INFLUENCE OF DIETARY SATURATED FAT ON MACRONUTRIENT SELECTION

by

Randall Jeffrey Kaplan

A thesis submitted in conformity with the requirements for the degree of Master of Science
Graduate Department of Nutritional Sciences, Faculty of Medicine
University of Toronto

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ABSTRACT

Dietary saturated fatty acids (SFAs) mediate behaviour, in the absence of nutrient deficiency, beyond brain development, and at fat levels consistent with North American consumption. The present experiments examined the influence of SFA chain length on macronutrient selection and the roles of glucose and insulin in mediating this feeding behaviour. Rats fed medium-chain triglyceride oil selected more protein and less carbohydrate than rats fed three other fats (hydrogenated coconut oil, fully hydrogenated soybean oil (HSB), and soybean oil). HSB-fed rats selected differently from previously reported long-chain SFA-fed animals, however, HSB was insufficiently absorbed. Although glucose and insulin concentration did not differ among diet groups, measures of insulin sensitivity correlated with selection. These results suggest that SFA chain length may influence macronutrient selection, but the specific relationship between chain length and selection remains undefined. A decrease in insulin sensitivity leads to an increase in protein and a decrease in carbohydrate intake.
ACKNOWLEDGEMENTS

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<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>EFA</td>
<td>essential fatty acid</td>
</tr>
<tr>
<td>HCO</td>
<td>hydrogenated coconut oil (enriched in iSFAs; 12:0 and 14:0)</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>5-hydroxyindole acetic acid</td>
</tr>
<tr>
<td>HSB</td>
<td>fully hydrogenated soybean oil (enriched in LSFAs; ≥16:0)</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine (serotonin)</td>
</tr>
<tr>
<td>ISFA</td>
<td>intermediate-chain saturated fatty acid (12:0 and 14:0)</td>
</tr>
<tr>
<td>LCT</td>
<td>long-chain triglyceride</td>
</tr>
<tr>
<td>LSFA</td>
<td>long-chain saturated fatty acid (≥16:0)</td>
</tr>
<tr>
<td>MAO</td>
<td>monoamine oxidase</td>
</tr>
<tr>
<td>MCT</td>
<td>medium-chain triglyceride (enriched in MSFAs; 8:0 and 10:0)</td>
</tr>
<tr>
<td>MSFA</td>
<td>medium-chain saturated fatty acid (8:0 and 10:0)</td>
</tr>
<tr>
<td>MUFA</td>
<td>monounsaturated fatty acid</td>
</tr>
<tr>
<td>PUFA</td>
<td>polyunsaturated fatty acid</td>
</tr>
<tr>
<td>SBO</td>
<td>soybean oil (enriched in PUFAs)</td>
</tr>
<tr>
<td>SFA</td>
<td>saturated fatty acid</td>
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CHAPTER 1

INTRODUCTION
1. INTRODUCTION

The influence of nutrient deficient diets (including protein, fat, vitamin, and mineral deficiencies) on brain function has been well documented through studies examining the effect of such diets on behavioural outcomes (for review, see Greenwood & Craig, 1987). The most convincing evidence suggests that dietary inadequacies during periods of rapid brain growth lead to physiological changes in the brain that are manifested by impaired behavioural performance. With respect to the influence of dietary fat on brain function, essential fatty acid (linoleic and α-linolenic acid) deficiency has been shown to lead to both physiological and functional changes in developing animals. In contrast, although the developed brain has been shown to be sensitive to dietary fat manipulations, the functional significance of these specific changes is not clear.

The broad purpose of the present research was to examine the impact of variations in dietary fatty acid composition on brain function in mature animals (i.e., developed brain), in the absence of nutrient deficiencies, and at levels similar to those consumed by North Americans. The current literature indicates that under these conditions, the brain, and subsequent behaviour can be altered by dietary fatty acid composition. Specifically, the concentration of saturated fatty acids (SFAs) in the diet has been shown to be the important component of dietary fat in mediating both macronutrient selection (a type of feeding behaviour) and cognitive behaviour (learning and memory). These data suggest that the impact of dietary SFA content on the brain is general and widespread, since several widely variable behaviours can be altered.

The mechanism involved in mediating dietary SFA-induced behavioural changes remains undefined. Several studies have undertaken the task of attempting to identify the underlying mechanism, but the obvious explanations, such as the influence of dietary fat on neural membrane fatty acid composition, and neurotransmitter
metabolism have failed to result in a clear explanation. Although it is postulated that the central nervous system must be involved in the mechanism such that behaviour could ultimately be altered, it is possible that the central nervous system is influenced by the periphery. That is, dietary fat may act on the central nervous system via an indirect peripheral pathway. Alternatively, and possibly, more likely, a combination of peripheral and central factors may be involved in mediating these behaviours.

In order to further understand the SFA effect on behaviour, the objective of this thesis was to address the role of SFA chain length in influencing macronutrient selection. Only the influence of dietary long-chain SFAs (≥16:0) on macronutrient selection has previously been examined. Furthermore, the role of dietary fat-induced changes in insulin sensitivity and glucose tolerance in the mechanism that mediates this feeding behaviour was investigated.
CHAPTER 2

LITERATURE REVIEW
2. LITERATURE REVIEW

2.1 Introduction

The purpose of this investigation was to determine the influence of dietary saturated fatty acid (SFA) chain length on macronutrient selection and to examine the underlying mechanism that mediates this feeding behaviour. Macronutrient selection represents one of several behaviours that have been shown to be mediated by both level and type of dietary fat consumed.

The literature review is organized into a number of parts. The first section deals with the recent data suggesting a role for dietary fat type in mediating brain function in the mature animal, in the absence of essential fatty acid (EFA) deficiencies. A discussion of the behavioural literature is presented next, followed by an argument for the importance of dietary SFAs in mediating several behaviours. Possible mechanisms that may explain dietary fat-induced behavioural changes are reviewed. Finally, a brief introduction to the present experimental work will be discussed.

2.2 Dietary fat and behaviour

Compelling evidence demonstrating the brain's sensitivity to dietary fat intake comes from the behavioural literature where alterations in a variety of animal behaviours have been reported. In general, the experimental approach has been to feed rats diets containing different fat sources for extended periods of time (weeks to months) and then test the rats using standard behavioural protocols common in the psychological literature. Two important issues must be kept in mind when evaluating this literature. First is the fact that exposure to the different experimental diets is chronic in nature. That is, the underlying assumption is that adaptation to the different fat sources is required prior to being able to observe the behavioural impact. This is in marked contrast to studies of other nutrients, such as amino acids, where the impact of
the amino acid supplementation is observed with acute, single dose administration (see for example Ng & Anderson, 1992; Anderson et al., 1994). Thus, in the case of the amino acids, the postulated mechanism relates to the fact that the enzymes required for neurotransmitter synthesis are not fully saturated with substrate under normal physiological conditions such that increasing brain levels of the amino acid will result in a concurrent enhancement of neurotransmitter levels and that the impact of this can be observed immediately in the behavioural outcomes of the animal (Greenwood & Craig, 1987). In contrast, the underlying hypothesis with regards to dietary fat intake is that the brain slowly adapts to different dietary fatty acids, with resulting changes in neuronal function which may be explained at the metabolic level, but that biochemical and behavioural outcomes will not be measurable within the context of an acute administration of the fatty acids.

Choice of dietary fat source is the second important consideration. Many studies are limited by the fact that comparisons were made between only two diets. Selection of appropriate dietary fats to examine a specific fatty acid or class of fatty acids is limited by the fact that as one component in the fat source changes, so do others. For example, Table 2.1 provides the fatty acid composition of commonly used experimental dietary fat sources. As can be seen, fats can be selected on the basis of being high or low in a specific characteristic (e.g., polyunsaturated fatty acid (PUFA) level) but the levels of the other fatty acids also vary. Results from these studies provide evidence that the brain is responsive to the composition of dietary fatty acids consumed. Nevertheless, it is impossible to attribute change to a specific characteristic or attribute of the fat source with true confidence. Further studies using fat blends or modified fat sources are required to determine the important attribute of the fat source mediating its effect.
Table 2.1 Fatty acid composition of commonly used experimental fats and oils (% composition)¹,²

<table>
<thead>
<tr>
<th>Fat</th>
<th>SFA</th>
<th>MUFA</th>
<th>PUFA</th>
<th>18:2n6</th>
<th>18:3n3</th>
<th>18:2n6/18:3n3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef Tallow</td>
<td>50</td>
<td>42</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>3.00</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>87</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>13</td>
<td>24</td>
<td>59</td>
<td>58</td>
<td>1</td>
<td>58.00</td>
</tr>
<tr>
<td>Lard</td>
<td>39</td>
<td>45</td>
<td>11</td>
<td>10</td>
<td>1</td>
<td>10.00</td>
</tr>
<tr>
<td>Flaxseed oil</td>
<td>9</td>
<td>20</td>
<td>66</td>
<td>13</td>
<td>53</td>
<td>0.25</td>
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<tr>
<td>Palm kernel oil</td>
<td>81</td>
<td>11</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Perilla oil³</td>
<td>8</td>
<td>18</td>
<td>73</td>
<td>15</td>
<td>58</td>
<td>0.26</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>9</td>
<td>12</td>
<td>75</td>
<td>74</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>14</td>
<td>23</td>
<td>58</td>
<td>51</td>
<td>7</td>
<td>7.29</td>
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<tr>
<td>Sunflower oil</td>
<td>10</td>
<td>20</td>
<td>66</td>
<td>66</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

¹ SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; 18:2n6 = linoleic acid; 18:2n-3 = α-linolenic acid

² All data adapted from United States Department of Agriculture (1979), except ³

³ Adapted from Peck (1994)

2.2.1 Essential fatty acid deficiency and behaviour

Linoleic (18:2n-6) and α-linolenic acid (18:3n-3) are commonly termed essential fatty acids (EFAs) since animals need adequate amounts of them in order to function normally, and are unable to synthesize them de novo in large quantities. Recent research suggests that animals can synthesize a small proportion of them from 16 carbon polyunsaturated fatty acids (Cunnane et al, 1995). Nevertheless, EFAs must be obtained from the diet to meet nutrient requirements. Deficiencies in EFAs have been shown to lead to various pathological symptoms including dry and scaly skin, kidney disorders, reproductive abnormalities (Burr & Burr, 1929; Burr & Burr 1930; Guesnet, 1986) and neurological and visual disturbance (Bjerve, 1991).

The majority of the studies that have investigated the influence of dietary EFA deficiency on behaviour have shown a general impairment on tests of learning and memory when EFAs were deficient in the diet (Lamptey & Walker, 1976; Lamptey & Walker, 1978; Morgan et al., 1981; Ruthrich et al., 1984; Yamamoto et al., 1987; Yamamoto et al., 1988; Bourre et al., 1989; Enslen et al., 1991; Reisbick et al., 1994;
Wainwright et al., 1994). Moreover, the evidence appears to be most compelling when the experimental manipulations occur during periods of rapid brain development. Indeed, most of this research has been conducted when feeding is commenced prior to conception, and continued throughout gestation, lactation, and the early post-weaning period. The dietary interventions are often continued throughout a number of generations (for review, see Wainwright, 1992).

Although the EFA deficiency studies have been instrumental in defining adequate EFA needs during the early stages of rapid cellular growth and membrane biosynthesis, it is difficult to extrapolate these results to the human as EFA deficiency only occurs in very unusual circumstances. Consequently, our interests lie in studying the effects of differences in dietary fat composition beyond periods of rapid development, without EFA deficiency, and at levels similar to those of the North American population.

2.2.2 Influence of dietary fat on behaviour in the absence of essential fatty acid deficiency

2.2.2.1 Pain sensitivity and thermoregulation

The first evidence demonstrating an influence of dietary fat on behaviour, in the absence of EFA deficiency, in post-developmental animals, comes from a study showing that body temperature regulation and pain sensitivity are influenced by different dietary fat sources (Yehuda et al., 1986). In these studies, dietary fat constituted 40% of energy to correspond to present North American consumption patterns. Rats fed a soybean oil (SBO) diet (20% (wt/wt); enriched in PUFAs) for three weeks were better able to maintain body temperature in a cold environment after a d-amphetamine challenge, and were less sensitive to pain than rats fed a lard diet (20% (wt/wt); enriched in SFAs). Dietary fat type appeared to be the only variable responsible for the behavioural alterations since the two diet groups did not differ with respect to body weight gain, caloric intake, basal colonic temperature or locomotor
activity. A similar study by Yehuda and Carasso (1987) supported the reproducibility of these results.

