem from methodological differences. (1) Most of the studies comparing the effect of MCT vs. LCT on body weight were performed on animals, mostly rats, and notably Zucker obese rats (Max et al., 1983). Although it is easier to manipulate diet in animal models, the metabolic response to MCT can be different from that in humans. (2) Adaptation to MCT diets in the long-term has been shown, which is manifested by a trend toward decreasing plasma ketone body concentration (Chanez et al., 1991; Crozier et al., 1987), and in some studies but not all an increasing fasting TG level (Hill et al., 1989) and a decreasing postprandial energy expenditure (White et al., 1999; White et al., 1996). These results may explain why MCT has marked impact on reducing body weight mostly with short-term feeding. (3) Young
animals have been reported to manipulate food energy and energy expenditure more efficiently than older animals. It is suggested that results obtained from young animals may not be able to be extrapolated to older animals. In our study, 3-wk-old rats were at their rapid growing stage. It is possible that the early onset of any disturbance in their growth rate (e.g., lower calorie intake in the LF-MCT group) may produce a more profound effect on body weight after a long feeding period. (4) The type of LCT used, being either corn, olive, soybean, sunflower, lard, or fish oil, may also account for the differences in body weight change. (5) It is plausible to consider the amount of dietary lipid as one of the contributing factors. Bach et al. (1996) have proposed that for MCT to prevent body weight gain, a threshold of 50% energy needs to be reached. This assumption may explain why 40% energy intake of MCT in our study failed to show an anti-obesity effect, but not when 15% energy of MCT was consumed. Due to the accompanying poor compliance and potential detrimental effect on blood lipid profile with such a high and long-term MCT intake, MCT may not be a favorable dietary treatment for obesity in humans.

Our results showed that during the low fat regimen, percent epididymal fat mass and body composition in rats fed MCT and LCT were similar. However, weight gain was markedly reduced with MCT. Since total calorie intake was also significantly lowered in rats fed MCT, changes in body weight can be secondary to the lower energy intake. Indeed, when body weight gain was adjusted for energy intake, no difference in feed efficiency ratio (g/kcal) was observed among fatty acids. Nevertheless, the effect of MCT on body weight gain reflects a rapid response. As early as the tenth day of the experiment, MCT had already induced a significantly lower body weight change as compared to MUFA and PUFA.

Contrary to the low fat group diets, higher MCT and LCT produced similar food energy intake, and were equally effective in partitioning food energy into body tissue. Since no effect of fatty acid type was found in FER at either 15% or 40% at intake, it is important to consider fat level in study comparisons, as well as in determining the causal effect of dietary fat on the development of obesity.

The effect of fat level on a parallel alteration of both body weight change and carcass lipid content was observed only in the SFA groups. Rats consuming the high fat diet had significantly higher body weight gain, which is a result of higher body fat mass. The possible explanation is an increased hepatic de novo lipid synthesis due to the high level of dietary MCT content. Decreased fat synthesis is usually
observed when a low fat diet is replaced with a high fat diet (Sul et al., 1998; Hillgartner et al., 1995). It has been noted that MCT are less efficient than LCT in down-regulating lipid synthesis when consumed at a high level (Bach et al., 1996; Chanez et al., 1991; Chanez et al., 1988). In parallel with this finding are alterations in enzyme activity and substrate concentrations that favor fatty acid synthesis after a MCT diet: less inhibition of acetyl-CoA carboxylase by medium-chain fatty acyl-CoA (Bortz et al., 1963); higher concentration of malonyl-CoA (Pégorier et al., 1988), and higher activity of lipogenic enzymes, such as fatty acid synthetase (Foufelle et al., 1992). Since this difference in weight gain was observed at the end of the 35-day study period, but not on the tenth day, the potential for high fat-MCT to induce a higher weight gain than low fat MCT may be time dependent. Adaptation to high level of MCT was also observed by Hill et al. (1993), who found that in rats with 45% energy intake from MCT, a higher body weight was detected at the last half, but not the first half of the six-month study, when compared to lard, corn and fish oil.

The effect of the degree of saturation of dietary fats on the magnitude of body weight and body fat mass alteration is also uncertain. A high SFA-containing diet as compared to a diet with high unsaturated fatty acid has been found to increase, decrease, or have no influence on body weight or body fat mass. Again, inconsistent results can be due to the various experimental designs among studies.

The present data show that when dietary fat supplies 15% of daily energy, SFA, MUFA and PUFA had similar feed efficiency ratio and body mass increments. However, with 40% of energy intake as fat, epididymal fat mass was higher in the SFA diet-fed group, whereas carcass body mass was higher in both SFA and MUFA dietary groups. The increased total carcass body fat but not epididymal fat with MUFA suggests that there is a variation in fat deposition among depots.

It is well established that average size and metabolic events (Lefebvre et al., 1998) can vary among fat depots in rats and humans. For example, visceral or upper body obesity is more closely linked to obesity-related disease. According to a study by Fried et al. in 1982, adult male Wistar rats had significantly larger cell size in perirenal than in epididymal and subcutaneous adipose tissues. When rats were fed different types of dietary fats, Hill et al. (1993) found larger retroperirenal and smaller inguinal adipose depots with lard than with corn oil consumption at 45% energy. Okuno et al. (1997) found no difference in perirenal fat but significantly lower epididymal fat when perilla oil rather than beef tallow and
olive oil was fed at 26.2% energy intake. These results imply that the distribution of body fat can be modified nutritionally, and that it is as important a determinant as the degree of body adiposity in inducing metabolic problems. However, it is not clear why each regional fat depot responds to nutritional and metabolic stimuli differently.

The lower body weight and body mass increment in rats fed n-3 PUFA is consistent with previous studies (Fickova et al., 1998; Pan et al., 1993), however, only in the high fat group. It is possible that the amount and the type of n-3 fat consumed may account for the discrepancies. A study by Awad et al. (1990) showed that fish oil, safflower oil and beef tallow had similar effects on food consumption, final body weight, adipose tissue lipolysis and lipogenesis when fed to adult rats for 4 months at 16% of total energy (14% fish oil plus 2% corn oil). However, Tsuboyama-Kasaoka et al. (1999) found a significant effect of 5-month 60% fish oil consumption on suppressing body weight gain and parametric white adipose tissue increment when compared with 11% high-oleic safflower oil in adult mice. Since the level of dietary fat in our study (15% energy) was similar to that of Awad’s, the anti-obesity effect of n-3 fatty acid may depend on the amount consumed.

Previous studies examining the effects of n-3 fatty acid on body weight or body composition mostly used fish oil, and in some cases, perilla oil (Okuno et al., 1997), which contains predominantly very long chain fatty acid (EPA and DHA) and ALA, respectively. Several of these studies suggest a beneficial effect of n-3 on body weight and composition (Mori et al., 1999; Cha et al., 1996). However, the effect of flaxseed oil, another major dietary source of ALA, is less well studied. Since cellular processes related to fish oil are usually attributed to its high contents of EPA and DHA, with the amount of EPA in excess of DHA, and that a high conversion of ALA to EPA has been shown to lower the production of DHA, as was observed in this study, further investigations are needed to examine the relative importance of EPA and DHA in the regulation of body lipid metabolism.

Differences in body composition can also result from an alteration of dietary fat content. Higher body adiposity was observed in high fat-fed animals that consumed SFA and MUFA, and in low fat-fed rats that consumed n-3. Since these differences are independent of energy input, high intake of SFA and MUFA and low intake of n-3 PUFA were more efficient in partitioning food energy into body fat.

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The finding that a diet containing low but not high amounts of n-3 caused a significantly higher body fat was surprising as high fat diets have a greater potential in promoting body fat gain. This unexpected result may be due to the existence of minor components other than fatty acids in flaxseed oil.

Flaxseed, which means “the most useful plant” in Latin, was recognized only recently for its health benefits, e.g., cardiovascular disease and certain cancer prevention (Thompson et al., 1996). Flaxseed oil is one of the most abundant sources of n-3 fatty acid, containing twice the amount found in fish oil. It also provides the richest source of phenolic compounds, such as the plant lignan, secoisolariciresinol diglycoside (SDG). SDG is a phytochemical that is used for mammalian lignan production via the gut microflora. Mammalian lignans possess both estrogenic and anti-estrogenic properties. Since phytoestrogens have been reported to alter hormone levels, and sex hormones are known to affect regional fat deposition, a disturbance of hormone balance may be responsible for the unpredicted results observed. Especially in the present study, dietary fats were provided to growing rats until puberty, during which time body development is more sensitive to hormone status, and changes in fat distribution and deposition are more likely to occur. If this is the case, further studies examining the effect of lignan per se, and the optional intake level of lignan and/or 18:3 on body lipid homeostasis are needed, as a high intake of 18:3 was more effective in lowering body fat deposition in our study. Since n-3 fatty acid consumption is low in most North American diets, flaxseed oil may provide an alternative way of increasing n-3 intake. However, safety issues regarding its daily dosage and its constituent phytochemicals has yet to be determined. Due to the limited conversion of 18:3 to EPA in the body, 18:3 may not be a total substitute for fish oil. Since the occurrence of liver lipid oxidation was caused less by flaxseed than by fish oil (Schumann et al., 2000; Javouhey et al., 1993), the optional ratio between fish oil and 18:3-rich flaxseed or perilla oil may provide a better health promoting effect than either of the dietary oils alone.

