HIGH-FAT FEEDING AND OBESITY IN RATS

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ABSTRACT

The general objective of this thesis was to investigate the effects of high-fat diets (67% of energy) containing high (butter), moderate (lard) or low levels (canola oil) of saturated fatty acids (SFA) on food intake, eating pattern, obesity development and its reversal in female Sprague-Dawley rats. Periods of 26 or 50 days of high-fat feeding were used in adult rats, and of 7 or 28 days in weaning animals; in adult rats, obesity reversal was evaluated following 28 days of low-fat feeding (27% of energy) and after 32 days of low-fat food restriction. The findings showed that: 1- Exposure for 26 days to low- or moderate-SFA high-fat diets resulted in comparable intake and body weight, while 26 or 50 days of feeding with high-SFA diet led to greater intake and body weight than low-SFA diet; 2- Obesity that developed with SFA-rich diet was accompanied with failure to adjust intake based on diet energy density and preserving body fat even after weight loss; 3- Weight loss was achieved by offering a restricted amount of a low-fat diet but not with ad libitum feeding; 4- Altered diurnal eating pattern was found with high-fat feeding and characterized by a smaller number of meals, longer inter-meal interval and enhanced satiety ratio, regardless of obesity status; SFA-rich diet fed obese rats ate larger meals overall; 5- In weaning rats, three-week exposure to high-fat diets shifted intake toward the light phase; this response was more prominent with high- than with low-SFA diet and was accompanied with greater body weight and altered eating pattern - larger diurnal than nocturnal meals were consumed at a higher rate - rather than overeating. In

conclusion, in adult female rats, a SFA-rich diet resulted in overeating and obesity, relative to a low-SFA diet. In weaning female rats, a SFA-rich diet also led to a greater body weight gain, but without overeating. These results underscore the role dietary fatty acid profile may play in developing obesity in early and adult life.

RÉSUMÉ

L'objectif général de cette thèse était d'examiner les effets de régimes riches en gras alimentaire (67% de l'énergie) à haute (beurre), moyenne (saindoux) ou faible (huile de canola) teneur en acides gras saturés (SFA) sur la consommation alimentaire, la séquence prandiale et nycthémérale, le développement de l'obésité et son inversion chez des rates Sprague-Dawley. Des rates adultes ont été exposées à un régime riche en graisse pendant des périodes de 26 ou 50 jours, et des périodes de 7 ou 28 jours pour des rates sevrées. Chez les animaux adultes, le renversement de l'obésité à été évalué après 28 jours avec un régime faible en graisses (27% de l'énergie) et suite à 32 jours avec un régime alimentaire restreint et faible en graisse. Les résultats de ces travaux ont montré que: 1- L'exposition durant 26 jours à un régime alimentaire riche en graisse mais à faible ou moyenne teneur en SFA a produit une consommation alimentaire et un poids corporel comparables, tandis que 26 ou 50 jours d'exposition au régime alimentaire riche en graisse et à haute teneur en SFA a mené à une ingestion alimentaire plus importante et un poids corporel plus élevé qu'un régime alimentaire à faible teneur en SFA; 2- L'obésité développée avec le régime alimentaire à haute teneur en SFA était accompagnée d'une incapacité d'ajuster l'ingestion alimentaire en fonction de la densité énergétique du régime et de la conservation du gras corporel même après la perte de poids; 3- Une perte de poids a été rendue possible avec un régime faible en graisse et offert en quantité restreinte mais pas avec l'alimentation à volonté; 4- Un régime riche en graisse a modifié la séquence

prandiale diurne, avec une diminution du nombre de repas, un intervalle entre chaque repas plus long et une satiété accrue sans égard à l'état de l'obésité; dans l'ensemble, les animaux obèses nourris avec le régime alimentaire à haute teneur en SFA ont ingéré de plus gros repas; 5- Chez les rates sevrées, après trois semaines d'exposition à un régime riche en graisse, l'ingestion s'est déplacée vers la phase lumineuse; cette réaction était plus importante avec le régime à haute teneur en SFA qu'avec le régime à faible teneur en SFA. Cette réponse était accompagnée d'un poids corporel plus élevé et d'une modification de la séquence alimentaire - de plus gros repas étaient consommés plus rapidement durant le jour qu'au cours de la nuit - mais sans surconsommation alimentaire. En conclusion, chez les rates adultes, un régime riche en gras alimentaire à haute teneur en SFA a produit une surconsommation alimentaire et de l'obésité, en comparaison avec un régime alimentaire à faible teneur en SFA. Chez les rates sevrées le régime à haute teneur en SFA a aussi produit un poids corporel plus élevé mais sans surconsommation. Ces résultats soulignent le rôle que peuvent jouer les acides gras alimentaires dans le développement de l'obésité tôt dans la vie et à l'âge adulte.

ADVANCE OF SCHOLARLY KNOWLEDGE

1. Original contribution to knowledge

The contributions of this doctoral dissertation to the knowledge in the field of dietary obesity are:

- A review paper describing the history of using high-fat diets to induce obesity in animals, and aiming to clarify the consequences of changing the amount and type of dietary fats on weight gain, body composition and adipose tissue cellularity. The review also explored contribution of genetics and sex, as well as the biochemical basis and the roles of hormones such as leptin, insulin and ghrelin in animal models of dietary obesity.
- High-fat diets were fed to adult rats at three levels of SFA - low, moderate and high within the same study, and the obesogenic effect of a high-fat diet was observed with a high-SFA fat-rich diet.
- For the first time, compared the ability of adult female rats fed diets with varying fatty acid composition to adjust for energy density of the diet, whether switched from low to high or the opposite. The studies showed the ability to adjust intake in animals fed a low-SFA diet, but failure to adjust with moderate and high-SFA diets.
- Investigated weight change of adult dietary obese female rats fed ad libitum or restricted on low-fat diets with varying fatty acid composition. A

significant weight loss was achieved with food restriction regardless of the dietary fatty acid composition.

- Examined, for the first time, the effect of short- and long-term exposure to low, moderate and high-SFA fat-rich diets on circadian rhythms of intake and meal pattern in adult female rats. Revealed that regardless of dietary fatty acid profile, an early exposure or later access to fat-rich diets did not alter the normal light-dark distribution of intake, but altered eating pattern during the light phase. With a high-SFA diet, larger meals were ingested and a greater body weight was reached. Eating pattern of adult rats subjected to food restriction on low-fat diets with different fatty acid composition showed a diurnal rather than nocturnal intake.
- Assessed eating pattern of weaning female rats at early and later exposure to low and high-SFA fat-rich diets. Three-week exposure shifted intake toward the light phase, and this response was more prominent with the high-SFA fat-rich diet which also induced greater weight gain but not overeating.

2. Research Manuscript published in refereed scientific journals

Hariri, N., Thibault, L. High-Fat Diet-Induced Obesity in Animal Models. *Nutrition Research Reviews* 2010, 23:270-299 (Chapter 2, Manuscript 1).

Hariri, N., Gougeon, R., Thibault, L. A highly saturated fat-rich diet is more obesogenic than diets with lower saturated fat content. *Nutrition Research* 2010, 30: 632-643 (Chapter 3, Manuscript 2).

3. Research Manuscripts accepted for publication in refereed scientific journals

Hariri, N., Thibault, L. Dietary obesity caused by a specific circadian eating. *Chronobiology International* 2011, in press (Chapter 4, Manuscript 3).

Hariri, N., Thibault, L. Diurnal feeding in young rats fed saturated fatty acid-rich diet. *Biological Rhythms Research* 2011, in press (Chapter 5, Manuscript 4).

4. Abstracts and presentations

Hariri, N., Azadi, B., Thibault, L. (2006). Effect of dietary fatty acid composition on the development of obesity and its reversal in rats. Presented as a poster in the 14th annual meeting of the Society for the Study of Ingestive Behavior (SSIB), Florida, USA (July 18-22, 2006). *Appetite*, 46 (3): 358.

Hariri, N., Gougeon, R., Thibault, L. (2008). Altered eating behaviors in adult rats prone to Dietary Induced Obesity (DIO). Presented as a poster in the 16th European Congress on Obesity (ECO), Geneva, Switzerland (May 14-17, 2008). *International Journal of Obesity,* 2008, 32 (1): S79.

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meeting of the Society for the Study of Ingestive Behavior (SSIB), Paris, France (July 15-19, 2008), *Appetite,* 51(2): 371.

CONTRIBUTION OF AUTHORS TO MANUSCRIPTS

Dr. Louise Thibault, the candidate's supervisor and the main investigator of this work, funded the experiments and supervised the development of the design of the studies. Dr. Thibault developed the writing of the Review article (Manuscript 1) and edited it. She supervised the execution of the experiments and interpreting the results, monitored the progression of the work through regular meetings, and edited Manuscripts 2, 3 and 4.

Dr. Gougeon, the candidate's committee member and the co-author of Manuscript 2, was involved in interpreting the data and reviewing the Manuscript. She also was a co-author of one of the abstracts (presented in the 16th European Congress on Obesity (ECO), Geneva, Switzerland).

The candidate reviewed the literature and collected the articles for writing the review article. The candidate contributed in designing the studies and was responsible for preparing the diets, executing the experiments, collecting data, performing the statistical analyses and interpreting the results. For Manuscript 2, the candidate assisted the lab technician with leptin and ghrelin measurements. For Manuscripts 3 and 4, the candidate was responsible for converting the 30-minute and 1-minute data of the Diet Scan to an Excel file, calculating meals according to the meal's definition used in the study and determining the parameters related to eating pattern. For Manuscript 4, the candidate contributed to developing the design of the study. The candidate wrote the manuscripts, made

corrections according to supervisor's suggestions and also prepared the figures and tables.

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DEDICATION

I dedicate this thesis to my dear parents, Nahid and Samad Hariri,

to my wonderful husband Javad Jalali,

and to my lovely sons Salar and Saman

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophin Hormone
ADP	Air Displacement Plethysmography
AGRP	Agouti-related peptide
BMI	Body Mass Index
CCK	Cholecystokinin
CLA	Conjugated Linoleic Acid
cm	Centimeter
CRF	Corticotrophin Releasing Factor
DEXA	Dual Energy X-rays Absorptiometry
DIO	Diet Induced Obesity
DIT	Diet Induced Thermogenesis
DLAM	Diet Scan Lab Animal Monitoring
g	Gram
GLP-1	Glucagon-Like Peptide 1
GIP	Gastric-Inhibitory Peptide
IMI	Intermeal interval
ICV	Intra-Cerebro Ventricular

kcal	Kilocalorie
kj	Kilojoule
LCFA	Long Chain Fatty Acids
LPL	Lipoprotein Lipase
MCFA	Medium Chain Fatty acids
MCT	Medium Chain Triglycerides
ml	Milliliter
MUFA	Monounsaturated Fatty Acid
ng	Nanogram
NPY	Neuropeptide Y
P	Period
pg	Picogram
PPAR $_{\gamma}$	Peroxisome Proliferator-Activated Receptor γ
PYY	Peptide YY
PUFA	Polyunsaturated Fatty Acid
RIA	Radioimmunoassay
RMR	Resting Metabolic Rate
RQ	Respiratory Quotient
SEM	Standard Error of Mean

SFA	Saturated Fatty acid
TRC	Taste Receptor Cells
WHO	World Health Organization

CHAPTER 1, INTRODUCTION

1.1. Background and rationale

Obesity is considered as the major leading cause of unnecessary deaths and diseases (type 2 diabetes, coronary heart disease and high blood pressure) (WHO 2006). The prevalence of obesity is increasing worldwide, and it was suggested that changes other than genetics, contribute to excessive energy storage and among them dietary patterns could play an important role (Jequier 2002; James 2008; Rosengren and Lissner 2008). The increase in fat intake and availability of high-fat diets have been blamed for this worldwide obesity (Bray and Popkin 1998; Schrauwen and Westerterp 2000; Jequier 2002; French and Robinson 2003) partly because of the high efficiency of utilizing and storing fat by the body, as well as passive overconsumption of energy related to the high energy density, post-ingestive effects and orosensory characteristics of fat-rich diets (Poppitt and Prentice 1996; Golay and Bobbioni 1997; Rolls 2000).

The use of high-fat diets for inducing obesity in animal models goes back to the middle of the last century (Ingle 1949; Fenton and Dowling 1953; Sclafani and Springer 1976). Diet induced obesity or "DIO" models resemble human obesity (Thibault et al. 2004) and are employed for investigating obesity outcomes. However, DIO models differ within and among laboratories in macronutrient composition.

Independently of percent contribution to energy of fat in the diet, fatty acid composition plays an important role in body weight regulation, and not all fat sources are known to be equally obesogenic (Bourgeois et al. 1983; Bell et al. 1997; DeLany et al. 2000; Storlien et al. 2001; Wang et al. 2002; Kien et al. 2005). Saturated fatty acids are known to be stored in adipose tissue rather than used as fuel while monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are less preferably stored (Moussavi et al. 2008). Therefore, obesity reports of high-fat DIO in animals are affected by the fatty acid content of the high-fat diet. Besides, some epidemiological studies have shown a lower total fat intake - g and % of energy - in their population of study compared to a generation ago despite the rise in obesity prevalence (Gray-Donald et al. 2000; Lissner et al. 2000). This suggests that the increased prevalence of obesity cannot be only attributed to total intake of fat; the types of dietary fatty acids might also play a role.

Feeding response differs when animals are fed diets of higher or lower energy density. Animals either adjust (Wade 1983) or fail to adjust food intake (Hill et al. 1989; Huang et al. 2004) when switched to a diet of different energy density. Whether fatty acid profile of the diet is involved in animals' ability to adjust intake is not yet clarified.

Low-fat diets and dietary restriction have been used for reversing dietary obesity in humans (Yu-Poth et al. 1999; Astrup et al. 2000). In some animal studies, ad libitum low-fat diets induced weight loss and reversed dietary obesity

(Bartness et al. 1992; Parekh et al. 1998; Huang et al. 2004), while in other studies this approach was not efficient (Wade 1983; Levin and Dunn-Meynell 2000). However, dietary fatty acid profile has not been investigated for reversing dietary obesity. On the other hand, clinical trials have shown that regardless of their macronutrient content, reduced-calorie diets result in significant weight loss (Gardner et al. 2007; Sacks et al. 2009).

Feeding is periodic and the circadian rhythmicity of intake and pattern of eating episodes including the size, frequency and duration of the meals, intermeal interval (IMI), satiety ratio, and the rate of eating are suggested to play an important role in the development of obesity in rodents (Mistlberger et al. 1998; Kohsaka et al. 2007; Arble et al. 2009) and humans (Ma et al. 2003; Toschke et al. 2005; Chapelot et al. 2006). Main feeding phase in humans is during the day, while rats are nocturnal animals that ingest 70-80% of their total 24 hour food intake during the dark phase (LeMagnen and Devos 1970).

Alterations in eating patterns have been reported by feeding high-fat diets (Farley et al. 2003; Donovan et al. 2007; Paulino et al. 2008). This was attributed to larger meal size due to low intra-meal satiety signals (Warwick et al. 2000; Synowski et al. 2005), decrease in sensitivity to food intake-lowering effect of cholecystokinin (CCK) which is a satiety signal (Savastano and Covasa 2005; Nefti et al. 2009), and less control over appetite due to less frequent meals which increase hunger and lead to higher energy intake (Taylor and Garrow 2001; Bellisle 2004). Eating at a circadian time that is different from the normal

nocturnal feeding phase in rodents has also been associated to higher risk of obesity (Mistlberger et al. 1998; Arble et al. 2009) and humans (Ma et al. 2003; Qin et al. 2003). The expression of clock genes such as C*lock, Bmal 1* and *Per* in suprachiasmatic nucleus of the hypothalamus and in most of the cells in the body have circadian rhythmicity and affects many of the physiological activities including lipid and glucose metabolism as well as eating (Rudic et al. 2004; Kaneko et al. 2009). Feeding high-fat diets and obesity attenuate the circadian expression of these clock genes in liver and adipose tissue (Kohsaka et al. 2007; Kaneko et al. 2009); therefore, affect metabolism and body weight homeostasis.

It is not well understood if alterations in diurnal and nocturnal distribution of intake and the pattern of eating episodes are dependent upon the duration of exposure to high-fat diets. As well, it is not clear whether high-fat diets with differing fatty acid composition similarly alter eating pattern and circadian rhythmicity of food intake are dependent upon their fatty acid content.

As many researchers have reported, there is an association between high-fat feeding, obesity and alteration in eating pattern. Since in many of these studies the duration of high-fat feeding had been long enough to induce obesity, the altered meal pattern resulting from high-fat feeding could not be differentiated from that resulting from obesity. Therefore, the nature of this association remains to be clarified.

1.2. Thesis objectives

The general objective of this thesis was to investigate in adult or young female rats, the effect of high-fat diets differing in their fatty acid profile on obesity development and its reversal, and on eating pattern.

The specific objectives were:

1. To examine the effect of high-fat diets containing high, moderate and low-SFA content on inducing and reversing dietary obesity in adult female rats.

    1.1. Is fatty acid composition of the diet an important factor in developing dietary obesity?

    1.2. How do rats adjust for energy density when exposed to high-fat diets with varying fatty acid composition?

    1.3. Is fatty acid composition of the diet involved in inducing weight loss?

    1.4. Is ad libitum low-fat feeding effective in inducing weight loss?

2. To compare diets with various SFA content in altering circadian rhythmicity of intake and pattern of eating episodes in adult female rats.

2.1. How does short- and long-term exposure to high-fat diets affect circadian rhythmicity of intake?

2.2. Is there any difference between eating pattern of rats when fed high-fat diets with high and low SFA content in the long-term?

3. To investigate, in weaning female rats, the effect of feeding high-fat diets on circadian eating pattern and body weight gain.

3.1. How is the pattern of eating affected by early and longer-term exposure to diets with high and low SFA content?

3.2. What is the relationship between altered eating pattern, high-fat feeding and body weight?

CHAPTER 2, REVIEW OF LITERATURE, MANUSCRIPT 1

This chapter of thesis is presented in a manuscript format and is published as a review paper in: **"Nutrition Research Reviews" 2010, 23:270-299.**

High-fat diet induced obesity in animal models

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2.1. Abstract

Epidemiological studies have shown a positive relationship between dietary fat intake and obesity. Since rats and mice show a similar relationship, they are considered an appropriate model for studying dietary obesity. The present paper describes the history of using high-fat diets to induce obesity in animals, aims to clarify the consequences of changing the amount and type of dietary fats on weight gain, body composition and adipose tissue cellularity, and explores contribution of genetics and sex, as well as the biochemical basis and the roles of hormones such as leptin, insulin and ghrelin in animal models of dietary obesity. The major factors that contribute to dietary obesity hyperphagia, energy density and post-ingestive effects of the dietary fat are discussed. Other factors that affect dietary obesity including feeding rhythmicity, social factors and stress are highlighted. Finally, we comment on the reversibility of high-fat diet induced obesity.

2.2. Introduction

Obesity is considered to be a major risk factor for chronic diseases such as coronary heart disease and hypertension, type II diabetes, and some types of cancer (WHO 2005). Its prevalence is increasing with 400 million obese and 1.6 billion overweight adults around the world (WHO 2005). Although genetics plays a role in regulation of body weight, body size and body composition and the metabolic response to feeding in humans (Bouchard and Tremblay 1997; De

Castro 2004; Farooqi and O'Rahilly 2005; Martinez-Hernandez et al. 2007; Ichihara and Yamada 2008) and in animals (Reuter 2007; Speakman et al. 2007), the increase in worldwide obesity in a short period of time cannot be explained by genetics; there are individual differences in genetic susceptibility to environmental factors such as diet (Jequier 2002; Martinez-Hernandez et al. 2007; Ichihara and Yamada 2008; James 2008; Rosengren and Lissner 2008).

Dietary fat intake often has been claimed as responsible for the increase in adiposity. Human studies have shown that high-fat diets (≥ 30% of energy from fat) can easily induce obesity (Bray and Popkin 1998; Hill et al. 2000; Schrauwen and Westerterp 2000; Jequier 2002; French and Robinson 2003). Epidemiological studies conducted in countries such as China, Canada and the United States have shown that, when the average amount of fat in the diet increases, the incidence of obesity also increases (George et al. 1990; Tucker and Kano 1992; Popkin et al. 1993; Saris et al. 2000). This has led to a worldwide effort to decrease the amount of fat in the human diet.

Diets rich in fat not only induce obesity in humans but also make animals obese (Rothwell and Stock 1984; Warwick and Schiffman 1992; Buettner et al. 2007). In both rats (Boozer et al. 1995; Ghibaudi et al. 2002) and mice (Bourgeois et al. 1983; Takahashi et al. 1999) a positive relationship has been found between the level of fat in the diet and body weight/fat gain. In the scientific literature it was first shown that rats consuming diets containing high proportions of fat gained weight faster than those on diets containing minimal fat content (Deuel

1944; Deuel 1947). In 1949, obesity was induced for the first time in rats by ad libitum feeding of a semi-liquid palatable diet (Ingle 1949). Then in 1953, Fenton and Dowling used high-fat diets with fat at 50% of total energy in weanling mice to induce obesity; they called it nutritional obesity (Fenton and Dowling 1953) but the model was later renamed as dietary obesity (Sclafani and Springer 1976).

Since under-reporting is an important bias in epidemiological studies on diet and obesity in humans (Poppitt et al. 1998; Voss et al. 1998; Hebert et al. 2003), animal models have been widely utilized for experiments on dietary obesity (Thibault et al. 2004; Reuter 2007; Speakman et al. 2007; Young and Kirkland 2007). Usually high-fat diets within the range of 30-78 % of total energy intake are used (Buettner et al. 2007) - either by adding a particular fat to the animal's diet or using an assortment of fat and sugar rich supermarket foods (cafeteria diet) - for studying obesity in rats (Mickelsen et al. 1955; Schemmel et al. 1969; Sclafani and Gorman 1977; Oscai 1982; Chang et al. 1990; Yaqoob et al. 1995; Ainslie et al. 2000; Harrold et al. 2000; Ghibaudi et al. 2002; Levin and Dunn-Meynell 2002; Woods et al. 2003) and mice (Herberg et al. 1974; Lemonnier et al. 1975; Bourgeois et al. 1983; Cunnane et al. 1986; Ikemoto et al. 1996; Bell et al. 1997; Huang et al. 2004). The use of high-carbohydrate/low-fat diets has not been found as efficient as high-fat/low-carbohydrate diets in inducing obesity (Harrold et al. 2000; Ellis et al. 2002; Ghibaudi et al. 2002).

It has been reported that despite the growing problem of obesity, Canadians and Americans are eating less fat than a generation ago (Gray-Donald et al. 2000;

Lissner et al. 2000). This shows that the increasing rate of obesity cannot be totally explained by high intakes of fat in the diet suggesting that type of fat may also play a role, although the results of the studies in humans and animals have not been conclusive (Moussavi et al. 2008). Some studies have reported that not all fats are obesogenic and the dietary fatty acid profile rather than the amount of energy from fat is an important variable in developing dietary obesity (Bourgeois et al. 1983; Bell et al. 1997; DeLany et al. 2000; Storlien et al. 2001; Wang et al. 2002; Kien et al. 2005), but there is some controversy on this matter since there are reports showing non-significant differences in final body weight and/or body weight gain of the animals consuming various fatty acids (Hill et al. 1993; Su and Jones 1993; Jones et al. 1995; Okuno et al. 1997; Cha and Jones 1998; Ellis et al. 2002; Okere et al. 2006).

Other factors that may contribute to obesity induced by a diet rich in fat include failure to adjust oxidation of fat to the extra fat in the diet (Schrauwen and Westerterp 2000), increase in adipose tissue lipoprotein lipase activity (Preiss-Landl et al. 2002), increased meal size and decreased meal frequency (Westerterp-Plantenga 2004), as well as overconsumption of energy attributed to high energy density of the diet (Poppitt and Prentice 1996; Blundell and Macdiarmid 1997; Golay and Bobbioni 1997; Rolls 2000), orosensory characteristics of fats and poorly satiating properties of the high-fat diets (Warwick and Schiffman 1992; Blundell and Macdiarmid 1997; Golay and Bobbioni 1997). Reviews of dietary obesity describe potential mechanisms of body weight and food intake regulation involving the central nervous system -

mainly the hypothalamus - neuropeptides such as ghrelin and neuropeptide Y, and hormones like insulin and leptin (Skelton et al. 2006; Kiess et al. 2008). Adipose tissue *per se* is considered to be an endocrine organ that secretes cytokines such as interleukin-6 and tumor necrosis factor alpha (TNFα); thus obesity could possibly be regarded as a chronic inflammatory disease (Sorisky 1999; Kershaw and Flier 2004; Skelton et al. 2006; Kiess et al. 2008).

Obesity occurs when energy uptake surpasses energy expenditure in the individual animal and so the stores of energy in body fat are enlarged, particularly in adipose tissues. Obesity involves both or either, an increase in the number of adipocytes (hyperplasia) and their size (hypertrophy) (Jequier 2002; Avram et al. 2005; de Ferranti and Mozaffarian 2008). Initially it was hypothesized that adipocyte number was determined in early childhood and the obesity developed during adulthood was a result of an increase in adipocyte size (Hirsch and Han 1969; Greenwood and Hirsch 1974). However, it is now known that hyperplasia is an ongoing event not limited to childhood. At any stage of life when adipocytes enlarge to the point of hypertrophy, they release factors such as TNFα and insulin-like growth factor that stimulate hyperplasia of the adipocytes (Prins and Orahilly 1997; Sorisky 1999; de Ferranti and Mozaffarian 2008). Conversely, recent studies on reversal of obesity in humans have found decreases not only in the size of the fat cells but also in their number: the loss of weight follow by apopotosis of adipocytes (Prins and Orahilly 1997; Avram et al. 2005).

This paper summarizes the present literature on factors that can play a role in development of obesity and explores mechanisms that have been proposed for obesity induced by a diet rich in fat. The adequacy of the paradigm of high-fat diets in animal models of human obesity will be discussed. The possibility of reversing dietary obesity in animal models will be explored. Physical activity is another important factor in obesity; however, this paper focuses on dietary factors only. Some reviews have been published about diverse areas of dietary obesity which have been cited in this introduction but the aim of this review is to summarize the range of relevant results and to provide a conclusive coverage of the different aspects of obesity from high-fat diets in non-human species.

2.3. Assessment of dietary obesity

In animal models as in humans, obesity can be assessed by criteria based on (1) gain of body weight or the Lee obesity index and/or (2) increase of body fat content. However standard thresholds for obesity have not been developed like body mass index (BMI) in human beings. In most studies, the degree of obesity has been evaluated by comparing body weight (or fat) of the experimental group fed high-fat/energy-dense diet with control animals that show normal growth while fed chow or low fat diets (Schemmel et al. 1969; Rothwell and Stock 1984; Harrold et al. 2000; Ghibaudi et al. 2002; Levin and Dunn-Meynell 2002; Woods et al. 2003). Researchers that have attempted to do so differed in the values that are 10%-25% greater body weight than age-matched control rats fed chow (normal pattern of body weight gain) as moderate obesity (Harrold et al. 2000;

Woods et al. 2003) and greater than 40% as severe obesity (Levin and Dunn-Meynell 2002).

The Lee index for assessing obesity in rats is similar to BMI in humans. It was defined by Lee in 1929 (Lee 1929) as the cube root of body weight (g) divided by the naso-anal length (cm) and multiplied by 1000. Lee considered values greater than 310 as an indicator of obesity. Since then some researchers have used Lee index to assess the levels of obesity in rats (Sclafani and Gorman 1977; Kanarek and Markskaufman 1979; Stephens 1980; Li et al. 1997; Sun et al. 2002; Li et al. 2004). Reliable correlations were found in some studies between the Lee index and fat content of the body (Bernardis and Patterson 1968; Bernardis 1970; Kanarek and Markskaufman 1979; Rogers and Webb 1980).

In human subjects body composition assessment with methods such as air displacement plethysmography (ADP) or dual energy X-rays absorptiometry (DEXA) gives a more precise idea of the degree of obesity than do anthropometric measurements alone (Lindsay et al. 2001; Tzotzas et al. 2008). For example children and adolescent males have smaller fat mass than females of a similar BMI, and this difference is more pronounced in the older age group; and so the relationship between BMI and the direct measures of adiposity is influenced by factors such as gender and age (Lindsay et al. 2001). Dual energy X-rays absorptiometry is also used in rats for assessing body composition (Ghibaudi et al. 2002; Holemans et al. 2004). In rats fed diets high in fat, a linear increase in body fat with increasing body weight has been shown (Schemmel et

al. 1969; Bourgeois et al. 1983). However results of the study of Woods et al. (Woods et al. 2003) showed that measuring body fat is a more sensitive criterion for assessing obesity in animals since rats fed a high-fat diet (40% of energy) for 10 weeks displayed a 10% increase in total body weight but a 35-40% increase in total body fat compared to the animals fed a low-fat diet.

In models of dietary obesity, animals are classified to prone and resistant based on their body weight, body weight gain, body fat, or norepinephrine (NE) concentrations in urine. Tulipano et al (2004) categorized Sprague-Dawley rats fed high-fat diet based on their final body weight, with rats in the highest quartile designated as obesity prone and those in the lowest quartile assigned as obesity resistant. In some studies upper (prone) and lower (resistant) tertiles of body weight gain (Levin and Dunn-Meynell 2002; Huang et al. 2004; Dourmashkin et al. 2006) or body fat (Dourmashkin et al. 2005) of the animals fed high-fat diets have been used for this classification. Prior to developing obesity while fed with chow, prone and resistant animals have also been identified based on high and low levels of urinary NE, respectively (Hassanain and Levin 2002; Michel et al. 2003).

2.4. High-fat diets

2.4.1. Energy density

In humans, a significant positive relationship has been found between the amount of dietary energy from fat and the proportion of the population who are

overweight (in epidemiological studies), and in clinical studies between the level of dietary fat and body weight gain as well as between the reduction in the dietary fat and weight loss (Lissner et al. 1987; George et al. 1990; Tucker and Kano 1992; Popkin et al. 1993). These associations have also been shown in animal studies (Bourgeois et al. 1983; Bartness et al. 1992; Boozer et al. 1995; Takahashi et al. 1999; Ghibaudi et al. 2002). This relationship in humans or in animal models of more dietary fat leading to greater obesity shows that fat content of the diet is an important factor in energy balance. In general, diets containing more than 30% of total energy as fat lead to the development of obesity.

Researchers have induced obesity by diets having different percentages and sources of fats in rats (Mickelsen et al. 1955; Schemmel et al. 1969; Sclafani and Springer 1976; Sclafani and Gorman 1977; Faust et al. 1978; Oscai 1982; Chang et al. 1990; Yaqoob et al. 1995; Loh et al. 1998; Ainslie et al. 2000; Harrold et al. 2000; Ellis et al. 2002; Ghibaudi et al. 2002; Levin and Dunn-Meynell 2002; Rolland et al. 2002; Jen et al. 2003; Woods et al. 2003), mice (Herberg et al. 1974; Lemonnier et al. 1975; Bourgeois et al. 1983; Cunnane et al. 1986; Ikemoto et al. 1996; Bell et al. 1997; Wang et al. 2002; Huang et al. 2004) and hamsters (Wade 1982). Furthermore, the characteristics of the diets used have differed within and between laboratories in macronutrient composition, energy density and orosensory properties. In many animal studies the composition of the control diet was not shown or a non purified chow control diet was used. This could have confounding effects arising from comparisons made with the high-fat diets.

Since the original observations of dietary obesity, obesity has been induced in animals by diets containing fat as low as 13% of total energy in a high energy diet (Harrold et al. 2000) (table, line 26) (which is more than the rat's requirement for fat: 5%) to as high as 85% of energy (Mickelsen et al. 1955) (table, line 1). Several researchers have reviewed the amount of fat required to induce obesity in animals. The most recent review was by Buettner et al. (Buettner et al. 2007) who summarized studies conducted between 1997 and 2007, and concluded that the best method to induce obesity in animals was to use semi-purified high-fat diets containing animal fats at 40% of energy, with low amount of n-3 fatty acids and low amount of plant oils rich in n-6 and n-9 fatty acids.

Interestingly, some recent studies have indicated that development of obesity is prevented in humans and rats when the increase in dietary fat is accompanied by an increase in protein (high protein/carbohydrate and low-carbohydrate/fat ratios) (Weigle et al. 2005; Pichon et al. 2006; Sinitskaya et al. 2007). This has been related to greater satiety with high protein diets, lower insulin levels with low carbohydrate diets and the energy required to convert amino acids in glucose compounds for gluconeogenesis (Pichon et al. 2006). High-protein diets were also found to increase CCK and decrease plasma levels of orexigenic hormone ghrelin (Blom et al. 2006; Potier et al. 2009), reduce gastric emptying (Blom et al. 2006) and increase central nervous system leptin sensitivity (Weigle et al. 2005; Saris 2006). Moreover, high-protein diets resulted in a decrease in fatty acid synthase enzyme activity in the liver that reduces hepatic lipogenesis (Pichon et al. 2006). The increase in circulating amino acids *per se* is

a satiety signal and inhibits food intake through suppressing agouti-related protein gene expression which is a neuropeptide in brain that increases appetite (Morrison et al. 2007; Potier et al. 2009). However Huang et al. (Huang et al. 2008) showed that increasing the dietary protein/carbohydrate ratio could not reduce the degree of obesity when obesity had already been induced in high-fat diet fed mice (at 40% of energy). Therefore they suggested that these diets might be efficient in preventing obesity but may not reverse obesity once established.

In the human diet, increase in dietary fat is usually accompanied by decrease in carbohydrate while the protein is relatively constant (for example fat: 35-45%, carbohydrate: 45-55%, protein: 15-20%). This is why a presumably positive relationship between level of fat of the diet and degree of obesity is usually found in epidemiological studies without controlling for dietary protein level.

2.4.2. Dietary profile of fatty acids

Fatty acid composition of the diet may play an important role in body weight regulation and cellularity of adipose tissue (fat cell volume and number) (Clarke 2000; Storlien et al. 2001; Ailhaud et al. 2006; Moussavi et al. 2008). Studies in humans have shown that SFA are more obesogenic than PUFA (Lichtenbelt et al. 1997; DeLany et al. 2000; Lawton et al. 2000; Piers et al. 2003; Kien et al. 2005). This idea has been supported by animal studies by showing either greater accumulation of body fat (Shillabeer and Lau 1994; Takeuchi et al. 1995; Yaqoob et al. 1995; Bell et al. 1997; Silva et al. 2006) (table, lines 19, 21,

16, 18 and 37; respectively) or higher body weight (Bourgeois et al. 1983; Takeuchi et al. 1995; Bell et al. 1997; Wang et al. 2002) (table, lines 11, 21, 33 and 18, respectively) on feeding with diets moderate or rich in SFA. A study conducted by Ellis et al. (Ellis et al. 2002) in 3-week old female Sprague-Dawley rats comparing diets rich in low-SFA corn oil or high-SFA coconut oil (40% of total energy) for 8 weeks, found higher fat cell number in animals fed coconut oil and greater fat cell size in the rats fed corn oil. Since hypertrophy of adipocytes is a prerequisite for hyperplasia, those results show that more severe form of obesity developed from feeding a diet high in SFA.

The obesogenic effect of SFA can be explained by the fact that SFA are poorly used for energy, and so remain to be acylated into triglycerides and stored in adipose tissue, whereas PUFA and MUFA are readily used for energy and so stored less (Storlien et al. 2001). In other words the effective energy content of a diet is greater when the fats in it are high in SFA. In addition the rate of oxidation of SFA decreases with increase of carbon chain length (DeLany et al. 2000). Furthermore, unlike MUFA and PUFA, SFA decrease resting metabolic rate (RMR) and diet induced thermogenesis (DIT) (Lichtenbelt et al. 1997; Clarke 2000; Piers et al. 2002; Soares et al. 2004; Casas-Agustench et al. 2009). Moussavi et al. (Moussavi et al. 2008) suggested that PUFA suppress the expression of lipogenic transcription genes while MUFA and SFA do not.

Another possible mechanism is that saturation of fatty acids decreases their suppressive effect on dietary intake: thus fats/oils containing high proportions of

linoleic acid are more satiating than fats/oils rich in oleic or stearic acid (Beardshall et al. 1989; Lawton et al. 2000; French and Robinson 2003). PUFA inhibit appetite more strongly than MUFA or SFA through an increase in the release of cholecystokinin (CCK) which augments other signals of satiety (Beardshall et al. 1989; Lawton et al. 2000). Another study however failed to confirm SFA induced less satiety than MUFA (Alfenas and Mattes 2003).

A study in adult male Wistar rats showed that feeding high-fat diets (60% of energy) for 8 weeks resulted in greater intra-thoracic fat mass in animals fed SFA-rich diet (cocoa butter) and greater intra-abdominal and epididymal fat mass in those fed PUFA (safflower oil) (Okere et al. 2006) (table, line 38). There are also reports of studies that did not show any specific effect of SFA and PUFA on body weight or fat mass (Jones et al. 1995; George et al. 2000) (table, lines 17 and 25, respectively).

Short chain (C2:0-C4:0) and medium chain (C6:0-C12:0) fatty acids are directly transported to the liver via portal system, are not dependent upon carnitine for entering the mitochondria and therefore are oxidized more and deposited less in adipose tissue than long chain fatty acids (C14:0-C24:0) (Moussavi et al. 2008; Nagao and Yanagita 2008; Takeuchi et al. 2008). Short and medium chain fatty acids also increase DIT and energy expenditure (Moussavi et al. 2008; Takeuchi et al. 2008). The lower obesogenic effect of medium chain triglycerides (MCT) which are composed of medium chain fatty acids (MCFA) was shown in many studies. Isoenergetic diets (with fat at 12% of total energy)

containing olive oil or MCFA (octanoic acid) offered for 23 days to overweight adult female Wistar rats led to a lower final weight and fat mass in MCFA-fed animals (Simón et al. 2000). Similarly, lower body weight and fat gain were found in adult male Sprague-Dawley rats fed MCT-rich high-fat diets (at 50% of energy) for 8 weeks than in rats fed high-fat diets based on long chain fatty acids (Takeuchi et al. 2006). Other studies in animals (Bray et al. 1980; Hwang et al. 1992; Noguchi et al. 2002) and in humans (Stubbs and Harbron 1996; Tsuji et al. 2001) reported similar findings.

The location of the terminal double bond of PUFAs may affect their action. Diets rich in n-3 fatty acids have been shown to prevent obesity better than other subclasses of PUFA (Clarke 2000; Moussavi et al. 2008). This effect has been reported in studies in humans (DeLany et al. 2000; Garaulet et al. 2001; Nogi et al. 2007), mice (Cunnane et al. 1986; Wang et al. 2002) and rats (Hill et al. 1993; Su and Jones 1993; Okuno et al. 1997; Cha and Jones 1998; Jen et al. 2003). In most of the animal studies lower fat deposition in subjects fed n-3 fatty acids was shown despite comparable food/energy intake among the groups (Cunnane et al. 1986; Su and Jones 1993; Okuno et al. 1997; Cha and Jones 1998; Wang et al. 2002) (table, lines 12, 33, 23, 22 and 14, respectively); therefore this effect can be related to the metabolic effects of n-3 fats. Suggested mechanisms involved in this effect of n-3 PUFAs are: (1) low expression of lipogenic transcription genes with diets high in n-3 PUFA (Lichtenbelt et al. 1997; Clarke 2000; Storlien et al. 2001; Wang et al. 2002); (2) increased concentrations of thromboxane A2, leukotriene B4 and some cytokines that are elevated by an increase in n-6 PUFA intake and

decrease in n-3 PUFA, and a low dietary n-6/ n-3 ratio is beneficial for preventing them (Simopoulos 2004); (3) inhibition of prostaglandin synthesis by n-3 PUFAs leading to suppression of terminal differentiation of adipocytes (Okuno et al. 1997).