2.2.2.2 Macronutrient selection

Given that both thermoregulation and pain sensitivity are mediated by the monoaminergic system, another behaviour mediated by the monoamines, feeding behaviour, has been assessed to determine the generalizability of this observation. The majority of researchers who have investigated feeding behaviour in animals have been concerned with the regulation of total food intake (Anderson, 1994). However, numerous studies have also investigated the ability of animals to regulate their intake of specific macronutrients. In an early study, Musten et al. (1974) allowed animals to self select between two simultaneously presented diets that only differed in protein and carbohydrate content. Diets were isocaloric and contained identical and adequate amounts of vitamins and minerals. This method allowed these researchers to measure both total energy consumed as well as the proportion of energy consumed from each macronutrient. Rats were able to precisely regulate protein intake at a constant proportion of energy, despite a wide range of dietary choices. This has led to the notion that not only total food intake, but also protein and carbohydrate intake are under physiological regulation.

Crane and Greenwood (1987) utilized a variation of this procedure in order to test the influence of different dietary fat sources on macronutrient selection (protein versus carbohydrate). Two groups of post-weanling rats were individually caged and given ad libitum access to a single cup diet for a 10-day experimental period to allow for physiological adaptation to the diets. Both groups were fed diets containing 40% of energy from fat (20% (wt/wt)). The fat source was SBO (enriched in PUFAs) for one group and lard (enriched in SFAs) for the other. Adequate linoleic and α-linolenic acid were added to the lard diet to avoid possible EFA deficiency. Both diets were isocaloric and contained identical amounts of all other nutrients.
Following the experimental period, rats were given the opportunity to select from two diets for the next 18 days. In each cage, the single cup diets were replaced with two food cups, one containing a high protein diet and the other, a high carbohydrate diet. Both the high protein and high carbohydrate diets were isocaloric with each other and with the experimental diet. The type and concentration of dietary fat in the selection diets was identical to that in the experimental diets. That is, the rats that were initially fed the 40% (kcal) SBO diet subsequently selected from a high protein diet containing 40% (kcal) SBO and a high carbohydrate diet also containing 40% (kcal) SBO. Likewise, the rats that were initially fed a 40% (kcal) lard diet subsequently selected from a high protein and a high carbohydrate diet each containing 40% (kcal) lard. This paradigm allowed the researchers to determine the effect of different dietary fat sources on subsequent macronutrient selection as both total food intake and food consumed from each macronutrient could be determined.

These researchers found that rats fed the lard diet selected a higher proportion of energy from protein and less from carbohydrate than rats fed the SBO diet. Furthermore, the two groups of animals did not differ with respect to total food intake, suggesting that the effect of dietary fat on feeding behaviour was specific for macronutrient selection.

The influence of dietary fat on feeding behaviour has been confirmed using fat sources including lard, beef tallow, and hydrogenated corn oil (enriched in SFAs) and SBO and corn oil (enriched in PUFAs) (McGee & Greenwood, 1989; Mullen & Martin, 1990; Mullen & Martin, 1991). Evidence indicates that physiological adaptation to the respective diets is necessary before macronutrient selection is altered and that sensory attributes of the diets, such as taste, are not important in mediating selection (McGee & Greenwood, 1989). Rats that were immediately presented with the selection diets, without prior adaptation to the different fat sources, did not significantly differ in macronutrient selection behaviour until the third week of feeding. Since an immediate dietary preference was not apparent, these results also
suggest that sensory attributes do not play a major role in mediating selection. In summary, rats fed diets enriched in SFAs subsequently select more protein and less carbohydrate than rats fed diets enriched in PUFAs.

Results from a number of studies that have investigated the influence of dietary fat type on macronutrient selection behaviour in rats are shown in Table 2.2. The studies by Crane and Greenwood (1987) and by McGee and Greenwood (McGee & Greenwood, 1989; McGee & Greenwood, 1990a; McGee & Greenwood, 1991) demonstrate that chronic feeding of a specific fat source, at 40% of energy, leads to a very consistent pattern of protein and carbohydrate selection. In all of these studies, rats were fed an initial experimental diet for 10 to 17 days followed by 10 to 18 days of selection feeding. Across seven independent experiments, rats fed diets with similar fatty acid compositions as 40% of energy selected both protein and carbohydrate within a very narrow range. These findings suggest that there exists a powerful mechanism that is specifically affected by differences in dietary fatty acid composition, and that exerts relatively precise control over macronutrient selection.

Table 2.2 Percent of energy selected as protein in response to dietary fat (in rats)

<table>
<thead>
<tr>
<th>Investigators</th>
<th>% energy from fat</th>
<th>experimental/feeding time</th>
<th>SFA source</th>
<th>% protein energy selected</th>
<th>PUFA source</th>
<th>% protein energy selected</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Crane &amp; Greenwood, 1987)</td>
<td>40</td>
<td>10 d / 18 d</td>
<td>lard</td>
<td>25</td>
<td>soybean oil</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>17 d / 10 d</td>
<td>lard</td>
<td>31</td>
<td>soybean oil</td>
<td>18</td>
</tr>
<tr>
<td>(McGee &amp; Greenwood, 1989)</td>
<td>40</td>
<td>14 d / 14 d</td>
<td>beef tallow</td>
<td>33</td>
<td>soybean oil</td>
<td>17</td>
</tr>
<tr>
<td>(McGee &amp; Greenwood, 1990a)</td>
<td>40</td>
<td>14 d / 14 d</td>
<td>beef tallow</td>
<td>26</td>
<td>soybean oil</td>
<td>18</td>
</tr>
<tr>
<td>(McGee &amp; Greenwood, 1991)</td>
<td>40</td>
<td>14 d / 15 d</td>
<td>beef tallow</td>
<td>24</td>
<td>soybean oil</td>
<td>17</td>
</tr>
<tr>
<td>(Mullen &amp; Martin, 1990)</td>
<td>40</td>
<td>14 d / 14 d</td>
<td>beef tallow</td>
<td>31</td>
<td>soybean oil</td>
<td>14</td>
</tr>
<tr>
<td>(Mullen &amp; Martin, 1992b)</td>
<td>79</td>
<td>14 d / 12 hrs</td>
<td>beef tallow</td>
<td>35</td>
<td>corn oil</td>
<td>16</td>
</tr>
<tr>
<td>(Mullen &amp; Martin, 1992b)</td>
<td>79</td>
<td>4 d / 12 hrs</td>
<td>beef tallow</td>
<td>51</td>
<td>corn oil</td>
<td>34</td>
</tr>
<tr>
<td>(Mullen &amp; Martin, 1992b)</td>
<td>79</td>
<td>1 d / 12 hrs</td>
<td>beef tallow</td>
<td>41</td>
<td>corn oil</td>
<td>20</td>
</tr>
<tr>
<td>(Grossman et al., 1994)</td>
<td>100</td>
<td>2 hrs / 12 hrs</td>
<td>beef tallow</td>
<td>42</td>
<td>corn oil</td>
<td>30</td>
</tr>
</tbody>
</table>

1 Results from a number of studies investigating macronutrient selection patterns of rats following the intake of various dietary fat sources. SFA = diet high in saturated fatty acids; PUFA = diet high in polyunsaturated fatty acids.
In contrast to the chronic nature of the initial feeding period in the noted studies, Mullen and Martin (Mullen & Martin, 1990; Mullen & Martin, 1992b; Grossman et al., 1994) have looked at the influence of acute feeding of dietary fat on selection patterns. An effect of acute fat exposure on selection appears to be evident when fat level is extremely high. Tube-feeding rats 2 mL of either beef tallow or corn oil only 2 hours prior to selection leads to differences in macronutrient selection behaviour over the next 12 hours (Grossman et al., 1994). Possibly, a different mechanism is involved when a very high fat diet is consumed for an acute period than when a lower level of fat is consumed for longer periods. In fact, following the consumption of 34% (wt/wt; 79% of calories) fat diets for 1 day, Mullen and Martin (1990) found differences in subsequent protein intake between rats fed beef tallow or corn oil diets. However, no differences between groups were found when 5% (wt/wt) fat diets were fed. Furthermore, in a separate experiment, these researchers found that rats that consumed a 34% (wt/wt) beef tallow diet for 18 hours subsequently selected more protein than rats fed 20% (wt/wt) or 5% (wt/wt) beef tallow diets. The selection profile of animals fed the 20% or 5% fat diets did not differ from each other.

Despite the consistency of the relatively high protein and low carbohydrate pattern of selection observed in rats fed high SFA diets, the noted studies do not indicate whether these animals are preferring protein or avoiding carbohydrate. Mullen and Martin (1992a) attempted to answer this question, and concluded that rats fed a high SFA diet avoid carbohydrate rather than prefer protein. These findings are important in order to help determine the mechanism involved in mediating selection, however, because of the disparity in the paradigms with previous studies, further work may be necessary before a firm conclusion can be drawn.

2.2.2.3 Cognitive behaviour

In addition to pain sensitivity, thermoregulation and feeding behaviour, the generalizability of the dietary fat effect to more complex behaviours has recently been examined. The first evidence that variations in the quality of dietary fat could
influence cognitive behaviour was observed using a spatial memory test, the Morris Water Maze, in which animals are required to learn the location of a submerged platform (Coscina et al., 1986; Yehuda & Carasso, 1987). Three weeks after consuming the same experimental diets as in other studies (i.e., Crane & Greenwood, 1987), rats fed the SBO diet demonstrated superior performance on this task in comparison to those fed the lard diet. Importantly, overall activity in an open field was not affected by the dietary fat source suggesting that the performance in the test of spatial memory was not related to a general change in motor skills. The differences in learning could also not be explained by changes in basal body temperature, energy consumption, body weight, or brain activity of choline acetyltransferase, the marker enzyme for cholinergic neurons.

The association between dietary fat intake and cognitive performance was explored in greater detail utilizing a variety of tasks which are sensitive to different aspects of learning and memory, rely on different brain regions for optimal functioning, and are sensitive to normal physiological changes such as those observed with aging. These studies provided comparable diets to those utilized previously (i.e., 40% energy from fat, adequate EFAs) (Crane & Greenwood, 1987) but extended the feeding time to three months prior to behavioural testing to ensure that maximal adaptation to the experimental diets had occurred. Impaired performance in animals fed the lard based diets relative to those fed SBO diets was now observed on three different cognitive tasks (Greenwood & Winocur, 1990). The deficits observed in the lard-fed animals were characterized by impairments in spatial memory (Olton’s radial arm maze), temporal memory over both short and long intervals (variable-interval delayed alternation task), and in the ability to learn various maze problems (Hebb-Williams maze). The failure to learn specific mazes in the Hebb-Williams task and spatial memory impairments tested by Olton’s task have been linked to learning and memory dysfunction regulated by the hippocampus and related structures (Olton, 1983; Kesner, 1986; Winocur & Moscovitch, 1990). In contrast, the general skill of maze learning
tested on the Hebb-Williams maze appears to be controlled by the frontal lobes (Winocur & Moscovitch, 1990), and the variable-interval task tests a wide range of functions that require the participation of several brain regions. Since deficits in the lard group, relative to the SBO group, were observed on all the tasks tested, the results suggest that the effects of dietary fat composition on brain function are non-selective affecting a number of brain regions and cognitive functions.

Compelling evidence has now been presented indicating that at the same level of total dietary fat, normal (i.e., non-deficient) variations in fatty acid composition can modulate a number of animal behaviours with varying degrees of complexity. The experimental paradigms thus far addressed do not allow identification of the characteristic of dietary fat that is important in mediating these behaviours. In order to determine the influence of specific components of fat on behaviour, more than two dietary fat sources must be used.