**3.4.3 Fatty Acid Composition**

The fatty acid composition of the diet has a profound effect on the fatty acid composition of the liver. Therefore, the relative proportions of total saturates, monoenes, n-3 PUFA and n-6 PUFA in the TG fraction and in total lipid of liver were similar to the composition of the diet. In the present study, either with low or high fat consumption, feeding rats safflower oil (high in 18:2n-6, LA) increased the level of

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total n-6 PUFA, LA, and AA, but not EPA and DHA. Rats fed flaxseed oil (rich in 18:3n-3, ALA) showed increased total n-3 PUFA, ALA, and EPA but reduced AA and DHA. This can be explained by the finding that both LA and ALA compete for the enzyme, Δ6-desaturase (Figure 3.14) (Teitelbaum et al., 2001; Nassar et al., 1986). Therefore, a large intake of LA inhibited the production of EPA and then DHA by competing with 24:5n-3 for Δ6-desaturase. Similarly, ALA in the flaxseed oil decreased the levels of AA by suppressing the conversion of LA to AA.

However, whether the inhibition of DHA production by LA and ALA is through their competition for the identical Δ6-desaturase remains debated (Innis et al., 1999; Marzo et. al, 1996). It has been suggested that a distinct Δ6-desaturase, selective for 24-carbon PUFA, may exist. This idea is supported by the findings that the inhibitory effect of 24:5n-3 on the desaturation of 18:3n-3 to 18:4n-3 is less than that of 18:3n-3 on 24:5n-3 desaturation to 24:6n-3 in rat hepatocytes (Geiger et al., 1993). Therefore, the lower proportion of DHA in liver total lipid with meals provided high ALA as compared to low ALA may be due to increased inhibition of 24:5n-3 desaturation by the higher level of ALA intake. On the contrary, the proportion of ALA in the liver TG and total lipid was greater when its presence in the diet was high.

Since the existence of DHA in the diet can inhibit LA and ALA desaturation (Brenner et al., 1975), it is suggested that the fraction of DHA in the flaxseed oil groups may be higher than if fish oil was used. In addition, under our experimental conditions, the rate of desaturation may be regulated by the competition between LA and ALA rather than feedback inhibition by DHA, under the assumption that the same Δ6-desaturase is used for the desaturation of LA, ALA and 24:5n-3. In a similar way, although our data showed that ALA of flaxseed oil produced a lower AA level, the magnitude of suppression may be lower than that of longer chain n-3 fatty acids (e.g., fish oil), as the latter appears to be more effective than ALA in inhibiting AA synthesis (Hwang et al., 1988).

A significantly higher DHA was found in the low ALA than in the high ALA group. As pointed out by Bourre et al. (1989) in rats, the DHA level may reach a plateau when the intake of ALA beyond 200mg/100g food. In our experiment, both low and high ALA groups, which provided 5.1g and 18.2g flaxseed oil/100g diet, or at least 266.7mg and 952mg ALA, respectively, (without the consideration of ALA from soybean oil) were over the 200mg threshold. Since DHA is an important mediator in several
Figure 3.14 Pathway for the metabolism of polyunsaturated fatty acids (Teitelbaum et al., 2001).
cellular processes, such as brain and retina tissue development, its concentration should be tightly regulated. Therefore, the higher extent of down-regulation of DHA levels by an excess ALA intake may represent a cell's defense mechanism. Adverse effects of large ingestion of n-3 fatty acids in experimental animals have been reported, such as reduced platelet aggregation, inhibition of AA metabolism (i.e., prostaglandin formation), and immunosuppression (Simopoulos, 1991). Even though these toxic effects were not examined here, we can not rule out the possibility that rats fed high levels of ALA might be developing these adverse consequences. For example, body fat accumulation was found to be lower in the group with higher rather than lower ALA ingestion which is opposite to the general idea that changes in body fat correlate with dietary fat level.

A counterbalance was also observed between 22:5n-6 and 22:6n-3 in liver total lipid. In all animals, 22:6n-3 was more abundant than 22:5n-6 even though both 20:4n-6 and 20:5n-3 shared the same enzyme system. Since 22:6n-3 is the predominant PUFA with 22 carbon chain length in most cellular membrane lipids, there may be a higher amount of 22:6n-3 than 22:5n-6 reside in the phospholipids fraction of rat liver.

An enhanced proportion of 22:5n-3 was observed in flaxseed oil fed animals. Since conversion of 22:5n-3 to 22:6n-3 has been reported for rat hepatocytes (Voss et al., 1991), this may provide an alternative pathway for DHA synthesis. However, the present study did not give definite answers to whether this pathway has any metabolic significance.

In the liver, fatty acid composition depends on dietary TG, de novo fatty acid synthesis primarily of palmitic acid, and desaturation and/or elongation of fatty acids. To date, it is still under debate if enzymes involved in elongation and desaturation act preferentially on MCFA (Leveille et al., 1967; Kritchevsky et al., 1966). This idea is supported by the study of Hinsch et al. (1975) who found that the rat liver mitochondrial chain elongation system has a higher affinity for MCFA. In our study, the levels of palmitic acid in the liver TG and liver total lipid were significantly higher in response to MCT than to MUFA and PUFA feeding at either low or high fat intake, and the concentration of total n-3 fatty acids was higher with MCT as compared to MUFA and n-6 PUFA in liver total lipid, suggesting that MCT were the more preferred substrates for elongation and desaturation.
For rats fed low fat diets containing MUFA and PUFA, higher liver palmitic acid levels were observed than for those fed high fat diets, suggesting the occurrence of high carbohydrate induced hepatic de novo lipogenesis. Whereas for rats fed SFA, similar palmitic acid levels in liver total lipid were found between low and high fat groups. This result coincides with the studies of Chanez et al. (1991; 1988) that high MCT intake had no inhibitory effect on hepatic lipogenesis as compared to low intake of MCT or high intake of other dietary fats.

The presence of trace amount of n-3 fatty acids in the livers of rats fed coconut and safflower oil diets, and of n-6 fatty acids in the livers of rats fed coconut, flaxseed and high-oleic safflower oil diets suggested chain elongation and desaturation in weaned rats, likely to be derived from exogenous soybean oil present in the diets at 1.1g and 1.3g per 100g diet for low fat and high fat, respectively. Soybean oil has replaced corn oil in the new AIN-93G rodent diet owing to its higher GLA level (1% and 8% of total fatty acids for corn oil and soybean oil, respectively). Previous studies have shown that DHA is important for fetuses and newborn rats for the development of brain and retina (Innis, 1991). The DHA concentration in rat liver is low by 2 weeks of age, increasing thereafter until 7 weeks of age, due to changes in DHA-synthesizing enzymes (Yonekubo et al., 1997). Since animals in our study were killed at 8 weeks of age, when their DHA-synthesizing ability was more mature, their DHA, and AA concentration as well, should be affected by dietary intake. This is evidenced by the presence of AA and DHA in all the animals studied. Further studies are needed to investigate if the concentration of DHA, EPA and AA found in animals without additional dietary supplement were of equal physiological importance, especially in the involvement of any metabolic alterations observed in the study.

3.4.4 Serum TG Levels

Previous studies on the effect of diet on plasma TG levels were mostly conducted in fasting blood samples. However, animals are usually in the postprandial phase, which, for example, represents an average of 18 hours per day in humans. Since most researchers (Patsch et al., 1992; Zilversmit, 1979), even though not all (Austin, 1991) suggested that non-fasting TG concentration appears to be a more accurate independent predictor of atherosclerosis than fasting TG concentration, the magnitude of the non-fasting TG response, which reflects more closely the free-living situation, was investigated.
A large body of studies has shown that high carbohydrate diets are associated with increased postabsorptive plasma TG level (Mittendorfer et al., 2001; Coulston et al., 1989; LaRosa et al., 1980; Ginsberg et al., 1976). However, the effect of macronutrient composition on the postprandial TG response is unclear. Some authors suggest that the degree of postprandial TG is influenced primarily by the amount of carbohydrate in the diet, with low carbohydrate, high fat diets improving nonfasting plasma TG levels (Wijk et al., 2001; Chen et al., 1995; Jepessen et al., 1995). The proposed mechanisms leading to the increased plasma TG during high carbohydrate diets are increased VLDL secretion, due to increased hepatic fatty acid availability as hepatic fatty acid oxidation is lowered (Mittendorfer et al., 2001), and decreased VLDL clearance (Parks et al., 1999). On the other hand, there are studies showing that increased non-fasting TG is positively correlated to dietary fat content (Anderson et al., 2000; Diwadkar et al., 1999; Cohen et al., 1988). The present finding showed that a significant difference in postprandial serum total TG concentration was found in the SFA groups, with high fat intake resulting in a higher TG level.

Many factors are involved in the regulation of plasma TG concentrations: production and secretion of chylomicrons from the intestine, production and secretion of VLDL from the liver, interconversion of TG molecules among lipoprotein particles, and tissue uptake of both TG-rich and TG-depleted lipoproteins. The exact mechanisms responsible for the high-fat induced hypertriglyceridemia by MCT were not examined in the present study, but it is suggested that the enhanced circulating TG may result from an increased VLDL secretion from the liver, where higher lipogenesis has been reported for rats fed a higher level of MCT (Chanez et al., 1991; Chanez et al., 1988).