The configuration of the double bonds of PUFA may also affect the development of obesity. Conjugated fatty acids are PUFAs that have at least one double bond separated by one single bond. Conjugated linoleic acid (CLA) was shown to prevent obesity, and this effect has been attributed to: lower energy intake by decreasing the expression of neuropeptide Y (NPY) and agouti-related protein, increased DIT, decreased pre-adipocyte differentiation via decreasing the expression of peroxisome proliferator-activated receptor ɣ (PPAR ɣ) which is a key factor for adipogenesis, and decreased lipogenesis through decreasing lipoprotein lipase activity and fatty acid synthase expression (Park 2009; Kennedy et al. 2010). The antiobesity effect of CLA was reported in studies conducted in rodents (Moon et al. 2009; Parra et al. 2010) and humans (Sahin et al. 2008; Norris et al. 2009). However, animal (Halade et al. 2010) and human (Ingelsson and Riserus 2008) studies have found that feeding CLA-rich diets might also lead to insulin resistance.

The studies mentioned varied in species, strain, age and/or sex of the animals used, which may explain some divergences among the results. Using different fats/fatty acids at various percentages from animal or plant origin and in a wide range of durations might have affected the results as well. For example, in

a diet containing 40% (Bell et al. 1997) or 58% (Wang et al. 2002) energy as beef tallow (48% SFA and 52% PUFA), the percentages of SFA and PUFA would be 19% and 21% (in the 40% diet) and 28% and 30% (in the 58% diet), respectively. These percentages might have been high enough to reveal the obesogenic effect of SFA when used for a 7 to 8-week period. The source of fat (plant origin versus animal origin) also might have affected the results for example, because SFAs with plant origin might not be as effective as SFAs with animal origin in developing obesity. Indeed, SFAs with plant origin mainly contain MCFA (lauric acid in coconut oil and palm kernel oil) rather than LCFA which are abundant in fats from animal origin (Van Nieuwenhuyzen and Adelmann 2007).

Taken together the findings indicate that rats and mice are appropriate models for studying the effects of various fatty acids in developing obesity.

2.5. Physiological mechanisms of high-fat diet induced obesity

2.5.1. Food efficiency and diet-induced thermogenesis

Some reports attributed obesity induced by high-fat diets to their high food efficiency (gram of body weight gain per kilocalorie of food consumed). Energy from fat has a larger effect on body weight gain than has energy from non-fat sources (Herberg et al. 1974; Wade 1982; Warwick and Schiffman 1992; Bray and Popkin 1998; Hill et al. 2000; Prpic et al. 2002; Roberts et al. 2002). Diet-induced thermogenesis (DIT) is the energy for digesting, absorbing and storing nutrients and produces a loss of energy for the body which is 2-3% for fats, 25-

30% for proteins and 6-8% for carbohydrates. Therefore, the efficiency of nutrient utilization differs among macronutrients and fats have an efficiency of 97-98%, whereas efficiency is 70-75% for proteins and 92-94% for carbohydrates (Warwick and Schiffman 1992; Hill et al. 2000; Jequier 2002; Saris 2006). In addition, it costs energy to build long-chain fatty acids (LCFA) from glucose or amino acids, whereas dietary fat contains LCFA pre-formed.

2.5.2. Energy density

Some studies have shown that a fat-rich diet induces obesity by increasing energy intake (Oscai 1982; Ainslie et al. 2000; Harrold et al. 2000; Ghibaudi et al. 2002; Woods et al. 2003). If weight of intake is not increased at least in proportion, this implicates the high energy density of high-fat diets.

Individuals with ad libitum access to diets with different energy densities ate the same amount of food by weight (in a meal or over a few days) (Poppitt and Prentice 1996; Rolls and Bell 1999; Rolls et al. 1999; Drewnowski 2003; Westerterp-Plantenga 2004). On the other hand, after 2 weeks of exposure, subjects learned to compensate for the higher energy density of the diet, and ate less weight of food (Stubbs and Whybrow 2004). Rats and mice have been labeled as hyperphagic when fed fat rich diet, which was based on animals ingesting more energy and not necessarily grams of food (Ainslie et al. 2000; Ghibaudi et al. 2002; Woods et al. 2003; Huang et al. 2004). Although in the mentioned studies, the grams of food ingested were not always reported, rats might have attempted to adjust their intake according to the energy density of the

fat-rich diet. While some of the high-fat diets were less dense in other macronutrients and micronutrients, rats couldn't fully adjust for the extra dietary energy while ingesting a minimal amount of the high-fat diet to meet their requirements (for example 5% protein for maintenance and 15% for growth) (Institute for Laboratory Animal Research 1995) with carrying extra energy. Therefore, high-fat diets used to induce obesity in animal models should meet macronutrient and micronutrient requirements of the animals, so that hyperphagia can be better interpreted.

2.5.3. Satiating effects of fat

Weak satiety signals from fat than from carbohydrates and proteins have been suggested to play a role in overconsumption of energy from fat rich diets (Blundell et al. 1996; Blundell and Macdiarmid 1997; Golay and Bobbioni 1997; Lucas et al. 1998). To clarify if the hyperphagia from fat rich diets is due to their post-ingestive effect rats were administered by gastric self-infusion for 16 days isoenergetic high-fat (59.9% of energy) and high carbohydrate (fat: 16.7% of energy) liquid diets (Warwick and Weingarten 1995). Rats self-infused more energy per day of the high-fat diet than of the high carbohydrate diet, thus when the orosensory effects are minimized a hyperphagia on high-fat diets remains. Poorly satiating post-ingestive effects of fat produced more frequent meals and resulted in large meals (Warwick and Weingarten 1995).

Post-ingestive effect of nutrients also may increase food intake by conditioning sensory preference (Lucas et al. 1998). In a 9-day study, adult

female Sprague Dawley rats were infused intragastrically with isoenergetic high-fat (59.6 % of energy) and high carbohydrate (14.6% of energy) diets paired with different flavors (cherry, grape or strawberry). The rats drank substantially more (38%) of the solution paired with the infusion of the high-fat diet than the solution paired with the infusion of high carbohydrate diet hence the post-ingestive effect of the diets high in fat enhances preference sensory features of high-fat diets (Lucas et al. 1998).

Various mechanisms have been suggested for a reduction in satiety signals with high-fat feeding and attenuation of suppression of energy intake by high-fat diets. These include (1) attenuated enterogastric inhibition of gastric emtpying and secretion of satiety hormones (CCK, PYY, and GLP-1) which are normally stimulated by the presence of fat in the small intestine, and thus decreased late satiety (Covasa and Ritter 2000; Little et al. 2007); (2) inhibition of fatty acid oxidation (Kahler et al. 1999; Scharrer 1999), and so goes that high-fat diets lower the rate of oxidation of fatty acids, hence they may increase intake; (3) insensitivity to the food intake reducing effect of apolipoprotein A-IV which is a peptide that decreases meal size (Tso and Liu 2004; Woods et al. 2004). Low energy dense diets have greater volume and so induce more stomach distension than diets with higher energy density (French and Robinson 2003).

2.5.4. Hormones

Signals from adipose tissue (leptin, adiponectin and resistin), stomach (ghrelin and obestatin), pancreas (insulin), and intestine (CCK, PYY and incretins

including glucagon-like peptide 1, GLP 1 and gastric-inhibitory peptide, GIP) are sent to brain to regulate energy balance (Gale et al. 2004; Crowell et al. 2006; Coll et al. 2007). This review reports the most extensively studied hormonal effects on energy balance (by reducing energy expenditure or increasing energy intake) associated with high-fat feeding.

2.5.4.1. Leptin

Leptin, first identified in 1994 by Rockefeller University scientists, is an important hormone in the control of food intake and body weight (Zhang et al. 1994). It is as an obese gene product produced by adipose tissue, generally in proportion to fat mass, with rises in plasma levels resulting in a decrease in food intake and increase in energy expenditure (Zhang et al. 1994; Halaas et al. 1995; Macdougald et al. 1995; Pelleymounter et al. 1995; Lin et al. 2000). Plasma leptin levels display a circadian rhythm. In humans leptin is increased during the night and peak values are reached at around 24:00 while minimum values are found at midday (Saad et al. 1998; Randeva et al. 2003). Studies in humans have shown that obesity is associated with higher concentrations of plasma leptin (Saad et al. 1998). Moreover, in healthy men leptin levels increased in response to a high-fat meal; however no differential effects among fatty acid chain length or saturation was reported (Poppitt et al. 2006).

Laboratory rats have similar circadian variations of plasma leptin although maximum levels are reached in the middle of their active phase (at night) and minimum levels in the middle of their resting phase (daytime) (Bodosi et al. 2004;

Chacon et al. 2005). In a study in weaning male and female normal FVB mice, 12 weeks of feeding high-fat diet (Western diet, Teklad Adjusted Calories Western-Type Diet, No. 88187; fat at 40% of total energy; Harlan-Teklad, Madison, WI, USA) produced 2.6 to 4.6 fold elevation in plasma leptin levels (measured between 9:00 and 11:00 am) relative to control mice fed chow, but intake of energy was not less than that of the chow-fed controls (Frederich et al. 1995). Higher leptin levels were also found after a 2-hour high-fat meal at dark onset compared to pre-meal levels in adult male obesity prone Sprague-Dawley rats (Leibowitz et al. 2006).

In adult male Osborne-Mendel rats, adapted to a high-fat diet (56% of energy) for 2 weeks, no reduction in food intake at 2, 4, 6 and 24 hours following intra-peritoneal injection of leptin (0.5 mg/ kg body weight) after an overnight fast was found (Lin et al. 2001). In contrast, when the rats had been adapted to a low-fat diet, the injection suppressed the food intake at all time points. Thus, the intake response to peripheral leptin was impaired by chronically high levels of fat intake (Lin et al. 2001). Harrold et al. (Harrold et al. 2000) found hyperleptinemia after a single week of feeding adult male Wistar rats a raised level of energy as fat (13% of energy). Levin and Dunn-Meynell (Levin and Dunn-Meynell 2002) showed that when adult male Sprague-Dawley rats were fed a high-fat diet (31% of energy) for 1 week and were then switched to 3 weeks of chow feeding, leptin levels (time of sampling not mentioned) were higher in rats that were prone to developing obesity on high-fat diet than in rats that were resistant to dietary obesity despite having comparable body weights. Both obesity prone and resistant

Sprague-Dawley rats fed high-fat diets (at 20% of total energy) showed resistance to the anorectic effect of centrally administered leptin (10μg, ICV), while control animals fed a low-fat diet (3% of total energy) decreased their caloric intake following leptin administration (Tulipano et al. 2004). Although in another study resistant animals did not show compromised responsiveness to the food-lowering effect of leptin when fed high-fat diets (Surwit and Collins 2001). Overall, these results indicate that high-fat feeding induces hyperleptinemia and leptin resistance and that this effect is independent of obesity-induced leptin resistance.

The mechanism thought to be involved in hyperleptinemia and leptin resistance on the high-fat diet involves hypothalamic leptin receptors and their signaling pathways (Frederich et al. 1995). Animals susceptible to dietary obesity have reduced hypothalamic leptin receptor gene expression and show an early leptin response to increase in dietary fat (Levin et al. 2003).

In contrast to this, Ainslie et al. (2000) showed that female Hooded Wistar rats aged between 20 and 22 weeks fed high-fat diet (36% of energy) for 4 weeks had significantly lower plasma leptin levels (measured after overnight fast) than control rats fed low-fat diets (6.5% of energy) (Ainslie et al. 2000). A more recent study showed that adult male Sprague-Dawley rats fed high-fat diet (60% of energy) for 2 weeks were hypersensitive to the food intake lowering effect of ICV administration of leptin (3μg); however, after 5 weeks on high-fat diet, rats became insensitive to this effect of injected leptin (Fam et al. 2007). Another study in weanling C57BL/6J mice led to similar conclusions (Lin et al. 2000). The

researchers suggested that early in high-fat feeding, animals are sensitive to the food lowering effect of leptin but despite the reduction in food intake animals become fat as a result of the increase in food efficiency, leading to an increase in plasma leptin levels that is followed by insensitivity to its action (Lin et al. 2000). This implies that leptin resistance after long-term feeding on a high-fat diet is an effect of the obese state rather than the cause of obesity development.

Animal studies found that the fatty acid composition of a high-fat diet may influence leptin levels in the circulation. Lower serum leptin levels (measured 3-6 h after initiation of the dark phase) were found in 8-week old lean male Wistar rats fed a diet rich in long chain SFA (cocoa butter at 60% of energy) than in animals fed a diet rich in long chain PUFA (safflower oil at the same percentage) or chow for 8 weeks (Okere et al. 2006). Although total body fat was similar across dietary groups, SFA-fed rats had less abdominal and epididymal fat, and more intra-thoracic fat compared to the other groups. Another study found that adult male Sprague-Dawley rats fed beef tallow-based diet for 10 weeks had lower leptin levels than animals fed safflower or fish oil, while fish oil-fed animals had the lowest amount of perirenal fat (Cha and Jones 1998). These studies suggest that the site of fat accumulation depends on the fatty acid profile of the diet, and various adipose tissue depots can differently contribute to circulating leptin. However, no differences were found between moderate-SFA and MUFA beef tallow, high-PUFA safflower oil and high-n-3 PUFA fish oil in the increased fasting leptin levels in adult male Sprague-Dawley rats fed these diets for 10 weeks (Hynes et al. 2003). Greater leptin levels were found in

weaning C57Bl/J6 male mice fed high-fat diets (at 58% of energy) based on beef tallow for 7 weeks than mice fed high-fat diets based on fish oil, safflower oil or animals fed low-fat diets (at 10% of energy); leptin levels were correlated with body fat as well (Wang et al. 2002). Similar results were found in other studies (Jang et al. 2003; Jen et al. 2003).

2.5.4.2. Ghrelin

Ghrelin is a peptide released by cells in the fundus of the stomach that stimulates the release of growth hormone from the pituitary and was identified by Kojima and colleagues in 1999 (Kojima et al. 1999). Ghrelin rises before and falls after each ad libitum meal and increases food intake (Tschop et al. 2000; Cummings et al. 2001). In humans ghrelin levels peak in the morning (8:00), at noon (12:00-13:00) and in the evening (17:00-19:00) and fall after each peak (Natalucci et al. 2005). Obese people have lower fasting ghrelin levels than lean people and reduced suppression of ghrelin secretion after a meal (English et al. 2002; Marzullo et al. 2004; le Roux et al. 2005; Reinehr et al. 2005). A fat-rich meal has a smaller suppressive effect on plasma ghrelin concentration than a carbohydrate rich meal regardless of obesity status (Tentolouris et al. 2004). So far, no effect of dietary fatty acid profile on total ghrelin levels has been reported (Poppitt et al. 2006; Lithander et al. 2008).

In rats there is a peak of plasma levels of ghrelin 5 hours after light onset (resting phase) which remains relatively high for 9 hours (Bodosi et al. 2004). There is also a second rise just before the beginning of the dark phase, followed

by a sharp drop and then a gradual rise during the remainder of the dark phase (Sanchez et al. 2004). Ghrelin gene expression and plasma ghrelin concentrations have been found to be lower in mice with dietary obesity than in their lean counterparts, coupled with a decrease in sensitivity to the orexigenic effects of ghrelin as well as impairment in suppression of ghrelin in response to a meal (Moesgaard et al. 2004; Perreault et al. 2004). A study was conducted by Liu et al. (Liu et al. 2004) in two strains of rats with different susceptibilities to develop obesity (Osborne-Mendel prone and S5B/P1 resistant) fed a diet high in fat (56% of energy) for 2 weeks. Ghrelin gene expression was increased in the stomach of fasted susceptible rats but plasma ghrelin concentrations remained unchanged, while in resistant rats both expression and plasma levels of ghrelin remained unchanged. This indicated that ghrelin may play a role in susceptibility to dietary obesity. In adult Long-Evans rats, 2 weeks of high-fat feeding (70% of energy) was associated with lower levels of ghrelin than was feeding on high-carbohydrate diet (Beck et al. 2002). In an attempt to distinguish between the effects of high-fat diet and of dietary obesity on ghrelin concentrations, Greeley et al. (Greeley et al. 2007) fed adult male Sprague-Dawley rats high (45% of energy) or low (12% of energy) fat diets for 3 weeks. Both groups were tested with triiodothyronine (T3) to prevent accumulation of fat. Decreased ghrelin levels in high-fat fed animals were not restored by T3 treatment, despite the fact that the groups had comparable weights. Moreover, duodenal and jejunal infusion of fat suppressed plasma ghrelin less than glucose and amino acids in adult male Sprague-Dawley rats (Overduin et al. 2005).

The mechanisms suggested for ghrelin's actions are twofold. It stimulates hypothalamic secretion of neuropeptide Y (NPY) that increases food intake, decreases fat oxidation and utilization of fat and plays a role in meal initiation (Cummings et al. 2001; Beck et al. 2002). Ghrelin also decreases utilization of fat (Tschop et al. 2000). High-fat diets are known to down-regulate ghrelin secretion (Beck et al. 2002; Moesgaard et al. 2004) and an inverse relationship between leptin and ghrelin has been reported (Beck et al. 2002). On the other hand, hypothalamic expression of ghrelin receptors was enhanced and ghrelin levels were greater in adult male Wistar rats fed a fat-rich meal (Sánchez et al. 2010). Thus, regulation of ghrelin concentration through fat intake remains inconclusive.

Since suppression of ghrelin levels after a meal is associated with postprandial satiety, the lower suppression of ghrelin secretion following high-fat diets might be an explanation for hyperphagia on high-fat diets. Thus, in an environment with abundant high-fat foods, impairment of ghrelin suppression after a meal leads to overconsumption of energy and induces obesity. Furthermore the obesity itself impairs the suppression of ghrelin secretion after a meal which further exacerbates the development of obesity.

2.5.4.3. Insulin

Obesity is associated with elevated basal plasma insulin levels and resistance to the metabolic effects of insulin (Lichtenstein and Schwab 2000; de Ferranti and Mozaffarian 2008). Independent of obesity, high-fat feeding itself contributes to impaired glucose tolerance and insensitivity to the blood glucose

lowering effect of insulin (Lichtenstein and Schwab 2000; Riccardi et al. 2004). The fatty acid profile of the diet plays a crucial role in insulin resistance dependent on a high-fat diet (Lichtenstein and Schwab 2000; Bray et al. 2002; Riccardi et al. 2004). In a human study, intake of SFA and MUFA was positively correlated with plasma levels of glucose and insulin (Lovejoy et al. 2001). Replacing SFA with MUFA had no beneficial effect on blood glucose and insulin levels during 4 weeks of high-fat feeding in adult overweight and obese men (Piers et al. 2003). On the other hand some studies have shown beneficial effects of MUFA intake on glucose homeostasis and insulin sensitivity (Vessby et al. 2001).

Animal studies have also shown that hyperinsulinemia and insulin resistance are induced by high-fat feeding (Barnard et al. 1998; Woods et al. 2003; Woods et al. 2004). In female C57BL/6J mice fed high-fat diets (at 10, 20, 30, 40, 50 and 60% of total energy) for 15 weeks, a linear relationship between the percent of dietary fat and glucose intolerance was found (Takahashi et al. 1999). This dose-dependent effect was also seen in weanling male Sprague-Dawley rats fed diets with different percentages of energy as fat (10, 32, 45%) (Ghibaudi et al. 2002).

Mechanisms of the hyperinsulinemia and insulin resistance with high-fat diets and obesity are discussed in reviews by Lichtenstein and Schwab (Lichtenstein and Schwab 2000), and Riccardi et al. (Riccardi et al. 2004). These authors suggest that decreases in insulin receptors, glucose transport and

metabolism are involved, plus reduction in liver and muscle glycogen synthase activity and storage of glucose as glycogen (Lichtenstein and Schwab 2000; Riccardi et al. 2004). These abnormalities thus develop when the intake of fat is more that 40% of total energy. Excessive amounts of adipose tissue (hypertrophy and hyperplasia) stress the endoplastic reticulum, resulting in secretion of cytokines and decrease in the responsiveness of the cells to insulin (de Ferranti and Mozaffarian 2008).

Differences among dietary fatty acids affect the composition of the cell membranes and this in turn influences the affinity of receptors for insulin and so its action on the cell (Lichtenstein and Schwab 2000; Storlien et al. 2000; Riccardi et al. 2004). Some studies have found that insulin secretion and sensitivity are enhanced as the degree of unsaturation of fatty acid increases especially with n-3 feeding, and thus feeding diets rich in SFA results in more insulin resistance than MUFA and PUFA (Clarke 2000; Lichtenstein and Schwab 2000; Storlien et al. 2000; Riccardi et al. 2004). In a study in 7-week old female C57BL/6J mice fed high-fat diets (60% of energy) composed of palm oil, lard, fish oil, perilla oil or rapeseed oil for 18 weeks, blood glucose levels were higher in all the high-fat fed animals 30, 60 and 120 minutes after an oral glucose challenge than in the group fed a high carbohydrate/low fat diet (fat: 11% of energy), but the increase in fasting blood insulin levels was only reliable in the group fed palm oil (Ikemoto et al. 1996). In weaning female Wistar rats, no difference in insulin levels was found between soybean oil and palm oil groups (Jen et al. 2003), whereas lower plasma insulin levels were found in adult male Wistar rats fed high-fat diet (60% of

energy) rich in SFA (cocoa butter) than in the control animals (10% of energy) (Okere et al. 2006). These disparities might be related to different fats used in these studies: palm oil and cocoa butter differ in SFA content and so diets will vary in SFA at the different percentages of total energy used in the studies. The same can be said for lard, soybean oil and safflower oil. Beneficial effects of n-3 PUFA on action of insulin are reported in many studies (Ikemoto et al. 1996; Cha and Jones 1998; Lombardo et al. 2007; Tsitouras et al. 2008).

Since human and animal studies have shown comparable relationships of hormones to obesity, these models can be used to clarify the uncertain areas such as effects of fatty acid profile of the diet on these hormones. However, relating hormone action to obesity itself requires demonstration of its effect on energy intake and/or expenditure.

2.6. Behavioral mechanisms of high-fat diet induced obesity

As discussed in the previous sections, one explanation why high-fat diets induce obesity is hyperplasia (Oscai 1982; Ainslie et al. 2000; Harrold et al. 2000; Ghibaudi et al. 2002; Woods et al. 2003) i.e. increased weight or volume of daily dietary intake. Effects of energy density were reviewed earlier. Possible lack of inhibitory effects of fats on intake ("satiety") was discussed above. Here the intake-facilitatory effects of sensory characteristics (or palatability) of high-fat diets will be considered (Warwick and Schiffman 1992; Blundell and Macdiarmid 1997; Golay and Bobbioni 1997; French and Robinson 2003). Feeding rhythmicity (Ma et al. 2003; Toschke et al. 2005), social environment (Sclafani

and Springer 1976; Redd and De Castro 1992; Scalera 1992; De Castro 1995; Feunekes et al. 1995; Brown and Grunberg 1996; Perez et al. 1997; Lopak and Eikelboom 2000; O'Connor and Eikelboom 2000; Herman et al. 2003; De Castro 2004; Lopak and Eikelboom 2004) and stress (Dallman et al. 2003; Dallman et al. 2004; Torres and Nowson 2007) may also promote obesity. Each of these will be reviewed below. Because social environment is not documented in relation to high fat intake, only feeding rhythmicity and stress will be reviewed below.

2.6.1. Sensory facilitation of intake

Facilitation of intake by the sensory characteristics of high-fat foods is an important influence on ingestion. Sensory stimulation from food consumption can influence energy intake directly (Yeomans et al. 2004), by promoting selection, consumption, digestion and absorption of a food (Yamaguchi and Ninomiya 2000). It also increases DIT (Swinburn and Ravussin 1994; LeBlanc and Labrie 1997). Foods high in fat are usually preferred by rats to those which are low in fat and are consumed in greater amounts as a result (Golay and Bobbioni 1997; Holt et al. 1999; French and Robinson 2003; Stubbs and Whybrow 2004). A variety of sensory properties contribute to this high palatability of fat-rich diets, mainly texture and odor (Warwick et al. 1990; Warwick and Weingarten 1995; Blundell and Macdiarmid 1997; Sclafani 2001).

In a study on adult male Long-Evans rats, Warwick and Weingarten (Warwick and Weingarten 1995) compared the sensory effects of a high-fat (59.9% of energy) and a high carbohydrate diet (fat at 16.7% of energy). In order

to minimize the post-ingestive effect of diets on intake, they used a preparation in which most of the ingested liquid food drained out of stomach via a fistula. When both diets were offered simultaneously, rats consumed more of the high-fat diet than the high carbohydrate diet, demonstrating a sensory preference. Warwick et al. (Warwick et al. 1990) concluded from a study in weanling female Sprague-Dawley rats that consuming high-fat diets early in life can lead to a sensory preference for this fat product which is relatively stable.

Evidence for sensory preferences for fats in rats and mice animal models are likely to be based on free fatty acids released from the triglycerides in food (Fushiki and Kawai 2005; Mizushige et al. 2007). Lingual lipase has such activity in rodents; taste receptor cells (TRC) in oral cavity of rats can easily detect these free fatty acids; these gustatory signals are transmitted to the brain where they cause release of neurotransmitters such as dopamine and endorphin (Gilbertson et al. 1997; Fushiki and Kawai 2005; Mizushige et al. 2007). Long chain PUFA stimulates TCR more efficiently and thus is more strongly preferred than other types of fatty acid (Gilbertson et al. 1997). Preference for fat is also found in humans, with textural, olfactory and gustatory cues being involved (Mattes 2005).

2.6.2. Rhythmicity of feeding

Rhythmicity in feeding (variation over time in total amount ingested, size and frequency of meals) may play a role in the development of obesity. In humans, a lower risk of obesity was reported in both adults and children with a high frequency of eating episodes (Fabry et al. 1966; Ma et al. 2003; Toschke et

al. 2005). A greater number of meals each day were consumed by obese women than healthy weight women in Sweden in a cross-sectional survey (Bertéus Forslund et al. 2002). However, similar meal patterns were found in obese and healthy weight Swedish men in a dietary survey (Andersson and Rossner 1996).

Time of eating also may play a role in the development of obesity. In humans, meals eaten late in the evening were suggested to be one of the risk factors of obesity (Ma et al. 2003; Qin et al. 2003). In free-living individuals food intake in the morning was more satiating and associated with less overall intake throughout the day than evening food (De Castro 2004). However, in another study, percent energy from evening food intake and weight changes were unrelated (Kant et al. 1997). Taylor et al. (Taylor et al. 2004) and Bellisle (Bellisle 2004) suggested that the effects of meal patterns on human obesity have yet to be clarified.

Unlike humans, rats are nocturnal animals that ingest 70-80% of their food during the dark phase (LeMagnen and Devos 1970). There are two peaks in meal frequency and rate of intakes: at the beginning of the night and towards the end i.e. dusk and dawn feeding (Prins et al. 1986; Clifton 2000). In adult male Wistar rats fed stock diet containing 10% energy as fat, the greater intake during the dark phase resulted in positive energy balance and fat deposition, with negative energy balance along with the oxidation of fat in the light phase over fourteen 24-hour cycles (LeMagnen and Devos 1970; LeMagnen 1988). Altered circadian rhythmicity of intake characterized by larger meal size and decreased meal

frequency has been found in genetically obese animals fed non-purified diets (Becker and Grinker 1977; Prins et al. 1986; Strohmayer and Smith 1987; Ho and Chin 1988; Fukagawa et al. 1992).

Some animal studies have found a relationship between sizes of meals and susceptibility to obesity. Adult male Sprague-Dawley rats ingested chow in larger meals had higher rate of weight gain when fed high-fat diets than rats that fed on chow in smaller meals (Drewnowski et al. 1984). When weaning male obesity prone Sprague-Dawley rats were fed high-fat diets (45% of energy) for 19 weeks, they ate larger meals than resistant animals (Farley et al. 2003). In adult inbred obesity prone and resistant rats fed chow, on the other hand, the obesity prone rats ingested smaller meals more frequently (Cottone et al. 2007). These results suggest that irregular meal pattern is not a cause of developing obesity in obesity prone animals. A 6-hour meal pattern analysis during the dark phase in adult male Sprague-Dawley rats exposed to isoenergetic high and low-fat diets (soy oil at 38% and 10% of total energy) for 2 weeks revealed comparable amounts of food ingested in the first meal, but less food ingested in the second and third meal of high-fat fed rats, as well as greater meal frequency, shorter IMI and lower rate of weight gain than animals fed the low-fat diet (Paulino et al. 2008). However when feeding period was prolonged to 8 weeks, the size of the second meal and IMI increased. Increased meal size and decreased meal frequency have also been found in rats acclimatized to a mixture of high-fat and high-carbohydrate diets (providing 38.5% of energy as fat) for 14 days and then fed a fat-rich diet (at 60% of total energy) for an additional 8 days (Synowski et al. 2005).

There is a shift of food intake from the dark phase to the light phase in genetically obese rats and mice (Becker and Grinker 1977; Ho and Chin 1988; Fukagawa et al. 1992). Mistlberger et al. (Mistlberger et al. 1998) reported higher weight gain in genetically obese Zucker rats when fed ad libitum than in those fed only during 14-hour dark phase, while both groups had similar food intakes. In addition, rats differ in their macronutrient selection during the light-dark cycle. It has been reported that when rats are offered a 2 or 3-way selection between macronutrients, they eat more carbohydrate at the beginning of the dark phase, and more protein and fat at the end of the dark phase and during the light period (Thibault and Booth 1999). Thus it is probable that, with high-fat feeding, more food will be ingested in the light period that may further facilitate development of obesity.

Obesity prone rats respond more than resistant animals with an increase in meal size. This might account for hyperphagia with high-fat feeding in dietary obesity. Further research is needed to find out the cause/effect relationship between eating patterns and obesity.

2.6.3. Stress

Many studies have shown that long-term stress increases food intake and promotes weight/fat gain in humans (Laitinen et al. 2002; Branth et al. 2007). In addition, obesity was found to be associated with depression (Rivenes et al. 2009). Higher levels of obesity in depressed people as well as higher prevalence of depression in overweight and obese women and extremely obese men

(BMI≥40) were found (Allison et al. 2009; Zhao et al. 2009). Depressed people with eating disorders often describe themselves as chronically stressed and usually are obese, suggesting that they eat more when stressed in an attempt to cope with the situation and feel better (Dallman et al. 2003). Energy-dense foods with high-fat and sugar are known as "comfort food" and are more often eaten during stress (Laitinen et al. 2002; Dallman et al. 2004; Pecoraro et al. 2004). On the other hand, some people show loss of appetite during stress (Oliver and Wardle 1999). It has been suggested that this difference is based on the dieting history of the individuals: usually dieters increase and non-dieters decrease their intake while in a stressful situation (Oliver and Wardle 1999).

A different pattern of responsiveness to stress has been shown in a variety of rodent models (Rowland and Antelman 1976; Marti et al. 1994; Levin et al. 2000; Michel et al. 2003). Rowland and Antelman (Rowland and Antelman 1976) discovered that in adult female Sprague Dawley rats mild stress induced by 6 daily sessions (10-15 minutes) of pinching of the tail for 5 days at equal intervals while they had free access to sweetened milk and tap water resulted in greater food intake and body weight gain than in the control animals. However, chronic exposure of adult male Sprague-Dawley rats to an immobilization stressor led to a decrease in food intake, independent of the duration of the stress, while handling stress did not result in change in food intake (Marti et al. 1994).

Obesity prone and resistant animals are also different in their responsiveness to stress. A study was conducted by Levin et al. (2000) in 2.5-month old

selectively bred male obesity prone and resistant Sprague-Dawley rats fed a high-fat diet (31% of energy) for 1 week. They were then randomly assigned to stress group or control while fed the high-fat diet for 3 weeks and then the high-fat diet plus Ensure (Ross Products Division, Medical Supplies Depot, AL, USA) for another 2 weeks. Rats in the stress group had daily exposure to different stressors for 5 weeks, which were restraint for 15 minutes, moving the animal to the cage of another, exposure to another male rat for 10 minutes, 2 minutes swimming or saline injection. Results showed that stressed obesity resistant rats gained less weight without any decrease in energy intake with little effect of the stressors on body weight gain and energy intake of obesity prone animals. Adding Ensure to the high-fat diet increased energy intake and rate of weight gain in resistant animals, but cumulative weight gain over 5 weeks was still lower in stressed rat than in control animals. Weight gain and intake of prone rats was unaffected by the addition of Ensure. It was suggested that resistant rats had a lowered sympathetic activity compared to their unstressed controls which was shown by lower norepinephrine levels in their urine.

The effect of high-fat diet on weight gain after stress was investigated in a study in 3-4 month old male obesity prone and resistant Sprague-Dawley rats that were restrained once for 20 minutes, and after release were presented either a high-fat diet (at 31% of energy) or chow for 9 days (Michel, Levin et al., 2003). Stressed prone rats fed high-fat diet gained more weight than unstressed prone rats fed the same diet while having similar food intakes. However, when stressed prone rats were fed chow, they gained less weight than unstressed prone rats fed

the same diet. These results showed that prone rats were less responsive to the weight reducing effect of immobilization stress when fed high-fat diet; at the same time were more responsive to this effect when fed chow. Immobilization stress had no effect on body weight gain in resistant rats fed either diet (Michel et al. 2003). In another study, adult male Sprague Dawley rats fed high-fat (at 40% of total energy) or low-fat diets (at 12% of total energy) for 4 days were divided in two groups of stressed (restraint tubes with no food and water access followed by tail blood sampling, 3-hour daily for 3 consecutive days) and mildly-stressed rats (moved to new cages, food and water deprived for the same period and blood sampled) (Legendre and Harris 2006). On the days of restraint, stressed rats lost weight regardless of the diet. High-fat fed mildly-stressed animals stopped gaining weight; however low-fat fed mildly-stressed rats gained weight throughout the experiment (Legendre and Harris 2006). Results showed that low- and high-fat diets resulted in similar body weight changes under a severe stress, whereas with a mild stress high-fat fed animals were more responsive to the weight-lowering effect of stress. In adult male Long-Evans rats, the weight loss resulting from chronic stress was regained after recovery from stress and body weight and fat gain were greater in high-fat fed rats than in chow-fed control animals (Tamashiro et al. 2006). Higher preference for high-fat feeding during chronic stress was reported in mice (Teegarden and Bale 2008).

Mechanisms that influence food intake during acute and chronic stress are different. Physiologically, the initial response of the body to an acute stress is secretion of corticotrophin releasing factor (CRF) from paraventricular nucleus of

hypothalamus that stimulates the secretion of adrenocorticotropin hormone (ACTH) from the anterior pituitary which in turn leads to the release of cortisol from adrenal cortex to provide energy for brain and/or muscles. Then cortisol itself makes a negative feedback for its further secretion. However, with a chronic exposure to stressor, the negative feedback does not work efficiently and thus induces an increase in food intake and body weight gain through increased secretion of glucocorticoids which elevate appetite, food intake and fat storage especially in abdomen (Dallman et al. 2003; Adam and Epel 2007; Torres and Nowson 2007). In adult male Wistar rats, a chronic stress of keeping rats in cages filled with water to a height of 2 centimeters for 5 days led to delayed gastric emptying during the first 24 hours of exposure, but after that it was accelerated and exceeded that of the control group by day 5. In addition, catecholamines were increased during the first 24 hours and then decreased while active ghrelin levels were high on day 3 and remained elevated until day 5 (Ochi et al. 2008). It was suggested that the increased sympathetic activity after 24 hours stimulated ghrelin secretion, and therefore the increased food intake found during chronic stress might be a result of enhanced plasma ghrelin. Plasma ghrelin levels were also found to be increased with acute stress (Kristenssson et al. 2006).

2.7. Susceptibility to obesity

There is a genetic background for susceptibility to obesity with interacting environmental factors; and the environment alone has an impact on the inherent risk of obesity in individuals (Speechly and Buffenstein 2000; De Castro 2004;

Karnehed et al. 2006; Marrades et al. 2007; Martinez-Hernandez et al. 2007). This has been shown in many studies in humans (Ichihara and Yamada 2008).

An underlying genetic predisposition to be obesity prone or resistant is also shown in animal models (Levin and Dunn-Meynell 2002; Levin et al. 2003). Rats and mice known as the standard models for studying dietary obesity are different in their susceptibility to obesity: outbred Sprague-Dawley rats, Wistar rats and C57BL/6C mice can be easily categorized to prone and resistant phenotypes with ad libitum access to high-fat diets (Buettner et al. 2007; Reuter 2007; Speakman et al. 2007). There are also strains known as genetically obese, such as Zucker fa/fa rat and ob/ob mice (Thibault et al. 2004; Speakman et al. 2007).

When exposed to high-fat diets, some animals are sensitive to high-fat diet induced obesity and become obese (obesity prone animals), while others resist to this obesogenic effect and grow normally (resistant animals) (Levin and Dunn-Meynell 2002; Levin et al. 2003). Some researchers have attributed this difference to higher energy intakes in obesity prone animals (Commerford et al. 2000; Commerford et al. 2001; Wang et al. 2002; Farley et al. 2003; Huang et al. 2004; Dourmashkin et al. 2005), while others have found similar intakes in prone and resistant animals, and suggested that susceptible animals were capable to store energy with greater efficiency (Levin and Dunn-Meynell 2000; Tulipano et al. 2004; Abdoulaye et al. 2006).

Suggested mechanisms for the difference between prone and resistant animals in responding to high-fat diets are that prone animals have lower fat

oxidation (Chang et al. 1990; Levin and Dunn-Meynell 2000; Hassanain and Levin 2002; Jackman et al. 2006), increased lipoprotein lipase activity (LPL) in their adipose tissue and no change in LPL of their muscles which favors fat storage in these animals (Pagliassotti et al. 1994; Dourmashkin et al. 2005; Jackman et al. 2006). However, Commerford et al. (Commerford et al. 2001) fed 7-week old male Wistar rats high-fat diets (45% of energy) for 1 or 5 weeks and found comparable fat accumulation and lipogenesis in prone and resistant rats after provided with an isoenergetic ^{14}C-labelled high-fat meal, suggesting that the increased energy intake is the main reason for accelerated weight gain in prone animals.

Dietary obese prone animals also had increased arcuate NPY mRNA expression (an orexigenic neuropeptide) (Levin and Dunn-Meynell 1997), decreased norepinephrine (NE) turnover and α_2-adrenoceptor binding in some parts of hypothalamus (ventromedial, dorsomedial and lateral) compared to resistant animals, as well as in pancreas and heart which shows a reduced sympathetic activity in these organs (Levin 1995). The reduction in NE turnover in pancreas leads to an increase in insulin release and development of obesity.