2.2.3 A role for saturated fatty acids in mediating behaviour

The components of dietary fat that were first targeted as possible mediators of post-developmental animal behaviour were the EFAs. The high SFA (lard, beef tallow) and PUFA (SBO, corn oil) diets shown to influence behaviour differentially, markedly differ in both relative and absolute amounts of n-6 and n-3 fatty acids. However, since these fat sources also differ in SFA, PUFA and monounsaturated fatty acid (MUFA) content, no conclusions could previously be made with respect to the importance of EFA content when only two diets were compared. To determine whether or not n-6 and n-3 fatty acids were important in mediating macronutrient selection, McGee and Greenwood (1990a) utilized four diets (n-6:n-3 ratio of 1 and 20, each at two different absolute amounts). Fatty acid composition of the diets were varied by blending fat sources. The relative or absolute amounts of n-3 and n-6 fatty acids were not associated with changes in subsequent protein and carbohydrate selection.
In contrast, Yehuda and Carasso (1993) found that the performance of post-weanling rats in the Morris water tank was modified by the ratio of EFAs administered in addition to a basal diet. The relevance of this study to the behaviour of animals fed diets adequate in EFAs can be questioned because the basal diet appears to have been severely deficient in EFAs. The dietary levels of EFAs fed were in mg/kg diet rather than in g/kg diet. Adult rats need approximately 12 g/kg diet of linoleic acid (Bourre et al., 1990) and 1.3 g/kg diet of α-linolenic acid (Bourre et al., 1993b) to meet dietary requirements. Alternatively, if the reported values should have been in g/kg (i.e., typographical error), then the animals’ intakes of EFAs from the basal diet would have been approximately 600-700 mg/d. In this case, the tested dose of EFAs (2.25 mg/d administered intraperitoneally) would only have increased daily exposure to EFAs (i.e., sum of dietary EFAs and that administered) by less than 1%. It is difficult to imagine a mechanism whereby such a small increase in exposure to EFAs would have such a profound effect on behaviour. Thus it would appear that partial correction of EFA deficiency is the most plausible explanation for the outcome observed.

The specific characteristic of dietary fat that mediates behaviour may be the SFAs, based on the macronutrient selection patterns of rats consuming various fat blends (McGee & Greenwood, 1990a). Six different diets were prepared, each containing 40% of calories from fat, representing a broad range of SFA content. A significant correlation between dietary SFA content and both percent of energy chosen as protein and as carbohydrate was found. Rats consuming diets with greater amounts of SFAs selected more protein and less carbohydrate than rats fed lesser amounts of SFAs. On the other hand, the influence on macronutrient selection of MUFA and PUFA content, the PUFA:SFA ratio, n-6 and n-3 fatty acid content, and the n-6:n-3 fatty acid ratio was shown to be very minimal. Total food intake did not differ over the 14-day selection period between the six dietary groups (see Figure 2.1), suggesting that the effect of dietary fat composition on feeding behaviour is specific for macronutrient selection.
More recently, Greenwood and Winocur (1996) found that the SFA content of the diet is also the most influential dietary component involved in influencing cognitive behaviour. Utilizing similar 40% fat diets, rats were fed various dietary fat blends for three months that ranged in SFA content and were designed such that the effects of MUFAs and PUFAs could also be investigated. A variable interval delayed alternation task was used as the behavioural parameter. Performance on this task showed that impairment, in both the ability to learn the basic alternation rule and in memory for specific events, was highly associated with SFA intake and independent of amounts of either MUFAs or PUFAs consumed. The association between percent of dietary SFAs and performance on the variable interval delayed alternation task is shown in Figure 2.2. A higher latency ratio represents poorer performance on the task up to 1.0 which represents the state where no learning or memory has occurred. A comparison of Figure 2.1 and 2.2 shows that the cognitive behaviour results are very similar to the results obtained with the macronutrient selection studies.

The consistency across the feeding and cognitive behaviour studies strongly indicates that the level of SFAs in the diet is the important dietary characteristic in mediating behavioural changes. Whether the specific mechanism responsible for these behavioural changes is a function of a direct or indirect influence of differences in dietary fatty acid composition on the brain is unclear. Nevertheless, SFAs must influence some function such that the brain, and ultimately behaviour, is altered.
Figure 2.1  Total food intake and percent of energy chosen as protein in response to varying levels of dietary saturated fatty acids (SFAs). Rats were fed one of six experimental diets (24% protein, 40% carbohydrate) for 2 weeks followed by a 2-week selection period. Rats selected from 2 diets (5% protein, 61% carbohydrate and 55% protein, 4% carbohydrate) containing the same dietary fat as during the experimental period. All diets were isocaloric containing 20% (wt/wt) dietary fat. (a) Total energy consumed during 2-week selection period. (b) Linear regression of percent of energy chosen as protein in response to dietary SFA content. \( r=0.92 \). Data adapted from McGee & Greenwood (1990a).
Figure 2.2. Linear regression of performance on a cognitive task in response to varying levels of dietary saturated fatty acids (SFAs). \( r = 0.93 \). Rats were fed one of five diets differing in SFA content and were tested on a variable delayed alternation task, which measures learning and memory parameters. Latency ratios decreasing from a value of 1.0 represent improved performance on the task. Values are mean ± SEM for days 13 to 15 of testing. \( N = 8/diet \) group. Data adapted from Greenwood & Winocur (1996).
2.2.4 More support for the role of saturated fatty acids in mediating behaviour

Further supporting the argument that dietary SFA content mediates behaviour are some of the studies that were initially designed to measure the influence of EFA deficiency on behaviour. A number of studies examining this phenomenon have used fat sources that differ in their content of one or both of the EFAs, and are similar in total content of SFAs (refer to Table 2.1). However, other investigators have utilized fat sources that differ in EFA content but also in SFA content. Thus, although these experiments were designed to determine the influence of EFA deficiency on behaviour, they may have also been looking at a SFA effect.

SBO and safflower oil are examples of two fat sources that would be beneficial in identifying the effects of α-linolenic acid. Both oils contain comparable amounts of SFAs and SBO contains adequate amounts of both linoleic and α-linolenic acid, whereas safflower oil is deficient in α-linolenic acid. Therefore, by comparing behavioural outcomes following consumption of these fats, investigators can conclude that behavioural differences are due to α-linolenic acid deficiency and not to SFA content. Many researchers have employed these two fat sources during periods of brain development and have indeed found behavioural deficits in the α-linolenic acid deficient groups (Lamptey & Walker, 1976; Enslen et al., 1991; Reisbick et al., 1994). Other equally beneficial dietary fats have been used (e.g., safflower versus perilla oil; sunflower versus SBO) to further demonstrate behavioural impairments when EFAs are deficient during developmental periods (Yamamoto et al., 1987; Yamamoto et al., 1988; Bourre et al., 1989).

In contrast to these fat sources that differ only in EFA composition, other researchers have used fat sources that differ in both EFA and SFA content. Nonetheless, the intent of these studies was only to look at EFA deficiency. For instance, Ruthrich et al. (1984) found cognitive impairments in adult rats fed EFA deficient diets and concluded that both the developing and the mature rat brain are sensitive to EFA deficiency. These findings seem contradictory to the hypothesis that
behaviour in the post-developmental animal is altered by SFAs but not by EFAs. However, Ruthrich et al. (1984) used a sunflower oil diet (rich in linoleic acid) and a hydrogenated palm kernel oil diet, which is linoleic acid deficient but also extremely high in SFAs. Therefore, considering the SFA literature for post-developmental animals, the behavioural impairments that were found in the hydrogenated palm kernel-fed group could have been the result of the high dietary content of SFAs rather than the low linoleic acid content.

Hydrogenated coconut oil, which is deficient in EFAs but also very high in SFAs, has been fed to animals during developmental periods to show the effects of EFA deficiency on behaviour (Lamptey & Walker, 1978; Morgan et al., 1981). In these studies, the high SFA-low EFA groups were impaired on some behavioural aspect compared to animals fed corn oil (enriched in linoleic acid). Nevertheless, the data interpretation suffers from the same concerns expressed above for palm kernel oil making it unclear as to the importance of the dietary changes in EFAs versus those in the SFAs. Since the dietary manipulations occurred during periods of rapid brain development, the effects could have been due to SFA content or could have been additive such that the EFA deficiency and the high SFA content of the fat sources were both important in inducing impairment. More research is needed to determine whether or not SFAs fed during periods of brain growth and development influence behaviour independently from the effects of EFA deficient diets during the same period, since this apparently has not been systematically tested.

2.2.5 Conclusions of behavioural studies

There is clear evidence indicating that when diets are fed for weeks or months the developed brain is affected by simple alterations in dietary fatty acid composition, even when EFA consumption is sufficient. Moreover, this effect is specifically linked to the content of SFAs in the diet and appears to be independent of the relative composition of dietary MUFAs, PUFAs, and n-3 and n-6 fatty acids. This influence of SFAs is manifested in a number of behavioural parameters indicating that the effect
may be relatively widespread involving numerous brain regions and neurotransmitter systems. The influence that specific dietary fatty acid manipulations have on specific behaviours appears to be quite precise and reproducible. When exploring possible mechanisms, the importance of both the widespread behavioural effects as well as the necessary adaptation time to the dietary fat source must be considered.

2.3 Possible mechanisms involved in mediating dietary fat-induced behavioural changes

The characteristic of dietary fat that is important in mediating behaviour appears to be the SFA content. What remains elusive, however, is how dietary SFAs affect the brain in such a way as to mediate behaviour since a direct role of SFAs in the brain has not been demonstrated. In keeping with the behavioural studies demonstrating a widespread effect of dietary SFAs, some studies have explored mechanisms which could impact throughout the brain and show little regional specificity, such as changes in membrane composition or brain fatty acid oxidation, while others have targeted specific neurotransmitter systems as a means of identifying an underlying mechanism which may be extended to other neurotransmitter systems. Furthermore, while the mechanism must involve the central nervous system (CNS) in order for behaviour to be affected, consideration must be given to the fact that the primary response may be occurring in peripheral tissues and that the mechanism may involve an indirect effect of dietary SFAs mediated through peripheral metabolic changes.

2.3.1 Brain membrane fatty acid composition

Not surprisingly, one of the first mechanisms explored was the possibility that dietary fatty acid intake was directly influencing neuronal function through changes in membrane phospholipid fatty acid composition. Previous research had clearly demonstrated qualitative changes in brain membrane phospholipid fatty acid composition associated with dietary EFA deficiency during stages of rapid brain development (Galli et al., 1971; Alling et al., 1972; Menon & Dhopeshwarkar, 1982;
Bourre et al., 1993a; Wainwright et al., 1994) and the depressed levels of the elongated metabolites of the EFAs in the brain were associated with the behavioural deficits (for review see Wainwright, 1992). Past periods of differentiation and growth, however, the brain appears to be more resistant to dietary EFA deficiency such that peripheral tissues show more rapid and extensive depletion of the EFAs and their metabolites in comparison to the brain (Alling et al., 1972; Dyer & Greenwood, 1991b; Bourre et al., 1992; Bourre et al., 1993b). These studies led to the hypothesis that brain membrane fatty acid profile is insensitive to dietary fatty acid intake beyond developmental periods.

In contrast, others have demonstrated changes in brain membrane phospholipid fatty acid profile beyond developmental periods in the absence of EFA deficiency. That is, changes in mitochondria, synaptosomal and microsomal membranes (Foot et al., 1982; Dyer & Greenwood, 1991b), as well as myelin (Dyer & Greenwood, 1991b) have been observed in postweanling rats following intake of different dietary fat sources, when EFAs were not deficient. Altered activity of certain membrane-bound enzymes (Foot et al., 1983) and electrical membrane properties of dorsal root ganglia (Scott et al., 1989) have also been demonstrated following similar dietary manipulations. Furthermore, the specific dietary ratio of n-6:n-3 fatty acids is reflected in the fatty acid composition of brain phospholipid membranes (Dyer & Greenwood, 1991b). The 18:2n-6 content of cardiolipin, a mitochondrial specific phospholipid, has been shown to be the most sensitive cellular organelle in the rat brain to dietary manipulations of EFAs (Yamoaka & Kito, 1988; Dyer & Greenwood, 1991a). Despite these findings, the magnitude of change may be too small to be physiologically or functionally relevant to the animal.