The effects of diets with various fatty acid compositions on the postprandial lipemic response is also complex. Although it has been suggested that dietary SFA, MUFA and n-6 PUFA affect postprandial plasma TG similarly (Mero et al., 1998; Williams, 1997), whereas long-chain n-3 fatty acids, especially fish oil, decrease the postprandial TG response (Zampelas et al., 1994), both greater (Heek et al., 1990) or less (Muesing et al., 1995; Edelin et al., 1968) TG response to SFA than to PUFA, and greater (Thomsen et al., 1999) or less (Higahi et al., 1997) TG response to SFA than to MUFA have been reported as well.

It is well documented that fish oil reduces fasting circulating TG dose dependently as compared to other types of dietary oils (Roche et al., 2000; Tinker et al., 1999). The explanations for the lower plasma
TG on fish oil are decreased VLDL synthesis (Harris et al., 1990), increased lipolysis of chylomicrons and VLDL by hepatic and lipoprotein lipases (Botham et al., 1997), and rapid removal of chylomicrons, VLDL and their remnants by the LDL receptor (Lambert et al., 1995) or LDL-receptor related protein (Brown et al., 1991). It is thought that the high contents of the very long chain n-3 PUFAs, EPA and DHA, contribute to the hypotriglyceridemia (Ikeda et al., 2001). Whether the intake of ALA, the precursor of EPA and DHA, produces the same plasma lipid response as fish oil is not clear. In this study, the influence of flaxseed oil, which is rich in ALA but not EPA and DHA, on lipid metabolism was evaluated. It was found that serum TG concentration did not differ among fatty acids either at low or high intake. In other words, n-3 fatty acids in the form of ALA may not improve plasma TG levels as compared to SFA, MUFA and n-6 PUFA in growing rats. Lack of alterations in plasma TG level by flaxseed oil have also been observed in healthy subjects (Kelley et al., 1993; Layne et al., 1996). However, Harris et al. (1997) found that in humans with a large intake, for example 7g per day, flaxseed oil conferred a hypotriglyceridemia effect. This result suggests that large amounts of flaxseed oil may be required to reduce circulating TG levels, as was suggested by Oomah (2001). If EPA and DHA are the components in fish oil contributing to the lower plasma TG level, whether the levels of EPA and DHA synthesized from ALA are as adequate as those found in fish oil-fed animals needs to be investigated.

3.4.5 Serum Cholesterol Levels

Considerable data are available concerning the effect of different diets on plasma cholesterol concentration. Current dietary guidelines for lowering plasma cholesterol level are to decrease total fat intake, especially saturated fatty acids, with the replacement of carbohydrate or MUFA (Kris-Etherton et al., 1999). Just as not all SFA affect plasma TG concentration similarly, various SFA do not affect plasma cholesterol concentration to the same degree. Lauric acid (12:0) and myristic acid (14:0) are considered cholesterol-raising, with myristic acid being the most potent SFA (Hajri et al., 1998; Hayes et al., 1991). The effect of palmitic acid (16:0) is unclear, and has been found to be either hypercholesterolemic (Snook et al., 1999) or neutral (Khosla et al., 1993), depending on the coexistence of cholesterol and 18:2n-6. Stearic acid (18:0) is neutral, whereas MUFA (18:1) and PUFA (n-6 and n-3) are hypocholesterolemic (Mensink et al., 1989; Mattson et al., 1985). Our results indicated that ALA, the n-3 fatty acid from

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flaxseed oil, exerted a dramatic hypocholesterolemic effect when compared to coconut oil. This effect became greater than that of SFA, MUFA and n-6 PUFA at 40% dietary energy. A possible explanation is that more EPA was synthesized from ALA, since EPA has been shown to decrease serum cholesterol in rats (Bravo, et al., 1995).

Feeding rats coconut oil, high-oleic and high LA safflower oil resulted in similar serum cholesterol levels. This finding may be due to variations in species, age, and diet composition. Rodents are known to be more resistant to the development of atherosclerotic lesions, which is due to the lack of cholesterol ester transfer protein (CETP) (Ha et al., 1982). CETP plays a key role in reverse cholesterol transport by promoting the exchange of cholesteryl ester from HDL for TG in LDL, VLDL and IDL. Differences in the remodeling of plasma lipoproteins can affect their clearance from the plasma, and transgenic mice expressing human CETP can induce a reduction in plasma cholesterol levels due to a decrease in HDL-cholesterol levels (Dinchuk et al., 1995).

It is also possible that the different characteristics of the dietary oils used contributed to our findings. Like olive oil, high oleic safflower oil has a high content of oleic acid but differs in TG composition and the concentration and distribution of minor fatty acids (Table 2.1 and 2.2). Olive oil has been suggested to be more beneficial than high-oleic sunflower oil in reducing atherosclerosis risk (Abia et al., 2001). One of the mechanisms is by increasing the ALA and DHA but not SFA levels in the sn-2 position of VLDL triacylglycerols (Ruiz-Gutiérrez, 1998). This modification of TG in VLDL can lead to altered lipoprotein metabolism and clearance (Abia et al., 1999). In addition, the minor components of olive oil, e.g., vitamin E, β-sitosterol, squalene and polyphenols (Jones et al., 1997; Chan et al., 1996), may further differentiate it from other MUFA-rich dietary lipids on the basis of plasma lipid regulation. This finding suggests that the hypocholesterolemic effect of MUFA in our experiment may be due to the type of high MUFA-containing dietary oil used. Since high-oleic acid safflower oil, a new vegetable source of MUFA, contains more LA but less palmitic acid than olive oil which gives it a hypocholesterolemic potential, additional analysis are needed to elucidate its effects on blood lipid regulation.

The other possible confounding factor is age. An age-associated increase in circulating total cholesterol level has been observed in both male and female rats (Lingelbach et al., 2000; Masoro et al.,
1983). However, exposing neonatal male rats to high cholesterol can maintain serum cholesterol at lower levels in adulthood (Reiser et al., 1972). Although there is little information concerning whether the early exposure to different types and levels of dietary fat may protect adult rats, owing to the evidence that dietary fat is as important a determinant as dietary cholesterol in influencing plasma cholesterol (Cheema et al., 1999; Hwa et al., 1992), it is plausible that this type of adaptation to dietary fat may exist. It would be interesting to examine if blunted serum cholesterol as well as serum TG levels among certain dietary groups, and the consistent suppression of cholesterol levels by n-3 PUFA persist until adulthood.

The dietary cholesterol concentration is also an important factor to consider. Studies in various species have shown that dietary cholesterol and fat can raise serum cholesterol in an individual or interactive manner (Hajri et al., 1998; Ohtani et al., 1990). Considering the cholesterol-free property of our experimental diets, the plasma cholesterol level may not be affected to a big extent. For example, a study in neonatal chicks showed a synergistic effect of 1% dietary cholesterol and 10% coconut oil on plasma total cholesterol, which is significantly higher as compared to either 2% cholesterol or 10% coconut oil alone (Castillo et al., 1998).

3.4.6 Liver Triacylglycerol and Total Lipid Levels

The study confirmed that liver lipid level was regulated influentially by the level of fat consumed. An increased dietary fat level led to an increased liver total lipid concentration.

Our data showed that liver TG levels paralleled those of serum TG. However, serum TG for high MUFA-fed animals was considered low when compared to hepatic TG levels. This result may be explained by a decreased secretion of VLDL-TG from the liver, and/or increased clearance of TG-rich lipoproteins from the circulation. Changes in extrahepatic lipoprotein uptake are more likely to be the major contributor as carcass body fat is higher in these rats.

In the high fat groups, rats given meals with high n-6 PUFA showed a relatively higher increase in liver total lipid, but not liver TG. Possibly, at high intake, liver fatty acids were preferentially incorporated into non-TG fractions of neutral lipids, such as phospholipids, cholesterol esters, diacylglycerol and monoacylglycerol. The decrease in liver TG associated with both n-6 and n-3 PUFA is in good agreement
with previous studies, and has been suggested to result from inhibition of hepatic lipogenesis, higher fatty acid oxidation, or lower hepatic fatty acid uptake and reesterification (Kitts et al., 1996; Iritani et al., 1998).

The higher liver lipid concentration in the high fat n-6 PUFA group is unexpected. As in our study, rats were in the postprandial state, it is therefore likely that differences in absorption efficiency may account for this effect. It has been shown that depending on the fatty acid composition and TG structure, dietary lipids may have different rates of digestion and absorption, which in turn influence fatty acid bioavailability, tissue uptake and distribution. The absorption of dietary fat is further complicated by the observation that a considerable portion of TG in the lymph is derived from endogenous sources (e.g., biliary phospholipids) (Porsgaard et al., 2000; Porsgaard et al., 1999; Shiau et al., 1985). Porsgaard et al. (2000) compared the absorption of dietary fats and found that at 8 hours after fat administration, a time frame similar to our experimental condition, olive oil and corn oil had similar absorption rates, and the absorption of high 18:3n-3 rapeseed oil was significantly lower than that of olive oil. Therefore, a higher TG output in lymph during early fat absorption may explain the higher levels of liver lipid in rats fed high MUFA and high n-6 PUFA in the present study. Since MCT are absorbed primarily via the portal vein and not incorporated into the lymph TG, the higher liver total lipid concentration accompanying the high fat intake in the SFA group can not be explained by differences in lymphatic transport. The moderate increase of liver total lipid in the high fat MCT rats as compared to high MUFA and high n-6 PUFA are likely to be due to the net result of enhanced fat absorption and the rapid hepatic fat oxidation.