2.8. Sex differences

In humans, there are differences between the two sexes in energy expenditure and requirements as well as in fat metabolism and fat distribution (Blaak 2001; Power and Schulkin 2008; Sweeting 2008). Greater storage of fat in lower body in females (gynoid) due to lower basal fat oxidation and greater

number of α_2 adrenoceptors, as well as decreased α_2 adrenergic sensitivity in abdominal region, all lead to more fat storage in the thigh region and less in abdomen compared to men who have greater storage of fat in upper body (android) (Blaak 2001; Power and Schulkin 2008; Sweeting 2008). Moreover women have more subcutaneous fat than men (Geary 2004). Despite all these differences, in a recent review of the genetic studies of obesity in different countries it was shown that overall obesity rates of males and females as determined by BMI were small and inconsistently different, with no indication of obesity in either sex being more prevalent (Sweeting 2008).

These differences can also been found in animal models of obesity (Thibault et al. 2004). In laboratory rats, males gain weight steadily throughout their lives while the body weight of female rats becomes stable in early adulthood (Charles River Laboratories). As a result, female rats are better models for studying obesity during adulthood since they are more like humans in their growth patterns. Besides more subcutaneous fat is found in females due to higher concentrations of estrogen and progesterone receptors in these depots while males have more visceral fat related to high concentrations of androgen receptors in this area (Mayes and Watson 2004).

The sex of the animals may also affect the cellular response of the adipose tissue to high-fat feeding. This was shown in adult rats fed cafeteria diets for 9 weeks which led to a more rapid development of obesity in female rats, and their weight difference with control animals became obvious after 5 days while in

males this became significant after 40 days (Sclafani and Gorman 1977). The same report showed that weight gain of male and female rats fed a supermarket diet is more similar to each other than that of rats fed chow. Therefore, the sex difference in weight gain normally seen in rats is reduced when animals are developing dietary obesity. Ten-week old female Golden Hamsters fed a fat-rich diet (52% of energy) ate significantly more energy and gained more weight than males (Wade 1982). Likewise, 10-day old female Wistar rats fed a cafeteria diet for 14 weeks gained more weight than their male counterparts fed the same diet suggesting less thermogenic capacity in females when fed the cafeteria diet (Rodriguez et al. 2001). A study in 3-month old Sprague-Dawley rats showed that female rats fed chow had higher food intake and greater increase in ghrelin and decrease in leptin levels than males following a 12-hour fast (Gayle et al. 2006). Moreover an interaction between sex and site of fat accumulation was found in 6-week old NMRI mice given different amounts of fat (17, 27, 43.5, 60% of energy) for 14 weeks, with more fat accumulation in retroperitoneal and parametrial sites in females, and in subcutaneous depot in males (Bourgeois et al. 1983).

All together, similar to humans, male and female rats have different body fat distribution which makes them appropriate models for studying adipose tissue. Besides, female animal models are better responders to high-fat feeding mimicking susceptibility to obesity in humans. However, in a recent review, male mice and rats are introduced as gold standards for studying dietary obesity (Reuter 2007). This might be because of the estrous cycle of the female animals which is

repeated every 4-6 days and can affect the food intake of the animal during this period (Andersen et al. 1997).

2.9. Reversibility

Animal studies showed that low-fat diets can induce weight loss in dietary obese rats. A reduction in energy intake and obesity reversal was found when adult male Wistar rats fed a high-fat diet at 60% of total energy for 17 weeks were switched to chow for 13 weeks (Hill et al. 1989). Decreased energy intake and obesity reversal with significant weight and fat loss were found in weaning male C57Bl/6 mice switched from 17 weeks of high-fat feeding (at 58% of energy) to low-fat feeding (at 11% of energy) for 17 weeks (Parekh et al. 1998) or after 13 weeks of high-fat feeding (at 59% of energy) to low-fat feeding (10% of energy) for 6 weeks (Huang et al. 2004). Likewise, a reduction in caloric intake and a complete reversal of diet-induced obesity was found when 13-week old female Wistar rats originally fed high-fat diets (at 30% or 60% of energy provided by Crisco; Proctor & Gamble, Cincinnati, OH, USA) for 8 or 14 weeks were switched to chow (Bartness et al. 1992).

In other studies, however, ad libitum low-fat feeding was not an efficient method to completely reverse dietary obesity. For example an initial decline in body weight followed by a plateau have been found in adult male obesity prone and resistant Sprague-Dawley rats after switching from 10 weeks of a high-fat diet (at 31% of energy) to chow feeding for 2 weeks (Levin and Dunn-Meynell 2000); however, when animals were restricted on chow to 50% of their caloric

intake for 3 weeks, their body weights reached the level of chow-fed control animals. Decline in body weight and a plateau was also found in adult male obesity prone Sprague-Dawley rats switched from 10 weeks of feeding a high-fat diet (at 31% of energy) to a 7 weeks of chow feeding, although their caloric intake was decreased while on chow (Levin and Dunn-Meynell 2002). Wade (1983) reported that young male and female Golden Hamsters fed high-fat diet (at 52% of energy) for 4 weeks and then switched to chow for 4 weeks (fat at 4.5% energy), had decreased their caloric intake and lost 80% of their weight gain from feeding on the fat-rich diet. Adult male obesity prone Sprague-Dawley rats fed a high-fat diet (at 31% of energy) for 12 weeks and then switched to chow for 1 week, decreased their energy intake (by 10-20%) but failed to lose weight (Levin and Keesey 1998).

Both hyperplasia and hypertrophy of fat cells are involved in developing obesity (Prins and Orahilly 1997; Avram et al. 2005; de Ferranti and Mozaffarian 2008). Eight weeks of energy restriction in normal weight adult male Sprague-Dawley rats did not result in hypoplasia despite the significant decrease in body weight and body fat (Miller Jr et al. 1983). When weaning male Sprague-Dawley rats made obese by feeding a diet containing chow, Crisco, sweetened condensed milk and sucrose solution for19 weeks and then food deprived for 8, 15 or 25 days, body weight and fat cell size decreased, but fat cell number was comparable to that of the chow-fed control animals (Yang et al. 1990). Another study in adult male C57Bl/6J mice fed high-fat diet (45% of energy, Research Diets) for 10 weeks and then switched to chow for 2 weeks have come to similar results (Shi et

al. 2009). Centrally administered leptin (10 μg, ICV) for 4 days in normal weight adult male Sprague-Dawley rats caused a decrease in the number of the inguinal fat cells (Gullicksen et al. 2003). Five days of leptin injection (5 μg, ICV) decreased the number of the fat cells in young (3-month old) male Sprague-Dawley rats, but not in mature (8-month old) animals (Qian et al. 1998). This was also found in a study in mice (Shi et al. 2009). However, a decrease in fat cell number was not found following dietary reversal of obesity in rats and mice (Miller Jr et al. 1983; Yang et al. 1990; Shi et al. 2009). These findings contrast with human studies that showed hypoplasia of adipocytes following reversal of obesity (Prins and Orahilly 1997; Avram et al. 2005).

2.10. Conclusions

The physiological mechanisms involved in high-fat diet induced obesity are overconsumption of high-fat diets due to their low satiating effects, the high efficiency of dietary fat in being stored in the body as well as the alterations in the hormones involved in energy balance, such as high-fat diet-induced hyperleptinemia and hyperinsulinemia accompanied by leptin and insulin resistance, and lowered suppression of ghrelin secretion following high-fat diets. Among the behavioral mechanisms, the sensory facilitation of intake with high-fat diets is well understood. Meal pattern analysis of high-fat fed animals in a pre-obese versus obese state could be useful to understand the development of obesity. An area for future research is to investigate whether different patterns of eating in animal models before obesity development can be a predictor of prone

and resistant phenotypes; and to assess their feeding circadian rhythms. There has been extensive research on the obesogenic effects of fatty acids with different degrees of saturation but no constant pattern of outcome under different conditions was found. More work is needed to prove that body weight can be regulated by fatty acid profile in high-fat diets. An important key point in designing animal studies is that high-fat diets meet animals' minimal nutrient requirements, especially for protein, vitamins and minerals, to eliminate the possibility of overconsumption of the diet to fulfill these nutrients needs. The ineffectiveness of low-fat diets at ad libitum to reverse dietary obesity induced by long-term high-fat feeding stresses the use of restricted regimens. This could help to investigate whether a significant and sustainable weight loss accompanied by decrease in fat cell number can be achieved.

2.11. Acknowledgements

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2.1. Table - Studies of high-fat diet induced obesity in animal models

Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION					Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Mickelson et al (1955)	rat/ Osborne Mendel/male/ 300 g/ adult/	g/100g 60 crisco(Proctor & Gamble, Cincinnati, OH, USA 25 casein 7 sucrose 1 starch 4 mineral salts 3 cottonseed oil containing: 2 vitamin A&D 1 vitamin E	63	85	g/100g 30 casein 61 sucrose 2 starch 4 mineral salts 3 cottonseed oil containing: 2 vitamin A&D 1 vitamin E	3	7	41	i	nr	-	-	-	1
Schemmel et al (1969)	rat/ Osborne Mendel/ male and female / 57.5±6.4 g (female) 51.6±3.1 g (male)/ 3.5 weeks	g/100g 60 crisco 25 casein liver 2 liver powder 0.25 DL methionine 5 mineral mix 2.2 vitamin mix 0.01 auremycin 2 fiber	60	78	grain	nr	nr	65	i	nr	(total) i	-	-	2
Herberg et al (1974)	mouse/ NMRI/ male/ 19.2±0.2 g (DIO diet) 19.3±0.2 g (control diet)/ 4 weeks	g/100g 38 soy oil 24 casein 10 starch 16 sucrose 5 powdered cellulose 1 vitamin mix 6 mineral mix	38	63	g/100g 20 protein 30 oatmeal 24 whole meal 13 flour 7 wheat 2.2 sweet whey 2.5 distillers solubles 0.2 vitamin mix 1.1 mineral mix	6.2	13	11-12	i	i	(epididymal and subcutaneous) i	d	(kcal) 0	3

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		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Lemonnier et al (1975)	mouse/ Swiss/ male and female/ nr/ weaning	g/100g 53 lard % of calories 72 lipid 22 protein 6 carbohydrate	53	72	% of total calories 9 lipid 22 protein 69 carbohydrate	4.5	9	10	i	nr	(genital) i	-	-	4
Sclafani and Springer (1976)	rat/ CFE/ female/ 234-239 g/ 17 weeks	g/100g 33 crisco 67 Purina chow + sweetened condensed milk + palatable supermarket foods :chocolate chip, cookies, salami, banana, marshmallows, milk chocolate, peanut butter	>33	>52.5	Purina chow (Company not specified)	4.5	9.6	8-9	nr	i	i	-	-	5
Sclafani and Gorman (1977)	rat/ CFE/ male and female/ 308 g/12 weeks (male) 233 g/13 weeks (female)	Purina chow + supermarket foods: marshmallows, cheese puffs, sugar coated cereal, chocolate cookies, peanut butter, bologna, sweetened condensed milk	nr	nr	Purina chow (Company not specified)	4.5	9.6	8.5	nr	i	-	-	-	6

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

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		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Faust et al (1978)	rat/ Sprague Dawley and OsborneMendel/ male/ nr/ 17 weeks	g/100 g 55 crisco 25 casein 13 dextrose 4 mineral salts 2.82 vitamin mix 0.12 L cystine 0.06 L cystein	55	76	Purina chow (Company not specified)	4.5	9.6	8.5	i (both strains)	nr	(retroperitoneal and gonadal) i (both strains)	-	-	7
Oscai (1982)	rat/ Wistar/ female/ nr/ weaning	g/100 g 4.84 corn oil 18.16 lard 18 casein 49.5 sucrose 2 Brewer's yeast 2 liver powder 4 Hegsted salt mixture 1.5 vitamin mix	23	42	Purina chow (Ralston Purina)	4.5	9.6	58	i	nr	(total) i	nr	-	8
Wade (1982)	Hamster/ Golden/ male and female/ 80-110 g/ nr	2 part Purina chow (Purina Rodent Chow, no 5001) 1 part vegetable shortening (Company not specified)	>33	>52.5	Purina chow (Purina Rodent Chow, no 5001)	4.5	9.6	4	nr	i	(total) i	nr	(kcal) 0	9

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Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION					Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Bourgeois et al (1983)	mouse/ NMRI/ male and female/ nr/ 4 weeks	g/100 g 5 lard 3.2 bran 0.3 DL methionine 4.3 mineral mix 2.4 vitamin mix 14 casein 70.8 wheat flour	5	6. 9	g/100 g 78.5 wheat flour 12.1 casein 3 bran 0.4 DL methionine 2.1 vitamin mix 3.9 mineral mix	3	6.1	13	0	nr	(retroperitoneal and parametrial) 0	-	-	10
		10 lard 3.5 bran 0.2 DL methionine 4.6 mineral mix 2.5 vitamin mix 17 casein 62 wheat flour	10	26.8					i (female only)	nr	0	-	-	
		20 lard 3.9 bran 0.1 DL methionine 5.2 mineral mix 2.9 vitamin mix 21.9 casein 45.5 wheat flour	20	43.5					i	nr	i	-	-	
		30 lard 4.2 bran 0.05 DL methionine 5.6 mineral mix 3.1 vitamin mix 27 casein 30.1 wheat flour	30	55.9					i	nr	i	-	-	

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Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Bourgeois et al (1983)	mouse/ NMRI/ male and female/ nr/ 4 weeks	g/100 g 30 lard 4.2 bran 0.05 DL methionine 5.6 mineral mix 3.1 vitamin mix 27 casein 30.1 wheat flour	30	55.9	g/100 g 78.5 wheat flour 12.1 casein 3 bran 0.4 DL methionine 2.1 vitamin mix 3.9 mineral mix	3	6.1	13	i	nr	(retroperitoneal and parametrial) i i vs beef tallow (male only) d vs beef tallow (female only)	-	-	11
		30 beef tallow 4.2 bran 0.05 DL methionine 5.6 mineral mix 3.1 vitamin mix 27 casein 30.1 wheat flour	30	55.9					i i vs sunflower oil (female only)	nr	i d vs lard, soybean oil (male only) i vs lard, soybean oil, sunflower oil (female only)	-	-	
		30 soybean oil 4.2 bran 0.05 DL methionine 5.6 mineral mix 3.1 vitamin mix 27 casein 30.1 wheat flour	30	55.9					i	nr	i i vs beef tallow (male only) d vs beef tallow (female only)	-	-	
		30 sunflower oil 4.2 bran 0.05 DL methionine 5.6 mineral mix 3.1 vitamin mix 27 casein 30.1 wheat flour	30	55.9					i d vs beef tallow (female only)	nr	i d vs beef tallow (female only)	-	-	

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

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Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Cunnane et al (1986)	mouse/ ob/ob and In/In/ male/ 22 g (In/In) 36 g (ob/ob)/ 6 weeks	g/100 g 10 evening primrose oil 20 casein 60 sucrose 5.5 cellulose 3.5 mineral mix 1 vitamin mix	10	21	Purina chow (Jackson laboratory, no 5001)	4.5	9.6	16	0 i vs cod liver oil (ob/ob only)	0 d vs cod liver oil (in/in only)	(epididymal) 0 vs cod liver oil	0	-	12
		10 cod liver oil 20 casein 60 sucrose 5.5 cellulose 3.5 mineral mix 1 vitamin mix	10	21					0 d vs evening primrose oil (ob/ob only	0 d vs evening primrose oil (in/in only)	0 vs evening primrose oil	0	-	
Chang et al (1990)	rat/ Wistar/ female/ nr/ nr	g/100 g 32.70 corn oil 29.20 casein 12.20 sucrose 12.15 dextrin 6.30 solka floc 2 vitamin mix 5 mineral mix 0.30 DL methionine 0.15 choline chloride	32	60	g/100 g 8.2 corn oil 22 casein 29.35 sucrose 28 dextrin 6.30 solkafloc 2 vitamin mix 5 mineral mix 0.20 DLmethionine 0.15 choline chloride	8.2	20	5	0 (obesity resistant only) i (obesity prone only) i (obesity prone vs obesity resistant)	i (obesity prone vs obesity resistant)	(retroperitoneal and parametrial i (obesity prone vs obesity resistant)	nr	(kcal) i (obesity prone vs obesity resistant)	13

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Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Su and Jones (1993)	rat/ Sprague Dawley/ male/ 65-85 g/ nr	g/100 g 22.4 fish oil 27.8 cornstarch 11.2 sucrose 22.4 casein 6.19 cellulose 1.24 vitamin mix 8.65 mineral mix	22.4	42	nr	nr	nr	12	0 vs other DIO diets	0 vs other DIO diets	(total) d vs beef tallow and olive oil	0 vs other DIO diets	nr	14
		22.4 safflower oil 27.8 cornstarch 11.2 sucrose 22.4 casein 6.19 cellulose 1.24 vitamin mix 8.65 mineral mix	22.4	42					0 vs other DIO diets	0 vs other DIO diets	0 vs other groups	0 vs other DIO diets	nr	
		22.4 olive oil 27.8 cornstarch 11.2 sucrose 22.4 casein 6.19 cellulose 1.24 vitamin mix 8.65 mineral mix	22.4	42					0 vs other DIO diets	0 vs other DIO diets	i vs fish oil	0 vs other DIO diets	nr	
		22.4 beef tallow 27.8 cornstarch 11.2 sucrose 22.4 casein 6.19 cellulose 1.24 vitamin mix 8.65 mineral mix	22.4	42					0 vs other DIO diets	0 vs other DIO diets	i vs fish oil	0 vs other DIO diets	nr	

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

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Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Hill et al (1993)	rat/ Wistar/ male/ 300 g/ 13-17 weeks	g/100 g 18.2 corn oil 1.3 safflower oil 20 casein 16.2 sucrose 16.2 cornstarch 0.3 DL methionine 5 fiber 1 vitamin mix 3.5 mineral mix	19.5	45	nr	nr	nr	25	0 vs other DIO diets	nr	(total) i vs fish oil	-	(kcal) 0 vs other DIO diets	15
		18.2 lard 1.3 safflower oil 20 casein 16.2 sucrose 16.2 cornstarch 0.3 DL methionine 5 fiber 1 vitamin mix 3.5 mineral mix	19.5	45					0 vs other DIO diets	nr	i vs fish oil	-	i vs fish oil	
		18.2 fish oil 1.3 safflower oil 20 casein 16.2 sucrose 16.2 cornstarch 0.3 DL methionine 5 fiber 1 vitamin mix 3.5 mineral mix	19.5	45					0 vs other DIO diets	nr	d vs corn oil and lard	-	d vs lard	

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Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Shillabeer and Lau (1994)	rat/ Sprague Dawley/ male/ 50-60 g/ 4 weeks	% of total calories 59 beef tallow 21 carbohydrate 20 protein	38	45	Purina chow (Company not specified)	4.5	9.6	26	0 0 vs other DIO diets	nr	(epididymal, and inguinal) i 0 vs other DIO diets	nr	(kcal) 0 vs other DIO diets	16
		59 safflower oil 21 carbohydrate 20 protein	38	45					0 0 vs other DIO diets	nr	0 0 vs other DIO diets	nr	0 vs other DIO diets	
		5 beef tallow 75 carbohydrate 20 protein	2.2	5					0 0 vs other DIO diets	nr	0 0 vs other DIO diets	nr	0 vs other DIO diets	
Jones et al (1995)	rat/ Sprague Dawley/ male/ 193±9.1 g/ nr	g/100g 19 beef tallow 31.2 cornstarch 11.2 sucrose 22.4 casein 6.2 cellulose 1.2 vitamin mix 8.6 mineral mix	20	40	nr	nr	nr	10	0 vs other DIO diets	nr	-	-	nr	17
		19 fish oil 31.2 cornstarch 11.2 sucrose 22.4 casein 6.2 cellulose 1.2 vitamin mix 8.6 mineral mix	20	40					0 vs other DIO diets	nr	-	-	nr	
		19 olive oil 31.2 cornstarch 11.2 sucrose 22.4 casein 6.2 cellulose 1.2 vitamin mix 8.6 mineral mix	20	40					0 vs other DIO diets	nr	-	-	nr	

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

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Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Jones et al (1995)	rat/ Sprague Dawley/ male/ 193±9.1 g/ nr	g/100g 19safflower oil 31.2 cornstarch 11.2 sucrose 22.4 casein 6.2 cellulose 1.2 vitamin mix 8.6 mineral mix	20	40	nr	nr	nr	10	0 vs other DIO diets	nr	-	-	nr	17 (cont'd)
Takeuchi et al (1995)	rat/ Sprague Dawley/ male/ 95-97 g/ 4 weeks	g/100g 20 lard 39.8 cornstarch 24 casein 5 sucrose 5 cellulose 1.2 vitamin mix 4.2 mineral mix 0.4 DL methionine 0.3 choline 0.002 α-tocopherol	20	39.4	nr	nr	nr	12	i vs high oleic safflower oil and linseed oil	nr	(epididymal, perinephrial and mesenteric) i vs high oleic safflower oil, safflower oil and linseed oil	nr	(kj) 0 vs other DIO diets	18
		20 high oleic safflower oil 39.8 cornstarch 24 casein 5 sucrose 5 cellulose 1.2 vitamin mix 4.2 mineral mix 0.4 DL methionine 0.3 choline 0.002 α-tocopherol	20	39.4					d vs lard	nr	d vs lard	nr	0 vs other DIO diets	

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

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Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Takeuchi et al (1995)	rat/ Sprague Dawley/ male/ 95-97 g/ 4 weeks	20 safflower oil 39.8 cornstarch 24 casein 5 sucrose 5 cellulose 1.2 vitamin mix 4.2 mineral mix 0.4 DL methionine 0.3 choline 0.002 α-tocopherol	20	39.4	nr	nr	nr	12	0 vs other DIO diets	nr	epididymal, perinephrial and mesenteric) d vs lard	nr	0 vs other DIO diets	18 (cont'd)
		20 linseed oil 39.8 cornstarch 24 casein 5 sucrose 5 cellulose 1.2 vitamin mix 4.2 mineral mix 0.4 DL methionine 0.3 choline 0.002 α-tocopherol	20	39.4					d vs lard	nr	d vs lard	nr	0 vs other DIO diets	
Yaqoob et al (1995)	rat/ Lewis/ male/ 65-85 g/ 3 weeks	g/100g 20 coconut oil 1 corn oil 0.0004 cholesterol 20 protein 49.5 carbohydrate 5 nonnutritive bulk 0.12 vitamin E	21	40.5	g/100g 14.6 protein 48.4 carbohydrate 18.6 non- nutritive bulk 0.01 vitamin E 2.4 unspecified oil 0.0001 cholesterol	2.4	6	10	i	i	(epididymal) i i vs other DIO diets	i 0 vs other DIO diets	(kcal) d vs olive oil, evening primrose oil, fish oil, control	19

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

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Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Yaqoob et al (1995)	rat/ Lewis/ male/ 65-85 g/ 3 weeks	g/100g 20 olive oil 1 corn oil 0.0004 cholesterol 20 protein 49.5 carbohydrate 5 nonnutritive bulk 0.12 vitamin E	21	40.5	g/100g 14.6 protein 48.4 carbohydrate 18.6 non- nutritive bulk 0.01 vitamin E 2.4 unspecified oil 0.0001 cholesterol	2.4	6	10	i d vs fish oil	i d vs fish oil	(epididymal) i d vs coconut oil	i 0 vs other DIO diets	(kcal) i vs coconut oil	19 (cont'd)
		20 safflower oil 1 corn oil 0.0004 cholesterol 20 protein 49.5 carbohydrate 5 nonnutritive bulk 0.12 vitamin E	21	40.5					i	i	i d vs coconut oil i vs evening primerose oil, fish oil	i 0 vs other DIO diets	0 vs other DIO diets	
		20 evening primrose oil 1 corn oil 0.0004 cholesterol 20 protein 49.5 carbohydrate 5 nonnutritive bulk 0.12 vitamin E	21	40.5					i d vs fish oil	i d vs fish oil	i d vs coconut oil, safflower oil	i 0 vs other DIO diets	i vs coconut oil	
		20 fish oil 1 corn oil 0.0004 cholesterol 20 protein 49.5 carbohydrate 5 nonnutritive bulk 0.12 vitamin E	21	40.5					i i vs olive oil and evening primrose oil	i i vs olive oil and evening primrose oil	i d vs coconut oil, safflower oil	i 0 vs other DIO diets	i vs coconut oil	

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

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Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Ikemoto et al (1996)	mouse/ C57BL/6J/ female/ nr/ 7 weeks	g/100g 32 palm 33.1 casein 17.6 sacrose 1.4 vitamin mix 9.8 mineral mix 5.6 cellulose powder 0.5 DL methionine	32	60	g/100g 4 safflower oil 23.7 casein 10 sucrose 50 starch 1 vitamin mix 7 mineral mix 4 cellulose powder 0.4 DL methionine	4	11	19	i	i	(parametrial) i	nr	(kcal) 0 d vs fish oil	20
		32 lard 33.1 casein 17.6 sacrose 1.4 vitamin mix 9.8 mineral mix 5.6 cellulose powder 0.5 DL methionine	32	60					i	i	i	nr	0 i vs perilla oil	
		32 fish oil 33.1 casein 17.6 sacrose 1.4 vitamin mix 9.8 mineral mix 5.6 cellulose powder 0.5 DL methionine	32	60					0	0	0	nr	i i vs palm oil, rapeseed oil, perilla oil	
		32 Perilla oil 33.1 casein 17.6 sacrose 1.4 vitamin mix 9.8 mineral mix 5.6 cellulose powder 0.5 DL methionine	32	60					i	i	i	nr	0 d vs lard, safflower oil, fish oil	

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		DIO diet	Amount of fat		Control diet	Amount of fat		Duration (weeks)	Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	Line number
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Ikemoto et al (1996)	mouse/ C57BL/6J/ female/ nr/ 7 weeks	g/100g 32 rapeseed oil 33.1 casein 17.6 sacrose 1.4 vitamin mix 9.8 mineral mix 5.6 cellulose powder 0.5 DL methionine	32	60	g/100g 4 safflower oil 23.7 casein 10 sucrose 50 starch 1 vitamin mix 7 mineral mix 4 cellulose powder 0.4 DL methionine	4	11	19	i	i	(parametrial) i	nr	(kcal) 0 d vs fish oil	20 (cont'd)
		32 soy-bean oil 33.1 casein 17.6 sacrose 1.4 vitamin mix 9.8 mineral mix 5.6 cellulose powder 0.5 DL methionine	32	60					i	i	i	nr	0	
		32 safflower oil 33.1 casein 17.6 sacrose 1.4 vitamin mix 9.8 mineral mix 5.6 cellulose powder 0.5 DL methionine	32	60					i	i	i	nr	0 i vs perilla oil	

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

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		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Bell et al (1997)	mouse/ Swiss Albino/ female/ nr/ 6 weeks	g/100g 14.4 beef fat 6 corn oil 24.1 casein 0.4 DL methionine 25.3 cornstarch 18.1 sucrose 6 cellulose 1.2 vitamin mix 4.2 mineral mix 0.2 choline 0.004 butylated hydroxytoluene	20.5	40.8	g/100g 5 corn oil 20 casein 0.3 DL methionine 50 cornstarch 15 sucrose 5 cellulose 1 vitamin mix 3.5 mineral mix 0.2 choline 0.001 butylated hydroxytoluene	5	11.5	8	i i vs canola oil	nr	(retroperitoneal i i vs canola oil	nr	(kj) 0	21
		14.4 canola oil 6 corn oil 24.1 casein 0.4 DL methionine 25.3 cornstarch 18.1 sucrose 6 cellulose 1.2 vitamin mix 4.2 mineral mix 0.2 choline 0.004 butylated hydroxytoluene	20.5	40.8					0 d vs beef fat	nr	0 d vs beef fat	nr	0	

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

2.1. Table - Studies of high-fat diet induced obesity in animal models

Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Okuno et al (1997)	rat/ Sprague-Dawley/ male/ nr/ 4 weeks	g/100g 12 beef tallow 20 casein 59 sucrose 4 cellulose 0.15 choline chloride 4 mineral mix 1 vitamin mix	12	26.2	nr	nr	nr	12	0 vs other DIO diets	nr	(epididymal and perirenal) i vs perilla oil	0 vs other DIO diets	nr	22
		12 oliveoil 20 casein 59 sucrose 4 cellulose 0.15 choline chloride 4 mineral mix 1 vitamin mix	12	26.2					0 vs other DIO diets	nr	i vs perilla oil	0 vs other DIO diets	nr	
		12 safflower oil 20 casein 59 sucrose 4 cellulose 0.15 choline chloride 4 mineral mix 1 vitamin mix	12	26.2					0 vs other DIO diets	nr	0 vs other DIO diets	0 vs other DIO diets	nr	
		12 perilla oil 20 casein 59 sucrose 4 cellulose 0.15 choline chloride 4 mineral mix 1 vitamin mix	12	26.2					0 vs other DIO diets	nr	d vs beef tallow, olive oil	0 vs other DIO diets	nr	

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

2.1. Table - Studies of high-fat diet induced obesity in animal models

Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Cha and Jones (1998)	rat/ Sprague-Dawley / male/ 209±6.5 g/ nr	g/100g 20 fish oil 15 casein 45 cornstarch 10 sucrose 5 cellulose 3.5 mineral mix 1 vitamin mix 0.18 L cystine 0.25 choline bitartrate 0.004 tert-butylhydroquinone	20	36	nr	nr	nr	10	0 vs other DIO diets	nr	(perirenal) d vs safflower oil, beef tallow	0 vs other DIO diets	nr	23
		20 safflower oil 15 casein 45 cornstarch 10 sucrose 5 cellulose 3.5 mineral mix 1 vitamin mix 0.18 L cystine 0.25 choline bitartrate 0.004 tert-butylhydroquinone	20	36					0 vs other DIO diets	nr	i vs beef tallow, fish oil	0 vs other DIO diets	nr	
		20 beef tallow 15 casein 45 cornstarch 10 sucrose 5 cellulose 3.5 mineral mix 1 vitamin mix 0.18 L cystine 0.25 choline bitartrate 0.004 tert-butylhydroquinone	20	36					0 vs other DIO diets	nr	i vs fish oil d vs safflower oil	0 vs other DIO diets	nr	

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

2.1. Table - Studies of high-fat diet induced obesity in animal models

Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Loh et al (1998)	rat/ Zucker / genetically obese and lean/ male/ lean: 112-11 3g, obese: 132-36 g/ 5 weeks	g/100g 35.8 soybean oil 23.5 casein 2.7sucrose 15 cornstarch 17.9 fiber 1 vitamin mix 3.5 mineral mix 0.3 L cystein 0.25 choline chloride	35.8	65	g/100g 6.85 soybean 19.5 casein 53.6 sucrose 10 cornstarch 5 fiber 1 vitamin mix 3.5 mineral mix 0.3 L cystein 0.25 choline chloride	6.8	15	8	0	0 d vs palm olein (only in obese)	(total) 0 d vs palm olein (only in obese)	nr	(kcal) i (only in obese)	24
		30 palm olein 5.8 soybean oil 23.5 casein 2.7sucrose 15 cornstarch 17.9 fiber 1 vitamin mix 3.5 mineral mix 0.3 L cystein 0.25 choline chloride	35.8	65					i (only in obese)	i (only in obese) i vs soybean oil (only in obese)	i (only in obese) i vs soybean oil (only in obese)	nr	i (only in obese)	
George et al (2000)	mouse/ C57B/6J/ female/ nr/ 9-10 weeks	g/100g cocoa butter	17.5	nr	Purina chow (Purina Rodent Lab Chow, no 5001)	8.25	nr	15	0 0 vs safflower oil	nr	-	nr	nr	25
		safflower oil	17.5	nr					0 0 vs cocoa butter	nr	-	nr	nr	

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

2.1. Table - Studies of high-fat diet induced obesity in animal models

Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Harrold et al (2000)	rat/ Wistar/ male/ 150 g/ 6 weeks	g/100g 33 nestle condensed milk 7 sucrose 33 ground pellet	6.3	13	standard pellet (CRM Biosure, Cambridge, U.K.) % of total calories 9 fat 65 carbohydrate 24 protein	4.3	9.2	8	i (high weight-gainers only) 0 (low weight-gainers only) i (high vs low weight-gainers)	i (high weight-gainers only) 0 (low weight-gainers only) i (high vs low weight-gainers	(epididymal and perirenal) i i (high vs low weight-gainers	nr	(kj) i i (high vs low weight-gainers	26
Ainslie et al (2000)	rat/ Wistar/ female/ 223-233 g/ 20-22 weeks	10 ml fat emulsion (Intralipid; Kabi Pharmacia, AB, Stockholm) + nonpurified lab diet (not specified)	20	36	nonpurified diet (not specified)	3	6.5	14	i	nr	(abdominal, infrarenal and subcutaneous) i	nr	(kj) i	27
Ghibaudi et al (2002)	rat/ Sprague-Dawley/ male/ 50-60 g/ weaning	% of total calories 45 fat 35 carbohydrate 20 protein (D12451; Research Diets, New Brunswick, NJ),	26	45	% of total calories 10 fat 70 carbohydrate 20 protein (D12450B; Research Diets)	4.6	10	26	i	i	(total) i	nr	(kcal) i	28
		32 fat 51 carbohydrate 17 protein (D12266; Research Diets)	17	32					0	0	0	nr	0	

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

2.1. Table - Studies of high-fat diet induced obesity in animal models

Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Levin and Dunn-Meynell (2002)	rat/ Sprague-Dawley (DIO and DR)/ male/ DR: 282-327 g DIO: 368-402 g/ 10-12 weeks	g/100g 8 corn oil 44 sweetened condensed milk 48 Purina chow (Purina Rat Chow, no 5008) % of total calories 31 fat 48 carbohydrate 21 protein	16.6	31	Purina chow (Purina Rat Chow, no 5008)	4.5	9.6	10	i (only in DIO) d vs Ensure	i (only in DIO) d vs Ensure	(epididymal, retroperitoneal, perirenal and mesenteric) i (only in DIO) d vs Ensure	nr	(kcal) 0 d vs Ensure	29
		DIO diet + chocolate flavored liquid diet (Ensure; Proctor & Gamble, Cincinnati, OH, USA) % of total calories 22 fat 64 carbohydrate 14 protein	11.1	22					i i vs DIO diet	i i vs DIO diet	i i vs DIO diet	nr	i i vs DIO diets	
Levin and Dunn-Meynell (2002)	rat/ Sprague-Dawley (DIO and DR)/ male/ 300-425 g/ nr	g/100g 8 corn oil 44 sweetened condensed milk 48 Purina chow(Purina Rat Chow, no 5001) % of total calories 31 fat 48 carbohydrate 21 protein	16.6	31	nr	nr	nr	4	i vs DR	nr	(total) i vs DR	nr	nr	30

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

2.1. Table - Studies of high-fat diet induced obesity in animal models

Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Ellis et al (2002)	rat/ Sprague Dawley / female/ 61 g/ 3 weeks	g/100g 20 corn oil 22.6 casein 45.4 dextrin 0.5 DL methionine 1.5 vitamin mix 3 mineral mix 4 cellulose	20	40	g/100g 2.4 corn oil 18.2 casein 70.5 dextrose 0.4 DL methionine 1.5 vitamin mix 3 mineral mix 4 cellulose	2.4	6	8	i i vs coconut oil (only in low fat diet)	i i vs coconut oil (only in low fat diet)	(total) % change i i vs coconut oil (only in low fat diet)	nr	(kcal) 0	31
		20 canola oil 22.6 casein 45.4 dextrin 0.5 DL methionine 1.5 vitamin mix 3 mineral mix 4 cellulose	20	40	2.4 canola oil 18.2 casein 70.5 dextrose 0.4 DL methionine 1.5 vitamin mix 3 mineral mix 4 cellulose	2.4	6		i	i i vs coconut oil (only in low fat diet)	i i vs coconut oil (only in low fat diet)	nr	0	
		20 coconut oil 22.6 casein 45.4 dextrin 0.5 DL methionine 1.5 vitamin mix 3 mineral mix 4 cellulose	20	40	2.4 coconut oil 18.2 casein 70.5 dextrose 0.4 DL methionine 1.5 vitamin mix 3 mineral mix 4 cellulose	2.4	6		i d vs corn oil (only in low fat diets)	i d vs canola oil, corn oil (only in low fat diets)	i d vs canola oil, corn oil (only ir low fat diets)	nr	0	
Rolland et al (2002)	rat/ Zucker (lean and obese)/ male/ obese: 227±12 lean:196±12/ 6 weeks	g/100g 17.1 butter 54.4 starch 20.4 casein 2 cellulose 5 mineral mix 1 vitamin mix 0.15 methionine	17.1	30	nr	nr	nr	11	i vs soybean oil (only in obese) d vs soybean oil (only in lean)	nr	(total) i vs soybean oil (only in obese)	i vs soybean oil	nr	32

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

2.1. Table - Studies of high-fat diet induced obesity in animal models

Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Rolland et al (2002)	rat/ Zucker (lean and obese)/ male/ obese: 227±12 lean:196±12/ 6 weeks	g/100g 14.1 soybean oil 56.5 starch 21.2 casein 2 cellulose 5 mineral mix 1 vitamin mix 0.15 methionine	14.1	27	nr	nr	nr	11	d vs butter (only in obese) i vs butter (only in lean)	nr	(total) d vs butter (only in obese)	d vs butter	nr	32 (cont'd)
Wang et al (2002)	mouse/ C57BL/6J/ male/ nr/ 3 weeks	g/100g 16.9 beef tallow 16.9 cornstarch 8.5 sucrose 25.4 casein 1.9 gelatin 5.1 bran 6.7 mineral mix 1.3 vitamin mix 0.3 methionine	16.9	58	g/100g 4.1 safflower oil 43.8 cornstarch 23.9 sucrose 18.8 casein 1.4 gelatin 3.8 bran 5 mineral mix 0.97 vitamin mix 0.23 methionine	4.1	10	7	nr	nr	(epididymal, perirenal and inguinal) i i vs fish oil	d vs safflower oil (week 5-7)	(kj) i 0 vs other DIO diets	33
		16.9 safflower oil 16.9 cornstarch 8.5 sucrose 25.4 casein 1.9 gelatin 5.1 bran 6.7 mineral mix 1.3 vitamin mix 0.3 methionine	16.9	58					nr	nr	0	i vs beef tallow (week 5-7)	i 0 vs other DIO diets	

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

2.1. Table - Studies of high-fat diet induced obesity in animal models

Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Wang et al (2002)	mouse/ C57BL/6J/ male/ nr/ 3 weeks	g/100g 16.9 fish oil 16.9 cornstarch 8.5 sucrose 25.4 casein 1.9 gelatin 5.1 bran 6.7 mineral mix 1.3 vitamin mix 0.3 methionine	16.9	58	g/100g 4.1 safflower oil 43.8 cornstarch 23.9 sucrose 18.8 casein 1.4 gelatin 3.8 bran 5 mineral mix 0.97 vitamin mix 0.23 methionine	4.1	10	7	nr	nr	(epididymal, perirenal and inguinal) d d vs beef tallow	nr	i 0 vs other DIO diets	33 (cont'd)
Jen et al (2003)	rat/ Wistar/ female/ nr/ 3 weeks	g/100g 40 soybean oil 26 casein 0.65 cornstarch 0.217 maltodextrin 20 maltose dextrin 6.5 cellulose 0.008 butylhydroquinone 4.5 salt mix 1.3 vitamin mix 0.039 vitamin E 0.39 L cystine 0.325 choline bitartrate	40	65.4	g/100g 7 soybean oil 20 casein 32.1 cornstarch 10.72 maltodextrin 20 maltose dextrin 5 cellulose 0.008 butylhydroquinone 3.5 salt mix 1 vitamin mix 0.03 vitamin E 0.3 L cystine 0. 25 choline bitartrate	7	15.9	6	i i vs fish oil	nr	(abdominal fat) i i vs fish oil	nr	(kcal) 0 d vs palm oil	34
		33 palm oil 7 soybean oil 26 casein 0.65 cornstarch 0.217 maltodextrin 20 maltose dextrin 6.5 cellulose 0.008 butylhydroquinone 4.5 salt mix 1.3 vitamin mix 0.039 vitamin E 0.39 L cystine 0.325 choline bitartrate	40	65.4					i i vs fish oil	nr	i i vs fish oil	nr	i i vs soybean oil, fish oil	