To help understand whether such neuronal membrane fatty acid alterations in the mature brain are functionally relevant, the role played by these changes in influencing behaviour has been investigated. Using the macronutrient selection paradigm of previous experiments, McGee and Greenwood (1989) examined the
phospholipid fatty acid composition of synaptosomal membranes. As explained previously, the high SFA (beef tallow) group chose more protein and less carbohydrate than the high PUFA (SBO) group. Concurrently with these behavioural differences, these investigators found that the different dietary fat sources did indeed influence the phospholipid fatty acid profiles. Phosphatidylcholine was the most sensitive to differences in dietary fat composition and phosphatidylinositol was most resistant. However, a cause-and-effect relationship could not be concluded from this study.

In a series of subsequent experiments, McGee and Greenwood (1990b) showed that although changes in neural membrane fatty acid composition were induced by dietary fatty acid composition, these changes did not associate with macronutrient selection. Dietary SFA content strongly influenced subsequent macronutrient selection patterns, yet variations in dietary EFAs did not influence selection. In contrast, membrane fatty acid profiles significantly correlated with the EFA content of the experimental diets. Thus, alterations in membrane fatty acid composition occurred in the absence of differences in feeding behaviour. Furthermore, no significant relationship was found between the influence of six diets (differing in levels of SFAs) on selection and the influence of these diets on synaptosomal membrane fatty acids. Therefore, these results support the view that although alterations in neural membrane fatty acid composition occur in response to dietary fat treatments, these changes are not responsible for mediating macronutrient selection.

Similarly, changes in neural membrane fatty acid profiles have not been found to explain cognitive behavioural changes following dietary fat manipulations (Greenwood & Winocur, 1996). Cognitive impairment was associated with levels of dietary SFAs, and independent of dietary MUFA and PUFA levels. Again, brain membrane phosphatidylcholine fatty acid composition was influenced by dietary treatment, but these changes were not associated with the changes in cognitive performance. These studies (McGee & Greenwood, 1990b; Greenwood & Winocur, 1996) have shown that different behavioural parameters are highly correlated with
dietary intake of SFAs, and independent of changes in brain membrane fatty acid composition. Thus, it appears that the changes in membrane composition associated with variations in diet occur in tandem with the behavioural changes but that a cause and effect relationship is not likely.

2.3.2 Brain fatty acid uptake and oxidation

Brain uptake and oxidation of fatty acids may be another way in which the brain can be directly affected by dietary fat. It has been reported that brain uptake and oxidation of fatty acids are responsive to feeding status, and may therefore be involved in regulating feeding behaviour (Kasser et al., 1985; Kasser et al., 1986). Hypothalamic uptake of palmitate and hypothalamic fatty acid oxidation rates have been shown to be much greater in hungry compared to satiated rats (Kasser et al., 1985; Kasser et al., 1986). Furthermore, fatty acid oxidation inhibitors have been shown to stimulate an increase in feeding (Wang et al., 1994).

Fatty acid oxidation may be involved in regulating macronutrient selection following acute dietary fat treatment (Grossman et al., 1994). Consistent with other studies (e.g., Crane & Greenwood, 1987), Grossman et al. (1994) found that macronutrient selection behaviour differed between rats fed beef tallow or corn oil. This difference between diet groups was eliminated when an injection of mercaptoacetate (a fatty acid β-oxidation inhibitor) was administered prior to diet fat treatment.

Another report (Wang et al., 1994) found that dietary PUFAs (corn oil), compared to dietary SFAs (beef tallow) led to an increase in brain uptake of palmitate and lateral hypothalamic fatty acid oxidation. These authors (Wang et al., 1994) also found that injections of mercaptoacetate caused corn oil-fed rats to increase food intake, and had no effect on beef tallow-fed rats. This may be explained by noting that the rate of whole-body fatty acid oxidation is higher for corn oil (Jones et al., 1985; Leyton et al., 1987), so a greater response to a fatty acid oxidation inhibitor could be expected. These investigators have further suggested that since both mercaptoacetate
Cromer, 1993) and beef tallow have been shown to increase protein intake in rats, SFAs and mercaptoacetate may act through similar mechanisms. If both treatments act through similar mechanisms, then beef tallow-fed rats would not be expected to respond to mercaptoacetate treatment, which is exactly what was found.

These studies indicate that dietary fatty acid composition influences brain uptake and oxidation of fatty acids. In turn, such dietary induced changes may alter behaviour. More research is needed in this area since the amount of fat and feeding time needed to alter such brain functions have not been consistent with the diet treatments needed to alter behaviour.

2.3.3 Serotonin

Neurotransmitter systems have been suggested as another possible component of the mechanism that regulates dietary fat-induced behavioural alterations. There exists an abundance of literature implicating several neurotransmitters and neuropeptides as possible mediators of feeding behaviour (for review see Leibowitz, 1986; Bray, 1992). The role of the brain neurotransmitter, serotonin (5-hydroxytryptamine, 5-HT), as a mediator of both total food intake and macronutrient selection has probably received the greatest attention (Anderson, 1994). The data on food intake regulation suggest that increases in levels of 5-HT induces satiety (Shor-Posner et al., 1986; Duhault et al., 1993; Leibowitz et al., 1993). Increasing 5-HT levels either directly or indirectly has been shown to lead to a specific increase in protein intake and/or a decrease in carbohydrate intake, whereas decreasing 5-HT levels causes the opposite pattern of macronutrient selection (Ashley & Anderson, 1975; Li & Anderson, 1984b; Shor-Posner et al., 1986; Morris et al., 1987; White et al., 1988; Leibowitz et al., 1989; Currie, 1993; Duhault et al., 1993; Leibowitz et al., 1993). Although challenges to the serotonin hypothesis of macronutrient regulation (suggested by Anderson, 1979; Anderson, 1988) have been made (Peters et al., 1984; Peters & Harper, 1987; Fernstrom, 1987; Anonymous, 1992), based on the connection of 5-HT to feeding behaviour as well as to other functions including attention, pain
sensitivity, sleep, sexual behaviour, learning and memory (Tenen, 1967; McEntee & Crook, 1991; Steckler & Sahgal, 1995), it follows logically to investigate whether or not brain neurotransmitter systems are involved in altering behaviour patterns following fatty acid consumption.

The following discussion suggests that a number of inconsistencies implying a role for serotonin in mediating dietary-fat induced behavioural changes are evident. Consequently, it appears that serotonin may not play a major role in affecting macronutrient selection following the long-term intake of a 40% (kcal) fat diet. Crane and Greenwood (1987) investigated whether dietary fat mediates macronutrient selection via alterations in serotonin metabolism. These authors found that rats fed a high SFA (lard) diet select more protein and less carbohydrate than rats fed a high PUFA (SBO) diet. Monoamine oxidase (MAO; an enzyme involved in the degradation of serotonin) activity in brain mitochondria from rats consuming the SBO diet was significantly lower than in those fed the lard diet. However, no differences in absolute levels of either 5-HT or its metabolite 5-hydroxyindole acetic acid (5-HIAA) were observed between the two dietary groups. These results indicated that although neuronal function could be influenced by dietary fat, simple alterations in 5-HT metabolism did not appear to influence macronutrient selection.

Additional evidence supports the conclusions that serotonin is not involved in mediating the effects of dietary fat on behaviour. Following the discovery that the SFA content of dietary fat is the important component mediating protein and carbohydrate selection (McGee & Greenwood, 1990a), these authors studied the influence of different levels of dietary SFAs on serotonin metabolism (McGee & Greenwood, 1991). In agreement with the previously mentioned findings (Crane & Greenwood, 1987), this study found no differences in steady-state hypothalamic levels of tryptophan (increases the synthesis of brain 5-HT (Fernstrom & Wurtman, 1971a), 5-HT, or 5-HIAA as a function of dietary fat treatment. 5-HT turnover was also unaltered by differences in dietary fat composition. Taken together, these findings
suggest that the effect of SFAs on feeding behaviour are not related to changes in steady-state 5-HT metabolism.

In contrast to these findings, Mullen and Martin (1992b) have suggested a role for serotonin in the control of macronutrient selection following dietary fat intake. These investigators found that rats fed a high SFA diet (beef tallow) had higher serum insulin levels (increases plasma and brain tryptophan levels (Fernstrom & Wurtman, 1971b; Fernstrom & Wurtman, 1972)) and showed elevated serotonin in the raphe area compared to rats fed a high PUFA diet (corn oil). In addition, they found that fenfluramine, a 5-HT agonist, depressed food intake more so in rats fed beef tallow than in rats fed corn oil. The discrepancy may be due to the different methods employed. The diets used by McGee and Greenwood contain less total fat and are fed for much longer time periods than those of Mullen and Martin (see Table 2.2). Thus, serotonin may be involved in mediating macronutrient selection following the acute consumption of a very high fat diet, but may not be involved in mediating selection following the chronic consumption of a lower fat diet.

There are some concerns with the data that support a role for serotonin in the acute situation. Although Mullen and Martin (1992b) found differences in 5-HT levels in the raphe area between diet groups, these were not accompanied by differences in 5-HIAA levels. Furthermore, neither 5-HT nor 5-HIAA levels were different in the ventromedial hypothalamus, suggesting that while steady-state 5-HT levels may be affected in the cell body that these alterations are not apparent in the terminal areas. Hence, it is difficult to speculate how differences in 5-HT neurotransmission would be expressed. Additionally, a previous study by these same investigators (Mullen & Martin, 1991) showed that there was no difference among three dietary groups (hydrogenated corn oil, beef tallow, and SBO) with respect to tryptophan uptake into hypothalamic or raphe areas. Finally, although Mullen and Martin have shown that animals fed different high fat diets for as little as 2 hours (Grossman et al., 1994) select macronutrients differently, the data implicating a role for serotonin was shown after a feeding period of 7 days (Mullen & Martin, 1992b). Thus, until a difference in
serotonin levels, associated with terminal projections and synaptic release, can be shown in tandem with differences in feeding behaviour, the data remain unclear.

2.3.4 Evidence of possible mechanisms involving peripheral involvement

2.3.4.1 Vagus nerve

As noted earlier, the mechanism involved in mediating dietary fat-induced behavioural changes must ultimately involve the CNS, but the impact may be via an indirect, peripheral route. One recent finding suggests that the effect of saturated fat on macronutrient selection may be mediated by a more indirect mechanism. Severing the hepatic branch of the vagus nerve was shown to eliminate the SFA effect on macronutrient selection (Grossman et al., 1994). Although this study implies that signals from the liver are important in mediating selection, it should be noted that not all of the vagal signals are abolished when this surgery is performed (Nijima, 1983, cited in Grossman et al., 1994) and in addition to the liver, the hepatic branch of the vagus also innervates nonhepatic sites (Berthoud et al., 1993, cited in Grossman et al., 1994). Since the vagus provides the innervation of most of the digestive tube, including regions that control swallowing, propulsion, storage, digestion, and absorption of nutrients (Powley & Berthoud, 1986), it is not surprising that it should play an important role in the regulation of food intake. In fact, the role of the vagal system in regulating both stimulatory and inhibitory effects on total food intake (for reviews see Powley & Berthoud, 1986; Ritter et al., 1992) and macronutrient selection (Scalfani & Kramer, 1983; Li & Anderson, 1984a) has been well-established. The hepatic innervation may be important in communicating information about the energy availability of the animal to the brain. In addition, an influence of dietary fat on the release of and/or the response to gut peptides could be relayed to the brain via other vagal innervations. Whatever the mechanism, this study appears to suggest that dietary fatty acids may influence a peripheral site such that an indirect pathway to the CNS via afferent nerves may be important in mediating behaviour.
One shortcoming of the vagus nerve as an important component of the mechanism that mediates dietary fat-induced behavioural alterations is that its role in mediating behaviours other than feeding behaviour is not readily obvious. Based on the similarities between the results that have been found with different behaviours, it is hypothesized that whatever mediating mechanism is involved, there is likely a common element linking the SFA effect on macronutrient selection with the effect on cognition, thermoregulation and pain sensitivity. This mechanism may also be responsible for mediating a wider, yet untested set of behaviours. Thus, for any mechanism to be plausible, it would be expected to not only be specifically influenced by SFAs but that it would mediate a widespread set of behaviours as well.