On the other hand, rats fed SFA had the highest liver TG, but lower liver total lipid concentration than MUFA and n-6 PUFA, suggesting higher incorporation of fatty acids into liver TG. Hepatic TG can be derived from dietary lipids, fatty acid released by adipose tissue lipolysis, and hepatic de novo lipogenesis (Degrace et al., 1998). Increased dietary source and liver fatty acid synthesis, as well as reduced partitioning into phospholipids are the likely explanations for the enhanced liver TG levels of rats fed high SFA diets. Since the liver TG levels paralleled serum TG levels, it is suggested that the higher serum TG levels in rats fed high MCT as compared to low MCT may be through increased hepatic VLDL TG secretion, resulting from increased hepatic TG synthesis. At least two lines of evidence suggested that an increased secretion of endogenous VLDL rather than exogenous chylomicron was the source of the
increased serum TG level. First, MCT are absorbed mainly through the portal vein rather than the lymphatic pathway, and lower lymphatic fatty acid output has been reported with MCT feeding (Degrace et al., 1998). Second, endogenous oleic acid (18:1) produced from stearic acid through Δ9-desaturation but not exogenous oleic acid, has been linked to enhanced VLDL TG secretion (Legrand et al., 1997; Legrand et al., 1996). Our fatty acid composition data clearly showed a significantly higher proportion of oleic acid in the liver total lipid and TG of rats fed SFA than of rats fed high PUFA safflower oil and flaxseed oil.

3.4.7 Serum Leptin Levels

The dietary manipulations appeared to have no significant influence on serum leptin concentration in this study. However, there was a trend for the high fat diets to induce higher serum leptin levels except for the MUFA dietary group. This result suggests that during development, serum leptin may increase following high fat intakes. The increased leptin might decrease food intake, increase fat oxidation and energy expenditure, and help to maintain body energy balance. The well controlled serum leptin level may explain the moderate effect of dietary management on food intake and body weight gain.
CHAPTER 4

EFFECTS OF TYPE AND LEVEL OF DIETARY FATS ON PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GENE EXPRESSION IN GROWING RATS.

4.1. INTRODUCTION

There is increasing evidence that an interaction between genes and nutrients plays an important role in the development of obesity. One of the recently discovered genes involved in lipid metabolism is PPAR, a transcription factor that appears to be activated by a number of structurally diverse compounds, including naturally occurring lipids. Studies have shown that various dietary fats can induce different levels of body weight or body fatness (Hill et al., 1993; Birt et al., 1990), in vitro PPAR activation (Gottlicher et al., 1992), and PPAR expression. The finding that PPAR is involved in the transcriptional regulation of genes related to energy metabolism (Jump et al., 1999) led to the hypothesis that dietary fats may determine body nutrient partitioning between oxidation and storage through their direct regulation on PPAR, which in turn modulates the activation of several lipid metabolism-related genes.

To date, very little is known about the long-term effect of fat type and level on PPAR gene expression in vivo, as well as on developing adipocytes. The objective of the present study was thus (1) to examine if the expression levels of PPARα (in liver) and PPARγ (in adipocyte) can be modified by type and level of fat, and (2) to identify if any change in PPAR expression is associated with concomitant alterations in the expression of its target genes involved in lipid homeostasis (e.g., lipoprotein lipase for PPARγ, and acyl-CoA oxidase for PPARα), and both in in vivo studies via the use of rapidly growing rats.

Lipoprotein lipase is an enzyme expressed mostly in adipose tissue and muscle that hydrolyzes TG from TG-rich lipoproteins to free fatty acids (FFA) and glycerol. The FFA released can be used as fuel in muscle or stored as TG in adipose tissue. The balance between adipose tissue fat storage and muscle fat...
oxidation is critical for obesity development. An increased lipoprotein lipase activity in adipose tissue has been found in obese subjects (Kern et al., 1990), and in high fat-induced obesity in rats (Kusunoki et al., 2000).

Acyl-CoA oxidase is the rate-limiting enzyme in peroxisome β-oxidation, and plays an important role in oxidizing very long chain fatty acids (C>20). As these proteins are key regulators of energy balance, examining their mRNA levels may provide information regarding the mechanisms by which dietary fatty acids regulate body energy homeostasis.

4.2. METHODS

4.2.1 Experimental Considerations

The study was approved by the Ohio State University Animal Care and Use Committee, including the protocol for sacrificing animals. According to the study by Lemberget et al. (1996b), hepatic PPARα concentration exhibits a diurnal rhythm. To control for the diurnal variation of PPARα, rats were sacrificed between 9am and 12pm, i.e., 1 to 4 hours before the dark cycle began, when both the mRNA and protein levels of hepatic PPARα were high. At autopsy, epididymal fat pads and liver were excised, weighed, immediately frozen in liquid nitrogen, and stored at -70°C until analysis.

Different fatty acids have been shown to exhibit different effects in inducing PPAR in vitro. In general, long and very long chain (C₁₈-C₃₂) polyunsaturated fatty acids and their metabolites have a higher ability than medium and short chain fatty acids, and monounsaturated and saturated fatty acids (Jump et al., 1999; Gottlicher et al., 1992). Thus, dietary fats used in the study were chosen according to their PPAR activating potential. Coconut oil, which contains predominantly medium chain fatty acids (C₄-C₁₂), and high-oleic safflower oil rich in 18:1n-9, were classified as low PPAR activating groups, while high n-6 PUFA safflower oil (rich in 18:2n-6) and high n-3 PUFA flaxseed oil (rich in 18:3n-3) were classified as high PPAR activating groups. It was our intention to use these dietary oils to investigate whether various fatty acids could affect in vivo PPAR expression to the degree predicted in in vitro studies. This intention is due to the more complicated fatty acid metabolism in the in vivo system, in which both exogenous (from

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dietary source) and endogenous (from *de novo* synthesis and from lipoproteins of dietary, hepatic and extrahepatic origins) fatty acids are possible ligands for PPAR, as compared to an *in vitro* system, in which exogenous fatty acids are the primary source.

4.2.2. Animals and Diets

Forty-four 21-day-old male Sprague-Dawley rats obtained from Harlan (Indianapolis, IN) were housed individually under a 12-12 h light-dark cycle, lights on at 1am. Animals were acclimated to their new environment with an *ad libitum*-fed rat Chow diet for two to five days (Figure 4.1) before random assignment to one of the eight diets, low-fat diet: SFA (n=5), MUFA (n=6), n-6 (n=5), n-3 (n=6), and high-fat diet: SFA (n=6), MUFA (n=5), n-6 (n=6), n-3 (n=3). All diets provided the same level per unit food energy of protein, vitamins, minerals, and fiber (Table 4.1).

The high fat diets were formulated by replacing carbohydrate with experimental lipids. The food products providing the experimental fats in both diets were as follows: high SFA, coconut oil (Dyets, Bethlehem, PA); high MUFA, high oleic, safflower oil (Hein Food Group, Uniondale, NY); high n-6, safflower oil (Dyets, Bethlehem, PA), and high n-3, flaxseed oil (Spectrum Essentials, Petaluma, CA). Briefly, the diet contained 17% energy as protein, 68% (LF) or 43% (HF) as carbohydrate, and 15% (LF) or 40% (HF) as fat. The ratio of simple to complex carbohydrate was kept constant and was based on the rodent AIN-93G diet to avoid effects of taste preference and rate of carbohydrate metabolism on energy intake (corn starch: Dyetrose: sucrose= 3.97: 1.32: 1, g/kcal). Each diet contained 6 g linoleic acid/kg diet as 11.3 g soybean oil (Dyets, Bethlehem, PA) per kg diet to provide essential fatty acid. t-Butylhydroquinone (TBHQ) was added at 0.02 g/100 g lipid to prevent lipid oxidation. Food intake and body weight were monitored daily throughout the experiment.

4.2.3 Isolation and Analysis of mRNA

Total RNA was isolated using TRIZol reagent (Appendix F). The yield and quality of the RNA were assessed by the 260 to 280nm optical density ratio and by electrophoresis using 1% denatured agarose gel. RNA was analyzed by RT-PCR after deoxyribonuclease (DNase) treatment. The amplified products
(20μl) were resolved in 2% agarose gel, stained with ethidium bromide, and visualized using an U.V. transilluminator. Signal intensities were quantified by Alphalmager densitometer (Alpha Innotech Corporation, San Leandro, CA) (Appendix G).

### 4.2.4 Statistical Analysis

Data were analyzed using the Statistical Analysis System (SAS)-PC for WINDOWS (version 8.0; SAS Institute Inc., Cary, NC). Values were expressed as mean±SD. Two-way ANOVA was used to assess effects of type and amount of fat on all variables. If no interactions were observed, comparison of group mean was then performed by t-test to investigate the influence of level of fat, and one-way ANOVA followed by Tukey’s multiple range test to analyze the effect of type of fat. The level of significant difference was set at p<0.05.