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

2.1. Table - Studies of high-fat diet induced obesity in animal models

Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Jen et al (2003)	rat/ Wistar/ female/ nr/ 3 weeks	g/100 g 33 fish oil 7 soybean oil 26 casein 0.65 cornstarch 0.217 maltodextrin 20 maltose dextrin 6.5 cellulose 0.008 butylhydroquinone 4.5 salt mix 1.3 vitamin mix 0.039 vitamin E 0.39 L cystine 0. 25 choline bitartrate	40	65.4	g/100g 7 soybean oil 20 casein 32.1 cornstarch 10.72 maltodextrin 20 maltose dextrin 5 cellulose 0.008 butylhydroquinone 3.5 salt mix 1 vitamin mix 0.03 vitamin E 0.3 L cystine 0. 25 choline bitartrate	7	15.9	6	0 d vs soybean oil, palm oil	nr	(abdominal fat) 0 d vs soybean oil, palm oil	nr	0 d vs palm oil	34 (cont'd)
Woods et al (2003)	rat/ Long-Evans/ male and female/ 250-350 g/ 9-10 weeks	g/100g 19 butter oil 1 soybean oil 16.4 casein 30.3 cornstarch 11.5 dextrose 8.9 sucrose 5.8 cellulose 5.2 mineral mix 1.17 vitamin mix 0.21 Lcystine 0.29 choline bitartrate	20	40	g/100g 3 butter oil 1 soybean oil 14 casein 45.5 cornstarch 15.5 dextrose 10 sucrose 5 cellulose 4.5 mineral mix 1 vitamin mix 0.18 Lcystine 0.25 choline bitartrate	4	8	10	i	nr	(total) i	nr	(kj) i	35

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

2.1. Table - Studies of high-fat diet induced obesity in animal models

Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Huang et al (2004)	mouse/ C57BL/6J/ male/ nr/ 3 weeks	% of total calories 59 fat 14 carbohydrate 27 protein (not specified)	38.9	59	% of total calories 10 fat 63 carbohydrate 27 protein (Company not specified)	4.6	10	13	nr	i (only in obesity prone)	-	nr	(kcal) i	36
Silva et al (2006)	rat/ Wistar/ male/ nr/ 3 weeks	g/100g 7 soybean oil 15 casein 67.9 cornstarch 5 cellulose 3.5 mineral mix 1 vitamin mix 1.8 Lcystine 0.25 choline	7	16	nr	nr	nr	3-4	d vs palm oil and hydrogenated vegetable oil	nr	(epididymal) d vs palm oil and hydrogenated vegetable oil	0 vs other DIO diets (starting week 3)	-	37
		5 palm oil 2 soybean oil 15 casein 67.9 cornstarch 5 cellulose 3.5 mineral mix 1 vitamin mix 1.8 Lcystine 0.25 choline	7	16					i vs soy oil and canola oil	nr	i vs soy oil, canola oil and hydrogenated vegetable oil	0 vs other DIO diets (starting week 3)	-	
		6 canola oil 1 soybean oil 15 casein 67.9 cornstarch 5 cellulose 3.5 mineral mix 1 vitamin mix 1.8 Lcystine 0.25 choline	7	16					d vs palm oil and hydrogenated vegetable oil	nr	d vs palm oil and hydrogenated vegetable oil	0 vs other DIO diets (starting week 3)	-	

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

2.1. Table - Studies of high-fat diet induced obesity in animal models

Author (s)/ Year	Specie/ Strain/Sex/ Weight/Age	DIET COMPOSITION						Duration (weeks)	CHANGES IN BODY WEIGHT AND DIETARY INTAKE					Line number
		DIO diet	Amount of fat		Control diet	Amount of fat			Final/daily body weight (g)	Body weight gain (g)	Body fat (g)	Food intake (g)	Energy intake	
			g/100 g diet	% of total energy		g/100 g diet	% of total energy							
Silva et al (2006)	rat/ Wistar/ male/ nr/ 3 weeks	g/100g 6 hydrogenated vegetable oil 1 soybean oil 15 casein 67.9 cornstarch 5 cellulose 3.5 mineral mix 1 vitamin mix 1.8 Lcystine 0.25 cholin	7	16	nr	nr	nr	3-4	i vs soy oil and canola oil	nr	(epididymal) i vs soy oil and canola oil d vs palm oil	0 vs other DIO diets (starting week 3)	-	37 (cont'd)
Okere et al (2006)	rat/ Wistar/ male/ 329±9.7/ 8-9 weeks	cocoa butter (Research Diets, New Brunswick, NJ)	nr	60	Purina chow (Teklad)	4.7	10	8	0 0 vs other DIO diet	0 0 vs other DIO diet	(abdominal, epididymal and intra-thoracic) i i vs safflower oil (intra-thooracic)	nr	(kcal) 0 0 vs other DIO diet	38
		safflower oil (Research Diets, New Brunswick, NJ)	nr	60					0 0 vs other DIO diet	0 0 vs other DIO diet	d d vs safflower oil (epididymal and abdominal) 0 i vs cocoa butter (epididymal and abdominal) d vs coca butter (intra-thooracic fat)	nr	0 0 vs other DIO diet	

i: increase vs control diet or as specified; d: decrease vs control diet or as specified; 0: no change or difference vs control diet or as specified; nr: not reported; - : not measured; DR: dietary obesity resistant

BRIDGE 1

Fat storage in response to high-fat feeding is shown to be dependent upon fatty acid composition of the diet, with SFA more preferably stored in the body and MUFA and PUFA rather used as fuel. The literature reports on diet-induced obesity in animal models show that high-fat diets used in different laboratories varied in fat percentage of the diet and their fatty acid composition. Most studies have contrasted diets in which SFA was predominant (not necessarily high) with those mainly containing MUFA or PUFA (low in SFA). Considering the fact that the quantity of SFA in the diet might play an important role in body weight regulation and fat storage, the objective of the first study was to examine the effect of high-fat diets containing high, moderate and low SFA content on inducing obesity in adult female rats. Besides, this study also aimed to investigate whether fatty acid profile of the diet was involved in animals' ability to adjust intake according to energy density, and if it modulates body weight loss.

CHAPTER 3, MANUSCRIPT 2

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A highly saturated fat-rich diet is more obesogenic than diets with lower saturated fat content

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3.1. Abbreviations

ANCOVA; analysis of covariance

ANOVA; analysis of variance

MUFA; monounsaturated fatty acids

PUFA; polyunsaturated fatty acids

SFA; saturated fatty acids

3.2. Abstract

The present study tested the hypothesis that a saturated fatty acid (SFA)-rich diet is more obesogenic than diets with lower SFA content. In eight female Sprague-Dawley rats fed a low-SFA canola or a moderate-SFA lard-rich diets at 67% of energy for 26 days, body weight gain, final body weight, obesity index and food and energy intake were comparable. Twenty-nine rats were fed canola or high-SFA butter-rich diets (67% of energy) or chow for 50 days, then high-fat feeding was followed by ad libitum low-fat feeding (27% of energy) for 28 days and by a food restricted low-fat diet for 32 days. High-fat feeding resulted in a greater body weight gain ($p<0.04$), final body weight ($p<0.04$), and energy intake ($p<0.008$) in butter-fed rats than in canola and chow-fed controls, after 26 or 50 days. Ad libitum canola and butter low-fat diets or chow feeding resulted in similar weight change, while food restricted low-fat diets led to comparable weight loss and final weight. Canola-fed animals adjusted their intake based on diet energy density, while lard and butter-fed animals failed to do so. Abdominal

fat (p=0.012) and plasma leptin (p=0.005) were higher in chow-fed controls than in canola-fed rats, but comparable to those of butter-fed rats. Prone and resistant phenotypes were detected with high-fat feeding. In conclusion, only feeding the high SFA butter-rich diet led to obesity development, failure to adjust intake based on the energy density and preserving body fat even after weight loss. The high availability of SFA-rich foods in today's obesogenic environment could contribute to develop and maintain obesity.

Key words: Obesity, Dietary Fats, Fatty Acids, Weight Loss Diet, Abdominal Fat, Leptin, Rat.

3.3. Introduction

Obesity is a major health problem with an increasing prevalence throughout the world (World Health Organization 2006). Excessive energy intake and reduced physical activity are important variables in the development of obesity. Dietary patterns, mainly those favoring fat intake, often have been blamed for the increase in adiposity (James 2008; Rosengren and Lissner 2008). High-fat diets have been used to induce obesity in animals in a model first called nutritional obesity, but later renamed as dietary obesity (reviewed in Hariri and Thibault 2010). Animal models of genetic obesity have identified single genes mutations involved in the control of energy homeostasis, although inherited human genetic predisposition involves multiple genes (Bouchard and Tremblay 1997; De Castro 2004). The paradigm of dietary obesity in animal is an appropriate model for

studying human obesity in the context of an environment where energy-dense foods and diets are highly available.

Fatty acid composition of dietary fat may play an important role in body weight regulation. Studies in animals and in humans have shown that polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) are more readily used for fuel, whereas saturated fatty acids (SFA) are more likely to be accumulated in adipose tissue (DeLany et al. 2000; Storlien et al. 2001; Kien et al. 2005). Experimental demonstration of this principle remains inconclusive. No constant pattern of outcome was found in rats fed high-fat diets with different saturation of fatty acids (Awad et al. 1990; Hill et al. 1993; Su and Jones 1993; Shillabeer and Lau 1994; Jones et al. 1995; Okuno et al. 1997; Cha and Jones 1998; Kloss et al. 2005; Buettner et al. 2006). However, most of the available data used male animals fed diets containing moderate (beef tallow, lard, or palm oil) or low (olive oil, safflower oil or corn oil) concentrations of SFA (reviewed in Hariri and Thibault 2010).

Dietary obesity was reversed by using diets low in fat fed ad libitum (Hill et al. 1989; Levin et al. 1989; Bartness et al. 1992; Parekh et al. 1998; Huang et al. 2004), while in other studies feeding low-fat diets resulted in a plateau in body weight (Wade 1983; Levin and Dunn-Meynell 2000; Levin and Dunn-Meynell 2002). Besides, it was shown that feeding responses differ when animals were fed diets of higher or lower energy density. For example, animals adjusted intake when switched to a diet of higher energy density by decreasing food intake and

maintaining energy intake (Wade 1983), or failed to adjust and reduced energy intake when switched to a lower energy dense diet (Hill et al. 1989; Huang et al. 2004).

Leptin and ghrelin are important hormones in the control of food intake and body weight. Leptin is an obese gene product produced by adipose tissue, and rises in plasma levels result in a decrease in food intake and increase in energy expenditure (Halaas et al. 1995). Ghrelin is a peptide released by cells in the fundus of the stomach, which rises before and falls after each ad libitum meal and increases food intake (Tschop et al. 2000).

Although there has been extensive research on dietary obesity, more work is needed to prove that fatty acid profile in high-fat diets can regulate body weight. Thus we conducted the present study to test the hypothesis that a SFA-rich diet is more obesogenic than diets with lower SFA content. The main objectives were to: 1- examine the effect of high-fat diets containing high, moderate and low-SFA content on food intake and body weight gain, 2- test reversal of obesity with low-fat diets, 3 - analyse the feeding response when animals are switched to diets of higher or lower energy density, and 4- measure abdominal fat, plasma leptin and active and total ghrelin levels after weight loss. We used genetically similar adult female Sprague-Dawley rats fed low-SFA canola oil (7%), moderate-SFA lard (43%), and high-SFA butter (68%) high-fat diets at 67% of energy to test obesity development, and then canola and butter in low-fat diets at 27% of energy to test obesity reversal. Female rats' body weight becomes stable in early adulthood,

whereas male rats continue to gain weight throughout their lives (Charles River Laboratories). Human subjects of both sexes have a growth pattern more like that of female rats. The estrous cycle of female animals synchronizes when they live together (McClintock 1981). Total body weight gain was also used to categorize animals as obesity prone, resistant and intermediate. Lee obesity index (Lee 1929) was measured, as reliable correlations were found between this index and body fat (Kanarek and Markskaufman 1979; Rogers and Webb 1980).

By using the animal model of dietary obesity that shares many features with human obesity (Thibault et al. 2004), these experiments will provide much important data on how dietary fatty acid profile affects the development and treatment of obesity.

3.4. Methods and materials

3.4.1. Animals and diets

Adult female Sprague-Dawley rats weighing 250-310 g (13 weeks of age) obtained from Charles River Laboratories (St-Constant, Quebec) were used for these experiments. They were housed in individual cages, under controlled temperature (22-25^{0}C) and humidity (70%) and with a 12:12 light-dark cycle for which the lights were on from 06:00 to 18:00. Prior to the experiments, all the rats had ad libitum access to Purina chow and tap water for 7-10 days to allow them to adjust to their new environment. The research procedures were approved by the McGill University Animal Care Committee. The high and low-fat diets were prepared in our laboratory (Table 1). The high-fat diets were isoenergetic and

provided 67% of energy from fat; the protein content was adequate for the rats' maintenance requirements (5 g/100 g of diet; (Institute for Laboratory Animal Research 1995)). The low-fat diets were isoenergetic, providing 27% of energy from fat.

Eight rats were randomly assigned to two experimental groups fed either high-fat lard (N=4) or canola (N=4) -based diets (Figure 1). Animals had free access to their diets and tap water for 26 days. Another group of twenty rats were randomly assigned to two experimental groups fed high-fat butter (N=10) or canola (N=10) -based diets. An age-matched female control group (N=9) was fed chow ad libitum throughout the study (Figure 1). The animals had free access to the diets and tap water for 50 days and then were switched to ad libitum low-fat diet feeding to induce weight loss. Because body weight remained stable after 28 days on this diet, food was restricted to 60% of intake for 32 days at which time they were euthanized by decapitation with CO_2 anasthesia at one of two different periods: in the morning immediately after lights were turned on or in the evening immediately before lights were turned off. This was done to compare ghrelin levels at these two time points identified as the trough and peak of circadian variation of ghrelin (Bodosi et al. 2004; Sanchez et al. 2004). Leptin was also measured at these time points even if its circadian rhythmicity differs from that of ghrelin; plasma leptin levels are expected to be higher in the middle of rats' active phase and reach a minimum in the middle of their resting phase (Bodosi et al. 2004; Chacon et al. 2005). Abdominal fat (retroperitoneal, perirenal and

mesenteric) was removed and blood was collected in tubes containing K2-EDTA and stored at –80°C for analyses.

3.4.2. Measurements

Body weight and food intake were measured daily throughout the study using a digital Mettler PJ 3000 balance. Food intake was corrected for spillage on a daily basis. Food efficiency was calculated as grams of body weight gain per kilojoule of food consumed (LeBlanc and Labrie 1997). Lee obesity index was assessed on day 19 of exposure to the canola and lard-rich diets and on day 29 of exposure to the canola and butter-rich diets or chow. It was measured under CO_2 anesthesia and calculated by dividing the cube root of the body weight (g) to the naso-anal length (cm) multiplied by 1000 (Lee 1929). Following 50 days of high-fat feeding, rats in the highest tertile of total body weight gain were considered obesity prone (canola: N=3, body weight gain: 53-55 g; butter: N=3, 97-159 g), and those in the lowest tertile were considered obesity resistant (canola: N= 2, 3-8 g; butter: N=5, 41-57 g). The other animals were designated as being in the intermediate group (canola: N=5, 20-39 g; butter: N=2, 78 g). Previously published work used a similar categorization to compare variation of body weight gain within genetically similar animals fed diets rich in fat (Levin and Keesey 1998; Levin and Dunn-Meynell 2000; Levin and Dunn-Meynell 2002; Huang et al. 2004; Dourmashkin et al. 2006). Total body weight gain of the chow-fed animals was ranging from -5 to 44 g. Lee obesity index was also measured following 32 days of restricted low-canola and butter feeding as well as in chow-

fed rats. Abdominal fat was weighed using Mettler PJ 3000 balance. Plasma leptin, active ghrelin (acylated form of ghrelin responsible for its biological characteristics) and total ghrelin (acylated + deacylated forms) were assessed using RIA kits and iodine isotope 125 (Linco Research Company, Missouri, USA). Serum samples (100 µl) were added to 100 µl of the assay buffer, and then 100 µl of antibody (leptin or ghrelin) was added and incubated for 24 hours (LINCO Research Company). The tracer was then added (100 µl) and after a second 24-hour incubation and addition of a reagent and/or a carrier, the samples were read by a gamma reader and the amount of leptin or ghrelin in the samples was calculated.

3.4.3. Statistical analyses

Statistical analyses were performed to test the main effects of diet, period, time of the day and phenotype on the dependent variables. Data were first analyzed for diet main effect to make within and between groups comparisons for the first (canola or lard) and the second group of animals (canola, butter or chow). One-way repeated measures analysis of covariance (ANCOVA) was used to analyse body weight, and one-way ANCOVA was used for final body weight, daily body weight gain and obesity index, with diet as the main effect and initial weight as the covariate. For daily food and energy intakes one-way repeated measures analysis of variance (ANOVA) was used, and for mean food and energy intakes, food efficiency and initial weight analyses were performed using one-way ANOVA with diet as the main effect. Within-subjects comparisons measured

the effect of period (chow versus high-fat diet or chow versus high-fat diet versus low-fat diet) and diet and their interaction on the above dependent variables by using two-way repeated measures ANOVA (intake data) and ANCOVA (body weight). For leptin, active and total ghrelin, analyses were performed using two-way ANOVA with diet and time of the day as the main effects, and testing their interaction. In rats fed canola and butter-rich diets or chow, the above dependent variables were also assessed using two-way ANOVA and ANCOVA with phenotype (prone, intermediate and resistant) and diet as the main effects, and testing their interaction. In the analyses, chow-fed animals were considered as a group. For post-hoc analyses of the significant main effects, multiple comparisons were conducted by using Scheffe's test. In addition, correlation analyses were performed. Data are presented as means ± SEM, and all differences were considered statistically significant if $p<0.05$. SAS version 8.02 was used for analyzing the data. ANOVA results for high-fat canola and butter-based diets and chow feeding are presented in Table 2.

3.5. Results

3.5.1. Body weight and Lee obesity index

3.5.1.1. High-fat feeding

Initial weight of the animals fed canola and lard was comparable at baseline (317.0±7.7 and 293.0±7.7 g, respectively). Daily body weight and weight gain, final weight and food efficiency were greater in animals fed lard for 26 days than

canola, but these differences did not reach statistical significance (daily body weight gain: 1.81±0.33 and 1.12±0.33 g/day, final weight: 352.1±8.6 and 334.4±8.6 g, respectively, all values were adjusted for initial weight; food efficiency: 0.0057±0.0007 and 0.0043±0.0007 g/kJ, respectively). Within-subject comparisons revealed a significantly greater final weight following 26 days of high-fat lard feeding than after 10 days of chow feeding at baseline ($p<0.0001$), while no significant difference between final body weight at these two time points was found in rats fed canola. Lee obesity index was similar in rats fed lard or canola-rich diets (308.7±5.1 and 310.3±5.1, respectively), and was not correlated with body weight gain and final weight.

Initial weight of the animals fed canola, butter and chow was comparable at baseline (287.9±6.4, 295.5±6.4 and 287.3±6.7 g, respectively). Greater daily body weight, daily body weight gain and final weight were found in rats fed the butter-based diet ($p<0.05$, $p<0.04$ and $p<0.04$, respectively) than in animals fed canola or chow for 26 days (experimental day 33) (daily body weight gain: butter: 2.1±0.3 g/day, canola: 0.7±0.3 g/day, chow: 0.8±0.2 g/day; final weight: butter: 346.2±8.9 g, canola: 307.5±8.9 g, chow: 311.9±6.5 g); or 50 days (experimental day 57) (daily body weight gain: butter: 1.4±0.2 g/day, canola: 0.7±0.2 g/day, chow: 0.5±0.2 g/day; final weight: butter: 363.1±10.5 g, canola: 323.8±10.5 g, chow: 318.0±7.6 g) (Figure 2). A significant correlation was found between initial and final weights in canola and butter-fed rats over 50 days of high-fat feeding. ($r=0.86$, $p=0.002$ and $r=0.85$, $p=0.002$, respectively). In control animals this correlation approached significance ($r=0.66$, $p=0.051$).

High-fat butter feeding for 26 days resulted in greater food efficiency (0.006±0.0007 g/kJ) than canola or chow feeding (0.0024±0.0007 and 0.0029±0.0007 g/kJ, respectively, $p<0.02$). High-fat butter feeding for 50 days also resulted in greater food efficiency (0.0038±0.0007 g/kJ, $p=0.02$) than chow feeding (0.0019±0.0005 g/kJ), with food efficiency of the canola-based diet (0.0026±0.0007 g/kJ) in between. Lee obesity index measured after 28 days on high-fat diets was comparable in rats fed butter, canola and chow (320.4±4.6, 312.0±4.6 and 311.2±3.4, respectively). A positive correlation was found between obesity index and body weight gain in both butter ($r=0.81$, $p=0.004$) and canola-fed rats ($r=0.63$, $p=0.049$), but not in control animals ($r=0.57$, $p=0.109$). Correlation between obesity index and final weight was significant in all dietary groups (butter: $r=0.74$, $p=0.014$; canola: $r=0.69$, $p=0.027$; chow: $r=0.88$, $p=0.001$).

Prone, resistant and intermediate animals fed high-fat diets and control rats had comparable initial weights. Fifty days of high-canola or butter feeding resulted in a greater body weight gain and final weight in butter-fed prone animals (2.3±0.2 g/day, 410.2±8.6 g) than in butter-fed resistant and intermediate rats (body weight gain: 1.0±0.1 and 1.5±0.2 g/day, respectively, $p<0.003$; final weight: 341.1±6.0 and 368.0±9.3 g, respectively, $p<0.0001$), in prone, resistant and intermediate animals fed canola (1.1±0.2, 0.1±0.2 and 0.5±0.1 g/day, respectively, $p<0.0001$; 344.0±7.6, 296.6±9.7 and 317.0±5.9 g, respectively, $p<0.0001$) and in control rats (0.52±0.1 g/day, 316.5±6.3 g, $p<0.0001$; all values were adjusted for initial weight). Food efficiency of the high-fat diets was

significantly greater in prone rats (0.005±0.0002 g/kJ) than in resistant, intermediate and chow-fed animals (0.0017±0.0002, 0.0031±0.0002 and 0.0019±0.0002 g/kJ, respectively, $p<0.0004$) regardless of the diet. Obesity index measured after 28 days of high-fat feeding was comparable in prone, resistant and intermediate animals (320.8±4.3, 311.9±4.4 and 319.1±4.2, respectively) and control rats (310.9±3.3).

3.5.1.2. Low-fat feeding ad libitum or restricted

With ad libitum low-fat feeding for 28 days (experimental days 58- 85), a significant main effect of diet on daily body weight gain was found (chow: 0.6±0.2 g/day, canola: 0.2±0.2 g/day, butter: 0.1±0.2 g/day; $F(2,24)=3.46$, $p=0.048$), however multiple comparisons did not show significance. In addition, when adjusted for initial weight, animals reached comparable final body weight (353.3±4.4, 342.2±5.9 and 338.4±6.2 g, respectively). Food efficiency was lower in rats fed the low-fat butter diet than in control animals (0.00007±0.0007 and 0.0024±0.0005 g/kJ, respectively, $p=0.022$), with food efficiency of the low-fat canola-fed rats in between (0.0001±0.0007 g/kJ).

Across 32 days of restricted low-fat feeding (experimental days 86-117), originally high-fat butter and canola-fed groups had lower daily body weight gain (-1.4±0.1 and -1.3±0.1 g/day, respectively, $p<0.0001$) than chow-fed rats (0.2±0.1 g/day). A significant main effect of diet on obesity index ($F(2,24)=4.84$, $p=0.017$) was found, with animals fed butter or canola having lower obesity index (308.6±3.95 and 308.8±3.90, respectively; $p<0.044$) than rats fed chow

(319.4±2.87). Following restricted low-fat feeding obesity index was lower in resistant rats (304.7±3.6) than in control animals (319.0±2.7, p=0.004), with obesity index of prone and intermediate rats in between (310.4±3.5 and 311.9±3.4, respectively).

3.5.2. Weight and energy of intake

3.5.2.1. High-fat feeding

In rats fed lard and canola for 26 days, mean food (13.63±0.90 and 12.08±0.90 g/day, respectively) and energy intake (305.89±20.25 and 271.25±20.25 kJ/day, respectively) did not differ significantly. Within-subject comparisons revealed a significant decrease in food intake of the animals switched from chow to canola-based diet (19.43±0.94 and 12.08±0.94 g/day, respectively; $F(1,6)=30.85$, $p=0.001$) or from chow to lard-based diet (17.10±0.86 and 13.63±0.86 g/day, respectively; $F(1,6)=8.07$, $p=0.03$). However, energy intake was maintained in rats fed canola (chow: 276.48±17.11 kJ/day, canola: 271.25±17.11 kJ/day), but it was increased in animals switched to lard (chow: 243.34±16.82 kJ/day, lard: 305.89±16.82 kJ/day; $F(1,6)=6.92$, $p=0.039$).

Over 26 days of high-fat feeding, food intake was greater in chow-fed control animals (18.6± 0.7 g/day) than in rats fed canola or butter-based diets (11.1± 1.0 and 15.9± 1.0 g/day, respectively, $p<0.047$) (Figure 3), while energy intake of animals fed butter was greater (348.61±20.9 kJ/day) than that of the other groups (canola: 243.28±20.9 kJ/day, control: 264.59±15.05 kJ/day;

$p<0.008$). Across 50 days of high-fat feeding, mean food intake of the canola-fed rats (11.0±0.7 g/day) was significantly less than that of the animals fed butter (16.6±0.7 g/day, $p=0.0002$) or chow (18.4±0.5 g/day, $p<0.0001$) (Figure 3). A significant main effect of diet on mean energy intake was also found during that period, with butter-fed rats ingesting more energy (367.42±15.05 kJ/day) than canola and chow-fed animals (244.11±15.05 and 260.83±10.87 kJ/day, respectively; $p<0.0001$). Comparing food intake of animals during 7 days of chow-feeding at baseline and first 7 days of high-fat feeding showed a significant decrease of intake in animals switched from chow to canola-based diet (Figure 4) with maintained energy intake (238.26±13.79 and 264.18±13.79 kJ/day, respectively). Animals switched from chow to high-fat butter-based diet maintained their food intake (Figure 4), and therefore energy intake was increased after the switch (from 249.13±13.79 to 369.09±13.79 kJ/day, $p<0.0001$).

Across 50 days of high-fat feeding, a greater food intake in butter-fed prone and intermediate rats (18.4±0.8 and 17.4±0.9 g/day, respectively) and control animals (18.4±0.7 g/day) than in resistant animals fed butter (14.3±0.6 g/day, $p=0.0003$) and prone, resistant and intermediate animals fed canola (12.0±0.8, 9.9±0.9 and 11.1±0.6 g/day, respectively, $p<0.0009$) was found. Energy intake was greater in butter-fed prone (406.71±13.79 kJ/day, $p<0.0001$), intermediate (385.81±17.14 kJ/day, $p<0.0001$) and resistant animals (333.15±10.87 kJ/day, $p<0.0009$) than in canola-fed rats (prone: 265.01 kJ/day, resistant: 225.3±17.14 kJ/day, intermediate: 246.6±10.87 kJ/day) and control animals (261.25±11.7 kJ/day).

3.5.2.2. Low-fat feeding ad libitum

Food and energy intake were similar across diets during ad libitum low-fat feeding (Figure 3). However, intakes during the last 7 days of high-fat feeding and first 7 days of low-fat feeding showed a greater food intake in animals fed canola after the switch (Figure 4), which resulted in a maintained energy intake (from 251.22±10.45 to 271.7±10.45 kJ/day), while animals fed butter maintained their intake after the switch, therefore decreasing their energy intake (from 378.29±10.45 to 247.04±10.45 kJ/day, $p<0.0001$). Intakes over 50 days of high-fat feeding versus 28 days of low-fat feeding showed that rats fed canola-based diet increased food intake after the switch (from 11.1±0.6 to 18.3±0.6 g/day, $p<0.0001$) with a concomitant greater energy intake (from 246.2±11.7 to 275.04±11.7 kJ/day, $p=0.046$), whereas butter-fed rats maintained food intake (from 16.2±0.6 to 17.6±0.6 g/day) and then had a lower energy intake (from 354.05±11.7 to 264.59±11.7 kJ/day, $p<0.0001$). Weight and energy of intake were not affected by phenotype during ad libitum low-fat feeding.

3.5.3. Abdominal fat

When euthanized at the end of restricted feeding on the low-fat diets, canola-fed rats had a smaller amount of abdominal fat than control animals, with butter-fed rats in between (Table 3). A positive correlation was found between abdominal fat and final body weight in all dietary groups (chow: $r=0.95$, $p=0.0002$; canola: $r=0.75$, $p=0.012$; butter: $r=0.89$, $p=0.0005$). The correlation between abdominal fat and obesity index was significant in rats fed canola

(r=0.86, p=0.001) or butter-based diets (r=0.67, p=0.034), but not in controls. Butter-fed prone rats had a greater amount of abdominal fat (42.9±3.6 g) than butter-fed resistant and intermediate animals (11.0±2.8 and 22.6±4.4 g, respectively, $p<0.002$), canola-fed prone, resistant and intermediate animals (10.1±3.6, 14.0±4.4 and 21.4±2.8 g, respectively, $p \leqslant 0.0001$) and control animals (30.16±3.1 g, $p<0.028$).

3.5.4. Leptin and Ghrelin

A significant main effect of diet on plasma leptin ($F(2,22)=6.42$, $p=0.006$) revealed greater leptin levels in chow-fed rats (8.8±1.2 ng/mL) than in canola-fed animals (2.6±1.6 ng/mL, $p=0.005$), with intermediate leptin levels in butter-fed rats (5.3±1.6 ng/mL) (Table 3). A positive correlation was found between plasma leptin and abdominal fat in all dietary groups (chow: $r=0.92$, $p=0.001$; butter: $r=0.92$, $p=0.0001$; canola: $r=0.63$, $p=0.049$). Only butter-fed animals showed significant positive correlations between leptin and obesity index ($r=0.78$, $p=0.007$) and leptin and final weight ($r=0.74$, $p=0.015$) as well as leptin and food intake during previous high-fat feeding ($r=0.67$, $p=0.03$). Butter-fed prone animals had higher levels of leptin (10.4±1.6 ng/mL) than butter-fed resistant (1.9±1.2 ng/mL, $p=0.0005$) and canola-fed prone, resistant and intermediate animals (1.8±1.6, 2.1±2.6 and 4.7±1.2 ng/mL, respectively, $p<0.0017$).

A significant interaction between diet and time of day was found for total ghrelin ($F(2,22)=4.03$, $p=0.032$), with butter and canola-fed rats having higher

total ghrelin levels in the morning than in the evening (Table 3). Active ghrelin levels did not differ with time of day or across diets. Total and active ghrelin levels were not affected by phenotype.

3.6. Discussion

The present study tested canola, lard and butter, respectively low, moderate and rich sources of SFA, widely consumed in the human diet, in an animal model of dietary obesity. As predicted, results confirmed the hypothesis that a SFA rich diet is more obesogenic than diets with lower SFA content. Indeed, we found that only feeding with a high-butter diet led to obesity development and the characteristics related to it: failure to adjust intake based on the energy density of the diet and preserving body fat even after weight loss.

Body weight gain tended to be higher in rats fed the lard-rich diet than in the canola group. However this difference was not significant, nor was final weight. Other studies failed to find a difference in body weight of adult rats fed high-fat diets with moderate or low SFA content. For example, composition of fatty acids in high-fat diets (33-45% of energy) with safflower oil (low in SFA), fish oil (moderate in SFA), or beef fat (moderate in SFA) fed to adult male Sprague-Dawley rats for 4 weeks (Awad et al. 1990), and with lard (moderate in SFA) or corn oil (low in SFA) fed to adult male Wistar rats for 6 months (Hill et al. 1993) did not affect body weight of the animals. Similarly, adult male Wistar rats fed a high-fat lard-based diet (42% of energy) did not gain weight reliably more than another group fed an olive oil-based diet (low in SFA) for 12 weeks

(Buettner et al. 2006). Comparing high-fat diets with moderate (beef tallow) and low-SFA content (safflower, olive and soybean oil) led to similar results (Su and Jones 1993; Shillabeer and Lau 1994; Jones et al. 1995; Okuno et al. 1997; Cha and Jones 1998).

High-fat butter feeding resulted in a greater daily body weight gain, final body weight, food efficiency and energy intake than chow feeding. These results are in agreement with the poor utilization of SFA for energy and their preferential storage in white adipose tissue (Storlien et al. 2001). SFA also increase food efficiency by decreasing resting metabolic rate and diet induced thermogenesis (Clarke 2000; Casas-Agustench et al. 2009).

To our knowledge, only a few studies have reported that a high degree of saturation of dietary fatty acids promoted the development of obesity versus moderate or low saturated fatty acids in genetically similar animals. Adult male Wistar rats fed a butter-based diet (52% of energy) for 5 weeks reached a greater body weight than rats fed corn oil at the same percentage (Alsaif and Duwaihy 2004). In weaning male Wistar rats, feeding high-fat diets (27% of energy) based on SFA-rich coconut oil and cocoa butter for 8 weeks led to greater body weight gain than feeding high-fat diets based on canola or soybean oil (Calder et al. 1994). The present work supports and extends to adult female Sprague-Dawley rats previous findings that highly saturated fatty acids in a fat-rich diet are more effective at inducing dietary obesity than moderately or poorly saturated fatty acids.

After weight loss on the restricted low-fat diet, abdominal fat of butter-fed rats was not significantly different from that of chow-fed controls. In the literature, abdominal fat was mostly measured in male rats fed ad libitum with high-fat SFA-rich diets which led to a greater body fat content (Yaqoob et al. 1995; Alsaif and Duwaihy 2004; Kloss et al. 2005; Hsu and Huang 2006). We found three studies that measured body fat following weight loss in originally high-fat-fed animals. Weaning male C57Bl/6 mice fed coconut oil-based high-fat diet (58% of energy) for 4 months and then switched to low-fat coconut oil ad libitum feeding (11% of energy) for the same duration had fat pad weight comparable to that of the control animals fed the low-fat diet throughout the study (Parekh et al. 1998). The present work extends to butter feeding in adult female Sprague-Dawley rats this finding that reversing dietary obesity developed with SFA-rich diet preserves body fat. Portillo et al (Portillo et al. 1998) found in 12-week old female Wistar rats originally fed a high-fat diet based on coconut oil (60% of energy) for 7 weeks then switched to 40% energy restriction on a lower-fat diet (olive oil, 20% of energy) or to ad libitum feeding with a normal fat control diet (olive oil, 12% of energy) for 3 weeks, less adipose tissue with energy restriction than with ad libitum feeding. This concurs with the present study, where restricted low-fat canola-fed animals had less abdominal fat than controls. However, Bartness et al (Bartness et al. 1992) found comparable parametrial and retroperitoneal fat pads in control animals and in 13-week old female Wistar rats originally fed high-fat diets (low-SFA vegetable shortening at 60% of energy) for 8 or 14 weeks and switched to ad libitum chow for the same period. This

difference could be attributed to the much lower percentage of SFA in canola (7%) than in vegetable shortening (25%) and ad libitum feeding versus food restriction in the present study.

Fat-rich diets generally induce hyperphagia in male and female rodents regardless of the degree of saturation (Ainslie et al. 2000; Harrold et al. 2000; Ghibaudi et al. 2002; Wang et al. 2002; Woods et al. 2003; Huang et al. 2004). However, SFA would exert a weaker control over appetite than MUFA and PUFA through a decrease in the release of cholecystokinin (which is known to promote satiety (Lawton et al. 2000). Texture and odor are also known to contribute to the high acceptability of fat-rich diets (Warwick et al. 1990; Warwick and Weingarten 1995; Blundell and Macdiarmid 1997). In the present work, high-fat feeding resulted in a greater energy intake in butter-fed rats than in canola and chow-fed animals. The texture or ease of consumption of the fat-rich diets could also have been related to their intake. Even though we have added pectin to improve the texture of the diets, mixing chow with canola oil gave a greasy and sticky texture, with a denser and more consistent texture for the chow and butter mixture which could ease consumption.

Canola-fed animals from both groups adjusted their intake based on the energy density of the diet, while lard and butter-fed animals failed to do so. Wade (Wade 1982) found that hamsters switched from chow to a high-fat vegetable shortening-based diet adjusted their intake. Failure to adjust intake was found in adult male Wistar rats and weaning male C57BL/6 mice switched from 13-17

weeks of feeding a high-fat diet (60% of energy) based on lard or a mixture of beef tallow and safflower oil to a low-fat diet (chow or a safflower-based diet at 10%) for 6-13 weeks (Hill et al. 1989; Huang et al. 2004).

Ad libitum canola and butter low-fat diets or chow feeding resulted in similar weight change and final body weight, while restricting the low-fat diets led to comparable weight loss and final weight. Other studies also showed that with ad libitum low-fat feeding animals lost some weight but their body weight plateaued and weight loss was only possible with restricting the amount of low-fat food offered (Levin and Keesey 1998; Levin and Dunn-Meynell 2000; Levin and Dunn-Meynell 2002).

The positive correlation between Lee obesity index and body weight gain and abdominal fat found in canola and butter-fed rats suggests that obesity index can be helpful for assessing the obesity induced by long-term high-fat feeding. Woods et al (Woods et al. 2003) found that body weight alone is not a very strong predictor of obesity in rodents and body fat is known to be a better criterion. Similar to our results, Sefcikova et al. (Sefcikova et al. 2008) found a positive correlation between Lee obesity index and body weight gain in adult male Sprague-Dawley rats fed high-fat diets at 30% of energy (Research Diet, 53316 Test Diet, fat source not mentioned) for 28 days. A significant correlation between Lee obesity index and body fat was found in weaning and mature male and female Theiller's Original (T/O) mice made obese by gold thioglucose and bipiperidyl mustard (Rogers and Webb 1980), and in Holtzman rats made obese

by ventromedial hypothalamic lesions (Bernardis and Patterson 1968; Bernardis 1970).

Plasma leptin was highest in controls, lowest in restricted low-fat canola-fed rats and intermediate in butter-fed rats. Leptin was thus reduced with chronic underfeeding although positively correlated with abdominal fat in all dietary groups. It was shown that energy restriction reduces leptin levels (Cha and Jones 1998; Gregersen et al. 2003; Hynes et al. 2003). Our study extends these findings with a significantly lower plasma leptin while on a restricted low-fat low-SFA diet but not with a low-fat high-SFA diet. Circulating leptin crosses the blood-brain barrier, where its act centrally through leptin receptors in the hypothalamus by decreasing neuropeptide Y (NPY) and agouti-related peptide (AGRP) and increasing pro-opiomelanocortin or POMC expression, leading to a reduction in food intake (Angelopoulos et al. 2005).