2.3.4.2 Absorption and metabolism of different chain length saturated fatty acids and effects on feeding behaviour

Saturated fatty acids are generally divided into short- (≤6:0), medium- (8:0 and 10:0) and long-chain SFAs (≥12:0) depending on the number of carbons in the fatty acid molecule. For the purposes of the present thesis, however, the following terms will be used: medium-chain SFAs (MSFAs): 8:0 and 10:0; intermediate-chain SFAs (ISFAs): 12:0 and 14:0; and long-chain SFAs (LSFAs): ≥16:0. The term “intermediate-chain SFAs” has been used because SFAs of 12 and 14 carbons have been shown to act differently from longer and shorter chain SFAs.

There are clearly differences in the absorption and metabolism of SFAs depending on chain length. Such differences may be involved in mediating feeding behaviour, including macronutrient selection. However, for the most part, only dietary fat sources enriched in LSFAs have been examined (e.g., lard, beef tallow) for their influences on macronutrient selection. Compared to LSFAs, MSFAs have a much lower melting point and are relatively more water soluble (Bach & Babayan, 1982). With respect to absorption, medium-chain triglycerides (MCTs) are more rapidly and completely hydrolyzed than long-chain triglycerides (LCTs) in the intestinal lumen by pancreatic lipase (Holt, 1967; Harkins & Sarett, 1968a). In addition, bile salts
stimulate hydrolysis in LCTs, but have little influence on the hydrolysis of MCTs. The products of MCT hydrolysis are absorbed primarily as free fatty acids, and thus absorbed faster than those of LCTs, and as fast as glucose (Bach & Babayan, 1982).

LSFAs are reesterified to LCTs, which are incorporated into chylomicrons, and then delivered into the lymph (Isselbacher, 1968; Scheig, 1968). LCTs are largely taken up by adipose tissue, and reach the liver indirectly (Wiley & Leveille, 1973). In contrast, fatty acids released from MCTs are usually not reesterified, and remain as free fatty acids. Thus, following MCT ingestion, total triglyceride production is low, although the chylomicron triglycerides that are synthesized do contain small amounts of MSFAs (Swift et al., 1990). Nevertheless, since they are not significantly incorporated into chylomicrons, MSFAs leave the intestine faster than LSFAs (Bach & Babayan, 1982). For the most part, they are transported as an albumin complex to the liver via the portal bloodstream, rather than via the lymph (Isselbacher, 1968; Scheig, 1968). Therefore, MSFAs reach the liver in greater abundance than do exogenous LSFAs (Bach & Babayan, 1982). In fact, it has been shown that in a relatively linear fashion, the shorter the fatty acid, the more is absorbed via the portal bloodstream, rather than via the lymph (Senior, 1968). In other words, more MSFAs than ISFAs, and more ISFAs than LSFAs are absorbed via portal blood.

With respect to metabolism, MSFAs cross the double mitochondrial membrane very rapidly and the process is not dependent on carnitine, whereas LSFAs are carnitine dependent (Babayan, 1987). Compared to LCTs, MCTs are poorly utilized for tissue lipid synthesis but are readily catabolized to CO₂, acetate, and ketones by the liver (Scheig, 1968; Greenberger & Skillman, 1969). There is a relatively linear relationship between increasing SFA chain length and rates of fatty acid oxidation (Leyton et al., 1987). That is, the oxidation of shorter chain SFAs is more rapid than the oxidation of longer chain SFAs. In fact, the oxidation of octanoic acid (8:0) to CO₂ by the liver occurs approximately ten times faster than that of palmitic acid (16:0) (Harkins & Sarett 1968a).
A number of studies have shown selective effects of different fatty acid chain lengths on total food intake, although macronutrient selection has apparently not been investigated. Rats supplemented with MCT oil diets showed a cumulative decrease in short-term food intake compared to rats fed a diet containing dietary LCTs (Furuse et al., 1992). Similar results have also been found using chickens (Furuse et al., 1993). It appears that satiety, and not palatability, is affected by chain length (Furuse et al., 1992). It has also been shown that MCT oil was the most powerful stimulator of cholecystokinin secretion in rats, among four triglycerides (Douglas et al., 1990). Since cholecystokinin has been shown to be a stimulator of satiety signals (see Leibowitz, 1986; Bray, 1992), the reduced food intake may be caused by enhanced plasma cholecystokinin. However, Furuse et al. (1992) concluded that the responsibility of endogenous cholecystokinin is minimal.

The many differences between SFAs of varying chain lengths, including their varying rates of oxidation and routes of absorption may contribute to differences in the regulation of feeding behaviour. The LSFAs are oxidized at a slower rate than ISFAs, which are in turn oxidized slower than MSFAs. As is the case with portal blood absorption, these differences appear to be linearly related. Based on this data, it seems reasonable to suggest that if rates of fatty acid oxidation and/or routes of absorption are involved in the mechanism that mediates dietary-fat induced macronutrient selection, then any differential effects that these different fatty acids may have on macronutrient selection would also occur in a relatively linear fashion. That is, the influence of ISFAs would be expected to be intermediate between those effects seen with MSFAs and with LSFAs. In addition, it can reasonably be argued that animals fed fat sources high in the shorter chain fatty acids may select similarly to animals fed low SFA sources (enriched in PUFAs) (i.e., select a high carbohydrate, low protein diet relative to LSFA-fed animals), since PUFAs are also oxidized at a more rapid rate than LSFAs (Leyton et al., 1987).
2.3.5 Possible relationship between dietary fat-induced changes in insulin sensitivity, brain uptake and utilization of glucose and behaviour

The role of SFAs in influencing insulin sensitivity may be involved in the mechanism by which SFAs mediate behaviour. The mechanism of action would involve an indirect peripheral effect of dietary fat on insulin response, which could impact on glucose uptake and utilization by the brain and ultimately lead to behavioural changes. Although this system may be important in mediating behaviour, a number of other components most likely contribute to the underlying mechanisms as well.

2.3.5.1 Decrease in insulin sensitivity associated with saturated fatty acid intake

Consistent with the data suggesting that SFA content of dietary fats is the important mediator of behaviour, SFAs have also been implicated as mediators of insulin response. The fatty acid composition of a high-fat diet has been shown to be important in altering insulin concentration in rats. Specifically, a high SFA diet is linked to the development of insulin resistance (Storlien et al., 1991). Research by Clandinin et al. (1993) has also suggested a harmful role for SFAs since a high dietary PUFA:SFA ratio improved insulin response. The high PUFA:SFA ratio increases PUFA content of adipocyte plasma membrane phospholipids (Field et al., 1989) which increases insulin binding (van Amelsvoort et al., 1986; Field et al., 1989), insulin-stimulated glucose transport and glucose incorporation into lipids (lipogenesis) (Field et al., 1990; Pan & Berdanier, 1991). Hence, a low PUFA:SFA ratio will impair insulin sensitivity.

Research by van Amelsvoort et al. (1988) further supports a specific role for SFAs in regulating insulin. These investigators determined the influence of six different dietary fat sources on insulin-stimulated deoxyglucose uptake in rat epididymal fat cells, and concluded that the important mediator of the insulin response was not the amount of PUFAs or the ratio of PUFAs to SFAs, but rather the amount of
SFAs with 12, 14, or 16 carbon atoms. Higher dietary SFA content led to the lowest stimulation by insulin of deoxyglucose uptake, which again indicates that SFAs may decrease insulin sensitivity. Other evidence suggests that the same fat sources that have been shown to influence macronutrient selection also differentially affect blood insulin concentration. Rats fed a high beef tallow diet (Mullen & Martin, 1992b) or a high lard diet (Kauhan et al., 1994; Dulloo et al., 1995) have higher fasting plasma insulin concentrations than rats fed a high PUFA diet. A higher fasting plasma insulin concentration generally indicates a decrease in insulin sensitivity.

The majority of the literature supports the notion that insulin sensitivity responds to differences in dietary fatty acid composition. The same fat sources that influence behaviour differentially also appear to influence insulin concentration differentially. Moreover, consistent with the behavioural literature, dietary SFAs may play a more important role than other components of dietary fat in influencing insulin sensitivity.

### 2.3.5.2 Evidence for a role of glucose in mediating behaviour

The preceding discussion suggests that manipulations in dietary fatty acid composition lead to alterations in insulin sensitivity. For behaviour to be affected, such changes in peripheral insulin concentration must impact on some central process, possibly by influencing the supply of glucose to the CNS. Recent evidence suggests that circulating insulin can stimulate glucose uptake by the brain via the insulin-sensitive glucose transporter, GLUT 4 (Livingstone et al., 1995). GLUT 4 has been detected in the pituitary, the hypothalamus, the medulla (Brant et al., 1993) and the cerebellum (Rayner et al., 1994). Hence, via changes in insulin response, dietary fat may ultimately influence the energy supply to the brain, which could impact on behaviour.

Other literature suggests that insulin is present in the CNS and may be influenced by peripheral insulin concentration (for review see Wozniak et al., 1993). One hypothesis suggests that insulin is transported to the cerebrospinal fluid (CSF)
from peripheral tissues (for review see Plata-Salaman, 1991). Thus, dietary fat-induced changes in circulating insulin concentration may impact on central insulin via uptake into CSF, which could impact on behaviour. Finally, it should be noted that the origin of insulin in the CSF may be the result of uptake from the peripheral circulation and/or from locally produced insulin (Plata-Salaman, 1991). Thus, another possibility is that the regulation of centrally synthesized insulin concentration is dependent on peripheral events, which could affect subsequent brain function.

Numerous studies indicate that alterations in glucose concentration can affect several behaviours. Peripheral injections of glucose in rats (Gold, 1986; Stone et al., 1990; Rodriguez et al., 1993) and in mice (Messier & Destrade, 1988; Means & Fernandez, 1992; Kopf et al., 1993) have been shown to enhance memory on a number of different tasks. In accordance with the animal research, there are also a number of studies that show enhanced cognitive performance in humans following the intake of glucose-containing drinks, including improved memory (Benton, 1990; Benton & Owens, 1993) and faster reaction times (Benton et al., 1994). Furthermore, it appears that in both humans and animals the effects of glucose on cognition may be more pronounced in elderly subjects than in young individuals (Gonder-Frederick et al., 1987; Hall et al., 1989; Manning et al., 1993; Winocur, 1995). Another line of research that supports the findings that glucose can improve cognitive ability are studies that indicate that glucose can attenuate the effects of cognitive impairing factors in animals (Ragozzino et al., 1992; Stone et al., 1992; Walker & Gold, 1992; Ahlers et al., 1993).

Several researchers suggest that changes in circulating concentrations of glucose and insulin can influence behaviour. It has been suggested that peripheral glucose may mediate behaviour by influencing the uptake and utilization of glucose by the brain (Gold & Stone, 1988; Wenk, 1989), possibly via the GLUT 4 route mentioned above. In support of this notion, it has also been shown that central injections of glucose improve memory (Lee et al., 1988). Furthermore, a link between
the degree to which glucose influences behaviour and insulin resistance has been suggested (Winocur, 1995). Thus, a mechanism whereby dietary fat influences insulin sensitivity, which affects central concentrations of glucose, and ultimately leads to behavioural changes appears logical.

2.3.5.3 *Summary of the link between dietary fat-induced changes in insulin sensitivity and behaviour*

The evidence reviewed indicates that both a glucose load and the composition of dietary fat can influence behaviour. Furthermore, it is possible that dietary fat composition can influence central glucose concentration, via a change in insulin sensitivity. However, a connection between dietary fat composition, insulin sensitivity and behaviour has not yet been tested. A diagram of the possible connection is illustrated in Figure 2.3. Dietary fat may influence several behaviours by first affecting glucose metabolism and/or insulin sensitivity, which in turn affects the glucose supply to the CNS. As one prevailing hypothesis on the role of glucose in enhancing cognitive function suggests, such alterations in brain glucose concentration may influence the biochemistry of one or more different neural systems (Wenk, 1989). The role of glucose in the brain as a precursor for the formation of acetylcholine and many other neurotransmitters may be important (Wenk, 1989). These changes may be ultimately manifested as altered behaviour.