<table>
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<th>4</th>
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<th>6</th>
<th>37</th>
<th>38</th>
<th>39</th>
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<td>MUFA</td>
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</table>

**Figure 4.1 Experimental protocol timeline.** Rats arrived on day 0. Semi-purified powder diets were fed for 35 days, starting from day 2 (SFA), day 3 (MUFA), day 4 (n-6 PUFA), and day 5 (n-3 PUFA). Rat Chow diet was fed before the experimental diets. Rats were killed and samples were taken on day 37 (SFA), day 38 (MUFA), day 39 (n-6 PUFA), and day 40 (n-3 PUFA).
<table>
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<th>Fat Level</th>
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</tr>
<tr>
<td>L-cysteine</td>
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</tr>
<tr>
<td>corn starch</td>
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<td>31</td>
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<tr>
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<td>soybean oil</td>
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</tr>
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<td>18.2</td>
</tr>
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</tr>
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<td>fat (% energy)</td>
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<td>40</td>
</tr>
</tbody>
</table>

Table 4.1 Composition of experimental diets. The four experimental oils used were coconut oil high in SFA, safflower oil rich in MUFA or n-6 PUFA, and flaxseed oil rich in n-3 PUFA. Antioxidant was added at 0.02g/100g lipid.
4.3 RESULTS

4.3.1 Liver PPARα mRNA levels

Liver PPARα expression was significantly higher in rats fed LF than HF diets in the SFA, MUFA, and n-6 groups, but not in the n-3 group (Figure 4.2). Within the LF groups, a significantly higher expression was found in SFA- and MUFA- fed rats as compared with n-3-fed rats (p<0.005). A similar PPARα expression was observed among SFA, MUFA and n-6, and between n-6 and n-3. Two-way ANOVA showed that the main effect of level was significant (p<0.05) but was not the same for groups within the same fat type, as indicated by the significant interaction (p<0.05).

4.3.2 Liver ACO mRNA levels

ACO is a downstream gene of PPARα in the liver. Thus, liver ACO mRNA levels were measured to confirm the in vivo expression of PPARα in rats fed a low (LF) and high (HF) amount of different types of dietary fats. As expected, only the overall effect of fat level on ACO gene expression was significant (p<0.05), as analyzed by two-way ANOVA. In general, HF diets were characterized by having significantly higher levels of ACO mRNA than LF diets (p<0.05). The only exception is in MUFA groups (p=0.07), in which the increase was not statistically significant (Figure 4.3).

4.3.3 Epididymal Fat PPARγ mRNA levels

The significant effect of fat level on PPARγ expression was observed only in rats fed a LF SFA diet. LF-SFA caused a marked decrease in PPARγ expression (p<0.05) when compared to all other LF groups and HF SFA group (Figure 4.4). Statistical analysis shows that there were a main effect of level, and type-by-level interaction, but no main effect of type (p=0.07) on PPARγ gene expression in epididymal adipose tissue.

4.3.4 Epididymal Fat LPL mRNA levels

For LPL mRNA levels, the major differences among fatty acids were found in the LF groups (Figure 4.5). n-3 induced a significantly higher LPL mRNA level as compared to SFA, MUFA and n-6 (p<0.05). Statistically, the overall effect of type (p=0.02) and level (p<0.0001), and the interactive effect of type-by-level (p=0.0008) were all significant.
Figure 4.2 Liver PPARα mRNA levels (PPARα/β-actin) in rats fed a low (LF) and high (HF) amount of different types of dietary fats. Data are subjected to two-way ANOVA for significance of effects due to level and type of dietary fat. Mean values with different superscripts are significantly different among fat types (p<0.05). Mean values with * are significantly different within fat types (p<0.05). Error bars are SD. Blots represent RT-PCR results of PPARα and β-actin from a single animal.
Figure 4.3 Liver ACO mRNA levels (ACO/β-actin) in rats fed a low (LF) and high (HF) amount of different types of dietary fats. Data are subjected to two-way ANOVA for significance of effects due to level and type of dietary fat. Mean values with * are significantly different within fat types (p<0.05). Error bars are SD. Blots represent RT-PCR results of ACO and β-actin from a single animal.
Figure 4.4 Adipose tissue PPARγ mRNA levels (PPARγ/β-actin) in rats fed a low (LF) and high (HF) amount of different types of dietary fats. Data are subjected to two-way ANOVA for significance of effects due to level and type of dietary fat. Mean values with different superscripts are significantly different among fat types (p<0.05). Mean values with * are significantly different within fat types (p<0.05). Error bars are SD. Blots represent RT-PCR results of PPARγ and β-actin from a single animal.
Figure 4.5 Adipose tissue LPL mRNA expression (LPL/β-actin) in rats fed a low (LF) and high (HF) amount of different types of dietary fats. Data are subjected to two-way ANOVA for significance of effects due to level and type of dietary fat. Mean values with different superscripts are significantly different among fat types (p<0.05). Mean values with * are significantly different within fat types (p<0.05). Error bars are SD. Blots represent RT-PCR results of LPL and β-actin from a single animal.
4.4 Discussion

4.4.1 Liver PPARα and ACO mRNA Levels

Except for the MUFA groups, high fat feeding resulted in a significantly increased liver ACO gene expression. Our results are consistent with previous studies that demonstrated that in rodents, high fat intake caused an induction of peroxisomal β-oxidation (Reddy et al., 1994; Neat et al., 1981; Neat et al., 1980).

It is pertinent to point out that although the rat liver peroxisome is the main organelle involved in the oxidation of very long chain fatty acids (C>20), it also participates in the metabolism of long chain dicarboxylic acids, prostaglandins, xenobiotic compounds, and bile acid precursors (Schulz, 1991). In addition, retroconversion of adrenic acid (22:4n-6) to arachidonate (20:4n-6), 22:5n-3 to EPA (20:5n-3), and DHA (22:6n-3) to 24:6n-3 is also subject to peroxisomal β-oxidation (Williard et al., 1998; Hagve et al., 1986; Osmundsen et al., 1991). It is unclear which process is more important under our experimental conditions. The in vivo situation further makes it difficult to assess. It is possible that less efficiency in β-oxidizing any or all of the lipid molecules may account for the non-significant effect of the high fat MUFA diet in inducing ACO expression, as compared to its corresponding low fat group.

Even though in rodents, increased liver peroxisomes by peroxisome proliferators usually accompanies ACO induction via a stimulation of PPARα, we failed to find a parallel alteration of ACO and PPARα. One explanation is an autoregulation of PPARα by its fatty acid ligands.

When rats were fed an adequate amount of dietary fat, both mitochondrial and peroxisomal β-oxidation operate to reestablish energy balance. However, an increased liver fat overload, as occurred during high fat consumption, may exceed the hepatocyte’s competence to oxidize fatty acid. The unmetabolized energy may stimulate PPARγ-mediated adipose tissue fat storage while attenuating PPARα-mediated fat oxidation. This may explain why a significantly lower liver PPARα mRNA level was found when rats were fed a high fat diet, with the exception for n-3 fatty acid fed animals.
This assumption is supported by the investigations of Bojes et al. (1996; 1994), who found that peroxisome proliferators can inhibit liver β-oxidation. It was shown that inhibition of acyl-CoA synthetase by exposure to peroxisome proliferators increased the level of free fatty acids, with a concomitant diminished β-oxidation.

Further support of this idea comes from the fact that other receptors, including the nuclear receptors, are subject to ligand-mediated autoregulation. For example, glucocorticoid and L-triiodothyronine (T₃) can down-regulate the glucocorticoid receptor (Rosewicz et al., 1998) and thyroid receptor (Ortiz-Caro et al., 1987), respectively. If this is the case for the down-regulation of PPARα under high ligand supply, further studies are needed to investigate the mechanisms underlying the ligand-induced suppression of receptor, i.e., whether the suppression occurs through a reduced transcription or increased turnover, at the mRNA or protein levels.

An alternative possibility is that PPAR is identified as a phosphoprotein, and phosphorylation of PPAR by insulin is associated with increased transcriptional activity (Shalev et al., 1996). Therefore, an increased insulin secretion, which usually accompanies low fat, high carbohydrate diets, may explain the higher PPARα levels observed in the present study.

Another possible explanation for the high fat suppressed PPARα expression is provided by the fact that one of the byproducts of peroxisomal β-oxidation, H₂O₂, is deleterious to cells (Reddy et al., 1989). Disproportional alterations in H₂O₂ producing enzymes and oxidative stress eliminating enzymes catalase and glutathione peroxidase have been shown in peroxisome proliferator-treated liver cells (Yeldandi et al., 2000). The lower PPARα expression may represent a cell’s defense system, i.e., a feedback regulation of PPARα by the products of its target gene ACO to try to minimize the H₂O₂ production.