Rats fed restricted low-canola and butter diets had greater morning than evening total plasma ghrelin, whereas control rats fed chow ad libitum had similar levels. Animals were given canola and butter-based low-fat diets at the beginning of the light cycle (10:00) every day, hence raising the possibility that they ate the restricted amount of food during daytime thus reversing feeding phase. Plasma ghrelin rises before and falls after ad libitum meals and increases food intake (Tschop et al. 2000; Cummings et al. 2001). Ghrelin's orexigenic effects are attributed to increased central NPY and AGRP (Angelopoulos et al. 2005). In restricted animals, greater morning than evening ghrelin values could be

indicative of a completely empty stomach, in contrast with ad libitum state (Perreault et al. 2004). Because rats were periodically restricted from highly desired foods (restricted food was offered once a day), bingeing may have occurred when these foods were available, which greater ghrelin could be indicative of. Other studies found greater total and active ghrelin in adult male Wistar rat or in female Sprague-Dawley rats and C57/B6 mice fed restricted amounts of chow (Reimer et al. 2010: morning values; Gualillo et al. 2002; Yang et al. 2007: sampling time not mentioned).

We identified prone and resistant phenotypes with feeding high-fat diets, with food metabolized more efficiently in prone animals, although obesity was only developed with butter feeding. However, type II error cannot be excluded because of the relatively small number of animals in each sub-group. Future work should be conducted with a larger sample size to better delineate phenotypes. Besides, we studied female rats, which restrict the generalizability of our findings. It would be important to replicate our findings with male animals.

The present work is the first report that contrasted the development of obesity with low, moderate and high SFA fat-rich diet in adult female Sprague-Dawley rats. We found that obesity is promoted by a diet rich in saturated fats and body fat is preserved after weight loss. The high availability of SFA-rich foods in today's obesogenic environment could contribute to develop and maintain obesity. The ineffectiveness of ad libitum low-fat diets to reverse dietary obesity emphasizes the use of restricted regimens.

3.7. Acknowledgment

This work was supported by a grant to Louise Thibault by the Natural Sciences and Engineering Council of Canada (NSERC RGPIN 39636-01). The authors wish to thank Roger Cue for his helpful advice for statistical analyses and Behnam Azadi for his technical assistance in performing the biochemical assays. The authors had no conflicts of interest.

Table 3.1. Diet composition (g/100g)

	Purina chow [a]	High-fat diets			Low-fat diets	
		Canola[b]	Lard [c]	Butter [d]	Canola	Butter
Total protein	18.1	10.6	10.6	10.6	16	16
Total carbohydrate	57.3	33.7	33.7	33.7	51	51
Total fat:	4.5[*]	39.8	39.8	39.8	11	11
from chow	4.5	2.6	2.6	2.6	4	4
from other sources	----	37.2	37.2	37.2	7	7
SFA	----	2.6	16	25.3	0.5	4.8
MUFA	----	22.7	17.5	10.4	4.3	2
PUFA	----	11.9	3.7	1.5	2.3	0.3
Fiber	3.4	2	2	2	3	3
Vitamins and minerals	3.7	2.2	2.2	2.2	3.3	3.3
Pectin [e]	----	4	4	4	4	4
Percent of energy from fat	12	67	67	67	27	27
Energy (kJ/g)	14.2	22.2	22.2	22.2	15.1	15.1

[*] : Provided by fish meal, beef tallow, soybean meal, corn and wheat.
[a] : Charles River rodent chow 5075, St-Constant, Quebec.
[b] : President's Choice, Canada.
[c] : Tenderflake, Maple Leaf, Canada.
[d] : My Country, Lactantia, Canada.
[e] : MP Biomedicals, Inc., USA

Table 3.2. ANOVA results for ad libitum high-fat canola and butter-based diets and chow feeding

One-way ANOVA with diet as the main effect

	Diet	Covariate	Day	Diet*day
Initial weight	F(2,26)=0.50, p=0.6134	n/a	n/a	n/a
Daily body weight (26 days)	F(2,24)=6.71, p=0.005	F(1,24)= 153.50, p<.0001	F(25, 650)= 59.33, p<.0001	F(50, 650)= 12.29, p<.0001
Daily body weight (50 days)	F(2,24)=5.85, p=0.009	F(1,24)= 84.50, p<.0001	F(49,1274)=58.43, p<0.0001	F(98,1274)= 8.04, p<0.0001
Final body weight (26 days)	F(2,24)= 4.55, p=0.021	F(1,24)=54.47, p<0.0001	n/a	n/a
Final body weight (50 days)	F(2,24)=5.66, p=0.001	F(1,24)=50.27, p<0.0001	n/a	n/a
Daily body weight gain (26 days)	F(2,24)= 4.55, p=0.0211	F(,24)= 4.06, p=0.0553	n/a	n/a
Daily body weight gain (50 days)	F(2,24)=5.57, p=0.01	F(1,24)=6.53, p=0.0173	n/a	n/a
Daily food intake	F(2, 25)= 35.99, p<0.0001	n/a	F(49,1269)= 1.87, p=0.0003	F(98,1269)= 2.82, p<0.0001
Daily energy intake	F(2, 25)=7.21, p=0.0034	n/a	F(49,1271)=Infty, p<0.0001	F(98,1271)=Infty, p<0.0001
Mean food intake (26 days)	F(2,25)=22.76, p<0.0001	n/a	n/a	n/a
Mean food intake (50 days)	F(2,25)=37.62, p<0.0001	n/a	n/a	n/a
Mean energy intake (26 days)	F(2,25)=5.31, p=0.012	n/a	n/a	n/a
Mean energy intake (50 days)	F(2,25)=15.92, pp<0.0001	n/a	n/a	n/a
Lee obesity index (day 29)	F(2,24)=1.20, p=0.318	F(2,24)=6.95, p=0.015	n/a	n/a
Food efficiency	F(2,25)=3.36, p=0.05	n/a	n/a	n/a
Abdominal fat	F(2,23)=4.72, p=0.0191	F(1,23)=10.56, p=0.0035	n/a	n/a

Table 3.2. ANOVA results for ad libitum high-fat canola and butter-based diets and chow feeding (cont'd)

Two-way ANOVA with diet and phenotype as main effects, and their interaction

	Diet	Phenotype	Covariate	Diet*phenotype
Initial weight	F(1,21)=2.04, p=0.1681	F(3,21)=2.27, p=0.1097	n/a	F(3,21)=0.69, p=0.5711
Final body weight (50 days)	F(1,20)=57.26, p<0.0001	F(3,20)=24.35, p<0.0001	F(1,20)=46.90, p<0.0001	F(3,20)=7.66, p=0.001
Daily body weight gain (50 days)	F(1,20)=54.31, p<0.0001	F(3,20)=23.95, p<0.0001	F(1,20)=0.08, p=0.7802	F(3,20)=7.37, p=0.002
Mean food intake (50 days)	F(1,21)=66.67, p<0.0001	F(3,21)=29.16, p<0.0001	n/a	F(3,21)=10.91, p=0.0002
Mean energy intake (50 days)	F(1,21)=105.82, p<0.0001	F(3,21)=14.42, p<0.0001	n/a	F(3,21)=15.99, p<0.0001
Lee obesity index (day 29)	F(1,20)=6.91, p=0.0161	F(3,20)=1.41, p=0.2695	F(1,20)=1.49, p=0.2370	F(3,20)=1.16, p=0.3494
Food efficiency	F(1,21)=26.93, p<0.0001	F(3,21)=23.08, p<0.0001	n/a	F(3,21)=2.82, p=0.0638
Abdominal fat	F(1,21)=7.91, p=0.0108	F(3,21)=9.68, p=0.0004	n/a	F(3,21)=12.17, p<0.0001

NA: not applicable; Infty: infinity.

Table 3.3. Abdominal fat, leptin and total and active ghrelin plasma levels following 32 days of restricted low-fat or ad libitum chow feeding

		Chow	Canola	Butter
Abdominal fat (g)	----	30.9±3.3[a]	15.9±4.4 [b]	22.9±4.5[ab]
Leptin (ng/mL)	morning	6.2±1.8	1.8±1.9	5.5±2.0
	evening	11.4±1.6	3.5±2.1	5.1±2.0
Total ghrelin (pg/mL)	morning	3582±390	4537±413[A]	4503±436[A]
	evening	3090±352	2316±470[B]	2160±436[B]
Active ghrelin (pg/mL)	morning	254.2±47.0	298.3±49.8	268.2±52.7
	evening	246.0±42.6	313.9±56.7	243.2±52.7

Data are presented as mean±SEM. Different lower case letters indicate values statistically different among the dietary groups ($p=0.012$), and different capital letters indicate values statistically different between morning and evening within each dietary group (canola: $p=0.0002$, butter: $p<0.0001$).

3.8. Figure captions

Figure 3.1. Experimental design.

Figure 3.2. Daily body weight (mean ± SEM) of rats fed chow, canola or butter-rich diets.

Figure 3.3. Daily food intake (mean ± SEM) of rats fed chow, canola or butter - rich diets.

Figure 3.4. Ad libitum food intake (mean±SEM) of rats switched from baseline chow-feeding (7 days) to high-fat canola or butter-based diets (first 7 days) and from high (last 7 days) to low-fat canola or butter-based diets (first 7 days). Within each dietary group, different lower case letters indicate values statistically different between chow and high-fat feeding and different capital letters indicate values statistically different between high and low-fat feeding at $p<0.0001$.

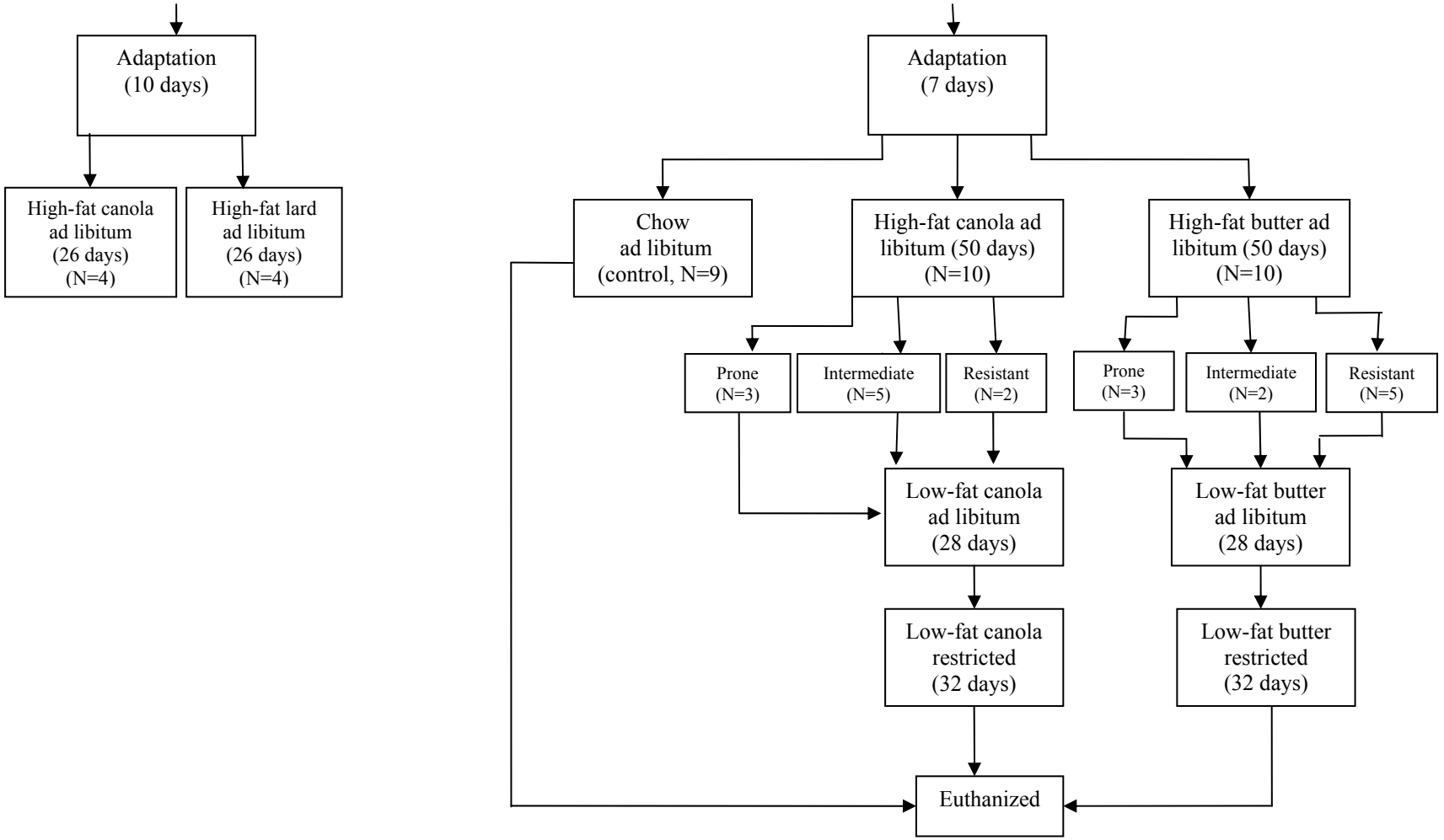
Adaptation
(10 days)
High-fat canola
ad libitum
(26 days)
(N=4)
High-fat lard
ad libitum
(26 days)
(N=4)
Adaptation
(7 days)
Chow
ad libitum
(control, N=9)
High-fat canola ad
libitum (50 days)
(N=10)
High-fat butter ad
libitum (50 days)
(N=10)
Prone
(N=3)
Intermediate
(N=5)
Resistant
(N=2)
Prone
(N=3)
Intermediate
(N=2)
Resistant
(N=5)
Low-fat canola
ad libitum
(28 days)
Low-fat butter
ad libitum
(28 days)
Low-fat canola
restricted
(32 days)
Low-fat butter
restricted
(32 days)
Euthanized

Figure 3.1.

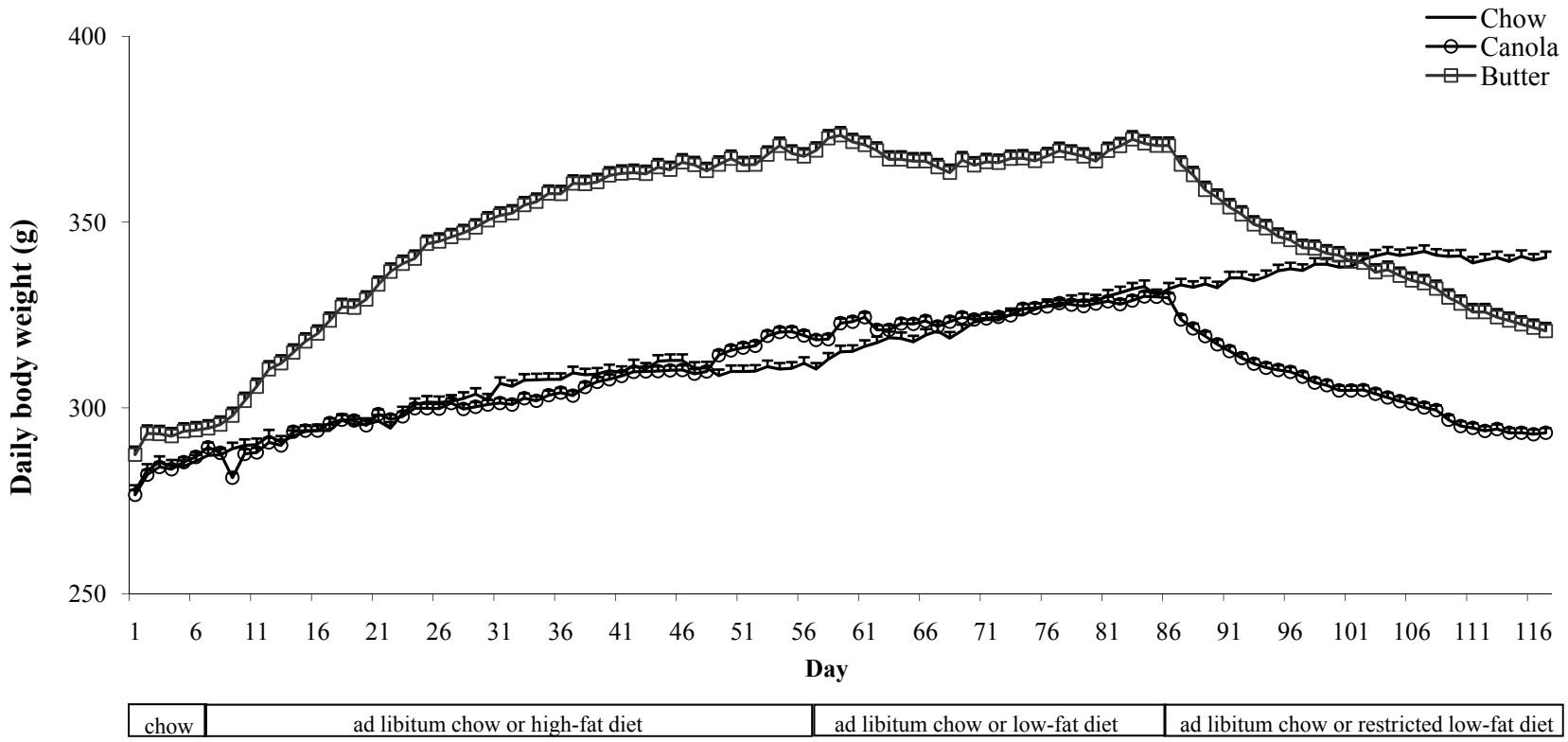
Chow
Canola
Butter
Daily body weight (g)
400
350
300
250
1
6
11
16
21
26
31
36
41
46
51
56
61
66
71
76
81
86
91
96
101
106
111
116
Day
chow
ad libitum chow or high-fat diet
ad libitum chow or low-fat diet
ad libitum chow or restricted low-fat diet

Figure 3.2.

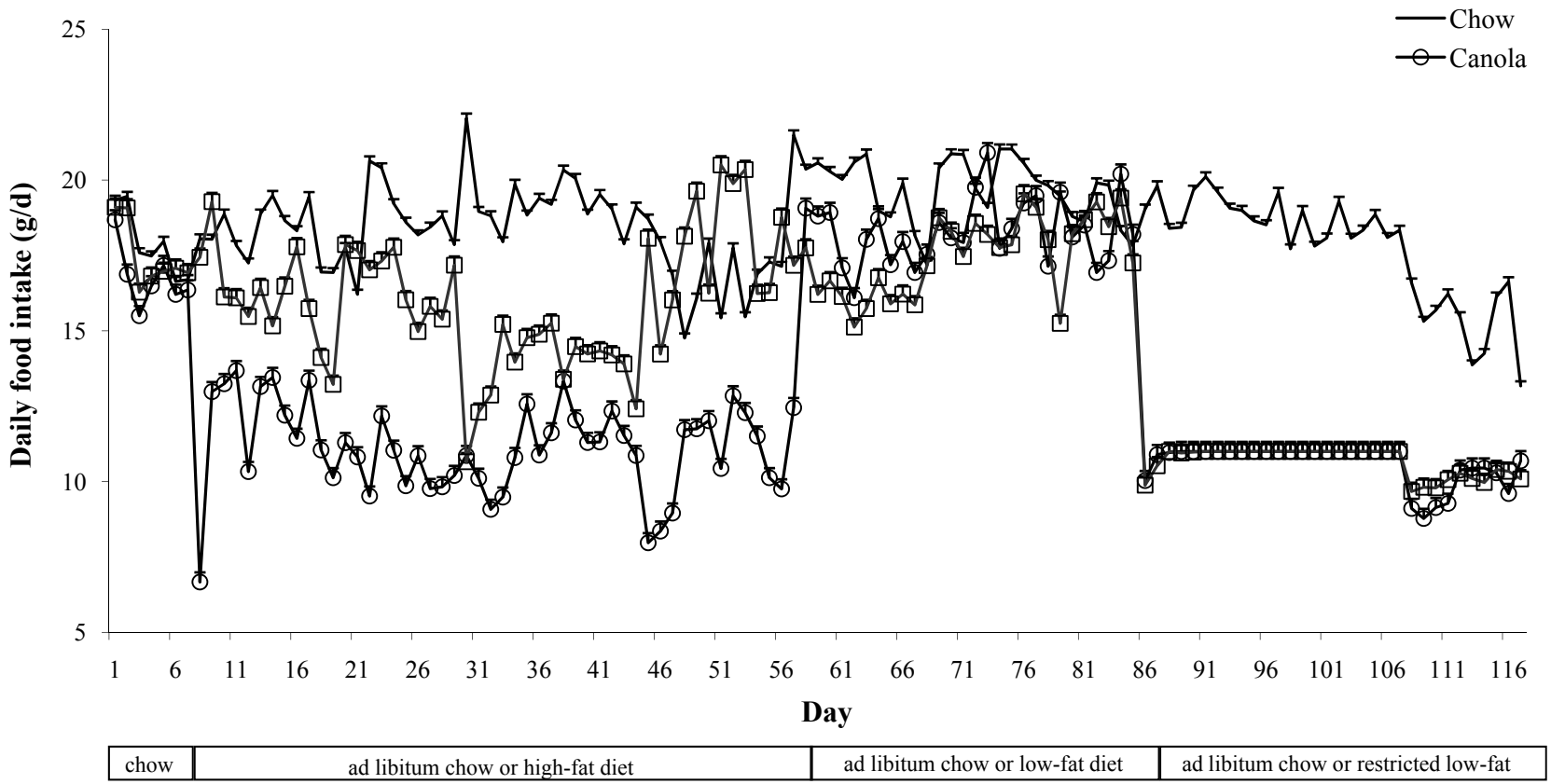
Chow
Canola
Daily food intake (g/d)
25
20
15
10
5
1
6
11
16
21
26
31
36
41
46
51
56
61
66
71
76
81
86
91
96
101
106
111
116
Day
chow
ad libitum chow or high-fat diet
ad libitum chow or low-fat diet
ad libitum chow or restricted low-fat

Figure 3.3.

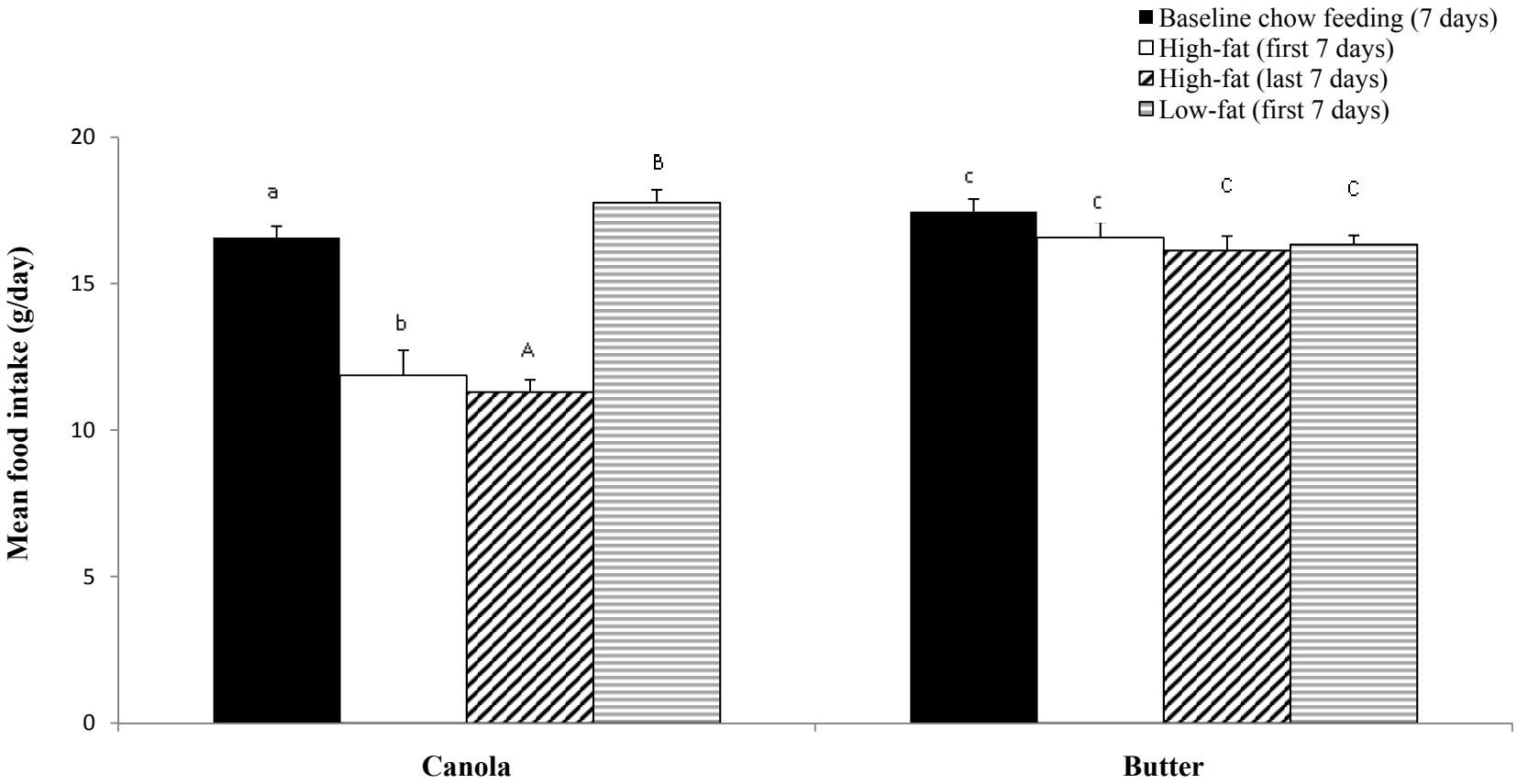
Baseline chow feeding (7 days)
High-fat (first 7 days)
High-fat (last 7 days)
Low-fat (first 7 days)
Mean food intake (g/day)
20
15
10
5
0
a
b
A
B
c
c
C
C
Canola
Butter

Figure 3.4.

BRIDGE 2

A greater obesity induced by high-SFA butter feeding and not moderate-SFA lard in Manuscript 2 revealed that the obesogenic effect of a fat increases according to its SFA content. This led us to investigate the factors - other than overconsumption of energy - involved in the obesogenic effect of SFA. Previous studies have shown that high-fat feeding was associated with alteration in the circadian rhythmicity of eating pattern. To investigate this relationship, other studies have compared diets high and low in dietary fat; however, no study has investigated the effect of diets of varying fatty acid profile on eating pattern alteration. Besides, some studies have evaluated the meal pattern following food restriction in rats, while eating pattern during long-term food restriction remains to be clarified. Therefore in the third Manuscript we aimed to verify whether high, moderate and low-SFA diets differently alter the eating pattern of adult female rats. We also examined if these alterations are involved in obesity induced by high-fat feeding. We then assessed the pattern of eating in obese rats subjected to food restriction on a low-fat diet.

CHAPTER 4, MANUSCRIPT 3

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Dietary obesity caused by a specific circadian eating pattern

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4.1. Abstract

The eating pattern is altered by high-fat diet-induced obesity. To clarify whether this is dependent on the fatty acid profile of the diet, we conducted two studies on adult female Sprague-Dawley rats fed normal-fat chow or high-fat diets with varying fatty acid composition. Eating pattern and body weight were assessed in rats fed canola-based (low in saturated fatty acids) or lard-based (moderate in saturated fatty acids) diets for 7 days, and in animals fed chow, or canola-, or butter-based diets (rich in saturated fatty acids) for 43 days. These parameters were also determined when restricted amounts of low-fat canola or butter-based diets were consumed for 25 days. Early exposure to canola or lard high-fat feeding or a prolonged access to canola or butter-based fat-rich diets (relative to chow feeding) did not alter the normal light-dark distribution of food and energy intake. All animals ingested most of their food during the dark phase. However, feeding the high-fat canola and butter-based diets produced an altered eating pattern during the light phase characterized by a smaller number of meals, longer intermeal interval, and enhanced satiety ratio, and consumption of shorter-lasting meals than chow-fed animals. Relative to canola or chow feeding, butter-fed animals consumed a lower number of meals during the dark phase and had a higher eating rate in the light phase, but ate larger meals overall. Only butter feeding led to overeating and obesity. When given a restricted amount of low-fat canola or butter-based diet at the start of the light phase, rats ate most of their food in that phase and diurnal rather than nocturnal feeding occurred with

restriction. These findings underscore the role of saturated fatty acids and the resulting eating pattern alteration in the development of obesity.

Key words: High-fat diet; Saturated fatty acids; Dietary obesity; Circadian eating pattern; Rat.

4.2. Introduction

Biological function and behavior, including eating, exhibit 24-h patterns that are driven by an endogenous circadian clock mechanism (Duguay and Cermakian 2009). The parameters of the feeding rhythm or eating pattern are the size and frequency of meals as well as the time of eating. Some human studies involving both children and adults have revealed lower risk of obesity with frequent daily eating episodes (Fabry et al. 1966; Ma et al. 2003; Toschke et al. 2005), as well as increase in adiposity with omitting a meal in adults (Chapelot et al. 2006). However, Andersson and Rossner (1996) found similar eating patterns in obese and normal-weight individuals, while other studies found greater number of meals in obese subjects (Bertéus Forslund et al. 2002), and greater eating frequency associated with higher energy intake and obesity (Howarth et al. 2007). The relationship between eating meals late in the evening and obesity remains unclear (Kant et al. 1997; Ma et al. 2003; Qin et al. 2003).

In animal studies, obesity and feeding high-fat diets were found to attenuate the circadian expression of clock genes (Kaneko et al. 2009; Kohsaka et al. 2007), therefore, affecting metabolism and body weight homeostasis.

Eating at an "improper" circadian time has been associated with higher risk of obesity in humans (Ma et al. 2003; Qin et al. 2003) and rodents (Arble et al. 2009; Mistlberger et al. 1998). In 5-week old female genetically obese Zucker rats fed chow at ad libitum for 60 days, weight gain was greater compared to animals fed chow only during the dark phase (14 h), even though both groups had similar food intakes (Mistlberger et al. 1998). Similarly, higher body weight was found in adult male C57BL/6J mice fed a high-fat diet (lard as 60% of total calories) during the light phase only, than those fed the same diet in the dark phase, in spite of consuming a similar amount of calories (Arble et al. 2009).

A relationship between long-term, high-fat feeding and meal pattern has been found in rodents, such as shorter and larger meals ingested by weaning male Sprague-Dawley rats with 5 weeks of high-fat feeding (lard as 60% of energy) than when fed low-fat diets (same fat as 10% of energy) for the same duration (Furnes et al. 2009). Increased meal size and intermeal interval (IMI: the interval of time from the last meal to the next meal) were found in adult male Sprague-Dawley rats after 8 weeks than after 2 weeks of high-fat feeding (soybean oil as 38% of energy) (Paulino et al. 2008).

Animal studies have found a relationship between eating pattern and susceptibility to obesity. Adult female Sprague-Dawley rats that ate chow in larger meals (gorgers) gained weight at a higher rate when fed high-fat/high-sugar diets (chocolate chip cookies and sweetened condensed milk plus chow) for 16 weeks than rats that ate chow in smaller meals (nibblers) (Drewnowski et al.

1984). Male inbred obesity-prone rats at 11week of age ate chow at more frequent intervals, but had smaller and shorter meals and IMI than resistant rats during the light and dark phases (Cottone et al. 2007). However, when the meal pattern of the first 2 h of the dark phase was separated from the remaining hours of that phase, prone and resistant anima ate meals of similar size and duration, but this resulted in hyperphagia in prone rats due to the shorter IMI.

In the literature, most of the available data on feeding rhythms with high-fat diets in genetically similar animals have been obtained from a single high-fat diet containing low or moderate concentrations of saturated fatty acids (Hariri and Thibault 2010). In order to better understand how high-fat feeding affects food intake rhythmicity, and whether the fatty acid profile of the diet accounts for that effect, we conducted a first study on adult female Sprague-Dawley rats to examine the light/dark distribution of food and energy intake at early exposure to isoenergetic high-fat diets (at 67% of total energy) based on canola oil (low in saturated fatty acids [SFA]) or lard (moderate in SFA). In this study, the canola- and lard-based diets induced similar body weight gain and final weight after 26 days. Therefore, we conducted a second study contrasting canola oil with a fat rich in SFA (butter) in relation to a control group fed chow. We examined the distribution of food and energy intake across light and dark phases and the meal pattern of adult female Sprague-Dawley rats fed ad libitum with high-fat diets (at 67% of total energy) rich in canola oil or butter (high in SFA) for 50 days, or fed restricted low-fat canola oil or butter-based diets for 32 days.

4.8. Materials and methods

4.3.1. Animals and diets

Adult female Sprague-Dawley rats weighing 260-310 g (12-14 weeks of age) obtained from Charles River Laboratories (St-Constant, Quebec, Canada) were used for the experiments. They were housed in individual cages, under controlled temperature (22-25^{0}C), relative humidity (70%), and 12:12 light–dark phase with lights-on from 06:00 to 18:00 h. The studies were conducted from May to October. All the rats had ad libitum access to Purina chow (3.4 kcal/g; Charles River rodent chow 5075, St-Constant, Quebec, Canada) and tap water for 7-10 days prior to the experiments to adapt to the new environment. The research procedures were approved by the McGill University Animal Care Committee, and conformed to international ethical standards (Portaluppi et al. 2010).

In Experiment 1, eight rats were randomly assigned to two experimental groups fed either high-fat lard-based (43% SFA; N = 4) or canola oil-based (7% SFA; N = 4) diets. Animals had free access to their diets and tap water for 10 days. In Experiment 2, a total of 29 rats were randomly assigned to three experimental groups fed high-fat butter-based (68% SFA; N = 10) or canola-based (N = 10) diets or chow (controls; N = 9). Animals had free access to their diets and tap water for 50 days. In order to induce weight loss, on day 51 the high-fat fed animals were switched to ad libitum low-fat diet feeding. Because body weight maintenance was observed with the ad libitum low-fat feeding for 28 days, the amount of food offered was restricted. During the next 32 days, the low-fat

diet was limited to 60% of the intake during the ad libitum low-fat feeding period (11 g). The control group was fed chow ad libitum throughout the study.

The high-fat diets were isocaloric and provided 67% of energy from fat (5.3 kcal/g) and were prepared in our laboratory: 58.8% (g/100g) Purina chow, 4% pure pectin to emulsify fat (MP Biomedicals, Inc., USA), and 37.2% fat consisting of lard (Tenderflake, Maple Leaf, Canada), canola oil (President's Choice, Canada), or butter (My Country, Lactantia, Canada). The protein content of the high-fat diets was adequate for their maintenance, i.e., 5 g/100 g of diet (Institute for Laboratory Animal Research, 1995). The low-fat diets were also isocaloric, and 27% of their energy was provided by fat (3.6 kcal/g): 89% (g/100g) Purina chow, 4% pure pectin, and 7% fat consisting of either butter or canola oil.

4.3.2. Diet Scan system

The Diet Scan system (AccuScan Instruments Inc., Columbus, Ohio, USA) is a computerized data acquisition system designed to continuously record and study food and water ingestion patterns of small animals. Diet Scan cages, made of clear acrylic materials (41.75 X 41.75 X 31.5 cm^3), were designed with a diagonal divider in order to house two animals separately. Each animal had access to a feeder through a square opening in the cage. The feeders, filled with the diets, were placed on electronic scales (Ohaus Port-O-Gram-C301P and A&D-EW300A). Access to water was through a drinking spout connected to a drinking bottle that was positioned by a holder on a scale plate. All the scales were

connected to an analyzer device linked to an IBM computer and programmed with the Diet Scan Lab Animal Monitoring System Software (DLAM); data collection recordings took place on each scale at 30-min or 1-min intervals throughout the 24 h. A Diet Scan template was then used to reorganize the recorded information into data files in the form of Microsoft Excel spreadsheets.

4.3.3. Measurements

Body weight and food intake were measured daily at 10:00 h throughout the study using a digital Mettler PJ 3000 balance; fresh food and water were then provided.

In Experiment 1, at the end of the adaptation period, rats were transferred to the Diet Scan for 13 days: 3 days on chow and 10 days on canola-based (N = 4) and lard-based (N = 4) diets, and food intake was measured at 30-min intervals. For body weight analysis, data of the last 7 days of high-fat feeding while in Diet Scan were used. Data of the last 3 days were used to calculate 24-h intake, the nocturnal/diurnal distribution of intakes, and 4-h food intake during the dark phase (early: 18:00-22:00 h, middle: 22:00-02:00 h, late: 02:00-06:00 h).

In Experiment 2, after 40 days of high-fat feeding a subset of eight rats from each dietary group (canola and butter) and eight rats from the control group were transferred to the Diet Scan and their food intake was measured at 1-min intervals for 10 days. The Diet Scan data of the last 7 days were used for body weight analysis. Data of the last 3 days were used for assessing the 24-h intake,

the nocturnal/diurnal distribution of intakes, and 4-h food intake during the dark phase to compare with the first group fed canola or lard. Data of the last 7 days were also analyzed for meal calculation. Following 50 days of ad libitum canola and butter high-fat diet feeding, rats in the highest tertile of total body weight gain were considered obesity-prone, those in the lowest tertile obesity-resistant, and the other animals intermediate. Diet Scan measurements were also made at 1-min intervals for 10 days following 22 days of restricted low-fat feeding of the two subsets of eight rats fed canola or butter as well as in control animals. Diet Scan data of the last 7 days were used for the analysis.

A meal was defined as changes in the weight of food ≥ 0.3 g, which lasted ≥ 1 min and was separated from other feeding episodes by ≥ 10 mins (Langhans and Scharrer 1987). Diurnal and nocturnal food intake, meal size, frequency, and duration, as well as IMI, satiety ratio (ratio of IMI to the preceding meal size [g and kcal]), and eating rate (meal size [g and kcal] divided by meal duration) were calculated using the data of Diet Scan.

4.3.4. Statistical analyses

In canola and lard-fed rats, one-way ANCOVA was used for analyzing final body weight and daily body weight gain and one-way ANOVA for mean daily food and energy intake with dietary fat as the main effect and initial weight as the covariate. For diurnal, nocturnal, and 4-h food intakes, analyses were performed using two-way, repeated-measures ANOVA with diet and phase as the main effects and testing of their interaction. The above-mentioned variables were

analyzed in animals fed canola or butter-based diets or chow by one-way ANCOVA, and two-way, repeated-measures ANOVA with the same main effects and covariate. Analyses for meal size, number, duration, plus IMI, satiety ratio, and eating rate were performed using two-way, repeated-measures ANOVA with diet and phase as the main effects and testing of their interaction. Three-way, repeated-measures ANOVA was applied for analyzing the mentioned variables with phenotype, diet, and phase as the main effects, and testing of their interaction, with chow-fed animals considered as the control group. For all the significant main effects, multiple comparisons were conducted by Scheffe's test. In addition, correlation analyses were performed. Data are presented as mean ± SEM, and all differences were considered statistically significant if $p<0.05$. SAS version 9.1 was used for analyzing the data.