**Figure 2.3.** Diagram of possible mechanism by which dietary fat may mediate behaviour. Dietary fat influences glucose and insulin concentration, ultimately affecting glucose uptake by the brain. Alterations in brain glucose uptake influence a number of neural systems eventually leading to changes in behaviour.

To determine the importance of the mechanism that mediates dietary fat-induced behaviour changes, future investigators must consider a number of factors.
The literature provides evidence that dietary SFA content is a key factor important in regulating behaviour. This component of fat must also mediate insulin and glucose concentration if the hypothesis that the relationship between fat composition and behaviour is mediated by this mechanism is to hold true. As discussed earlier, van Amelsvoort et al. (1988) concluded that the mediating factor influencing insulin response following dietary fat intake was the concentration of long-chain SFAs. In addition, to be consistent with the literature, a change in insulin sensitivity in response to increased SFA intake must occur over an adaptation period of weeks to months. Finally, the impact of a change in insulin sensitivity on brain uptake and utilization of glucose must affect several brain regions and a widespread set of behaviours.

2.4 Conclusions

Simple differences in dietary fatty acid composition, beyond periods of rapid brain development, without EFA deficiency, and at levels similar to those of the North American population influence a number of behaviours including feeding behaviour and cognitive ability. With respect to macronutrient selection and cognition, the intake of SFAs appears to be the specific fat characteristic that is important in mediating these behaviours. However, only LSFAs have been examined and the individual influences of SFAs of varying chain lengths on behaviour are not known.

Although a variety of potential mechanisms have been explored, the underlying mechanism responsible for these dietary fat-induced behaviour changes remains elusive.

2.5 Introduction to experimental work

The data implicating a role for SFAs in mediating behaviour have all looked at fat sources enriched in LSFAs. It may therefore be beneficial to look further into what specific aspect of saturated fat is causing the effect. One way of examining this is to investigate the influence of MSFAs, ISFAs and LSFAs on macronutrient selection.
The purpose of the first experiment undertaken was to examine the role of SFAs of varying chain length in mediating macronutrient selection. In previous studies which suggested that SFA concentration was the important component of dietary fat in mediating selection, dietary cholesterol and peroxide products were also considered as potential variables since they varied concurrently with SFA concentration (McGee & Greenwood, 1990a). To eliminate these potential confounding variables, all fat sources were composed of vegetable oils (contain no dietary cholesterol) and very highly saturated fat sources were utilized (have very little opportunity to develop peroxides).

The correlation between SFA chain length and protein and carbohydrate selection was examined to determine the role of rates of fatty acid oxidation and/or routes of absorption in the mediating mechanism. A correlation between SFA chain length and macronutrient selection would suggest that oxidation and absorption may be important in mediating this behaviour, whereas a non-significant relationship would suggest that these parameters are not important in mediating selection.

The second experiment was conducted to reproduce the behavioural components of the first experiment, and to examine the importance of insulin sensitivity and glucose tolerance as mediators of dietary fat-induced macronutrient selection behaviour.
CHAPTER 3

HYPOTHESIS AND OBJECTIVES
3. HYPOTHESIS AND OBJECTIVES

3.1 Hypothesis

The hypothesis tested in this thesis was that following the long-term consumption of nutrient-sufficient diets, dietary saturated fatty acid chain length influences macronutrient selection in post-weanling rats.

3.2 Objectives

1. To determine if a correlation between dietary saturated fatty acid chain length and macronutrient selection exists.
2. To determine if the influence of dietary saturated fatty acid chain length on macronutrient selection correlates with its influence on plasma glucose concentration, plasma insulin concentration and/or glucose tolerance.
CHAPTER 4

MATERIALS AND METHODS
4. MATERIALS AND METHODS

4.1 Maintenance of animals

Male Wistar rats (Charles River, St. Constant, Quebec) were used for all experiments. Rats were housed singly in stainless steel wire-mesh cages (necessary in order to measure food spillage) in a temperature-controlled environment (22 ± 1°C). Animals were maintained on a 12-hour light cycle and had ad libitum access to all diets and water. All protocols were approved by the University Animal Care Committee.

4.2 Experimental and selection diets

All diets were fed from food cups that were secured with a spring to minimize spillage. All diets were isocaloric, nutritionally adequate purified granular mixtures, with no deficiencies in 18:2n-6 or 18:3n-3 (Bourre et al., 1990; Bourre et al., 1993b). This was accomplished by adding a safflower oil-flaxseed oil mixture to each diet, except for the soybean oil diets. All diets contained a similar ratio of 18:2n-6:18:3n-3 fatty acids. L-methionine was added to all diets (2.5g/kg diet). All diets contained equal amounts of choline bitartrate, minerals, and vitamins per kg diet. Diet composition is shown in Table 4.1. Standard AIN-76 vitamin and mineral mixes were used (Tables 4.2 and 4.3). Protein and carbohydrate composition of the diets were adjusted by varying the amounts of casein and cornstarch. Cellulose content was adjusted to keep the diets isocaloric.

Specific fatty acid profiles were obtained by blending fat sources. Fatty acid composition of each diet is shown in Table 4.4. Fatty acid composition of fat sources (fully hydrogenated soybean oil (HSB), soybean oil (SBO), flaxseed oil, and safflower oil) were determined by gas chromatography as described below. Fatty acid compositions of medium-chain triglyceride oil (MCT) and hydrogenated coconut oil (HCO) are based on the values provided by the manufacturers.
Table 4.1 Diet Composition

<table>
<thead>
<tr>
<th></th>
<th>Experimental Diet</th>
<th>Selection Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24% Protein(^1)</td>
<td>5% Protein(^3)</td>
</tr>
<tr>
<td></td>
<td>36% Carbohydrate(^5)</td>
<td>55% Carbohydrate(^5)</td>
</tr>
<tr>
<td></td>
<td>(g / kg) (kcal)(^6)</td>
<td>(g / kg) (kcal)(^6)</td>
</tr>
<tr>
<td>Casein(^1)</td>
<td>287.5 1026</td>
<td>60.0 214</td>
</tr>
<tr>
<td>Cornstarch(^2)</td>
<td>384.5 1538</td>
<td>587.5 2350</td>
</tr>
<tr>
<td>Cellulose(^1)</td>
<td>88.5 113.0</td>
<td>48.5</td>
</tr>
<tr>
<td>Vitamins(^1)</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Minerals(^1)</td>
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<td>35.0</td>
</tr>
<tr>
<td>L-Methionine(^1)</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Choline Bitartrate(^1)</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Fat (40% kcal)(^3)</td>
<td>190.0 1710</td>
<td>190.0 1710</td>
</tr>
<tr>
<td></td>
<td>1000.0 4274</td>
<td>1000.0 4274</td>
</tr>
</tbody>
</table>

\(^1\) Purchased from Harlan Teklad (Madison, WI). Casein: 87% protein, 1% fat
\(^2\) A.E. Staley MFG. Co. (Decatur, IL).
\(^3\) MCT Oil as fat source: 203.5 g / kg fat; cellulose decreased by 13.5 g / kg
\(^4\) % Protein = % of kcal from casein
\(^5\) % Carbohydrate = % of kcal from cornstarch
\(^6\) Protein = 4 kcal/g; Carbohydrate = 4 kcal/g; Fat = 9 kcal/g except MCT = 8.3 kcal/g
(Harkins & Sarett, 1968b). Vitamin mix, mineral mix, and L-methionine make a small caloric contribution (not included in calculation)
<table>
<thead>
<tr>
<th>Component</th>
<th>g / kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamin HCl</td>
<td>0.60</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.60</td>
</tr>
<tr>
<td>Pyridoxine HCl</td>
<td>0.70</td>
</tr>
<tr>
<td>Niacin</td>
<td>3.00</td>
</tr>
<tr>
<td>Calcium Pantothenate</td>
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</tr>
<tr>
<td>Folic Acid</td>
<td>0.20</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.02</td>
</tr>
<tr>
<td>Vitamin B12 (0.1% trituration in mannitol)</td>
<td>1.00</td>
</tr>
<tr>
<td>Dry Vitamin A Palmitate (500,000 U/g)</td>
<td>0.80</td>
</tr>
<tr>
<td>Dry Vitamin E Acetate (500 U/g)</td>
<td>10.00</td>
</tr>
<tr>
<td>Vitamin D3 Trituration (400,000 U/g)</td>
<td>0.25</td>
</tr>
<tr>
<td>Menadione Sodium Bisulfite Complex</td>
<td>0.15</td>
</tr>
<tr>
<td>Sucrose, fine powder</td>
<td>981.08</td>
</tr>
</tbody>
</table>

1 Harlan Teklad (Madison, WI) Vitamin Mix, AIN-76A 40077
Table 4.3 Composition of Mineral Mix\(^1\)

<table>
<thead>
<tr>
<th>Component</th>
<th>g / kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Phosphate, dibasic</td>
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</tr>
<tr>
<td>Sodium Chloride</td>
<td>74.00</td>
</tr>
<tr>
<td>Potassium Citrate, monohydrate</td>
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</tr>
<tr>
<td>Potassium Sulfate</td>
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</tr>
<tr>
<td>Magnesium Oxide</td>
<td>24.00</td>
</tr>
<tr>
<td>Manganous Carbonate</td>
<td>3.50</td>
</tr>
<tr>
<td>Ferric Citrate</td>
<td>6.00</td>
</tr>
<tr>
<td>Zinc Carbonate</td>
<td>1.60</td>
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<tr>
<td>Cupric Carbonate</td>
<td>0.30</td>
</tr>
<tr>
<td>Potassium Iodate</td>
<td>0.01</td>
</tr>
<tr>
<td>Sodium Selenite</td>
<td>0.01</td>
</tr>
<tr>
<td>Chromium Potassium Sulfate</td>
<td>0.55</td>
</tr>
<tr>
<td>Sucrose, finely powdered</td>
<td>118.03</td>
</tr>
</tbody>
</table>

\(^1\) Harlan Teklad (Madison, WI) Mineral Mix, AI\textsuperscript{N}-76 170915
Table 4.4 Fatty Acid Composition of Diets

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>HSB&lt;sup&gt;1&lt;/sup&gt;</th>
<th>HCO&lt;sup&gt;2&lt;/sup&gt;</th>
<th>MCT&lt;sup&gt;3&lt;/sup&gt;</th>
<th>SBO&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of fat (w/w)</td>
<td>% of total (w/w)</td>
<td>% of fat (w/w)</td>
<td>% of total (w/w)</td>
</tr>
<tr>
<td>6:0</td>
<td>0.67</td>
<td>0.13</td>
<td>2.54</td>
<td>0.52</td>
</tr>
<tr>
<td>8:0</td>
<td>7.73</td>
<td>1.47</td>
<td>59.49</td>
<td>12.11</td>
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<tr>
<td>10:0</td>
<td>0.30</td>
<td>0.06</td>
<td>5.23</td>
<td>0.99</td>
</tr>
<tr>
<td>12:0</td>
<td>0.85</td>
<td>0.16</td>
<td>37.80</td>
<td>7.18</td>
</tr>
<tr>
<td>14:0</td>
<td>0.61</td>
<td>0.12</td>
<td>13.54</td>
<td>2.57</td>
</tr>
<tr>
<td>16:0</td>
<td>12.31</td>
<td>2.34</td>
<td>8.07</td>
<td>1.53</td>
</tr>
<tr>
<td>18:0</td>
<td>70.47</td>
<td>13.39</td>
<td>10.71</td>
<td>2.03</td>
</tr>
<tr>
<td>18:1</td>
<td>2.37</td>
<td>0.45</td>
<td>3.87</td>
<td>0.74</td>
</tr>
<tr>
<td>18:2</td>
<td>11.05</td>
<td>2.10</td>
<td>11.15</td>
<td>2.12</td>
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<tr>
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<td>1.14</td>
<td>0.22</td>
<td>1.15</td>
<td>0.22</td>
</tr>
<tr>
<td>20:0</td>
<td>0.57</td>
<td>0.11</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>22:0</td>
<td>0.31</td>
<td>0.06</td>
<td>0.02</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Saturated, total 85.42 16.23 83.81 15.92 84.68 17.23 13.65 2.59
Medium chain 0.30 0.06 12.95 2.46 80.76 16.43 0.22 0.04
Intermediate chain 1.46 0.28 51.34 9.75 0.02 0.00 0.00 0.00
Long chain 83.86 15.90 18.84 3.58 1.36 0.28 13.43 2.55
Unsaturated, total 14.56 2.77 16.17 3.07 13.59 2.77 86.33 16.40

<sup>1</sup> HSB (enriched in ≥16:0) = 83.95% Fully Hydrogenated Soybean Oil (Thomas J. Lipton Inc.), 14.16% Safflower Oil (Baldwin's Natural Foods), 1.89% Flaxseed Oil (Baldwin's Natural Foods)

<sup>2</sup> HCO (enriched in 12:0 and 14:0) = 83.95% Hydrogenated Coconut Oil (Harlan Teklad), 14.13% Safflower Oil, 1.92% Flaxseed Oil

<sup>3</sup> MCT (enriched in 8:0 and 10:0) = 85.01% Medium Chain Triglyceride Oil (Mead Johnson Canada), 13.22% Safflower Oil, 1.77% Flaxseed Oil

<sup>4</sup> SBO (enriched in PUFAs) = 100.00% Soybean Oil (Noah's Natural Foods)
4.3 Diet preparation

All powder ingredients (choline bitartrate (ground with mortar and pestle), L-methionine, vitamin mix, mineral mix, cornstarch, cellulose and casein) and melted fat blends were added to a mixing bowl. A Hobart mixer was used to homogeneously mix the ingredients.