Since a receptor’s transcriptional activity does not always correlate with the level of mRNA, we can not exclude the possibility that there might be an increased activity of PPARα in spite of a lower mRNA level. Further studies examining the regulation of PPAR at both the level of gene expression and receptor activity will be helpful to elucidate the molecular control of PPAR in lipid metabolism.
Differential expression of PPAR subtypes may also be an important determinant. Even though the predominant isoform in the liver is PPARα, recent obesity studies in mice (Boelsterli et al., 2002; Edvardsson et al., 1999) suggested that hepatic PPARγ may be the subtype that is upregulated in obesity. For example, elevated liver PPARγ levels have been found in obese mice that consumed a high fat diet (Vidalpuig et al., 1996), and in obese PPARα knockout mice (Costet et al., 1998). It is therefore possible that the PPARγ-mediated gene expression may override the transcriptional effect of PPARα under certain conditions, such as high fat feeding.

It is important to note that even though the proportion of LA, GLA, AA and EPA, the identified PPARα ligands found in in vitro systems, was higher in the n-6 and n-3 PUFA treated rats, it is not necessarily related to an increased magnitude of PPARα induction. Even though a study has shown that both free fatty acids and their ethyl esters are potent ligands for PPAR (Schmidt et al., 1996), the high incorporation of these fatty acids and their derivatives and metabolites into cell membranes, and/or their high association with fatty acid binding protein, may make them less accessible for PPARα activation.

PPAR activity depends not only on the availability of ligands, but also on the presence of transcription cofactors, receptor phosphorylation via cell-surface signal transduction pathways, and possibly crosstalk with other nuclear factors and hormones. In the case of ligand activation, for example, intracellular free fatty acids are involved in the activation of a number of nuclear receptors besides PPARα, such as SREBP and HNF-4. Alternatively, fatty acids may regulate cellular events via the second messenger cascade through the cyclooxygenase and the lipoxygenase pathways. This complexity of liver lipid metabolism suggests that even though PPAR is an important mediator, its ultimate effect results from an interplay between PPAR and other factors. How these factors interact to contribute to the regulation of liver lipid homeostasis or PPARα, is an active area of research.

4.4.2 Epididymal Adipose Tissue PPARγ and LPL mRNA Levels

Our results showed that LPL gene expression was markedly induced by high fat diets in the SFA and MUFA-fed rats. This effect paralleled the higher carcass body fat observed in these two groups, suggesting that increased LPL transcription following a high SFA and MUFA meal enhanced adipose tissue fatty acid uptake which led to increased body fat storage. This higher capacity of adipose tissue fatty acid

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uptake, mainly through LPL-mediated lipolysis of circulating TG-rich lipoproteins, may also explain the similar levels of serum TG among fatty acids despite a higher liver TG concentration found in rats fed high SFA and MUFA diets.

Since LPL is an early marker of adipocyte differentiation (Ntambi et al., 2000), an increased adipose tissue LPL expression may imply that increased fat storage was caused by both increased adipose tissue differentiation, and increased plasma fatty acid uptake. In addition, increased glucose uptake and subsequent triacylglycerol synthesis in the adipose tissue may also account for the enhanced adipose tissue expansion. This is supported by the evidence that marked glucose metabolism in fat cells was observed in young rather than in adult animals and humans (DiGirolamo et al., 1981).

It is not clear why 15 energy % as opposed to 40 energy % intake of flaxseed oil led to higher body fat accumulation. However, this could be explained at least in part by an increased adipose tissue LPL expression. Since depot-specific variation in adipocyte metabolism has been identified, it is likely that other adipose locations could also participate in the whole body lipid metabolism, thus resulting in the various levels of body fat deposition that can not be predicted solely by the epididymal fat LPL expression.

Rats fed a diet with 15% SFA had lower epididymal fat PPARγ mRNA level than rats fed 40% SFA or 15% MUFA or PUFA. Even though adipose tissue fatty acid composition was not examined, according to previous studies, the fatty acid composition of adipose tissue is probably similar to that of dietary fat, except for the MCT group, in which MCFA are rapidly oxidized in the liver and are thus absent in the adipose tissue (Seidelin 1995; Amelsvoort et al., 1988; Beynen et al., 1980). As is the case for the liver, it is not known if a high proportion of a particular fatty acid that is the in vitro ligand for PPARγ could provide the cell sufficient free fatty acids for PPARγ activation. Moreover, the regulation of adipose tissue lipid metabolism is also complex, involving not only PPARγ but also other mediators, such as C/EBP, ADD-1, hormones and cytokines (Morrison et al., 2000). Therefore, it may be necessary to evaluate other in vivo contributing factors in lipid metabolism as well when identifying the role of PPARγ in adipose tissue fat accumulation.
CHAPTER 5

CONCLUSIONS

5.1 Conclusion

This study provides evidence that both dietary fat level and type are involved in the regulation of body energy balance in weanling rats. Although high dietary fat has been shown to increase body weight partly due to increased food energy intake, in the present study, weanling rats fed a high fat diet did not increase voluntary energy intake, and had similar elevations of body weight gain, except for rats fed the high MCT-containing SFA diet. This result suggests that lipid is an important dietary component for development, and that growing rats may exhibit self-adjustments in calorie intake in response to changes in the energy density of the diet. More specifically, the high demand for dietary fat by growing animals and the high capacity of fat oxidation during rapid development may protect young animals from obesity.

Our data demonstrated that diets containing 15% of total energy as MCT had the same effect as MUFA and PUFA in preventing body fat accumulation. However, when provided at a high level (40% en), high MCT diets were more likely to cause excess body fat gain than diets high in PUFA, even under similar calorie consumption. These results may be attributed to enhanced removal of circulating TG-containing lipoproteins by the adipose tissue LPL, which was activated via the nuclear receptor PPARγ. Since no correlation between liver PPARα and ACO gene expression was observed, the morphological and biochemical changes responsible for the different degrees of body adiposity may be independent of changes in liver PPARα, at least at the gene expression level.

The fatty acid composition of the TG fraction and total lipid of the liver was sensitive to dietary fat modulation. As in previous studies, a high level of LA consumption suppressed the production of EPA and DHA from ALA, and a high level of ALA suppressed the conversion of LA to AA. Since LA, ALA and
AA are putative ligands for PPAR in vitro, their presence inside the cells may be an important element in determining PPAR transactivation activity. However, this study did not provide evidence as to whether the relative intracellular concentrations of LA, ALA, AA and their metabolites were related to their ability to function as PPAR activators in vivo, as their bioavailability was not investigated.

Long-term dietary intake of flaxseed oil rich in ALA reduced postprandial liver TG, liver total lipid, and serum cholesterol levels in young rats. This result suggests that flaxseed oil may serve as an alternative source of dietary n-3 PUFA, especially in areas where fish or fish oil supply is limited. However, as higher deposition of body fat was found with low intake but not high intake of flaxseed oil, the health benefits of flaxseed oil consumption remains to be determined.

5.2 Limitations

Although results of animals studies may not be applied directly and precisely to humans, they can be used to elucidate more detailed underlying mechanisms. However, species differences in PPARα expression cannot be ignored when extrapolating results to humans, as less abundant PPARα in humans could be associated with less response to its ligands, and a concomitant diminished activation of its target gene expression. In addition, rodent models have been reported to respond differently than humans to dietary lipid interventions influencing plasma lipids. Future studies using animal models with plasma lipoprotein metabolism similar to that of humans, such as hamsters and pigs, may provide more persuasive results.

In the present study, the independent effects of each dietary fat on body energy regulation were investigated. However, in real-life conditions, individuals are likely to consume foods with a combination of these dietary lipids. Also, self-selection of food items rather than manipulated diets as in the laboratory settings can affect appetite or food intake, and further influence the causal effect of dietary fat on body fat regulation.

Even though the ligand binding domain of PPAR is rather large, and a number of molecules have been found to bind to and activate PPAR in vitro, including fatty acids and their metabolites, whether these compounds represent genuine ligands in vivo remains enigmatic. For example, the presence and stability of
15-deoxy-12, 14-prostaglandin J₂ and leukotriene B₄ in a living cell have been questioned. The present study was conducted under the assumption that fatty acids serve as physiological ligands for PPAR. However, detailed information about the ligands for PPAR in vivo awaits investigation.
APPENDIX A

BODY COMPOSITION ANALYSIS

Sample Preparation

1. Record carcass wet weight (WtA) before freezing.
2. Autoclave frozen carcasses in individually sealed beakers at 121°C for 15 minutes.
3. When cooled, record autoclaved carcass weight.
4. Homogenize carcass with 100ml water in a Waring blender, rinse out blender with another 100ml.
5. Record total weight (WtB). Stored at 4°C if necessary.
6. Aliquots of approximately 20g (Wtc) of the homogenate are analyzed for water and fat content.