4.9. Results

4.4.1. Ad libitum high-fat feeding

4.9.1.1. Body weight

Body weight gain of the rats fed the canola and lard-based diets was comparable across 7 days of early exposure to high-fat feeding (2.7 ± 0.3 and 3.3 ± 0.3 g/day, respectively), which resulted in a similar weight of the animals (345.9 ± 7.4 and 348.1 ± 7.4 g, respectively; values adjusted for initial body weight of 317.0 ± 7.7 and 293.0 ± 7.7 g, respectively). Body weight gain across the 7 days was similar in rats that had been fed canola, butter, or chow for 43 days

(0.49 ± 0.60, 0.07 ± 0.63, and 0.06 ± 0.46 g/day, respectively). However, a main effect of diet ($F(2,24)=5.66$, $p=0.001$) showed that animals fed butter reached a greater weight (363.1 ± 10.5 g) than those fed canola and chow (323.8 ± 10.5 g, $p=0.043$ and 318.0 ±7 .6 g, $p=0.003$, respectively; values adjusted for initial weight of 295.5 ± 6.4, 287.9 ± 6.4, and 287.3 ± 6.7 g, respectively).

4.9.1.2. Food and energy intake

Twenty-four-hour food and energy intake: After 7 days of high-fat feeding, mean food and energy intake were comparable in rats fed canola-based (14.8 ± 0.7 g; 78.9 ±3 .8 kcal) or lard-based (15.0 ± 0.7 g; 79.9 ± 3.8 kcal) diets. Following 47 days of high-fat feeding with canola, or butter, or chow, a main effect of diet ($F(2,19)=10.29$, $p=0.0009$) revealed that canola-fed rats ate less (10.3 ± 1.2 g) than animals fed butter or chow (17.7 ± 1.2 g/day, $p=0.002$ and 16.8 ± 0.8 g/day, $p=0.0002$, respectively). Energy intake ($F(2,19)=12.60$, $p=0.0003$) was comparable in canola (55.2 ± 5.7 kcal) and chow-fed animals (57.2 ± 4.0 kcal), but it was greater in rats fed the butter diet (93.0 ± 5.9 kcal, $p<0.001$).

Diurnal and nocturnal food and energy intake: Early exposure to high-fat canola or lard feeding revealed a significant main effect of phase ($F(1,27.7)=145.37$, $p<0.0001$) on food and energy intake, with greater nocturnal than diurnal intakes (nocturnal: 11.8 ± 0.4 g, 62.7 ± 2.3 kcal; diurnal: 3.0 ± 0.4 g, 16.1 ± 2.3 kcal), regardless of the diet.

Following chow, high-fat canola, or butter feeding, a significant main effect of phase ($F(1,134)=75.86$, $p<0.0001$) showed greater nocturnal (10.0 ± 0.4 g, 45.4 ± 2.7 kcal) than diurnal (4.8 ± 0.4 g, 24.3 ± 2.7 kcal) food and energy intake, regardless of the diet. At early exposure to the high-fat canola-based diet, nocturnal intake contributed to 75.7 ± 5.9% of the 24-h intake, whereas prolonged exposure (47 days) led to a nocturnal intake representing 63.1 ± 4.0% of the daily intake; however, this decline was not statistically significant.

Early, middle and late parts of the dark phase food and energy intake: A significant diet by phase interaction ($F(2,15)=4.79$), $p=0.025$) showed in canola-fed animals greater intakes in the early (6.0 ± 0.6 g, 31.9 ± 3.4 kcal) than in the middle part of the dark phase (1.6 ± 0.6 g, 8.7 ± 3.4 kcal, $p=0.001$) and with intermediate intakes during the late part (3.6 ± 0.6 g, 19.0 ± 3.4 kcal) as shown in Figure 1A. In rats fed lard, food and energy intake were comparable across the three parts of the dark phase, and did not differ significantly from intakes of the canola-fed rats.

A significant diet by phase interaction ($F(4,63)=3.37$, $p=0.015$ and $F(4,63)=3.99$, $p=0.006$, respectively) revealed that butter-fed rats ingested more food and calories in the early (6.2 ± 0.5 g, 32.8 ± 2.8 kcal) than in the middle and late parts of the dark phase (2.8 ± 0.5 g, 14.7 ± 2.8 kcal and 1.9 ± 0.5 g, 9.9 ± 2.8 kcal, respectively, $p<0.022$). Energy consumed in the early part of the dark phase was greater in butter-fed than in chow-fed rats (14.4 ±2.8 kcal, $p=0.013$) as illustrated in Figure 1B.

Circadian rhythmicity of energy intake

The circadian rhythmicity of energy intake across days 8 to 10 of the early exposure to canola or lard ad libitum feeding is shown in Figure 2. During the light phase, canola-fed rats had more frequent intakes than rats fed lard, with peaks of intake found toward the middle and the end of this phase. The daytime intake of rats fed lard was characterized by low intakes at the beginning and a peak toward the end of this phase. In both groups, energy intake peaked at the beginning and at the end of the dark phase.

Figure 3 shows the circadian rhythmicity of energy intake across days 48 to 50 of high-fat canola, butter, or chow ad libitum feeding. During the light phase, butter-fed rats had greater but less frequent intakes compared to canola or chow-fed rats. All animals had a peak of intake at the beginning of the dark phase, which was more pronounced in animals fed butter, and was followed by lower intakes in all dietary groups.

Meal frequency: Chow, canola, and butter-fed animals consumed more frequent meals in the dark than light phase ($p<0.0001$) as summarized in Table 1. The significant diet by phase interaction ($F(2,163)=3.82$, $p=0.024$) also revealed that chow-fed animals ingested more frequent meals than canola (light: $p=0.036$, dark: $p=0.002$) and butter-fed rats (both phases: $p<0.0001$) in both phases, with the lowest nocturnal meal frequency found in rats fed butter.

A significant phenotype by diet by phase interaction ($F(4,297)=18.37$, $p<0.0001$) showed comparable number of meals ingested by butter-fed/obesity-prone animals in the light and dark phases (2.8 ± 0.5 and 3.7 ± 0.5 g, respectively), while meal frequency of the other phenotypes and dietary groups was greater in the dark than light phase (dark: butter/obesity-resistant: 6.3 ± 0.4 g, butter/obesity-intermediate: 6.7 ± 0.8 g, canola/obesity-prone: 8.9 ± 0.5 g, canola/obesity-resistant: 8.7 ± 0.8 g, canola/obesity-intermediate: 6.2 ± 0.4 g, chow: 9.5 ± 0.4 g; light: butter/obesity-resistant: 2.6 ± 0.4 g, butter/obesity-intermediate: 1.3 ± 0.8 g, canola/obesity-prone: 3.6 ± 0.5 g, canola/obesity-resistant: 2.9 ± 0.8 g, canola/obesity-intermediate: 3.7 ± 0.4 g, chow: 5.1 ± 0.4 g; $p<0.043$).

Meal size (g and kcal): A significant main effect of phase (g: $F(1,163)=6.45$, $p=0.012$; kcal: $F(1,163)=4.27$, $p=0.04$) revealed that diurnal meal size was significantly lower (1.45 ± 0.08 g, 7.06 ± 0.43 kcal) than nocturnal meal size (1.70 ± 0.08 g; 8.15 ± 0.43 kcal). In addition, a significant main effect of diet on meal size was found (g: $F(2,156)=30.26$, $p < 0.0001$; kcal: $F(2,156)=49.32$, $p<0.0001$), and animals fed the butter-based diet ate significantly more grams (2.6 ± 0.2) and calories (13.7 ± 0.8) of food in each meal than rats fed canola (1.0 ± 0.2 g, 5.3 ± 0.8 kcal; $p<0.0001$) or chow (1.1 ± 0.1 g, 3.8 ± 0.8 kcal; $p<0.0001$), regardless of phase.

A significant phenotype by diet by phase interaction ($F(4,301)=3.07$, $p=0.017$) showed that butter-fed/obesity-prone and resistant animals ingested

more calories in each meal in the light phase (14.0 ± 1.1 and 11.0 ± 1.0 kcal, respectively) than control animals (3.1 ± 0.9 kcal, $p<0.008$). In the dark phase, the calories of the meal were greater in butter-fed/obesity-prone and intermediate rats (13.9 ± 1.1 and 13.0 ± 1.3 kcal, respectively) than in control animals (4.4 ± 0.9 kcal, $p<0.017$).

A positive correlation between diurnal meal size and meal frequency in rats fed butter ($r=0.69$) approached significance ($p=0.055$). When all the animals were analyzed together, a negative correlation was found between nocturnal meal size and meal frequency ($r=-0.69$, $p=0.0002$) and between nocturnal meal size and satiety ratio ($r=-0.44$, $p=0.03$), while a positive correlation between meal size and IMI in the light ($r=0.55$, $p=0.006$) and dark phases ($r=0.58$, $p=0.003$) was found.

Intermeal interval: A significant diet by phase interaction ($F(2,161)=18.51$, $p<0.0001$) showed a longer diurnal IMI than nocturnal IMI in rats fed chow ($p=0.0001$), canola, and butter-based diets ($p<0.0001$) (Table 1). In addition, the diurnal IMI was longer in butter-fed animals than in canola- ($p=0.029$) and chow-fed rats ($p<0.0001$), while the nocturnal IMI was comparable in all the dietary groups.

In the light phase, a phenotype by phase interaction ($F(3,295)=11.99$, $p<0.0001$) showed that obesity-prone, resistant, and intermediate canola or butter-fed animals had longer IMI (307.2 ± 16.9, 315.5 ± 23.3, and 291.4 ± 23.2 min) than chow-fed rats (155.0 ± 14.6 min, $p<0.001$).

Satiety ratio: A greater diurnal than nocturnal satiety ratio was found in animals fed chow ($p=0.007$), canola and butter ($p<0.0001$) (Table 1). The significant diet by phase interaction (min/g) ($F(2,161)=6.79$, $p=0.002$) also showed that rats fed canola and butter-based diets had a greater diurnal satiety ratio than chow-fed animals ($p=0.006$ and $p=0.0002$, respectively); however, nocturnal satiety ratio did not differ among the dietary groups.

When expressed as min/kcal, a significant phase effect was found ($F(1,161)=164.30$, $p<0.0001$), with greater diurnal (59.4 ± 2.2 min/kcal) than nocturnal satiety ratio (19.1 ± 2.2 min/kcal). Diet had no significant effect on diurnal and nocturnal satiety ratio (chow: 37.2 ± 2.7 min/kcal, canola: 41.0 ± 3.8 min/kcal, butter: 39.4 ± 3.9 min/kcal).

A phenotype by phase interaction ($F(3,296)=5.16$, $p=0.002$) on satiety ratio (min/g) was found, with obesity-prone and resistant animals having a greater satiety ratio (334.5 ± 23.4 and 378.1 ± 32.4 min/g) than chow-fed animals (183.6 ± 20.3 min/g, $p<0.002$) in the light phase. When expressed as min/kcal, no effect of phenotype on satiety ratio was found.

Meal duration: A significant phase effect ($F(1,161)=69.13$, $p<0.0001$) showed a shorter diurnal (5.0 ± 0.3 min) than nocturnal meal duration (7.8 ± 0.3 min). In addition, a significant diet effect ($F(2,154)=8.28$, $p=0.0004$) revealed a shorter meal duration in rats fed canola-based and butter-based diets (6.0 ± 0.5 and 5.7 ± 0.5 min, respectively) than in rats fed the chow diet (7.5 ± 0.3, $p<0.043$).

A main effect of phenotype on meal duration ($F(3,15.1)=4.35$, $p = 0.021$) revealed that obesity-prone rats consumed shorter meals (5.3 ± 0.5 min) than chow-fed animals (7.5 ± 0.4 min, $p = 0.039$).

Eating rate: A significant diet by phase interaction ($F(2,160)=14.38$, $p<0.0001$) showed a greater diurnal than nocturnal eating rate (g/min) in rats fed the butter-based diet ($p<0.0001$), whereas chow and canola-fed rats showed comparable diurnal and nocturnal eating rates (Table 1). In addition, the diurnal eating rate was greater in rats fed butter than in animals fed canola and chow ($p<0.0001$), with similar nocturnal eating rates in the three dietary groups.

When eating rate is expressed as kcal/min, a significant diet by phase interaction ($F(2,125)=18.45$, $p<0.0001$) showed a greater diurnal than nocturnal eating rate in animals fed canola ($p=0.023$) and butter ($p<0.0001$), while chow-fed animals had the same eating rate in the light and dark phase. Moreover, butter-fed rats had a greater eating rate than animals fed canola or chow ($p<0.0001$) in the light phase. In the dark phase, eating rate was greater in butter-fed than in chow-fed rats ($p=0.0001$), with canola-fed animals in between.

A significant phenotype by diet by phase interaction on eating rate (g/min) ($F(4,287)=4.64$, $p=0.001$) showed a greater diurnal eating rate in butter-fed/obesity-prone than resistant rats (0.87 ± 0.04 and 0.51 ± 0.04 g/min, respectively, $p=0.0006$) and chow-fed animals (0.29 ± 0.04 g/min, $p<0.0001$). In the dark phase, the eating rate of the obesity-prone animals fed butter (0.44 ± 0.04 g/min) was also greater than that of chow-fed rats (0.15 ± 0.04 g/min, $p=0.027$).

When expressed as kcal/min, diurnal and nocturnal eating rates were greater in butter-fed/obesity prone rats (4.63 ± 0.24 and 2.31 ± 0.24 kcal/min, respectively) than in chow-fed rats (1.0 ± 0.21 and 0.51 ± 0.21 kcal/min respectively, $p<0.012$).

4.4.2. Restricted low-fat feeding

4.4.2.1. Body weight

Body weight change across 7 days was comparable in rats that had been fed a restricted amount of low-fat canola and butter-based diets for 25 days (-0.55±0.3 and -0.91±0.3 g/day, respectively). Following 32 days of restricted low-fat feeding, animals reached a similar weight (canola: 301.4±3.5 g, butter: 300.8±3.6 g; values adjusted for initial body weight).

4.4.2.2. Food and energy intake

Diurnal and nocturnal food and energy intake: A significant main effect of phase ($F(1,108)=963.39$, $p<0.0001$) revealed a greater intake in the light (8.5 ± 0.1 g, 30.5 ± 0.5 kcal) than dark phase (0.9 ± 0.1 g, 3.3 ± 0.5 kcal) in rats fed restricted low-fat canola or butter-based diets.

A significant phenotype by diet by phase interaction (food and energy: $F(3,197)=39.02$, $p<0.0001$) revealed greater diurnal food and energy intake in obesity-resistant rats fed butter (9.8 ± 0.3 g, 35.2 ± 1.1 kcal) than obesity-prone and intermediate animals fed the same fat source (7.3 ± 0.4 g, 26.2 ± 1.3 kcal and 5.1 ± 0.6 g, 18.5 ± 2.2 kcal, respectively; $p<0.0043$).

Circadian rhythmicity of energy intake

Figure 4 shows the circadian rhythmicity of energy intake across days 30-32 of restriction on low-fat canola or butter-based diets. Both dietary groups ate most of their energy in the light phase, with a peak of intake at 10:00 h (time when food was offered) followed by lower intakes throughout the light and dark phases.

Meal frequency: A significant main effect of phase ($F(1,108)=309.5$, $p<0.0001$) showed more frequent meals ingested in the light (4.6 ± 0.1) than dark phase (1.3 ± 0.1). In addition, a main effect of diet ($F(1,102)=7.70$, $p=0.007$) was found, with greater meal frequency in butter-fed rats (3.2 ± 0.1) than in canola-fed rats (2.7 ± 0.1, $p=0.007$). A significant phenotype by diet by phase interaction ($F(3,198)=4.86$, $p=0.003$) showed more frequent nocturnal meals in obesity-prone animals fed butter (2.7 ± 0.4) than in obesity-resistant rats fed the same fat source (0.3 ± 0.3, $p=0.024$).

Meal size (g and kcal): A significant main effect of phase ($F(1,108)=161.9$, $p<0.0001$) revealed larger meals ingested in the light (2.1 ± 0.1 g, 7.6 ± 0.3 kcal) than in the dark phase (0.5 ± 0.1 g, 1.7 ± 0.3 kcal). Across phenotype and diet, meal size was comparable during the light and dark phases (light: canola/obesity-prone: 2.2 ± 0.3 g and 7.9 ± 1.1 kcal, canola/obesity-resistant: 2.1 ± 0.5 g and 7.5 ± 1.9 kcal, canola/obesity-intermediate 2.3 ± 0.3 g and 8.2 ± 0.9 kcal, butter/obesity-prone: 1.4 ± 0.3 g and 5.0 ± 1.1 kcal, butter/ obesity-resistant: 2.6 ± 0.3 g and 9.5 ± 0.9 kcal, butter/obesity-intermediate: 1.2 ± 0.5 g and 4.4 ± 1.9 kcal; dark: canola/ obesity-prone: 0.2 ± 0.3 g and 0.9 ± 1.1 kcal, canola/obesity-

resistant: 0.4 ± 0.5 g and 1.5 ± 1.9 kcal, canola/obesity-intermediate: 0.6 ± 0.3 g and 2.2 ± 0.9 kcal, butter/obesity-prone: 1.0 ± 0.3 g and 3.5 ± 1.1 kcal, butter/obesity-resistant: 0.1 ± 0.3 g and 0.4 ± 0.9 kcal, butter/obesity-intermediate: 0.5 ± 0.5 g and 2.2 ± 1.9 kcal).

A positive correlation was found between diurnal meal size and IMI in both dietary groups (canola: $r=0.92$, $p=0.001$, butter: $r=0.99$, $p<0.0001$), meal duration (canola: $r=0.78$, $p=0.23$, butter: $r=0.96$, $p=0.0001$). The correlation between diurnal meal size and meal frequency was negative in rats fed canola ($r=-0.77$, $p=0.025$) and butter ($r=-0.81$, $p=0.15$).

Intermeal interval: A significant main effect of phase ($F(1,87)=14.13$, $p=0.0003$) showed longer IMI in the light (289.3 ± 16.6 min) than dark phase (178.0 ± 21.1 min). Across phenotype and diet, IMI was comparable during light and dark phases (light: canola/obesity-prone: 284.5 ± 58.2 min, canola/obesity-resistant: 321.3 ± 100.8 min, canola/obesity-intermediate 322.9 ± 50.4 min, butter/obesity-prone: 202.2 ± 59.2 min, butter/obesity-resistant: 329.9 ± 50.4 min, butter/obesity-intermediate: 211.3 ± 100.8 min; dark: canola/obesity-prone: 327.6 ± 67.2 min, canola/obesity-resistant: 322.5 ± 106.3 min, canola/obesity-intermediate: 104.2 ± 54.3 min, butter/obesity-prone: 107.0 ± 59.2 min, butter/obesity-resistant: 451.6 ± 69.2 min, butter/obesity-intermediate: 178.1 ± 100.8 min).

Satiety ratio: A significant main effect of phase (min/g: $F(1,111)=6.91$, $p=0.01$; min/kcal: $F(1,110)=7.48$, $p=0.007$) showed greater satiety ratio in the

light (373.3 ± 22.9 min/g, 103.7 ± 6.3 min/kcal) than dark phase (268.6 ± 28.9 min/g, 73.6 ± 8.0 min/kcal). A significant main effect of phenotype on satiety ratio (min/g and min/kcal) was found ($F(2,3.6)=9.23$, $p=0.038$ and $F(2,4.78)=7.30$, $p=0.035$) with obesity-resistant animals having greater satiety ratio (453.0 ± 31.0 min/g, 125.9 ± 9.5 min/kcal) than obesity-intermediate rats (289.0 ± 2.1 min/g, 103.3 ± 6.4 min/kcal, $p=0.035$), with satiety ratio of the obesity-prone animals in between 357.1 ± 21.2 min/g, 95.9 ± 6.3 min/kcal).

Meal duration: A significant main effect of phase ($F(1,108)=107.8$, $p<0.0001$) showed that meals of longer duration were ingested in the light (15.4 ± 0.7 min) than dark phase (4.4 ± 0.7 min). The duration of the meals did not differ significantly across phenotype and diet (light: canola/obesity-prone: 14.2 ± 2.1 min, canola/obesity-resistant: 16.0 ± 3.7 min, canola/obesity-intermediate 15.5 ± 1.9 min, butter/obesity-prone: 9.8 ± 2.2 min, butter/obesity-resistant: 21.8 ± 1.9 min, butter/obesity-intermediate: 9.0 ± 3.7 min; dark: canola/obesity-prone: 1.8 ± 2.1 min, canola/obesity-resistant: 5.4 ± 3.7 min, canola/obesity-intermediate: 7.2 ± 1.9 min, butter/obesity-prone: 8.0 ± 2.2 min, butter/obesity-resistant: 1.0 ± 1.9 min, butter/obesity-intermediate: 5.6 ± 3.7 min).

Eating rate: Eating rate of the animals was comparable in the light and dark phases (light: canola: 0.15 ± 0.01 g/min, 0.54 ± 0.03 kcal/min, butter: 0.13 ± 0.01 g/min, 0.47 ± 0.01 kcal/min; dark: canola: 0.13 ± 0.01 g/min, 0.48 ± 0.04 kcal/min, butter: 0.17 ± 0.01 g/min, 0.60 ± 0.04 kcal/min). A significant phenotype by diet by phase interaction on eating rate was found ($F(3,159)=4.53$,

p=0.005), with higher nocturnal eating rate in obesity-prone animals fed canola (0.23 ± 0.02 g/min, 0.81 ± 0.08 kcal/min) than in obesity-intermediate animals fed the same fat source (0.09 ± 0.02 g/min, 0.34 ± 0.06 kcal/min, p=0.041).

4.4.3. Restricted low-fat feeding versus ad libitum high-fat

During the restriction period while food was offered to rats at 10:00 h, we found that rats ate most of the food during daytime, thereby reversing their feeding phase. During that "diurnal feeding phase", restricted low-fat fed animals had a lower meal frequency (4.5 ± 0.4) and a greater satiety ratio (372.2 ± 18.3 min/g, 103.4 ± 5.0 min/kcal) than during their nocturnal feeding phase when fed ad libitum high-fat diets (6.7 ± 0.4, p=0.0004, and 99.6 ± 18.3 min/g, 19.6 ± 5.0 min/kcal, p<0.0001; respectively), regardless of the diet and phenotype. Meal duration and IMI were longer (14.3 ± 1.2 and 278.8 ± 22.4 min, respectively) during the "diurnal feeding phase" of restricted low-fat fed animals than when fed ad libitum (7.1 ± 1.2 and 106.1 ± 22.4 min, respectively, p<0.0007). Only obesity-prone and obesity-resistant animals in the butter group had a lower eating rate when restricted (prone: 0.15 ± 0.02 g/min, 0.53 ± 0.01 kcal/min; resistant: 0.12 ± 0.02 g/min, 0.42 ± 0.09 kcal/min) than when fed ad libitum (obesity-prone: 0.44 ± 0.02g/min, 2.33 ± 0.10 kcal/min, p<0.0003; obesity-resistant: 0.30 ± 0.02 g/min, 1.78 ± 0.11 kcal/min, p<0.009). However, meal size did not differ significantly across feeding periods (restricted low-fat feeding: canola/obesity-prone: 2.2 ± 0.4 g and 7.9 ± 1.8 kcal, canola/obesity-resistant: 2.1 ± 0.8 g and 7.5 ± 3.2 kcal, canola/obesity-intermediate 2.3 ± 0.4 g and 8.2 ± 1.6 kcal, butter/obesity-prone:

1.4 ± 0.4 g and 4.9±1.8 kcal, butter/obesity-resistant: 2.6 ± 0.4 g and 9.5 ± 1.6 kcal, butter/obesity-intermediate: 1.2 ± 0.8 g and 4.4 ± 3.2 kcal; ad libitum high-fat feeding: canola/obesity-prone: 1.1 ± 0.4 g and 5.9 ± 1.5 kcal, canola/obesity-resistant: 0.8 ± 0.8 g and 4.0 ± 3.2 kcal, canola/obesity-intermediate: 1.2 ± 0.4 g and 6.4 ± 1.6 kcal, butter/obesity-prone: 2.7 ± 0.4 g and 14.1 ± 1.8 kcal, butter/obesity-resistant: 2.6 ± 0.4 g and 13.9 ± 1.6 kcal, butter/obesity-intermediate: 2.8 ± 0.8 g and 14.8 ± 3.2 kcal).

4.5. Discussion

4.5.1. Effect of high-fat feeding

A prolonged exposure to canola and butter-based, fat-rich diets did not alter the normal light-dark distribution of food and energy intake, relative to chow feeding. All animals consumed most of their total amount of food during the dark phase (canola: 63.1%, butter: 63.6%, chow: 69.8%). Similar results were found at early exposure to canola or lard high-fat feeding (75.7% and 83.6%, respectively). However, feeding the high-fat canola and butter-based diets produced an altered eating pattern during the light phase, which was characterized by a smaller number of meals, longer IMI, and an enhanced satiety ratio (min/g). Overall, rats fed the high-fat diets consumed shorter meals than chow-fed animals. This altered eating pattern can be interpreted in terms of high-fat feeding and cannot be attributed to food and energy intake, or obesity. Indeed, canola- and butter-fed animals differed in their intakes as well as body weights; yet sharing some similarly altered circadian meal patterns.

The circadian distribution of food intake in 4-week old female C57BL/6J mice fed a high-fat diet (beef fat at 62% of energy) for 8 weeks did not differ from that of the control group fed a standard lab diet (10% of energy) (Yanagihara et al. 2006). In weaning male Sprague-Dawley rats fed the lard-based high fat (60% of energy) or low-fat (10% of energy) diets for 5, 17, or 33 weeks, nocturnal intake contributed to 79%, 70%, and 73% of the daily intake in high-fat fed rats and 83%, 72%, and 82% in low-fat fed animals (Furnes et al. 2009). On the other hand, Kohsaka et al. (2007) found that 4-week old male C57BL/6J mice fed high-fat diets (lard at 45% of energy) for 6 weeks consumed more food in the light phase and less in the dark phase than chow-fed control animals.

The present work supports the previous findings that eating pattern is altered with high-fat feeding. For example, when adult male Sprague-Dawley rats were fed high- or low-fat diets (soybean oil at 38% or 10% of energy) for 8 weeks, meal size and IMI (first 6 h of the dark phase) were greater with high-fat feeding compared to those parameters at week 2, while meal size and IMI were comparable in low-fat fed animals at these two time points (Paulino et al. 2008). After 5 weeks of feeding a fat-rich diet (lard at 60% of energy) in weaning male Sprague-Dawley rats, shorter and larger diurnal and nocturnal meals were ingested at a higher rate, with less frequent eating episodes in the dark phase, compared to a low-fat fed diet (same fat at 10% of energy) (Furnes et al. 2009).

4.5.2. Effect of fatty acid profile

It must be noted that feeding the high-fat, butter-based diet led to greater energy intake and final body weight relative to canola and chow feeding. This raises the possibility that the differing saturation of these two types of dietary fats could influence food intake and body weight gain, which is documented in the literature (reviewed in: Hariri and Thibault 2010). However, the role of the saturation of the fat on eating rhythms has been less investigated, and the present work provides some of this much needed information. Only butter feeding led to overeating and obesity, with less meals consumed during the dark phase, a higher eating rate in the light phase, and larger meals overall.

Feeding high- or low-fat diets (moderate-SFA, lard at 60% and 10% of energy, respectively) to weaning male Sprague-Dawley rats for 17 weeks resulted with high-fat feeding in altered meal patterning, characterized by eating less frequent meals in the dark phase and consuming larger meals at a higher rate in the light and dark phases , which led to greater weight and obesity development (Furnes et al. 2009). When the eating pattern of butter and chow-fed rats were compared in the present study, similar alterations in meal pattern were observed with SFA-rich butter, which resulted in the development of obesity following 50 days of high-fat feeding. Paulino and colleagues (2008) found that 8 weeks of high-fat or low-fat feeding based on low-SFA soybean oil (at 38% or 10% of energy, respectively) in adult male Sprague-Dawley rats led to similar meal size and IMI (over 6 h); however, rats fed the high-fat diet had a lower rate of weight

gain than low-fat fed animals. The present work also found comparable meal size in animals fed low-SFA canola or chow, although canola-fed rats had longer diurnal IMI.

The present work reports for the first time that a greater proportion of saturated fatty acid in a high-fat diet, such as butter fat, alters the circadian meal pattern in a way that is conducive to the development of obesity. It is of interest that we previously reported that after weight loss abdominal fat of butter-fed rats did not differ from that of chow-fed controls, suggesting that body fat is preserved with butter feeding (Hariri et al. 2010).

Mechanisms suggested for the induction of obesity by high-fat feeding and related alteration of eating patterns include: large meals being the result of weak intra-meal satiety signals and low satiation (Synowski et al. 2005; Warwick and Weingarten 1995; Warwick et al. 2000); eating large meals less frequently resulting in less fat oxidation and increase in 24-h respiratory quotient (RQ), which is related to availability of more carbohydrate and protein for oxidation, and the body's limited capacity to store them (Smeets and Westerterp-Plantenga 2008); decrease in meal frequency and increase in IMI enhancing the fluctuations in plasma glucose and insulin levels, which by themselves increase the sense of hunger (Smeets and Westerterp-Plantenga 2008).

The greater energy intake in butter-fed rats than in canola and chow-fed animals could have been possibly influenced by the texture of the diets. Because rodents prefer chewable textures, we have added an emulsifier (pectin) to the

high-fat diets. However, this provided a smoother texture with the canola oil and chow mix than the butter mixture.

4.5.3. Meal patterns of susceptible rats

In the present study, all obesity-prone animals ingested shorter meals; however, obesity was induced in butter-fed prone rats only. These animals ingested a comparable number of meals in both phases but had a greater eating rate than chow-fed animals. In adult male obesity-prone and obesity-resistant Sprague-Dawley rats fed high-fat diets (lard at 45% of energy) for 19 weeks, obesity induced in prone animals was accompanied with the ingestion of larger meals in both the light and dark phases compared to obesity-resistant or chow-fed animals (Farley et al. 2003). However, in the present work, there is the possibility of type II error, because of the small number of animals in each phenotype sub-group; therefore, for better contrasting the phenotypes, further studies should use larger number of animals.

Alteration in meal pattern with ingestion of less frequent but large meals were found in genetically obese Zucker rats or ob/ob C57BL/6J mice fed chow, sweetened condensed milk, or a liquid diet in light and dark phases (Becker and Grinker 1977; Fukagawa et al. 1992; Prins et al. 1986) or in the dark phase only (Strohmayer & Smith, 1987), and accompanied by a shift of intake to the light phase (Becker and Grinker 1977; Fukagawa et al. 1992; Ho and Chin 1988).

4.5.4. Meal patterns of rats when restricted

When given a restricted amount of low-fat canola or butter-based diet at the start of the light phase, rats ate most of their food in that phase, and diurnal rather than nocturnal feeding occurred with restriction. When a restricted amount of chow (66%) was offered to 8-week old male C3H mice 6 h after lights-on, animals ate 60% of their food within the first 3 h, and their feeding phases were reversed; while in control animals, daytime eating only contributed to 25% of their 24-h intake (Mendoza et al. 2005). In the present study, all rats responded to food restriction on a low-fat diet offered at early light by ingesting less frequent meals of longer duration, and with longer IMI and greater satiety ratio than with ad libitum high-fat feeding. Whether the present results could be repeated with restricted food access at another time of the nychthemeral cycle, e.g., early dark, remains to be determined. Moreover, the daily feeding pattern of control rats given a similarly restricted amount of chow at early light should be tested against that of rats fed a restricted low-fat diet. Similarly, high-fat diets offered at early dark (rather than early light in the present study) should be tested to replicate butter feeding specific to higher eating rate in the light phase and less meals during the dark phase. The food access schedule can alter biological rhythms, and daytime feeding has been used to investigate mechanisms controlling circadian rhythms. Our earlier review of the literature found no consistent effect of daytime feeding with a mixed diet on hormones, such as testosterone, androstenedione, and melatonin, or on clock genes in the master clock (Selmaoui and Thibault 2006). However, the circadian expression of clock genes and corticosteroid secretion in

peripheral tissues, such as the liver, was shown to be influenced by daily feeding cycles (Damiola et al. 2000; Koyanagi et al. 2006; Stokkan et al. 2001).

In the literature most studies have assessed the pattern of eating that followed food restriction that resulted in hyperphagia with ad libitum feeding (Brownlow et al. 1993; Del Prete et al. 1994). However, one study in 2-month old Sprague-Dawley rats fed low-fat pellets found that animals subjected to a short-term 7-day 50% food restriction offered at early dark increased meal size and decreased meal duration (Johansson and Elomaa 1986). The present work reports meal pattern following long-term (25 days) low-fat diet restriction leading to longer meal duration.

Both long-term canola and long-term butter feeding altered the diurnal eating pattern. However, rats fed the high-SFA butter-based diet consumed more energy, ingested larger meals in light and dark phases, and developed obesity. These findings underscore the specific effects of a high-SFA diet on eating pattern and its possible contribution to the development of obesity.

4.6. Conflicts of interest

The authors had no conflicts of interest.

4.7. Acknowledgment

This work was supported by a grant to Louise Thibault by the Natural Sciences and Engineering Council of Canada.

Table 4.1. Diurnal and nocturnal distribution of meal pattern (mean± SEM) following 43 days of chow or high-fat canola or butter-based diets ad libitum feeding.

	Meal frequency		Meal size (g) / (kcal)		IMI (min)		Satiety ratio (min/g) / (min/kcal)		Meal duration (min)		Eating rate (g/min) / (kcal/min)	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Chow	5.1±0.3 ^{aA}	9.5±0.3 ^{bA}	0.9±0.1 / 3.1±0.7	1.3±0.1 / 4.5±0.7	155.0±11.6 ^{aA}	69.8±11.6 ^{bA}	183.6±19.7 ^{aA} / 54.0±3.8	69.6±19.7 ^{bA} / 20.5±3.8	5.9±0.4	9.0±0.4	0.16±0.04 ^{aA} / 0.90±0.17 ^{aA}	0.16±0.04 ^{aA} / 0.53±0.17 ^{aA}
Canola	3.5±0.3 ^{aB}	7.5±0.3 ^{bB}	0.9±0.2 / 5.0±0.9	1.1±0.2 / 5.7±0.9	259.4±14.3 ^{aB}	96.4±14.3 ^{bA}	313.4±24.3 ^{aB} / 60.1±4.7	110.1±24.3 ^{bA} / 21.8±4.7	5.0±0.5	7.1±0.5	0.21±0.06 ^{aA} / 1.78±0.22 ^{aA}	0.16±0.06 ^{aA} / 0.94±0.22 ^{bAB}
Butter	2.5±0.3 ^{aB}	5.4±0.3 ^{bC}	2.5±0.2 / 13.1±0.9	2.7±0.2 / 14.2±0.9	342.9±14.4 ^{aC}	114.8±14.4 ^{bA}	344.1±24.6 ^{aB} / 63.9±4.8	84.7±24.6 ^{bA} / 15.0±4.8	4.1±0.6	7.2±0.6	0.80±0.06 ^{aB} / 4.29±0.22 ^{aB}	0.38±0.06 ^{bA} / 2.02±0.22 ^{bB}

For significant diet by phase interactions: different lower case letters indicate values significantly different between the phases within each dietary group. Different capital letters indicate values significantly different among the dietary groups within each phase. For significance refer to text.

4.8. Figure captions

Figure 4.1. Energy intake (kcal, mean ± SEM) at early exposure to ad libitum canola (N = 4) or lard (N = 4) diet (A), and following 47 days of chow (N = 8), canola (N = 8), or butter (N = 8) feeding (B) in the early, middle, and late parts of the dark phase. Different lower case letters indicate values statistically different across different parts of the dark phase within each dietary group. Different capital letters indicate values statistically different among dietary groups within each part of the dark phase. For significance refer to text.

Figure 4.2. Circadian rhythmicity of energy intake (kcal, mean ± SEM) at early exposure to ad libitum high-fat canola- (N = 4) or lard- (N = 4) based diets.

Figure 4.3. Circadian rhythmicity of energy intake (kcal, mean ± SEM) across days 48 to 50 of ad libitum chow (N = 8), high-fat canola- (N = 8) or butter- (N = 8) based diet feeding.

Figure 4.4. Circadian rhythmicity of energy intake (kcal, mean ± SEM) in rats fed ad libitum chow (N = 8) and restricted low-fat canola- (N = 8) or butter- (N = 8) based diets.