4.3.1 Fully hydrogenated soybean oil (HSB) diet

Since fully hydrogenated soybean oil (HSB) consists of almost one hundred percent long-chain saturated fatty acids, it is extremely hard in texture and difficult to incorporate into a homogenous diet. Even though the HSB was melted before incorporation into the diet mixture, the fat formed into small pellets such that the rats were able to avoid ingestion of the fat if preferred.

In order to avoid this problem, the HSB fat source was ground into a fine powder and then incorporated into the diet mixture. HSB was chipped from a solid block into small pieces. The pieces were then ground with a coffee grinder. A very fine fish net was used as a sieve to ensure the extreme fineness of the powder. The powder was ground further if it did not pass through the sieve. This HSB powder was then mixed with the other diet ingredients to form a completely homogeneous diet. The rats could no longer avoid eating the fat.

4.4 Statistical analyses

Statistical analyses were conducted with SAS 6.08 (SAS Institute, Inc., Cary, NC, USA) for the microcomputer. For all analyses, the acceptable level of significance (Type I error) was $P \leq 0.05$. Effects of diet fat treatment on protein and carbohydrate selection were determined by repeated measures analysis of variance (ANOVA). Where a significant effect of diet was evident, post-hoc comparison of group means was determined with Student-Newman-Keuls' test for multiple comparisons. Where initial body weights or body weights at the beginning of the selection period differed among groups, covariate analyses were performed to remove these differences as
confounding variables. Regression analysis was conducted using a linear regression procedure.

4.5 **Fatty acid analysis: gas chromatography**

All samples were analyzed in duplicate. Boron trifluoride (BF₃) in methanol (2 mL) was added to 15 to 20 mg of each dietary fat source to methylate the samples. Samples were flushed under a stream of nitrogen (N₂) for 15 sec and heated for 30 min at 90°C. Upon completion of methylation, the test tubes containing the fatty acid methyl esters were cooled in an ice bath. 2.0 mL of saline (0.9% NaCl) and 5.0 mL of hexane were added to each test tube. The tubes were vortexed and centrifuged at 1500 rpm for 5 min. The hexane layer (top layer) containing the methyl esters was transferred to a new, pre-weighed, test tube and dried under N₂. The methyl esters were diluted with dichloromethane (CH₂Cl₂) to 5 mg/mL and 100 μL were transferred to gas chromatography vials. CH₂Cl₂ (900 μL) was added to the solution to dilute to 0.5 mg/mL. The fatty acid composition of each dietary fat source was then analyzed on the gas chromatograph (Hewlett Packard 5890A GC with 7673A autosampler and 3393A integrator) using a DB-23 fused silica capillary column (30m x 0.25mm x 0.25μ) (S & W Scientific, Folsom, CA). Retention times were verified with purified standards. Results were expressed as percent of total fatty acids.

4.6 **Blood glucose analysis**

Blood was collected in eppendorf vials containing heparin and sodium fluoride (NaF) to obtain plasma. Following blood collection, samples were spun in a Beckman model TJ-6 centrifuge at 2500 rpm for 15 min at 4°C. Plasma was separated and frozen at -70°C. At the time of analysis, samples were thawed and re-spun in a centrifuge in order to separate any remaining impurities. A Beckman Glucose Analyzer 2 was utilized to determine the blood glucose values. The analyzer requires 10μl of plasma to be manually pipetted into enzyme reagent in a cup containing an
electrode that responds to oxygen concentration. Solid-state electronic circuitry determines the rate of oxygen consumption, which is directly proportional to the concentration of glucose in the sample. Samples were measured in duplicate, and in triplicate if there was a discrepancy in values between the first two measurements of ±3% or greater.

4.7 Blood insulin analysis

All plasma samples were sent to Dr. Margaret T. Behme (University Hospital, London, Ontario) for analysis. Plasma immunoreactive insulin was measured by radioimmunoassay with dextran-coated charcoal separation (Herbert et al., 1965) using ¹²⁵I-insulin (Amersham Canada Limited, Oakville, ON), rat insulin from Novo (Copenhagen, Denmark), and insulin antibody from P. Wright (Cambridge, U.K.).

4.8 Fecal analysis

To recover HSB as well as other lipid sources from feces, fecal lipid was extracted using a modification of the method of Folch et al. (1957). Feces were freeze-dried using a Labconco Freeze Dryer 18 until a constant weight was reached. Each sample was analyzed for lipid content in duplicate. A 0.5 g sample was weighed into a test tube and 0.5 mL of water were added. Methanol (5 mL) followed by chloroform (10 mL) were added to the tube. The tube was vortexed and heated in a hot-water bath (60°C) for 1 hour. Heating was necessary to completely extract HSB, since this fat source does not readily dissolve in the normal chloroform-methanol Folch solution. The sample was centrifuged at 1000 rpm for 10 min, and then re-heated at 60°C for 15 min in order to dissolve any particles that may have precipitated during cooling in the centrifuge. The solution was transferred with a Pasteur pipette to a second test tube. No filtering was used to avoid any cooling of the solution which would allow HSB to solidify and remain on the filter. The remaining solid residue was re-extracted with 5 mL of methanol and 10 mL of chloroform, vortexed and heated at 60°C for 1 hour. The first tube was centrifuged at 1000 rpm for 10 min and re-heated in the hot-water
bath (60°C) for 15 min. The solution was removed and added to the solution from the first extraction. The solid residue was discarded. KCl (0.88%) in water was added to the solution in a proportion of one-quarter of the total volume (about 7 mL). The tube was vortexed and centrifuged at 1500 rpm for 5 min to obtain two distinct layers. The bottom layer containing the purified lipid was transferred to a third, pre-weighed, test tube. The solution was dried with N₂ until only a solid lipid residue remained. Finally, the tube was freeze-dried over-night in a Savant Speed Vac Concentrator to ensure complete drying of the sample. The weight of total lipid was determined by difference.

By utilizing samples of diet containing known concentrations of fat, and comparing the results to another extraction method (Solvent extraction with Tecator Soxtec System HT; Hoganas, Sweden), it was determined that approximately 95% of lipids were recovered using the modified Folch extraction method.

Samples of diet were also analyzed using the method of Jeejeebhoy et al. (1970) for determining fecal fat content, but HSB was found to be insoluble in the heptane-diethyl ether-ethanol solvent that this method utilizes. Thus, it is recommended that this method not be used for extracting fat from the feces of animals that have consumed HSB.
CHAPTER 5

EXPERIMENT 1: Effect of Dietary Saturated Fatty Acid Chain Length on Macronutrient Selection
5. EXPERIMENT 1: Effect of Dietary Saturated Fatty Acid Chain Length on Macronutrient Selection

5.1 Introduction

Previous research suggests that dietary SFA content is the important characteristic of dietary fat in mediating subsequent macronutrient selection (McGee & Greenwood, 1990a). The MUFA, PUFA, n-3 and n-6 fatty acid content, as well as the PUFA:SFA ratio and the n-6:n-3 ratio were shown to not be important in mediating selection (McGee & Greenwood, 1990a). This evidence indicates that rats fed diets containing higher concentrations of dietary SFAs subsequently choose more energy as protein and less as carbohydrate than animals fed diets containing lower concentrations of SFAs.

The mechanism by which SFAs mediate macronutrient selection is unclear. It may be beneficial to look further into whether the SFA effect is only related to total SFA concentration, or if specific chain length SFAs play a specific role. The possibility that SFA chain length is important in mediating feeding behaviour seems plausible as the metabolic effects of various fatty acids differ in many respects. The purpose of Experiment 1 was to investigate whether dietary SFA chain length influences macronutrient selection. The effects of medium-chain saturated fatty acids (MSFA, 8:0 and 10:0), intermediate-chain saturated fatty acids (ISFA, 12:0 and 14:0) and long-chain saturated fatty acids (LSFA, ≥16:0) on this feeding behaviour were determined.

Animals fed fat sources enriched in LSFA (e.g., beef tallow (about 44% LSFA), lard (about 37% LSFA) (United States Department of Agriculture, 1979)) prefer protein (or avoid carbohydrate) compared to animals fed high PUFA fat sources (e.g., soybean oil, corn oil) (Crane & Greenwood, 1987; McGee & Greenwood, 1989; McGee & Greenwood, 1990a; Mullen & Martin, 1990; McGee & Greenwood, 1991; Mullen & Martin, 1992a; Grossman et al., 1994). Furthermore, one study has shown
that coconut oil (enriched in ISFAs; contains about 61% ISFAs (United States Department of Agriculture, 1979)) appears to act similarly to corn oil (Mullen & Martin, 1990). These observations indicate that the consumption of dietary fatty acids that are more rapidly oxidized (e.g., PUFAs and shorter chain fatty acids (Jones et al., 1985; Leyton et al., 1987)) leads to a relatively higher intake of carbohydrate and a lower intake of protein.

The objective of the present experiment was to determine whether a correlation exists between SFA chain length and macronutrient selection. The expectation was that rats fed a high LSFA diet (slowest rate of oxidation) would choose a higher protein diet than animals fed a high ISFA (medium rate of oxidation) diet which would in turn choose a higher protein diet than rats fed a high MSFA (faster rate of oxidation) diet. Such a correlation between chain length and selection would suggest that rates of fatty acid oxidation may be important in mediating selection. A correlation would suggest that the route of absorption of different chain length SFAs may also be involved in the mechanism. It has been shown that, in a linear fashion, the shorter the fatty acid, the more is absorbed via the portal bloodstream, rather than via the lymph (Senior, 1968). By specifically identifying how SFAs of varying chain length affect macronutrient selection, we will gain insight that may help explain which mechanism is involved in this, as well as other dietary fat mediated behaviours.

5.2 Experimental design

The experimental design is graphically represented in Figure 5.1. Rats were randomly assigned to one of four dietary groups. All rats consumed a single-cup experimental diet for an initial 14-day period. Each single-cup diet contained a fixed ratio of energy from protein, carbohydrate, and fat (24% protein (kcal from casein), 36% kcal from carbohydrate, 40% kcal from fat). All diets were isocaloric and nutritionally adequate. The four diets varied only in fatty acid composition.
**Figure 5.1.** Experimental design. Fat sources: 1) MCT = medium chain triglyceride oil (enriched in 8:0 and 10:0); 2) HCO = hydrogenated coconut oil (enriched in 12:0 and 14:0); 3) HSB = fully hydrogenated soybean oil (enriched in ≥16:0); 4) SBO = soybean oil (enriched in PUFAs).
The fat source was 1) enriched in LSFAs containing fully hydrogenated soybean oil (HSB); 2) enriched in ISFAs containing hydrogenated coconut oil (HCO); 3) enriched in MSFAs containing medium-chain triglyceride oil (MCT); or 4) enriched in PUFAs (low in saturated fatty acids) containing soybean oil (SBO). Furthermore, the HSB, HCO and MCT groups all contained similar amounts of total SFAs. Thus, only SFA composition differed among these three groups (see Table 4.4).