Moisture Measurement

1. Dry aluminum pans in a 100°C drying oven for 2 hours.
2. Cool aluminum pans in a desiccator for at least 1 hour.
4. Weigh approximately 20g of carcass homogenate into aluminum pans.
5. Dry samples in a 100°C drying oven for 18 hours.
6. Cool samples in a desiccator for at least 2 hours.
7. Record total weight of aluminum pan and sample.
8. Calculate percent of moisture of homogenate as follows:

\[
\% \text{H}_2\text{O}_{\text{homogenate}} = \frac{\text{Weight loss of sample after drying (g)}}{\text{Weight of sample before drying, } W_{tc} \text{ (g)}} \times 100\%
\]

9. Calculate percent of moisture of carcass as follows:

\[
\% \text{H}_2\text{O}_{\text{carcass}} = \frac{[(% \text{H}_2\text{O}_{\text{homogenate}}) \times (W_{tb})] + (W_{ta}) - (W_{tb})}{W_{ta}} \times 100\%
\]

Fat Measurement

1. Dry Whatman No.1 filter paper in a 100°C drying oven for 2 hours.
2. Cool filter paper in a desiccator for at least 1 hour.
3. Weigh filter papers.
4. Transfer dried samples from the aluminum pan to the filter paper with the aid of a spatula and petroleum ether.
5. Wrap samples in filter papers and place into Soxhlet thimbles.
6. Place thimbles along with the wrapped samples inside a Soxhlet extractor and extract for 8 hours.
7. After extraction, place thimbles in a ventilating hood for at least 2 hours.
8. Dry wrapped samples in a 100°C drying oven for 2 hours.
9. Cool wrapped samples in a desiccator for at least 1 hour.
10. Weigh and record.
11. Calculate percent fat of homogenate as follows:

\[ \% \text{fat}_{\text{homogenate}} = \frac{\text{Weight loss of dried sample after extraction (g)}}{\text{Sample before drying, } W_{tC} \text{ (g)}} \times 100\% \]

12. Calculate percent of moisture of carcass as follows:

\[ \% \text{fat}_{\text{carcass}} = \frac{\text{Fat of homogenate, } W_{tB}}{W_{tC}} \times \frac{W_{tA}}{W_{tC}} \]
APPENDIX B

FATTY ACID ANALYSIS

Total Lipid Extraction

1. Weigh approximately 1.0g of the partially frozen liver in 50ml teflon-lined screw cap tube.
2. Add 10ml methanol and homogenize tissue at a speed of ~4 for 15-20 seconds.
3. Add 20ml chloroform, cap and mix thoroughly.
   • Sample: solvent=1:20 (v/v)
   • Solvent, chloroform:methanol=2:1 (v/v)
4. Allow to stand for at least 1 hour at room temperature.
5. Add 6ml saline solution, cap and mix thoroughly.
   • saline: total volume=1:5 (v/v)
6. Centrifuge at ~1500rpm for 3 minutes.
7. Transfer the lower chloroform layer to a clean teflon-lined screw cap tube.
8. Dry down total lipids under nitrogen at 40°C.
9. Re-suspend total lipids in 2ml chloroform.
10. Keep in freezer until analysis.

TLC Separation of Neutral Lipids

1. Prepare developing tank with fresh solvent [Hexane: ethyl ether: glacial acetic acid= 70:30:1 (v/v/v)].
2. Apply 10µl standard and 100µl (low fat) or 50µl (high fat) of total lipid extract to TLC plate with the aid of a gentle flow of nitrogen.
3. Place TLC plate onto a plate holder and immerse the holder into the tank.
4. Place TLC plate in a hood to evaporate solvent.
5. Spray plate with 2,7-dichlorofluorescin
6. Dry plate in a hood.
7. Visualize and mark fluorescent bands of TG in a UV light box.
   An example of neutral lipid separation by TLC is displayed in Figure .

Sample Derivatization

1. Pipet 100µl of internal standard (~0.16mg/ml C17:0) into a disposal teflon-lined screw cap tube.
2. Evaporate solvent under nitrogen at 40°C.
3. For total lipids: add 100µl of the lipid extract in chloroform into tubes.
   For TG: remove TG fraction on TLC plate into tubes.
4. Add 2ml BF3-methanol (12% BF3). Flush with nitrogen, cap tightly and swirl to mix.
5. Heat at 95°C for 30 minutes. Swirl periodically during heating.
6. When cooled, add 2ml saline solution and mix.
7. Extract with 4ml hexane, vortex thoroughly and centrifuge at ~1500rpm for 3 minutes.
8. Transfer the upper hexane layer into a disposal teflon-lined screw-cap tube.
9. Dry sample under nitrogen at 40°C.
10. Re-suspend sample in 2ml (for total lipids) or 1ml (for TG) hexane along with a little portion of anhydrous sodium sulfate.

**GC Analysis**

1. Transfer hexane extract into GC autosampler vials and cap tightly.
2. Analyze fatty acid composition and quantity by GC (Hewlett-Packard 5890 series II) under the following conditions:
   - Injector temperature: 205°C
   - Detector temperature: 235°C
   - Oven temperature: 250°C
   - Run time: minutes

   An example of fatty acid separation by GC is given in Figure.
APPENDIX C

SERUM TRIACYLGLYCEROL (TG) ASSAY
(Adapted from Sigma Diagnostics Infinity Triglycerides Reagent Kit, 343-25P)

1. Warm samples and reagents to room temperature.
2. Mark test tubes for blank, standard (Sigma G 1394), controls and unknowns.
3. Pipet 1.0ml Triglyceride (GPO-Trinder) into each tube.
4. Add 10μl deionized water, standard, controls and unknowns to test tubes.
5. Mix gently by inversion.
6. Incubate at 37°C for 5 minutes.
7. Read absorbance at 540nm with absorbance of water as reference.
8. Calculate serum TG (mg/dl) as follows:

\[
\frac{TG_{unknown} - TG_{blank}}{TG_{standard} - TG_{blank}} \times \text{concentration of standard}
\]
APPENDIX D

SERUM CHOLESTEROL ASSAY
(Adapted from Sigma Diagnostics Infinity Cholesterol Reagent Kit, 401-500P)

1. Warm samples and reagents to room temperature.
2. Mark test tubes for blank, standard (Sigma C 0284), controls and unknowns.
3. Pipet 1.0ml Cholesterol Reagent into each tube.
4. Add 10µl deionized water, standard, controls and unknowns to test tubes.
5. Mix gently by inversion.
6. Incubate at 37°C for 5 minutes.
7. Read absorbance at 500nm with absorbance of water as reference.
8. Calculate serum cholesterol (mg/dl) as follows:

\[
\frac{\text{Chol}_{\text{unknown}} - \text{Chol}_{\text{blank}}}{\text{Chol}_{\text{standard}} - \text{Chol}_{\text{blank}}} \times \text{concentration of standard}
\]
APPENDIX E

SERUM LEPTIN ELISA ASSAY
(Adapted from Murine Leptin Enzyme-linked Immunosorbent Kit, DSL-10-2400)

1. Warm samples and reagents to room temperature.
2. Mark microtitration strips for standard, controls and unknowns.
3. Pipet 25µl standard, controls and unknowns into wells.
4. Pipet 50µl Assay Buffer into wells.
5. Incubate at room temperature (~25°C) for 3 hours while shaking on an orbital microplate shaker, at a fast speed (500-700 rpm).
6. Aspirate and wash 5 times with the Wash Solution.
7. Blot dry by inverting plate on an absorbent material.
8. Add 100µl of the freshly prepared (10-15 minutes before use) Antibody-Enzyme Conjugate Solution into wells.
9. Incubate at room temperature for 1 hour while shaking at fast speed (500-700 rpm) on an orbital microplate shaker.
10. Aspirate and wash 5 times with the Wash Solution.
11. Blot dry by inverting plate on an absorbent material.
12. Add 100µl TMB Chromogen Solution to wells.
13. Incubate at room temperature for 10 minutes while shaking at fast speed (500-700 rpm) on an orbital microplate shaker. Avoid exposure to direct sunlight.
14. Add 100µl Stopping Solution to wells.
15. Read absorbance at 450nm with absorbance of zero standard as reference. Absorbance at 600 or 620nm is set to correct for background.
APPENDIX F

TOTAL RNA ISOLATION
(Adapted from GibcoBPL TRizol reagent protocol, 15596-026)

Homogenization
1. Grind ~100mg tissue to powder in a mortar in liquid nitrogen.
2. Transfer sample to centrifuge tubes.
3. Add 1ml TRizol reagent.
4. Mix thoroughly.
5. Centrifuge at ≤12,000×g for 10 minutes at 2 to 8°C.
6. For liver samples:
   Transfer supernatant without pellet to clean tubes.
   For adipose tissue samples:
   Transfer homogenate without upper fat layer to clean tubes.

Phase Separation
1. Allow to stand at 15 to 30°C for 5 minutes.
2. Add 200μl chloroform.
3. Mix vigorously for 15 seconds.
4. Incubate at 15 to 30°C for 2 to 3 minutes.
5. Centrifuge at ≤12,000×g for 15 minutes at 2 to 8°C.
6. Transfer upper aqueous phase to clean tubes.

RNA Precipitation
1. For liver samples:
   Add 250μl isopropanol followed by 250μl high salt precipitation solution (0.8M sodium citrate and 1.2M NaCl).
   For adipose tissue samples:
   Add 500μl isopropanol.
2. Mix thoroughly.
3. Incubate at 15 to 30°C for 10 minutes.
4. Centrifuge at ≤12,000×g for 10 minutes at 2 to 8°C.