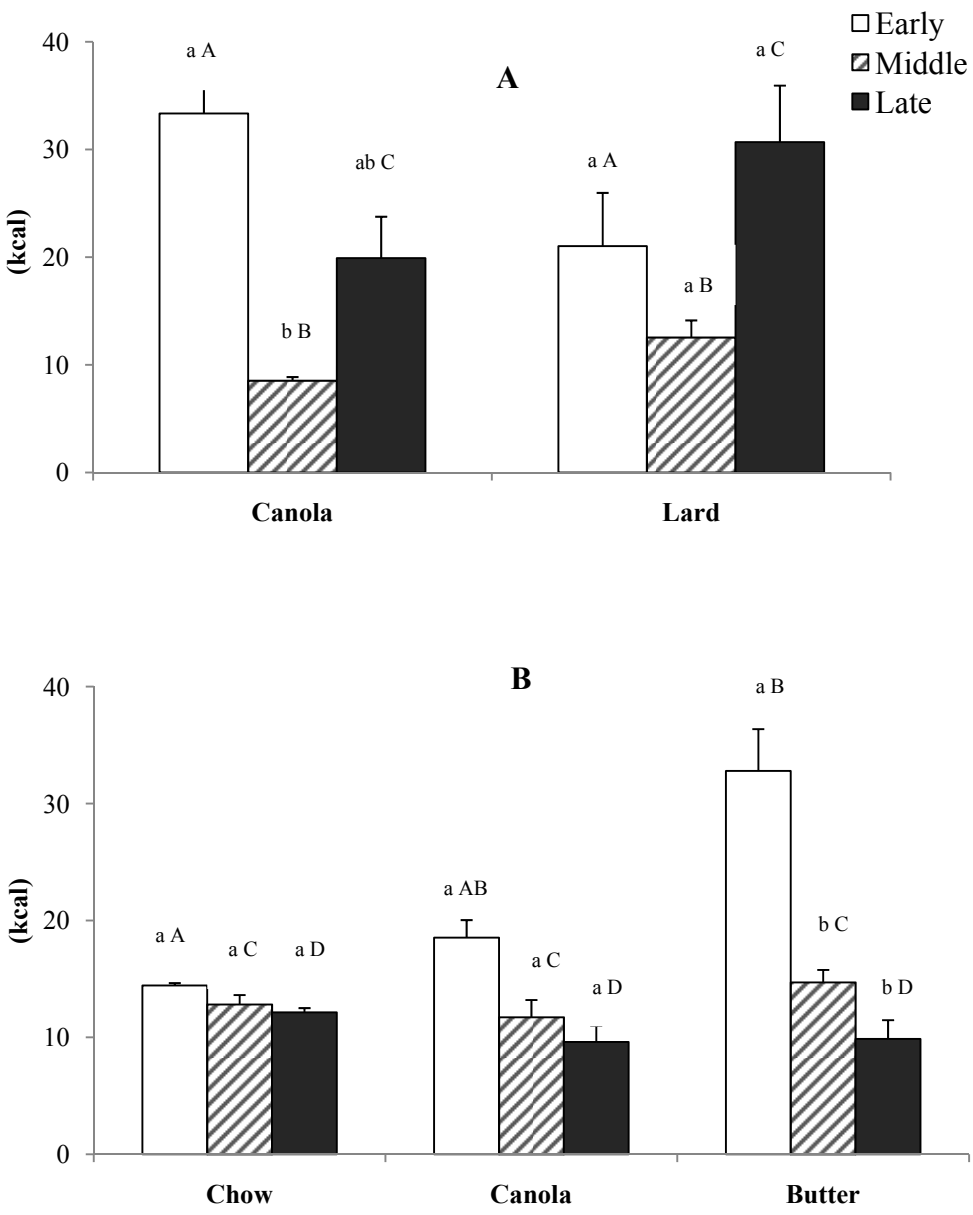
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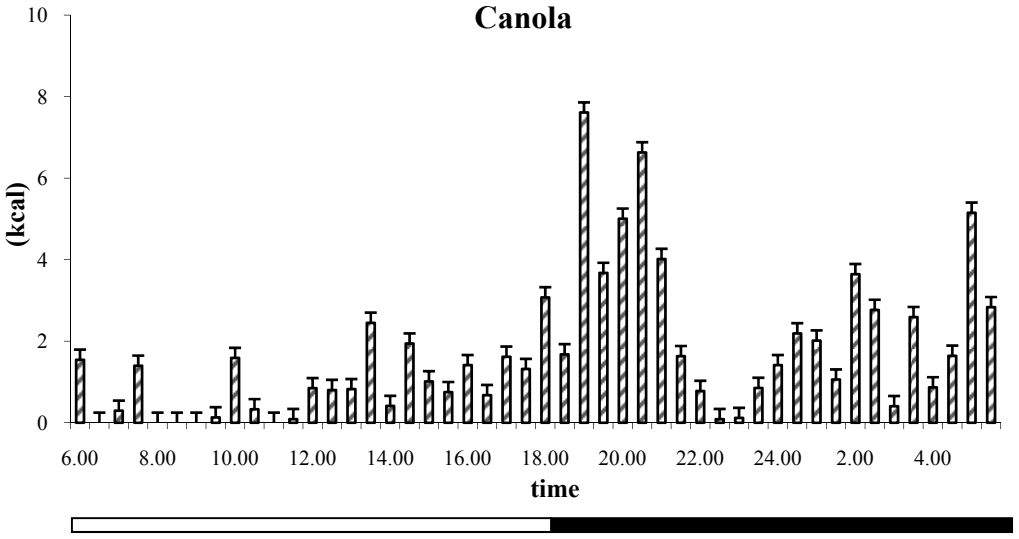
Figure 4.1.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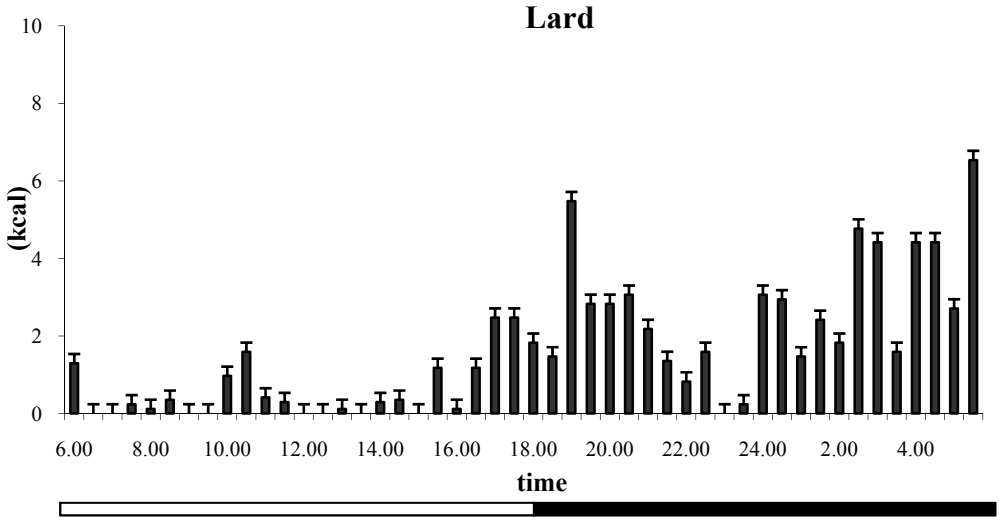
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Figure 4.2.

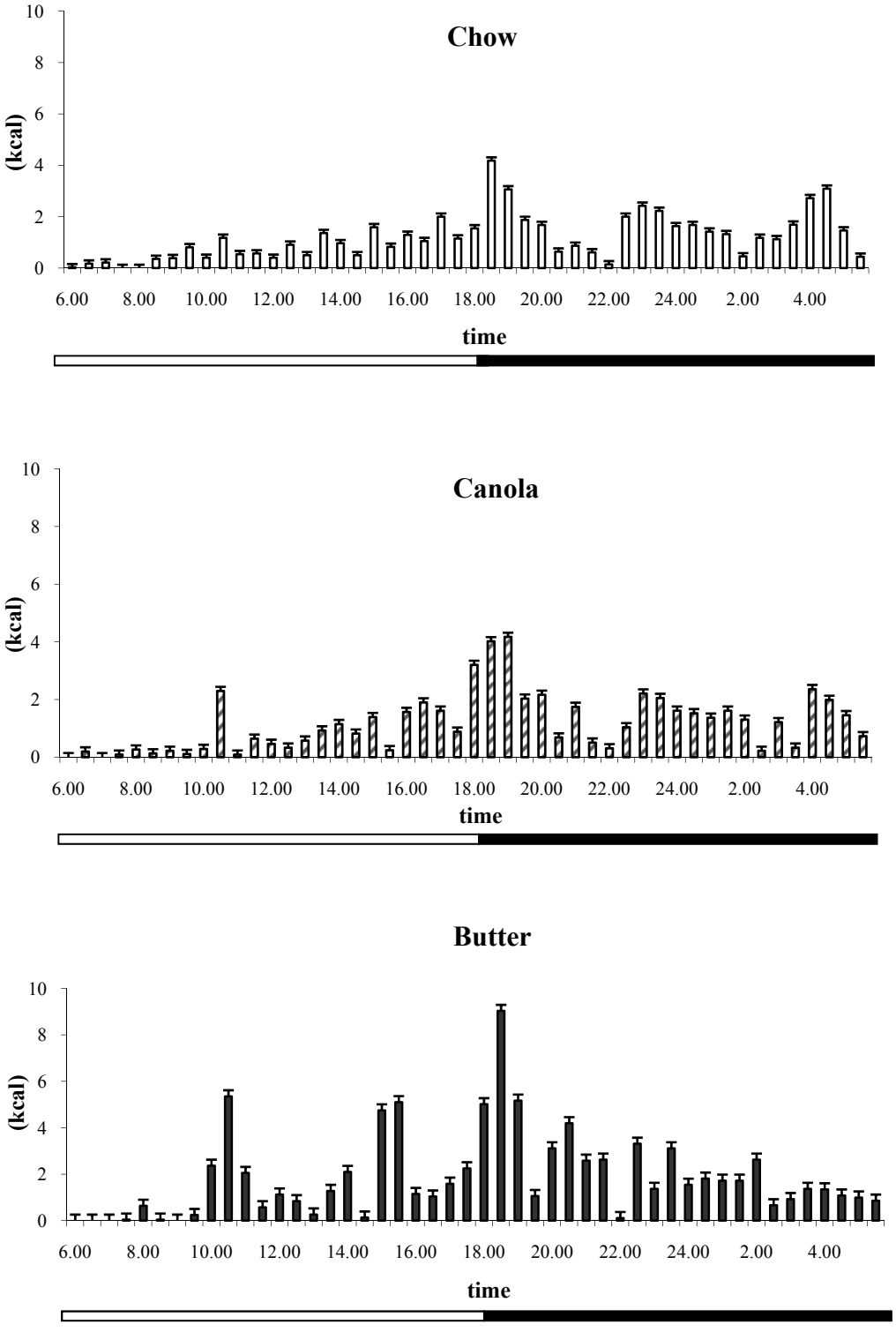
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Figure 4.3.

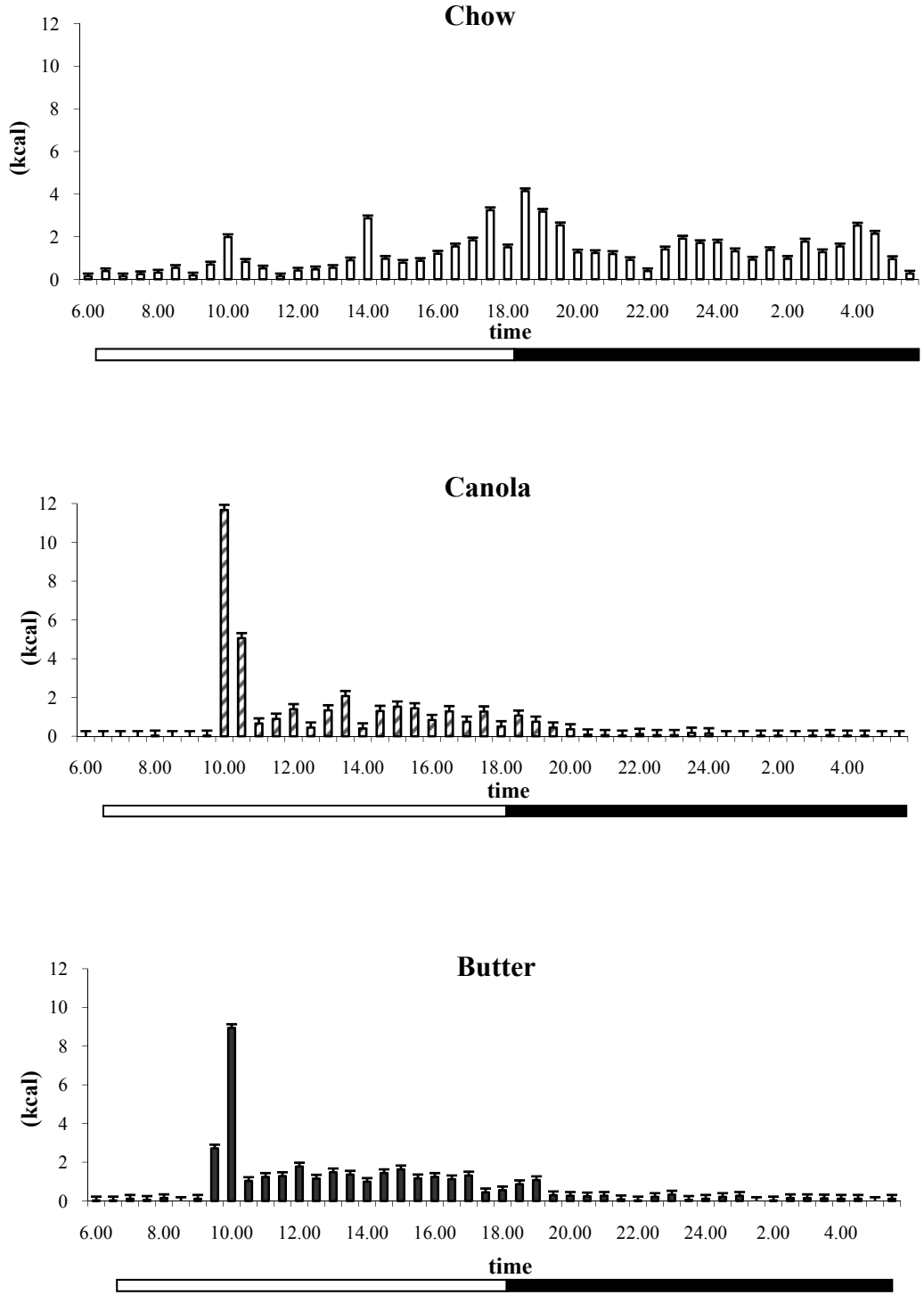
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Figure 4.4.

BRIDGE 3

The results of Manuscript 3 showed that fat-rich diets with high (butter), moderate (lard) and low (canola)-SFA content have different effects on eating pattern of adult rats. Long-term high-fat canola or butter feeding produced an altered eating pattern during the light phase characterized by a smaller number of meals, longer IMI and an enhanced satiety ratio. However, only butter-fed animals ate larger meals and developed obesity. Most studies in the literature have tested meal pattern alteration with high-fat feeding in either adult rats or in young animals. Therefore we aimed to conduct a study in weaning animals at early exposure to high-fat diet. In this study, we used high-fat diets with high and low-SFA content and assessed eating pattern and body weight gain after 3 days and 3 weeks of feeding. This allowed us to investigate whether high-fat feeding early in life leads to eating pattern and body weight alteration, and if dietary fatty acids are involved.

CHAPTER 5, MANUSCRIPT 4

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Diurnal feeding in young rats fed saturated fatty acid-rich diet

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Running head: Diurnal feeding with SFA-rich diet

Word count: 4250

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5.1. Abstract

This study investigated eating pattern in weaning female Sprague-Dawley rats fed high-fat diets of varying fatty acid composition. Rats were randomly assigned to canola- or butter-based diets and their eating pattern and body weight were assessed at early exposure (P1) and following 3 weeks (P2). In P2, a lower 24-hour intake, smaller meal size and shorter nocturnal meal duration were found in both dietary groups. In both groups 24-hour intakes were comparable throughout the experiment; however butter-fed rats gained more weight. With canola feeding, P1 nocturnal intake was greater than diurnal intake, but intakes were comparable in P2. Butter-fed rats had similar diurnal and nocturnal intakes in P1; in P2 diurnal was greater than nocturnal intake. Canola-fed rats had similar diurnal and nocturnal meal size and eating rate, whereas butter-fed rats ate larger meals at a higher rate in the light than in the dark phase. The present work brings new knowledge about exposure to high-fat diets early in life and later development of obesity.

Key words: Circadian eating pattern; Food intake High-fat diet; Body weight; Weaning rat.

5.2. Introduction

Many studies have shown that there is a relationship among eating pattern, feeding high-fat diets and obesity in rodents (Farley et al. 2003; Paulino et al. 2008; Furnes et al. 2009; Melhorn et al. 2010) and in humans (Bertéus Forslund et

al. 2002; Ma et al. 2003; Toschke et al. 2005; Chapelot et al. 2006; Howarth et al. 2007). Eating patterns that were studied include diurnal and nocturnal distribution of the eating episodes, as well as the size, frequency and duration of the meals, intermeal interval (IMI), satiety ratio, eating rate and snack frequency.

Short-term high-fat feeding was shown to alter meal size and frequency. In adult male 129/sv mice fed high-fat diets (low-SFA soy oil at 38% of energy) or isocaloric low-fat diets (soy oil at 10% of energy) for 15 days, high-fat fed animals had smaller meal size and meal frequency but comparable body weights (Donovan et al. 2007). In meal pattern analysis on days of 5-18 of exposure to isocaloric high and low-fat diets (soy oil at 38% and 10% of total calories) in adult male Sprague-Dawley rats, both groups ingested comparable amounts of food in their first meal, but high-fat fed rats ate less in their second and third meal, and had higher meal frequency, shorter IMI and lower rate of weight gain (Paulino et al. 2008). The smaller size of the meals associated with dietary fat intake can be explained by the fact that when entering intestine fat slows the gastric emptying, and stimulate the secretion of cholecystokinin (CCK), peptide YY (PYY) and glucagon-like peptide 1 (GLP-1), therefore suppressing intake (Little et al. 2007). However, altered eating patterns and increased meal size and eating rate and decreased meal frequency and satiety ratio were found in adult male Long Evans rats as early as 9 days after starting high-fat feeding (at 20% of energy, source not mentioned) (Melhorn et al. 2010).

Longer-term feeding with a fat-rich diet alters the greater intra-meal satiety induced by fat intake and a weak satiety signal is elicited. For example when feeding was continued for 8 weeks in the study by Paulino and colleagues (Paulino et al. 2008), the size of the second meal and IMI were increased. The results of other long-term studies are consistent with this: in a study in weaning male Sprague-Dawley rats fed diets high (moderate-SFA lard at 60% of energy) or low (at 10% of total energy) in fat for 5, 17 and 33 weeks, increased meal size and eating rate and decreased meal frequency and duration were found with 5 weeks of high-fat feeding (Furnes et al. 2009). Increased meal size has also been found after 19 weeks of high-fat feeding in adult male obesity prone Sprague-Dawley rats (Farley et al. 2003).

Frequent snacking was found to be related to obesity in humans (Zizza et al. 2001; Marmonier et al. 2002; Marin-Guerrero et al. 2008) and this was shown to be mainly related to the high availability of snacks rich in fat (Green et al. 2000) and sugar (Kerr et al. 2009) and large portion sizes (Rolls et al. 2004; Kerr et al. 2009). The concept of "snack" in humans is usually defined based on the food categories (high quality snack: milk or apple versus low quality snack: chocolate, candies, potato chips) and/or time of eating (consumed between 8:00-10:00 or 12:00-14:00 or 18:00-20:00 (Lennernas and Andersson 1999; Gregori and Maffeis 2007). Although the concept of "snack" might be different in animals, some studies have employed the same food categories used in humans as "snack foods" (Swithers et al. 2006; Jarosz et al. 2007). Since an eating episode that is not qualified as a "meal" can be identified as a "snack" in humans (Lennernas and

Andersson 1999), the food ingested in an amount less than a meal in rat can be considered as a "snack" and can resemble snacking behaviour in humans.

Fatty acid composition of the diet has an important effect on dietary obesity. It is known that SFA are more easily accumulated in adipose tissue rather than being used as fuel as compared to polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) (DeLany et al. 2000; Storlien et al. 2001; Kien et al. 2005). However, the effect of fatty acid profile of the diet on meal pattern is not yet studied.

The present work assessed eating pattern and body weight of young female Sprague-Dawley rats fed high-fat diets (at 67% of energy) based on low-SFA canola oil or high-SFA butter at early (days 4- 10) and later exposure (days 22-28).

5.3. Material and methods

5.3.1. Animals and diet

Twelve 3-week old weaning female Sprague-Dawley rats were used for this experiment. They were obtained from Charles River Laboratories (St-Constant, Quebec, Canada) and were housed in individual normal cages upon arrival. They were under controlled temperature (22-25^{o} C) and humidity (~ 70%) and with a 12:12 light-dark cycle with lights on from 06:00-18:00. To adapt to the new environment, all rats had ad libitum access to Purina chow (Charles River rodent chow 8075, Quebec, Canada) and tap water prior to the experiment for 10 days.

Then they were transferred to a Diet Scan system (period 1, P1), and after 1 day were randomly assigned to two experimental groups fed isocaloric fat-rich diets (67% energy from fat). The diets were made in our laboratory by using 58.8% (g/100g) Purina chow, 4% pure pectin (MP Biomedicals, Inc., USA) and 37.2 % fat of either butter (68% SFA; My Country, Lactantia, Canada) (N=6) or canola oil (7% SFA; President's choice, Canada) (N=6). The protein content of the high-fat diets was adequate for their growth (12 g/100 g of diet) (National Research Council 1978). After 10 days in the Diet Scan, they were transferred back to normal cages for 8 days followed by moving to the Diet Scan for another 10 days (period 2, P2). Animals had free access to their diets and tap water during the experimental period. The research procedures were approved by the McGill University Animal Care Committee.

5.3.2. Diet Scan system

The Diet Scan system (AccuScan Instruments Inc., Columbus, Ohio, USA) is a computerized data acquisition system designed to continuously record and study food and water ingestion patterns of small animals. Diet Scan cages, made of clear acrylic materials (41.75 X 41.75 X 31.5 cm^3), were designed with a diagonal divider in order to house two animals separately. Each animal had access to a feeder through a square opening in the cage. The feeders, filled with the diets, were placed on electronic scales (Ohaus Port-O-Gram-C301P and A&D-EW300A). Access to water was through a drinking spout connected to a drinking bottle that was held by a holder on a scale plate. All the scales were connected to

an analyzer device linked to an IBM computer and programmed with the Diet Scan Lab Animal Monitoring System Software (DLAM) for data collection recording every 30 minutes on each scale around the 24 h. A Diet Scan template was then used to reorganize the recorded information into data files in the form of Microsoft Excel spreadsheets.

5.3.3. Measurements

Body weight and food intake of the rats was measured daily (9:00-10:00) throughout the experiment using a digital Mettler PJ 3000 balance, fresh food and water were then provided. Food intake of the animals was also measured at 1 minute intervals while in Diet Scan. Spillage was collected and weighed. Data of the last 7 days were analyzed for meal calculation. A meal was defined as changes in the weight of food $\geq$ 0.3 g, which lasted $\geq$ 1 minute and was separated from other feeding episodes by $\geq$ 10 minutes (Langhans and Scharrer 1987). Food intake less than 0.3 g was considered as "snack". Diurnal and nocturnal food intakes as well as the size, frequency and duration of the meals, IMI (the interval from the last weight change of a meal to the first weight change of the next meal), satiety ratio (the ratio of IMI to the preceding meal size), eating rate (meal size divided by meal duration) and frequency of the snacks were calculated using the data of Diet Scan. On day 29 of exposure to the high-fat diets, naso-anal length of the rats was measured under CO_2 anaesthesia and Lee obesity index was calculated by dividing the cube root of the body weight (g) to the naso-anal length (cm) multiplied by 1000 (Lee 1929).

5.3.4. Statistical analyses

One-way ANCOVA was used for analyzing final body weight, body weight gain and obesity index and one-way ANOVA for mean daily food intake, with dietary fat as the main effect and initial weight as the covariate. For 24-hour, diurnal and nocturnal food intakes as well as meal size, number and duration, IMI, satiety ratio, eating rate and snack frequency analyses were performed using three-way repeated measures ANCOVA with diet, phase (light versus dark) and period (P1 versus P2) as the main effects and spillage as the covariate and with testing their interaction. For the significant main effects, multiple comparisons were conducted by using Scheffe's test. In addition, correlation analyses were performed. Data are presented as mean ± SEM, and all differences were considered statistically significant if $p<0.05$. SAS version 9.1 was used for analyzing the data.

5.4. Results

5.4.1. Body weight

Daily body weight gain of animals fed canola and butter-based diets were comparable across 7 days of early exposure to high-fat feeding (2.08±0.26 and 2.56±0.26 g/day, respectively, all values adjusted for initial weight), and both dietary groups reached similar weights after this period (144.1±2.6 and 148.9±2.6 g, respectively) (Figure 1). Moreover, canola and butter-fed rats gained comparable weight across days 21-28 of high-fat feeding (1.83±0.2 and 2.25±0.2

g/day, respectively), although butter-fed animals reached a greater final weight (201.9±3.7 and 181.9±3.7 g, respectively; p=0.005). Across 28 days of feeding, animals fed butter had greater daily body weight gain than canola-fed animals (2.82±0.14 and 2.09±0.14 g/d, respectively; p=0.005). However, both dietary groups had the same obesity index after 28 days (315.9±1.6).

5.4.2. Daily food intake

A significant main effect of period (p=0.009) showed that rats ingested less food and energy in P2 than in P1 (18.9±1.0 g/day, 100.4±5.0 kcal/day and 22.4±0.9 g/day, 118.9±4.7 kcal/day, respectively). Over 28 days, mean daily intake of the animals fed canola and butter-based diets was not significantly different (13.0±0.9 g/day, 68.7±5.0 kcal/day and 14.5±0.9 g/day, 77.1±5.0 kcal/day, respectively).

5.4.3. Diurnal and nocturnal intake

A significant diet by phase by period interaction (p=0.005) showed that rats fed canola ate more food (Figure 2) and energy in the dark than in the light phase in P1 (69.0±5.4 and 43.1±5.4 kcal, respectively; p=0.028), while their diurnal and nocturnal intake in P2 did not differ significantly (36.7±6.0 and 50.1±6.0 kcal, respectively). In butter-fed animals similar diurnal and nocturnal intakes were found in P1 (66.3±7.2 and 61.7±7.2 kcal, respectively), while in P2, they ingested more food (Figure 2) and energy in the light cycle than in the dark cycle (88.2±7.6 and 25.9±7.6 kcal, respectively; p<0.0001). Rats fed butter also had a

greater diurnal food and energy intake than animals fed canola in P2 (36.7±6.0 and 88.2±7.6 kcal, respectively; p=0.006). Besides, butter-fed animal had a lower nocturnal food and energy intake in P2 than in P1 (61.7±7.2 and 25.9±7.6 kcal, respectively; p=0.021).

5.4.4. Circadian rhythmicity of food intake

Meal frequency: A significant interaction of diet by phase was found ($p<0.0001$) (Table 1), and multiple comparisons showed that canola-fed rats ate less frequent meals in the light phase than in the dark phase ($p<0.0001$), while diurnal and nocturnal meal frequency did not differ in animals fed butter.

Meal size: A main effect of period (p=0.024) revealed that rats ingested smaller meals at P2 (1.7±0.1 g, 8.9±0.7 kcal) than in P1 (2.1±0.1 g, 11.1±0.7 kcal). An interaction of diet by phase (p=0.0004) showed that butter feeding resulted in larger meals ingested in the light phase than in the dark phase ($p<0.0001$), while canola fed-rats had comparable diurnal and nocturnal meal size (Table 1).

IMI: There was a significant diet by phase interaction ($p<0.0001$), with rats fed canola-based diet having longer IMI in the light phase than in the dark phase ($p<0.0001$) (Table 1). Moreover, diurnal IMI was longer with canola feeding than with butter feeding (p=0.036).

Satiety ratio: A diet by phase interaction ($p<0.0001$) showed that canola feeding elicited a greater satiety ratio (min/g and min/kcal) in the light phase than in the dark phase ($p<0.0001$) (Table 1).

Meal duration: A significant interaction of phase by period was found ($p=0.042$) and multiple comparisons revealed that shorter nocturnal meals were ingested by rats in P2 (7.9±0.9 min, $p=0.0005$) than in P1 (12.6±0.9 min). Besides, diurnal meals in P2 were longer than nocturnal meals (10.8±0.9 min, $p=0.042$).

Eating rate: A diet by phase interaction was found ($p<0.0001$), with butter-fed rats having greater eating rate (g/min and kcal/min) in the light than in the dark phase ($p<0.0001$) (Table 1).

Snack: There was a significant diet by phase interaction ($p=0.005$), and multiple comparisons showed that canola-fed rats ingested more snacks in the dark than in the light phase ($p<0.0001$) (Table 1).

5.4.5. Correlation

Correlation analysis showed that meal size was negatively correlated with meal frequency ($r=-0.82$, $p=0.047$) and positively correlated with IMI ($r=0.91$, $p=0.011$) in canola-fed rats during P1. In the overall analysis, daily food intake, diurnal intake, meal size and eating rate at P1 were all positively correlated with final weight (daily food intake: $r=0.67$, $p=0.034$; diurnal food intake: $r=0.75$, $p=0.012$; meal size: $r=0.77$, $p=0.009$; eating rate: $r=0.68$, $p=0.031$).

5.5. Discussion

Twenty-eight days of feeding high-fat diets based on canola oil and butter in young rats resulted in significant increase in body weight gain and final weight of the butter group only, while both groups had comparable intakes. This shows that the greater body weight gain with butter feeding in young rats of the present study was not related to higher energy intake in these animals.

The present findings also revealed that the diurnal and nocturnal food intake of rats fed butter-based diet became comparable as early as 3 days after feeding the high-fat diet; while in accordance with normal meal pattern in young rats (Mathews et al. 2000; Pu et al. 2000), diurnal intake was less than nocturnal intake in the canola group. Continuation of high-fat feeding led to comparable diurnal and nocturnal intakes in canola-fed rats, a shift of intake toward the light phase in butter-fed animals and a greater diurnal intake with butter than with canola feeding accompanied with a greater body weight gain.

The shift of intake to the light phase with 3 weeks of high-fat feeding is an important factor in developing obesity. Young rats ingest most of their food in the dark phase (Mathews et al. 2000; Pu et al. 2000), and this is in accordance with their activity phase, and eat less in the light phase which matches to their resting phase Six weeks of high-fat feeding (lard at 45% of total energy) in C57BL/6 male mice, led to a shift of intake toward light phase, while their activity phase was not shifted (Kohsaka et al. 2007). An alteration in the expression of the clock genes that are involved in glucose and lipid metabolism and have a circadian

rhythmicity was also found in these animals (Kohsaka et al. 2007), which shows that eating at an "improper" circadian time leads to storage of energy.

Other than the shift of intake, butter feeding also led to ingestion of larger meals at a higher rate in the light phase, and induced comparable diurnal and nocturnal meal and snack frequency, IMI and satiety ratio. Eating pattern measurement in weaning male Sprague-Dawley rats fed high (lard at 60% of energy) or low (same fat at 10% of total energy) -fat diets for 5 weeks showed a larger meal size and eating rate and a shorter meal duration in high-fat fed animals in light and dark phases, as well as a lower meal frequency in the dark phase (Furnes et al. 2009). The higher body weight gain in high-fat fed animals only reached significance after 6 weeks of high-fat feeding; while the only time that the total caloric intake was higher in high than that in low-fat fed animals was at week 5. The researchers concluded that the larger calorie per meal in high-fat fed animals was responsible for the higher weight gain in these animals rather than the higher calorie intake per day (Furnes et al. 2009). Increased meal size and eating rate, decreased meal frequency and satiety ratio and greater body weight gain have also been found in adult male Long Evans rats with 9 days of high-fat feeding (at 20% of energy, source not mentioned) than with chow feeding (Melhorn et al. 2010).

The difference between high-SFA and low-SFA diets in altering eating patterns may be related to the lower satiety signals and CCK secretion elicited with SFA, which leads to ingestion of larger meals (Beardshall et al. 1989;

Lawton et al. 2000; Maljaars et al. 2009). With less frequent large meals, more carbohydrate and protein are available; which with body's limited capacity to store them would cause a decrease in fat oxidation (Smeets and Westerterp-Plantenga 2008). Besides, low meal frequency increases the sensation of hunger and decreases the control over appetite (Bellisle 2004; Taylor et al. 2004).

The present study also revealed that from P1 to P2, rats decreased their 24-hour food intake and meal size, as well as the duration of the nocturnal meals. This reveals that with an energy-dense high-fat diet, young rats were attempting to adjust intake.

To our knowledge this is the first study comparing the eating pattern of young rats fed high-fat diets of varying fatty acid content at 2 different time points. This let us: 1- investigate the eating pattern changes while animals were aging and gaining weight on a high-fat diet, and 2- examine whether eating pattern alteration induced by high-fat feeding is dependent upon the fatty acid profile of the diet.

In conclusion, in weaning rats, three-week exposure to high-fat diets shifted intake toward the light phase; this was more prominent with high- than with low-SFA diet and accompanied with greater body weight and altered eating pattern rather than overeating. This work brings new knowledge about early exposure to high-fat diets and later development of obesity.

5.6. Acknowledgement

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Table 5.1- Diurnal and nocturnal distribution of meal patterns (mean± SEM) in rats fed high-fat canola or butter-based diets.

	Meal frequency		Meal size (g) / (kcal)		IMI (min)		Satiety ratio (min/g) / (min/kcal)		Meal duration (min)		Eating rate (g/min) / (kcal/min)		Snack frequency	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Canola	3.9±0.5 ^{A}	7.9±0.5 ^{B}	2.1±1.2 / 11.3±1.3	1.8±0.2 / 9.3±1.3	225.9±17.2 ^{aA}	86.4±17.2 ^{B}	300.6±30.6 ^{A} / 56.7±5.8 ^{A}	101.4±30.6 ^{B} / 19.1±5.8 ^{B}	11.7±1.2	9.9±1.2	0.19±0.02 / 1.03±0.12	0.18±0.02 / 0.93±0.12	1.2±0.5 ^{A}	2.8±0.5 ^{B}
Butter	5.6±0.7	6.8±0.7	2.7±0.3 ^{A} / 14.0±1.7 ^{A}	1.0±0.3 ^{B} / 5.4±1.7 ^{B}	118.4±23.4 ^{b}	108.6±23.5	132.3±41.6 / 25.0±7.9	116.9±41.8 / 22.1±7.9	11.7±1.7	10.6±1.7	0.30±0.03 ^{A} / 1.58±0.16 ^{A}	0.12±0.03 ^{B} / 0.64±0.16 ^{B}	3.1±0.6	3.2±0.6

For significant diet by phase interactions: different lower case letters indicate values significantly different between the dietary groups within each phase, and different capital letters indicate values significantly different between the phases within each dietary group. For significance refer to text.

5.7. Figure captions

Figure 5.1. Daily body weight (mean ± SEM) of rats fed canola or butter -rich diets.

Figure 5.2. Light and dark phases' food intake (mean ± SEM) of rats at their first exposure (Period 1) and following 3 weeks (Period 2) of feeding high-fat canola or butter-based diets. For significant diet by phase by period interactions: different symbols indicate values significantly different between dietary groups within each phase and period, different lower case letters indicate values significantly different across phase within each period and dietary group, different capital letters indicate values significantly different across period in each phase and dietary group. For significance refer to text.

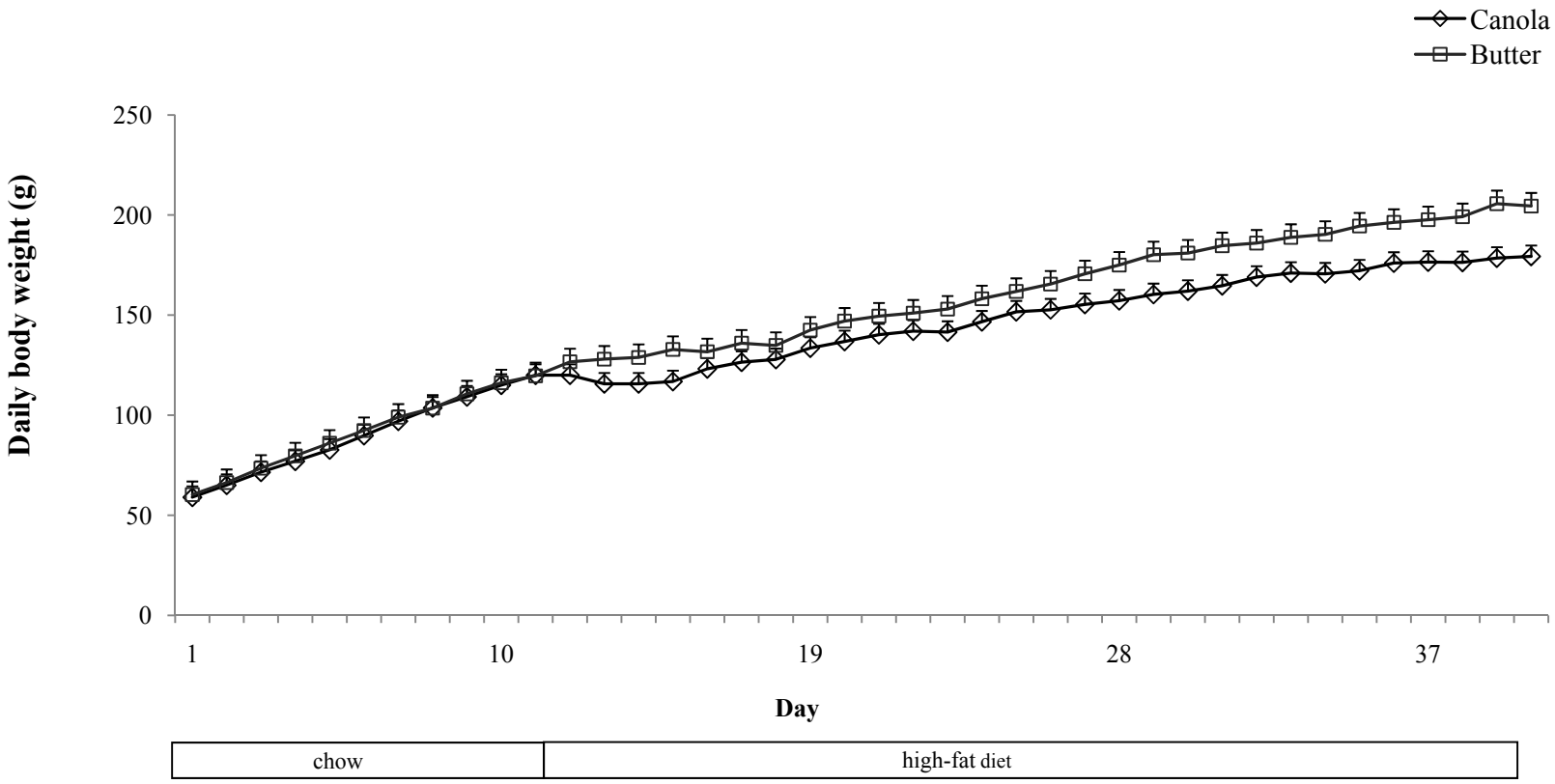
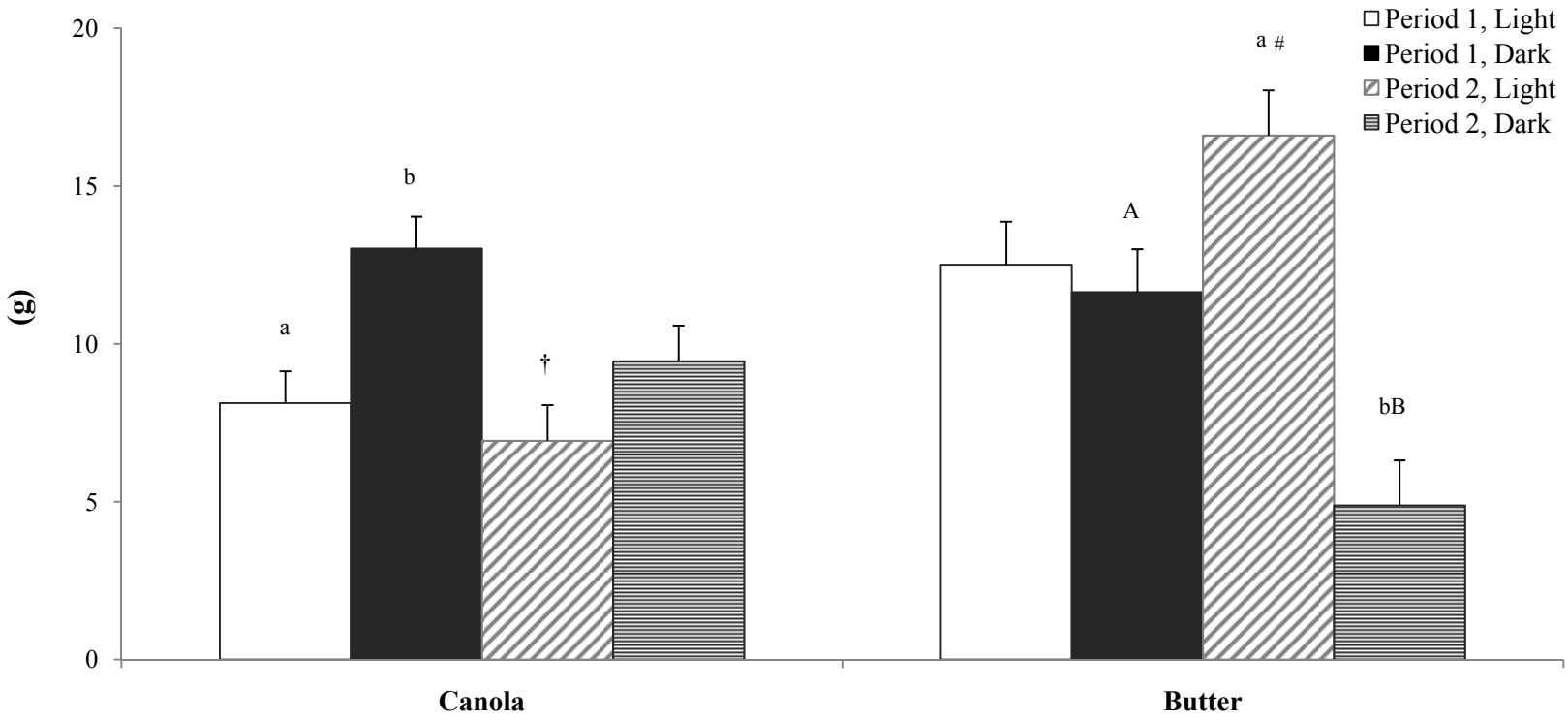

Canola
Butter
Daily body weight (g)
250
200
150
100
50
0
1
10
19
28
37
Day
chow
high-fat diet

Figure 5.1.


20
15
10
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0
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A
bB
Period 1, Light
Period 1, Dark
Period 2, Light
Period 2, Dark
Canola
Butter

Figure 5.2.

CHAPTER 6, OVERALL SUMMARY AND CONCLUSIONS

Obesity is prevalent around the world and the recent WHO term "globesity" (global obesity) shows the extent of the problem (WHO 2006). Because of the high prevalence of obesity and the complications related to it such as type 2 diabetes, hypertension and coronary heart diseases, obesity has become a worldwide concern. Since dietary pattern has shifted toward eating high-fat foods - known as "western diet" - the increase in fat intake is known to be responsible - at least partly - for the critical problem of global obesity (Bray and Popkin 1998; Saris et al. 2000).

There is an agreement in the literature that obesity can be induced by using high-fat diets in animal models (Buettner et al. 2007). It is also suggested that fatty acids are not the same in their obesogenic effect, with saturated fatty acids (SFA) having the greatest ability to induce weight and fat gain leading to obesity (Bell et al. 1997; Wang et al. 2002; Silva et al. 2006). On the other hand, some studies have shown that percent energy derived from fat is the most important factor in high-fat diet's obesogenic effect rather than their fatty acid profile (Jones et al. 1995; George et al. 2000).

The review of the available reports of animal models of dietary obesity (Manuscript 1) showed that research on the obesogenic effects of fatty acids with different degrees of saturation found no constant pattern of outcome under

different conditions. Besides, the role dietary fatty acids might play in reversing dietary obesity has not been investigated.

Therefore, in this thesis, we preformed studies using high and low-fat diets (at 67 and 27% of total energy, respectively) with different SFA content: low (canola), moderate (lard) and high (butter), in a rat model of dietary obesity.

First, we aimed to investigate the effect of these diets on food and energy intakes, body weight and obesity development as well as on weight loss in adult female Sprague-Dawley rats (Manuscript 2). Our results showed comparable body weights when feeding high-fat diets with low-SFA canola and moderate-SFA lard in a 26-day period. As well, obesity did not develop with 50 days of canola feeding, whereas the same feeding duration induced obesity in rats fed a high-SFA butter-based diet. This was attributed to:

1) Higher energy intake with the high-SFA diet:

- Our results showed a failure to adjust food intake when switched from chow to a high-SFA butter-based diet. A complete adjustment for diet with higher energy density with low-SFA canola feeding and an incomplete adjustment with moderate-SFA lard feeding - shown by an increase in the energy intake despite the decrease in food intake - were found. Failure to adjust intake was also found when animals were switched from SFA-rich high-fat to low-fat feeding. This shows that with the increase in the SFA content of the diet, rats fail to adjust their

food intake based on the energy density of the diet. In agreement with our findings, diets high in SFA are shown to have less control over appetite compared to those low in SFA (Beardshall et al. 1989; Lawton et al. 2000).

- Moreover among the orosensory characteristics of the diet, taste and texture are important attributes influencing intake. The higher intake with butter feeding can also be a result of texture differences between the diets and/or a distinctive flavor elicited by the butter-based diet.

2) High energy efficiency with high-SFA butter as a result of decreased RMR and DIT (Clarke 2000; Piers et al. 2002; Soares et al. 2004; Casas-Agustench et al. 2009) which provides greater overall energy content from the diet.

The findings of Manuscript 2 indicating obesity development with an SFA-rich diet, but not with diets with moderate and low SFA content, confirm that the quantity of SFA in a high-fat diet is important for obesity development. Therefore, the increase in the prevalence of obesity despite the decrease in the total fat intake, which has been reported by some epidemiological studies, may be explained (partially) by this result. In the future studies, investigating the quality of various fatty acids consumed by individuals rather than the total fat intake might provide a better understanding of this issue. Prevention strategies should emphasize decreasing the consumption of SFA when recommending lower intakes of fat.

The results of the present work investigating the effect of fatty acids on reversing dietary obesity showed that ad libitum access to the low- or high-SFA low-fat diets attenuated weight gain. A reversal of dietary obesity induced by long-term high-SFA feeding was only possible with food restriction. This underscores the use of restricted regimens in weight loss programs rather than ad libitum feeding on a low-fat diet. We also found that even after weight loss, high-SFA fed animals defended their obese state by retaining abdominal fat.

The literature showed that feeding high-fat diets result in alterations of eating pattern, and ingestion of large meals at a low frequency. However, it is not clear whether the high-fat diet-induced alteration in eating pattern was dependent upon the dietary fatty acid composition. Therefore, the third manuscript examined the effect of early or longer-term exposure to high-fat diets with the same fatty acids composition as in Manuscript 2 on circadian rhythmicity of intake and eating pattern in adult female Sprague-Dawley rats.

Early exposure or a prolonged access to low or high-SFA diets did not alter the circadian rhythm of intake. However, with long term high-fat feeding, the pattern of eating in the light phase was disrupted relative to low-fat chow feeding. This was shown by eating less frequent meals, with longer IMI and enhanced satiety ratio during daytime, regardless of obesity status. The SFA-rich diet led to ingestion of larger meals overall, less frequent nocturnal meals and higher diurnal eating rate, along with obesity. These findings show, for the first time, that meal

pattern is altered by high-fat feeding, with some characteristics attributable to the fatty acid profile.

Most studies in the literature have assessed the eating pattern of rats following food restriction to examine the restriction-induced hyperphagia on ad libitum feeding. Therefore we aimed to investigate eating pattern of adult rats in a long-term low-fat food restriction paradigm. The restricted amount of food was offered in the morning each day; however rats ate most of their food in that phase and diurnal rather than nocturnal feeding occurred. Compared to ad libitum high-fat feeding, an increased satiety ratio, longer meal duration and IMI and decreased meal frequency were found in restricted animals.

The results of Manuscripts 2 and 3 showed altered pattern of eating and obesity development with long-term high-fat feeding in adult rats. These findings and the lack of information on meal pattern alteration induced by high-fat feeding starting at a very young age led us to design our next study in weaning animals. Therefore, in that study (Manuscript 4), we used weaning rats to study their response to low and high-SFA high-fat diets, and assessed their pattern of eating episode at 2 different time points: first when rats had just started the high-fat diet and then, from week 3 to 4.

The results revealed that 3 weeks of high-fat feeding - based on either low-SFA or high-SFA - led to a shift of intake to the light phase (known as the resting phase). With early exposure to low-SFA high-fat diets, rats showed a greater nocturnal than diurnal food intake which is shown to be a normal circadian

rhythm of intake in rodents. However, 3 weeks of high-fat feeding based on low-SFA canola led to comparable diurnal and nocturnal intakes. The response of the rats to high-fat feeding was greater with high-SFA feeding: disturbed circadian rhythm of intake was shown by comparable diurnal and nocturnal intakes at early exposure to these diets and greater diurnal than nocturnal intake after 3 weeks. In addition, with the high-SFA diet, rats ate larger meal at a higher rate in the light phase and gained more weight. Another important result of Manuscript 4 was that despite the comparable food intake in rats fed high and low-SFA diets, greater body weight gain was found in high-SFA fed young rats. This strengthens the idea of altered eating pattern being involved in high body weight gain. A circadian rhythm in the expression of genes - known as clock genes - that are involved in many of the body's physiological pathways including fat and glucose metabolism has been found. Therefore, any disturbance in the circadian rhythm of intake and pattern of eating episodes can affect energy homeostasis. Thus, the greater body weight gain and altered eating pattern found with high-SFA feeding in our studies strengthens the significance of these diets in the development of obesity.

In conclusion, in adult female rats, a high-fat diet based on SFA-rich animal fat resulted in overeating and obesity, relative to a low-SFA diet. High-fat feeding induced alterations in diurnal eating pattern, with larger meals specific to the SFA-rich diet. In weaning female rats, an animal-based SFA-rich diet led to a greater body weight gain accompanied with an altered circadian pattern of eating rather than overeating. These results underscore the role dietary fatty acid profile may play in developing obesity early as well as later in life.

Like any other research, there were some limitations in these studies, which should be taken into account for future research:

- We attempted to control for the texture differences in our studies by adding pectin to the diets; however, still the canola and butter-based diets had different textures. Controlling for the texture differences (using manufactured animal foods) would help to clarify whether increased energy intake with high-SFA diets is related to the physiological aspects of SFA (such as their effect on appetite) or to the orosensory characteristics of the diet.

- Measuring abdominal fat before the start of high-fat feeding and following high-fat and low-fat diet feeding - in addition to measurement after restricted feeding - could have let us compare the storage of fat in rats that developed obesity and those that did not before and after weight reduction. This could be made possible by using DEXA for body fat assessments.

- Restricted low-fat feeding with food offered at early light led to diurnal rather than nocturnal feeding, with an altered eating pattern. Whether the present results could be repeated with an early dark access to food remains to be determined.

- In our eating pattern studies, concern might be raised over sample size. We used the maximum capacity of our Diet Scan system for the number of animals. Obviously, with an increase in the number of animals in each dietary group, the within- and inter-group variation would decrease and this is possible in Diet Scan

systems with greater capacity. Replicating these experiments with a larger number of animals would also be important.

- Butter is an animal source of SFA which contains palmitic acid as the predominant SFA. Some studies have shown that longer chain fatty acids (such as stearic acid, C18) are less easily absorbed than those with shorter chain length (palmitic acid, C16) (Sugano and Imaizumi 1995), and this might affect their obesogenic effect. Therefore, future studies should take SFA of different chain lengths into account.

Despite these limitations, this thesis presents enough evidence to understand the greater obesogenic effect of animal-based SFA through physiological and behavioral mechanisms. With obesity as a major global concern, the present findings provide important information that can help in prevention strategies for decreasing the prevalence of obesity as well as for reversing it, with focusing on dietary fatty acids rather than only total fat intake.

By using the animal model of dietary obesity that shares many features with human obesity this work has provided much important data on how dietary fatty acids affect the development and reversal of obesity. This thesis also opens new windows for future research. In the present studies, we applied the most commonly-used SFA-rich animal fats in humans - butter and lard - and the frequently-used oil - canola - which is low in SFA. Further studies could investigate the effect of fatty acids with moderate SFA content for longer durations (longer than 26 days that we have tested) in terms of altering eating

pattern and inducing obesity. High-SFA diets of plant origin could also be compared with those of animal origin for their effects on obesity.

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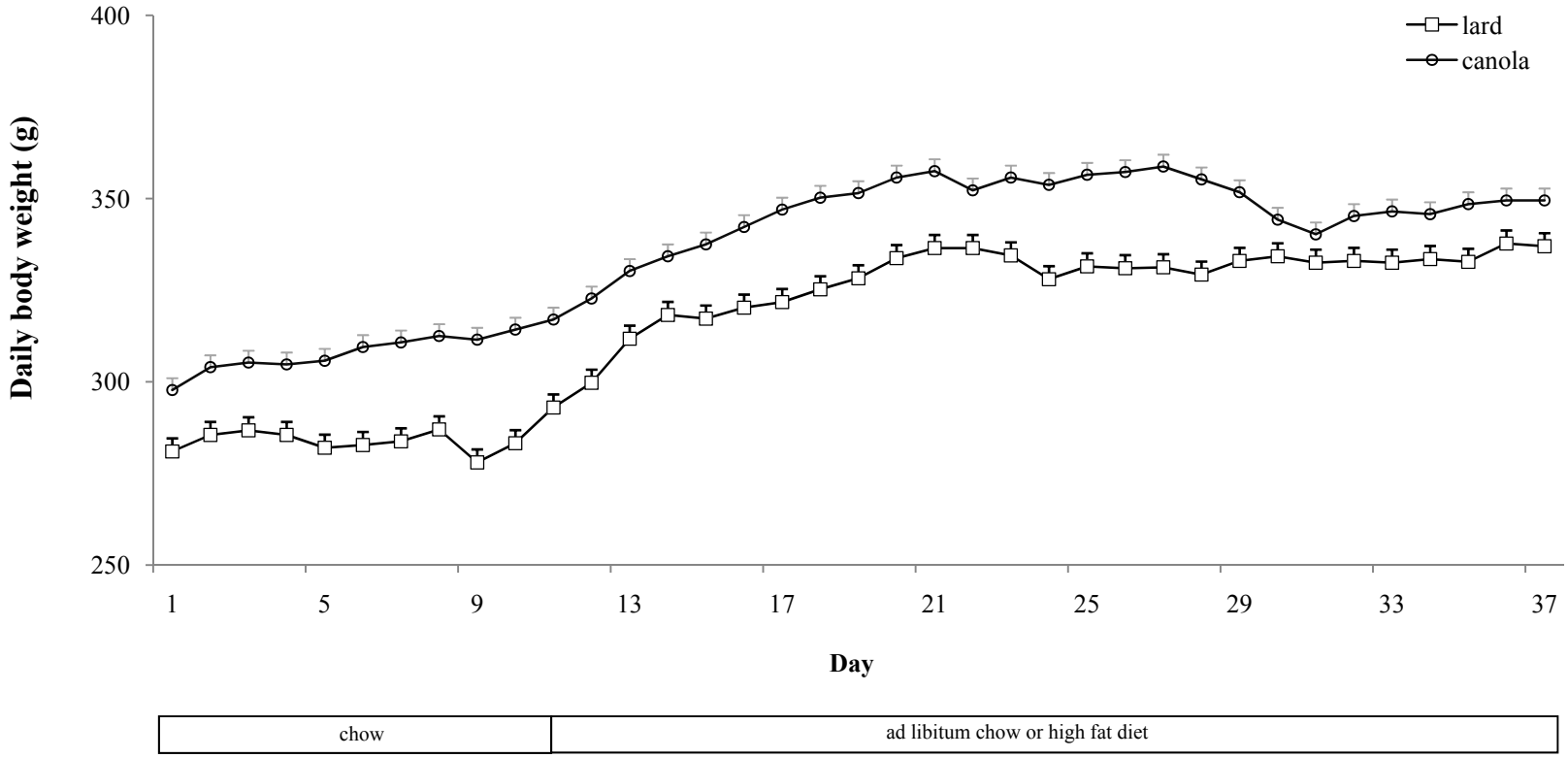
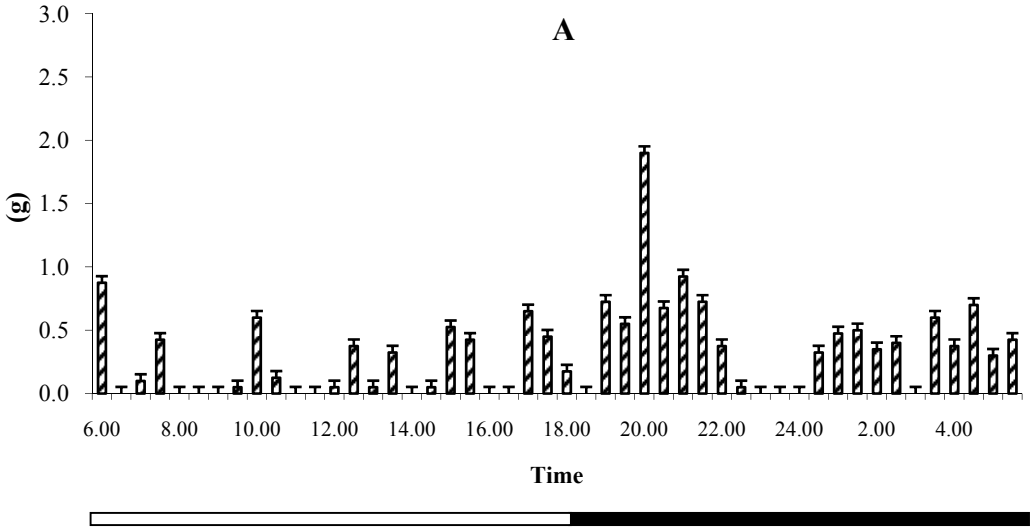
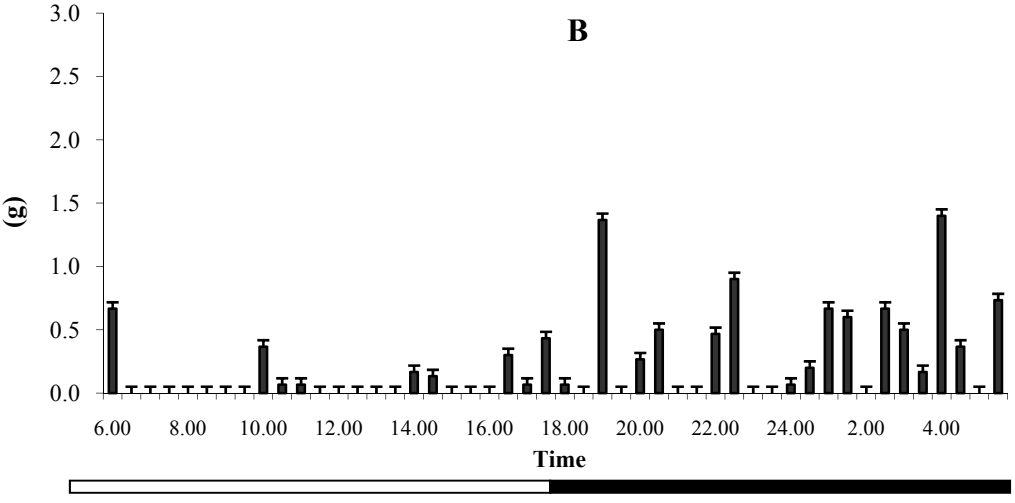
APPENDICES

Appendix 1. Daily body weight (mean ± SEM) of rats fed chow (10 days) and then switched to canola or lard -rich diets (26 days) (Manuscript 2).

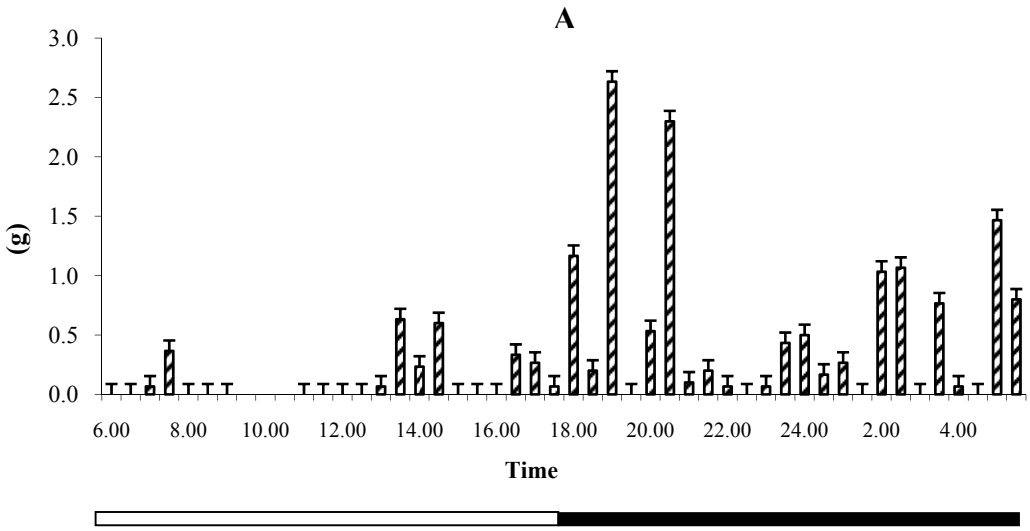


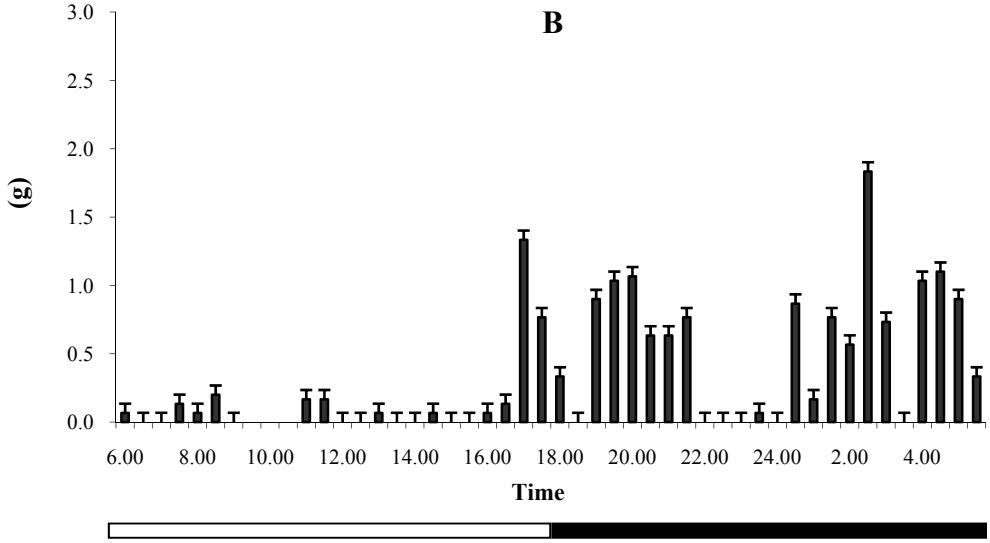
Appendix 2. Circadian rhythmicity of food intake on day 8 of high-fat feeding in rats fed canola (A) or lard (B) -based diets (Manuscript 3).



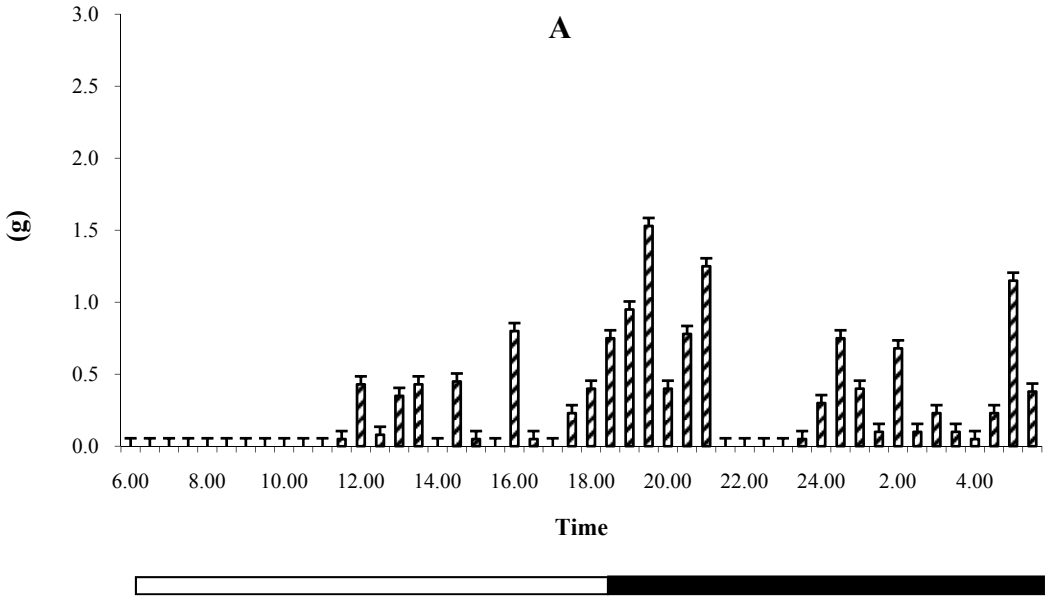


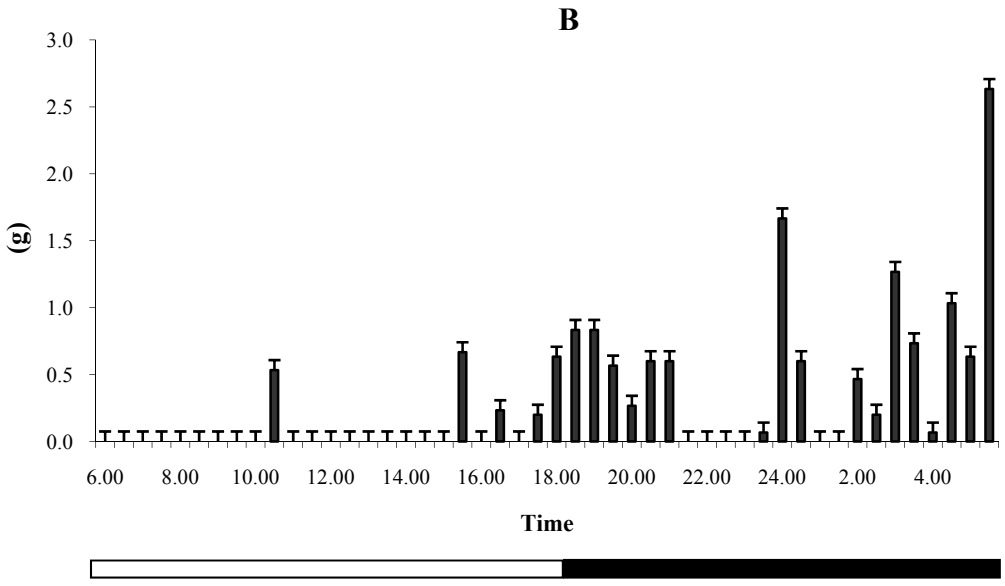
Appendix 3. Circadian rhythmicity of food intake on day 9 of high-fat feeding in rats fed canola (A) or lard (B) -based diets (Manuscript 3).



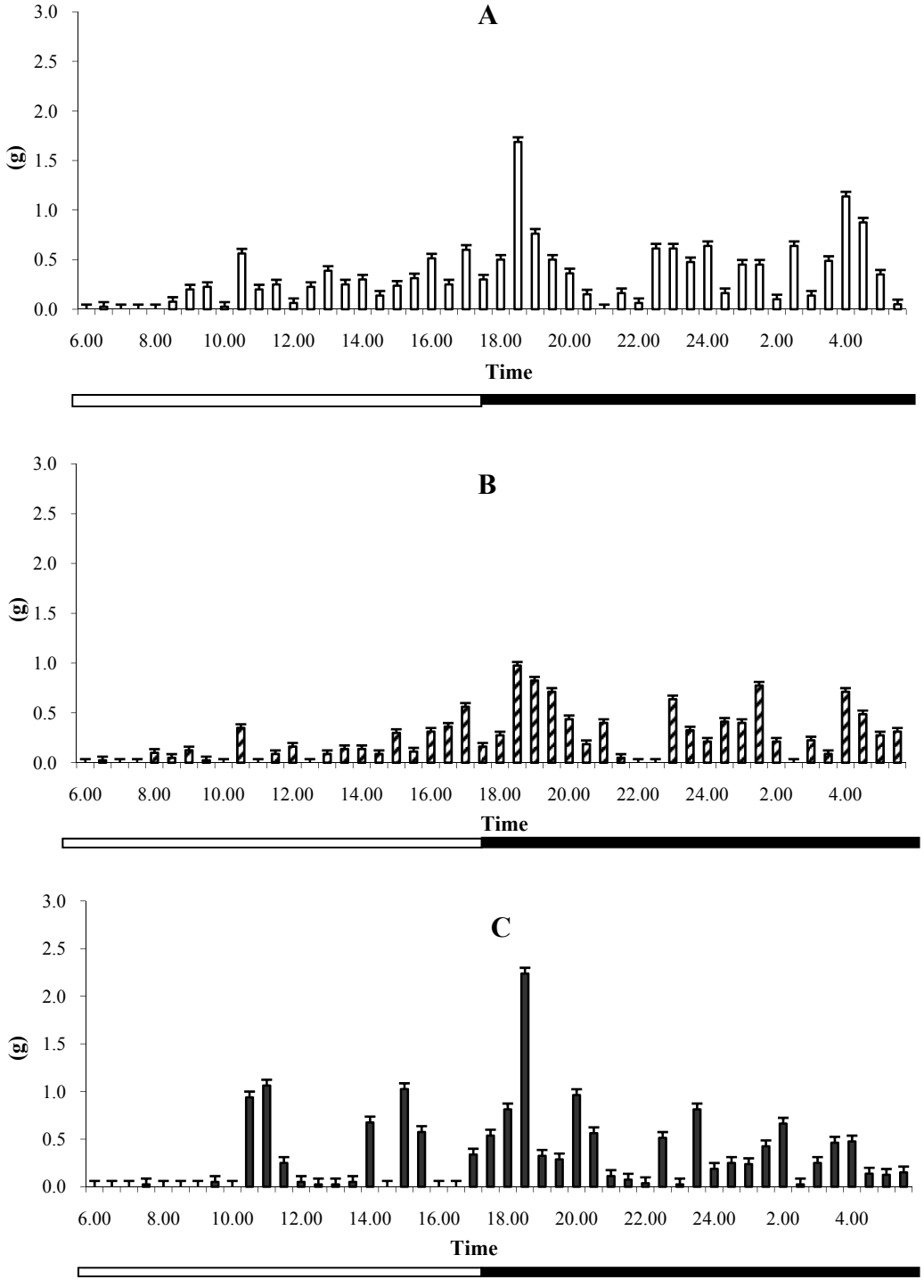


Appendix 4. Circadian rhythmicity of food intake on day 10 of high fat feeding in rats fed canola (A) or lard (B) based diets (Manuscript 3).

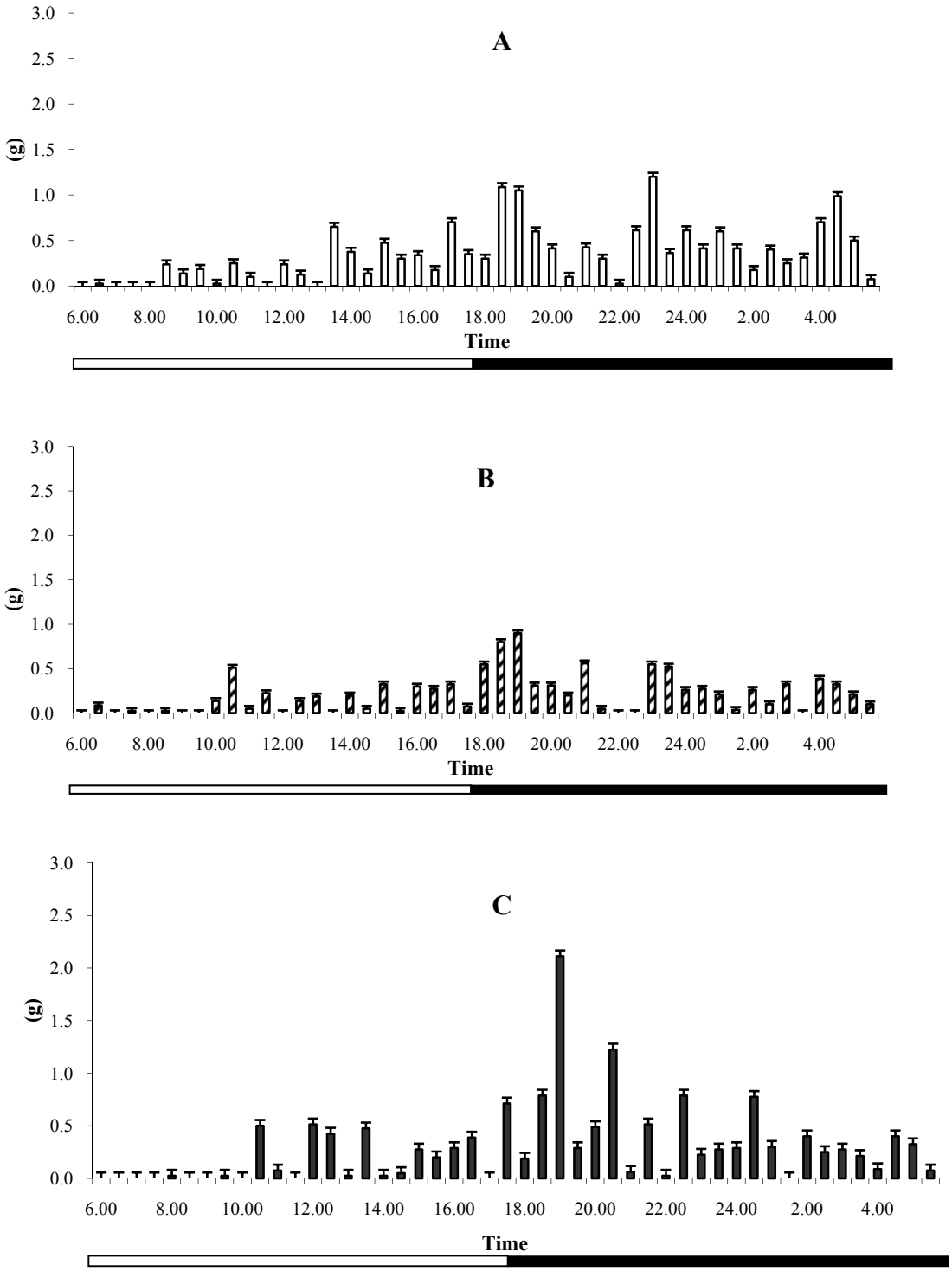




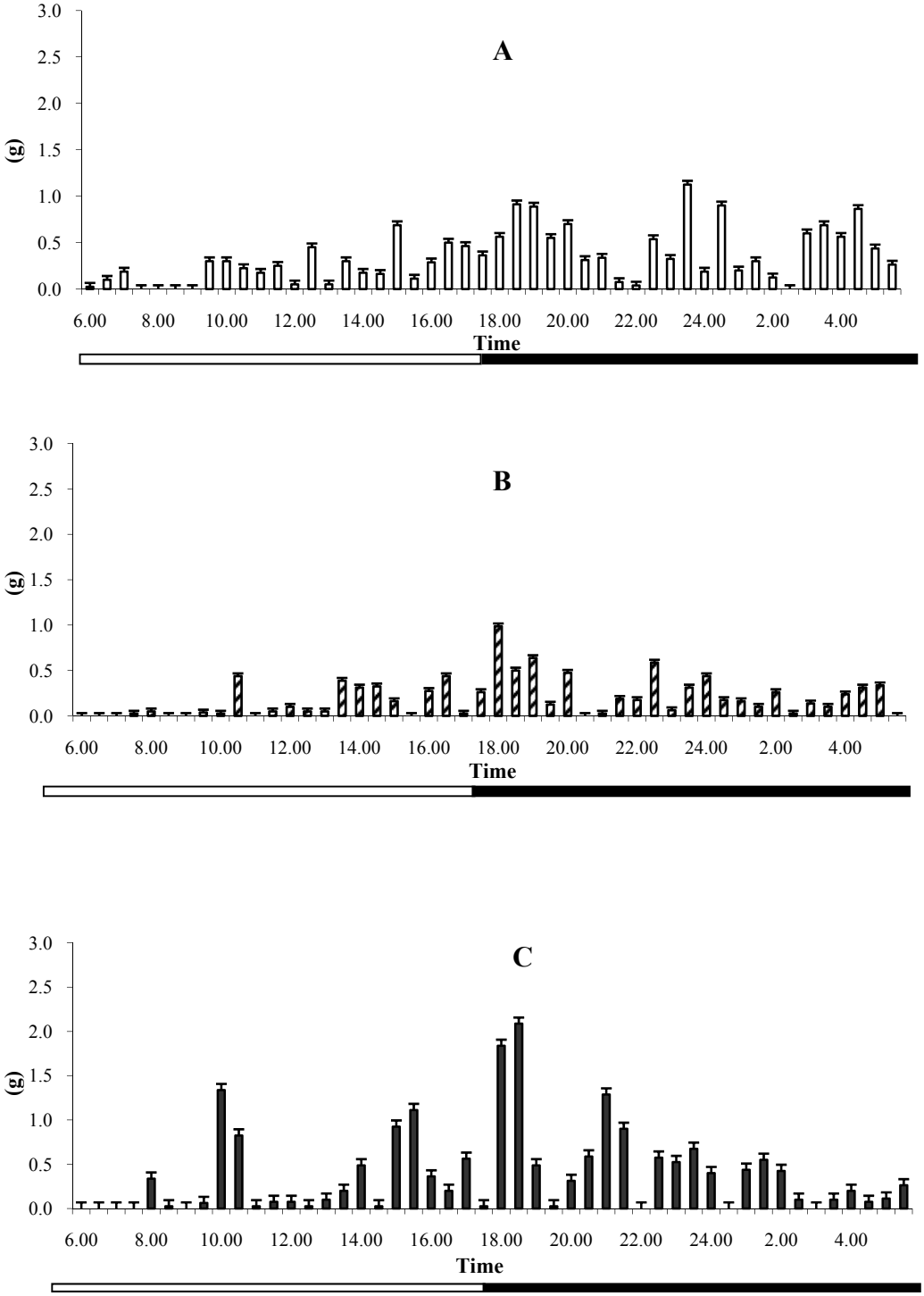
Appendix 5. Circadian rhythmicity of food intake (mean ± SEM) on day 48 of high-fat feeding in rats fed chow (A), canola (B) or butter (C) - based diets (Manuscript 3).



Appendix 6. Circadian rhythmicity of food intake (mean ± SEM) on day 49 of high-fat feeding in rats fed chow (A), canola (B) or butter (C) - based diets (Manuscript 3).



Appendix 7. Circadian rhythmicity of food intake (mean ± SEM) on day 50 of high-fat feeding in rats fed chow (A), canola (B) or butter (C) - based diets (Manuscript 3).



Appendix 8. Example of raw data retrieved from Diet Scan system (Day 10, Period 2, Manuscript 4)

File created on 11/28/06 at 01:27:20
day 10

DIETSCAN DATA FILE
SPECIFIC GRAVITY FOR LIQUID 1.000

SAMPLE	CHANNEL	SECONDS	MEASURE	UNITS
1	9	60	0.0	g
1	17	60	0.0	g
1	24	60	0.0	g
2	24	60	0.2	g
4	24	60	1.1	g
5	24	60	0.3	g
34	17	60	0.2	g
37	17	60	0.4	g
38	17	60	0.4	g
41	24	60	0.2	g
42	24	60	0.4	g
44	17	60	0.3	g
45	17	60	0.5	g
48	17	60	0.6	g
50	17	60	0.4	g
51	17	60	0.2	g
52	17	60	0.4	g
68	24	60	0.2	g
138	9	60	0.6	g
159	24	60	0.2	g
186	24	60	0.2	g
283	24	60	0.2	g
304	24	60	0.2	g
321	24	60	0.2	g
349	17	60	0.5	g
351	24	60	0.2	g
352	24	60	0.2	g
353	24	60	0.2	g
373	9	60	0.2	g
374	9	60	0.4	g

Appendix 9. Example of Diet Scan data transferred to excel sheet using a template (Day 10, Period 2, Manuscript 4)

	rat 1	rat 2	rat 3	rat 4	rat 5	rat 6	rat 7	rat 8	rat 9	rat 10
6:00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:07	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:14	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:23	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:34	1.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6:46	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00