RNA Wash
1. Remove supernatant.
2. Wash with 1ml 75% ethanol.
3. Centrifuge at ≤7500×g for 5 minutes at 2 to 8°C.
RNA Solubolization

1. Remove ethanol.
2. Briefly dry the RNA pellet.
3. Dissolve in RNase-free water.

Quantitation and Yield of Total RNA

1. Quantitate RNA by diluting 5μl of sample with 1ml of RNase-free water.
2. Read absorbance at 260 and 280.
3. Determine RNA purity by calculating \( \frac{A_{260}}{A_{280}} \) (within the range of 1.8-2.1).
4. Determine RNA concentration as follows:

   \[
   \text{RNA concentration (μg/μl)} = \frac{A_{260} \times 40 \times 200^*}{1000}
   \]

   * Dilution factor = 5μl/1ml = 200

5. Determine RNA integrity by electrophoretic separation of 15μg total RNA in 0.8-1.0% denatured agarose gel (0.8-1.0g agarose, 100μl formaldehyde, 2μl ethidium bromide, 100ml MOPS).
APPENDIX G

QUANTITATION OF mRNA BY RT-PCR

DNase Digestion
(Adapted from Sigma Deoxyribonuclease I, AMP-D1)

1. Add to an RNase-free PCR tube on ice:
   - 1μg RNA sample in 8μl water
   - 1μl 10× reaction buffer
   - 1μl DNase
2. Incubate at room temperature for 15 minutes.
3. Add 1μl Stop Solution.
4. Heat at 70°C for 10 minutes to denature both DNase I and RNA.
5. Chill on ice.

Reverse Transcriptase Reaction (RT)
(Adapted from Qiagen Omniscript Reverse Transcriptase kit, 205111)

1. Thaw RNA on ice.
2. Thaw 10× buffer, dNTP mix, primers, RTase and RNase-free water at room temperature.
3. Mix each solution by vortex <5 seconds, centrifuge briefly.
4. Set up reverse-transcription reaction on ice as follows:

<table>
<thead>
<tr>
<th>Vol. (μl)</th>
<th>Final conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNase-free water</td>
<td>4</td>
</tr>
<tr>
<td>10× buffer</td>
<td>2</td>
</tr>
<tr>
<td>dNTP mix</td>
<td>2</td>
</tr>
<tr>
<td>random primer (10μM)</td>
<td>2</td>
</tr>
<tr>
<td>RNase inhibitor</td>
<td>1</td>
</tr>
<tr>
<td>RTase</td>
<td>1</td>
</tr>
<tr>
<td>RNA template (add at last)</td>
<td>8</td>
</tr>
<tr>
<td>Total Vol.</td>
<td>20</td>
</tr>
</tbody>
</table>

5. Vortex ≤5 seconds, centrifuge briefly.
6. Incubate at 37°C for 60 minutes.
7. Chill on ice.

[Reagent Preparation]

- RNase inhibitor: Prepare fresh by diluting RNase inhibitor (GibcoBPL RNaseOUT Recombinant Ribonuclease Inhibitor, 10777-019) with ice-cold 1× buffer. Vortex ≤5 seconds, centrifuge briefly, keep on ice.
- Random primers: prepare a stock solution of 10μM by diluting random primers (GibcoBPL Random Primers, 48190-011) with TE buffer (3mM Tris, 0.2mM EDTA, pH7.0). Store at -20°C.
Polymerase Chain Reaction (PCR)
(Adapted from Qiagen HotStartTaq Master Mix Kit, 203443)

1. Mix each solution well, centrifuge briefly.
2. Set up PCR reaction at room temperature as follows:

<table>
<thead>
<tr>
<th></th>
<th>Vol. (µl)</th>
<th>Final conc.</th>
<th>Negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>target</td>
<td>internal</td>
<td>target internal</td>
</tr>
<tr>
<td></td>
<td>gene</td>
<td>standard</td>
<td>gene standard</td>
</tr>
<tr>
<td>Distilled water</td>
<td>17</td>
<td>16.5</td>
<td>21</td>
</tr>
<tr>
<td>HotStart Taq Master Mix</td>
<td>25</td>
<td>16.5</td>
<td>25</td>
</tr>
<tr>
<td>Primer* (forward, 5µM)</td>
<td>2</td>
<td>0.2</td>
<td>2</td>
</tr>
<tr>
<td>(reverse, 5µM)</td>
<td>2</td>
<td>0.2</td>
<td>2</td>
</tr>
<tr>
<td>(forward, 1µM)</td>
<td>2.25</td>
<td>0.045</td>
<td>2.25</td>
</tr>
<tr>
<td>(reverse, 1µM)</td>
<td>2.25</td>
<td>0.045</td>
<td>2.25</td>
</tr>
<tr>
<td>DNA template (add at last)</td>
<td>4</td>
<td>1µg</td>
<td>—</td>
</tr>
<tr>
<td>Total Vol.</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

* primer sequence and size listed in Table G.1.

3. Heat at 95°C for 15 minutes to activate DNA polymerase.
4. Start PCR cycling according to the following program:
   For PPARα (28 cycles), ACO (28 cycles), LPL (28 cycles), β-actin (26 cycles):
   - Denaturation 94°C×30 sec
   - Annealing 54°C×1 min
   - Extension 72°C×1 min
   For PPARγ (30 cycles):
   - Denaturation 94°C×30 sec
   - Annealing 52°C×1 min
   - Extension 72°C×30 sec

5. Add a final extension at 72°C for 10 minutes, then cooled down to 4°C.
6. Keep PCR product at -20°C.

PCR Product Analysis

1. Prepare 2% agarose gel with TAE buffer (10× TAE buffer: Tris-acetate 0.4M, EDTA 0.01M, pH8.3).
2. Mix 20µl PCR product with 4µl 6× gel loading solution (Sigma G7654).
3. Mix 4µl (0.65µg) DNA ladder (Sigma 25bp DNA Ladder, 10597-011) with 0.8µl 6× gel loading solution (Sigma G7654).
4. Run gel at 90V for ~1.5 hours.
5. Stain gel with ethidium bromide (0.5µg/ml in TAE buffer).
6. Determine signal intensity by exposing 1 second under UV light using AlphaImager Densitometer.
7. Determine relative intensity by calculating the ratio of target gene to β-actin.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence 5' to 3'</th>
<th>Size of PCR product (bp)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARα</td>
<td>5'-AAGCCATCTTCACGATGCTG-3'</td>
<td>510</td>
<td>Wang et al., 1999</td>
</tr>
<tr>
<td></td>
<td>5'-TCAGAGGTCCCTGAAACAGTG-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACO</td>
<td>5'-GCCCTCAGCTATGGTATTAC-3'</td>
<td>634</td>
<td>Wang et al., 1999</td>
</tr>
<tr>
<td></td>
<td>5'-AGGAACTGCTCTCAATGC-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPARγ</td>
<td>5'-AGCCCTTTACCACAGTTGATT-3'</td>
<td>425</td>
<td>Hattori et al., 1999</td>
</tr>
<tr>
<td></td>
<td>5'-AGACATCCACAGCAAG-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPL</td>
<td>5'-GC GGATTTCGTAGATGTC-3'</td>
<td>258</td>
<td>Garcia et al., 1999</td>
</tr>
<tr>
<td></td>
<td>5'-TG CTTGCTGAGGTTTTC-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>5'-CCGTGAAAAGATGACCCAGA-3'</td>
<td>98</td>
<td>Sloop et al., 1998</td>
</tr>
<tr>
<td></td>
<td>5'-GTACGACCAGAGCCATACAG-3'</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table G.1 Primer sequence and size of PCR products.


Belzung F., Raclot T., Groscolas R. Fish oil n-3 fatty acids selectively limit the hypertrophy of abdominal fat depots in growing rats fed high-fat diets. Am. J. Physiol. 1993; 33: R1111-R1118.


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Edelin Y.H., Kinsell L.W., Michaels G.D. Relation between dietary fat and fatty acid composition of “endogenous” and “exogenous” very low density lipoprotein triglycerides. Metabolism 1968; 17: 544-554.


Fickova M., Hubert P., Crémel G., Leray C. Dietary (n-3) and (n-6) polyunsaturated fatty acids rapidly modify fatty acid composition and insulin effects in rat adipocytes. J. Nutr. 1998; 128: 512-519.


Ijpenberg A., Jeannin E., Wahli W., Desvergne B. Polarity and specific sequence requirement of peroxisome proliferator-activated receptor (PPAR)/retinoid X receptor heterodimer binding to DNA. J. Biol. Chem. 1997; 272: 20108-20117.


Latruffe N., Vamecq J. Peroxisome proliferation and peroxisome proliferator activated receptors (PPARs) are regulators of lipid metabolism. Biochimie. 1997; 79: 81-94.


Layne K.S., Goh Y.K., Jumpsen J.A., Ryan E.A., Chow P., Clandinin M.T. Normal subjects consuming physiological levels of 18:3(n-3) and 20:5(n-3) from flaxseed or fish oils have characteristic differences in plasma lipid and lipoprotein fatty acid levels. J. Nutr. 1996; 126: 2130-2140.


Liimatta M., Towle H.C., Clarke S.D., Jump D.B. Dietary polyunsaturated fatty acids interfere with the insulin/glucose activation of L-type pyruvate kinase gene transcription. Molecular Endocrinology 1994; 8: 1147-1153.


Porsgaard T., Straarup E.M., Høy C.E. Lymphatic fatty acid absorption profile during 24 hours after administration of triglycerides to rats. 1999; Lipids 34: 103-107.


Shiau Y.F., Popper D.A., Reed M., Umstetter C., Capuzzi D., Levine G.M. Intestinal triglycerides are derived from both endogenous and exogenous sources. Am. J. Physiol. 1985; 248: G164-169.


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