After 14 days on the experimental diets, rats were offered a selection of two diets: a high protein diet (55% protein energy, 5% carbohydrate, 40% fat), or a high carbohydrate diet (5% protein, 55% carbohydrate, 40% fat). For each diet group, the fatty acid composition of selection diets was identical to that in their experimental diets. The two food cups were alternated in position daily. This selection period lasted for an additional 14 days.

Throughout, total food intake and the intake from the high protein and high carbohydrate diets were recorded on alternate days at the same time during the light cycle. Results are expressed in terms of the proportion of total energy consumed as protein or carbohydrate. Initial body weight measures were recorded, as well as every 4 days thereafter. Final body weight measures were also recorded at the end of each period.

On the basis of a power analysis, using estimates of effect size and variance from previous dietary selection studies, it was determined that 10 rats per group should be utilized. A total of 42 rats were used (11 rats were used in the HSB and MCT diet groups, 10 in the HCO and SBO groups).

5.3 Results

5.3.1 Body weight

Following random assignment of rats to diet groups, differences in initial body weights existed, with SBO animals having a greater body weight than HSB or HCO animals (Table 5.1). Covariate analyses were performed to adjust for differences in initial body weight and for differences in body weight at the start of the selection
Table 5.1 Body Weight Data for Rats Fed Various Diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Initial</th>
<th>End of Experimental</th>
<th>End of Selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT</td>
<td>92.2 ± 1.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>203.9 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>303.6 ± 4.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCO</td>
<td>86.9 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>204.5 ± 3.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>306.4 ± 6.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HSB</td>
<td>85.0 ± 3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>184.4 ± 4.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>253.9 ± 7.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBO</td>
<td>95.1 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>217.3 ± 2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>327.8 ± 5.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1 Rats consumed experimental diets (24% protein energy, 36% carbohydrate, 40% fat) for 14 days followed by selection diets for 14 more days. Rats selected from a high protein diet (55% protein, 5% carbohydrate) and a high carbohydrate diet (55% carbohydrate, 5% protein) both containing the same dietary fat as in experimental period. All diets were isoenergetic. Data analyzed by ANOVA. Values are mean ± SEM. N = 10 or 11/diet fat treatment. Values not sharing a common superscripted letter are significantly different (p<0.01).

2 MCT=medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO=hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB=fully hydrogenated soybean oil (enriched in ≥16:0); SBO=soybean oil (enriched in PUFAs)

3 Significant differences result of covariate analysis performed to rule out effect of initial differences in body weight.

4 Significant differences result of covariate analysis performed to rule out effect of differences in body weight at start of selection period.
period. Following the 14-day experimental period, rats in the HCO and SBO groups weighed more than MCT rats, which in turn, weighed more than HSB rats (P<0.01) (Table 5.1). This initial decrease in growth rate in rats fed MCT was anticipated from previous results (Harkins & Sarett, 1968b; Crozier et al., 1987; Chanez et al., 1991; Furuse et al., 1992). However, by the end of the 14-day selection period, body weight did not differ among the MCT, HCO, and SBO groups (Table 5.1). The elimination of the lower growth in MCT animals was also expected based on previous studies (Harkins & Sarett, 1968b; Bach et al., 1975; Bach et al., 1980). HSB rats continued to have lower body weights by the end of the selection period (P<0.01) (Table 5.1).

5.3.2 Total food intake

Over both the experimental period (data not shown) and the selection period, rats in the MCT, HCO, and SBO groups did not differ with respect to total food intake, and HSB rats consumed more food than the other rats (P<0.001) (see Table 5.2).

The rats fed the HSB diet did not completely consume the fat source in their diets. Since fully hydrogenated soybean oil has the unique composition of being almost one hundred percent saturated, it solidified into small pellets immediately following incorporation into the diets. Despite efforts to further grind the pellets into smaller pieces, it was evident by the amount of spillage of these pellets, that different rats within this diet group consumed variable amounts of the fat source ranging from most of it to almost none of it. Thus, although these rats consumed more total food than rats in the other groups, they most likely consumed less total calories, and as a result gained less weight as well.

5.3.3 Macronutrient selection

Since the HSB rats consumed variable amounts of the fat, the selection data for this group were difficult to interpret. Consequently, the other three dietary groups were compared with the data from the HSB group deleted.
Table 5.2 Total Food Intake and Macronutrient Selection During Selection Period

<table>
<thead>
<tr>
<th>Diet</th>
<th>Intake/14 days(^3) (g)</th>
<th>% of Energy(^4) from Protein</th>
<th>% of Energy(^4) from Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT</td>
<td>279.1 ± 5.1(^a)</td>
<td>35.3 ± 3.4(^a)</td>
<td>23.8 ± 3.5(^a)</td>
</tr>
<tr>
<td>HCO</td>
<td>302.9 ± 10.1(^a)</td>
<td>23.9 ± 4.0(^b)</td>
<td>35.5 ± 4.1(^b)</td>
</tr>
<tr>
<td>HSB</td>
<td>337.2 ± 12.1(^b)</td>
<td>22.1 ± 5.7</td>
<td>37.3 ± 5.8</td>
</tr>
<tr>
<td>SBO</td>
<td>317.3 ± 9.2(^a)</td>
<td>20.6 ± 2.9(^b)</td>
<td>38.9 ± 3.0(^b)</td>
</tr>
</tbody>
</table>

\(^1\) Rats consumed experimental diets for 14 days followed by selection diets for 14 more days. See Table 5.1 for further description. Data analyzed by ANOVA. Values are mean ± SEM. \(N\) = 10 or 11/diet fat treatment.

\(^2\) MCT=medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO=hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB=fully hydrogenated soybean oil (enriched in \(\geq\)16:0); SBO=soybean oil (enriched in PUFAs)

\(^3\) Significant differences result of covariate analyses performed to rule out effect of differences in body weight at start of selection period. Values not sharing a common superscripted letter are significantly different (\(p<0.001\)).

\(^4\) Significant differences result of analysis with HSB group deleted. Values not sharing a common superscripted letter are significantly different (\(p<0.01\)).
By the end of the selection period rats consuming the MCT diet chose more energy as protein and less as carbohydrate than rats consuming HCO or SBO (P<0.01) (Table 5.2). HCO and SBO rats did not significantly differ with respect to protein and carbohydrate intake. This pattern of selection did not fully emerge until day 8 of the selection period. By day 6 of the selection period, MCT rats consumed more energy as protein and less as carbohydrate than did SBO animals (P<0.05), but the macronutrient selection pattern of the HCO rats did not differ from either group. However, from day 8 through day 14 of the selection period, MCT rats chose more protein and less carbohydrate than SBO and HCO rats (p<0.02). SBO and HCO rats did not differ from day 8 onwards with respect to protein and carbohydrate selection. See Figure 5.2.

The rats in the HSB group, which appear to have actually been consuming a very low, almost fat-free diet consumed more carbohydrate than protein (not significantly different from rats fed the SBO or HCO diets). See Table 5.2 and Figure 5.2.
Figure 5.2. Percent of energy chosen as protein and as carbohydrate for each 2-day period during the 14-day selection period. Rats consumed one of 4 experimental diets for 14 days followed by selection diets for 14 more days. See Table 5.1 for further description. Data analyzed by ANOVA. Values are mean ± SEM. N = 10/11 per diet fat treatment. Values not sharing a common letter are significantly different (p < 0.05). Rats were fed one of four fat sources: medium chain triglyceride oil (MCT; enriched in 8:0 and 10:0), hydrogenated coconut oil (HCO; enriched in 12:0 and 14:0), fully hydrogenated soybean oil (HSB; enriched in ≥16:0), or soybean oil (SBO; enriched in PUFAs).
5.4 Discussion

Experiment 1 was designed to determine the role of SFA chain length in mediating macronutrient selection. The results demonstrate that rats fed MCT (enriched in MSFAs) select more protein and less carbohydrate than rats fed either HCO (enriched in ISFAs) or SBO (enriched in PUFAs). These data continue to show that dietary fat type influences macronutrient selection in rats. However, a clear association between SFA chain length and macronutrient selection cannot be established since rats fed HSB (enriched in LSFAs) did not uniformly consume this fat. Thus, reliable data was only obtained from two experimental diets (MCT and HCO), in addition to the reference group (SBO). Nevertheless, the results obtained with rats fed MCT and HCO suggest that SFA chain length may be involved in mediating macronutrient selection.

The suggestion that SFA chain length may be important in mediating macronutrient selection is based on previous experiments which suggest that dietary SFA content is the important characteristic of dietary fat in mediating selection (McGee & Greenwood, 1990a). This evidence indicates that rats fed diets containing higher concentrations of dietary SFAs subsequently choose more energy as protein and less as carbohydrate than animals fed diets containing lower concentrations of SFAs. Since these researchers implemented lard and beef tallow as the SFA sources, which are both enriched in LSFAs, the influence of specific chain length SFAs could not be determined. In contrast, in the present study, three dietary fat sources (MCT, HCO, and HSB) which contain similar concentrations of total SFAs but differ in their relative concentrations of different chain length SFAs were utilized. SBO (low SFA-high PUFA content) was used as a reference fat for comparison.

Clearly, rats fed MCT for 14 days subsequently chose more protein and less carbohydrate than rats fed either HCO or SBO. The animals that were fed the high LSFA diet in the present experiment (HSB) did not uniformly consume the fat source, and their macronutrient selection profile is therefore unclear. Total food intake over
the selection period did not differ among the three dietary groups, which indicates that the differences in feeding behaviour were specific to protein and carbohydrate selection (see Table 5.2).

One possible explanation for these results is that there is something specific about MCT that is important in mediating selection. The metabolism of MCT (which has been compared to carbohydrate metabolism because of the rapid absorption and oxidation of this fat source (Bach & Babayan, 1982)) and the relatively high glycerol content of this fat may have influenced the MCT-fed animals to select a relatively high protein-low carbohydrate diet. If these factors are indeed important, these data would suggest that while MCT may play a unique behavioural role, the results would not support the hypothesis that SFAs are the important component mediating selection.

An alternative explanation, however, is that the SFA chain length composition of MCT and HCO is important in mediating the differences in feeding behaviour between these two fats. In support of this argument, animals that consumed MCT and HCO selected differently, and the most prominent feature of these two diets is that both contain the same amount of total SFAs, but differ in their relative compositions of different chain length SFAs. Furthermore, research by Mullen and Martin (1990) suggests that the fatty acid composition of coconut oil (high ISFAs) may be important in mediating selection following the short-term feeding of this fat. These researchers found that rats fed a coconut oil diet selected protein at an intermediate level compared to animals fed corn oil (high PUFAs) or beef tallow (high LSFAs) (coconut oil-fed rats did not select a diet which was significantly different from rats fed corn oil or beef tallow). Further research using a fat source that is enriched in LSFAs and can be properly incorporated into the diet is needed to more clearly determine whether or not SFA chain length is important in mediating macronutrient selection.

It is interesting to note that although a trend in selection patterns is evident after two days of selection, the eventual patterns do not significantly appear until after eight days of selection (see Figure 5.2). In particular, the MCT group appeared to
continually select more and more protein and less and less carbohydrate for the first ten days of selection until finally leveling off. Thus, the mechanism involved appears to take a period of time to fully influence the rats' feeding behaviour.

Since HSB was not uniformly consumed by the animals, an attempt to hypothesize possible associations between SFA chain length and selection behaviour, based on comparisons with previously published data was made. The selection profile observed with the MCT rats in the present study is similar to the profile observed with rats in previous experiments fed lard or beef tallow diets (enriched in LSFAs) (Crane & Greenwood, 1987; McGee & Greenwood, 1989; McGee & Greenwood, 1990a; Mullen & Martin, 1990; McGee & Greenwood, 1991; Mullen & Martin, 1992b; Grossman et al., 1994). That is, in all of these previous studies (see Table 2.2), rats fed the high LSFA source selected more protein and less carbohydrate than the rats that were fed the high PUFA source (SBO or corn oil). Thus, the relationship that can be suggested, based on the present results and those of previous studies, is that rats fed diets enriched in MSFAs (MCT group) and LSFAs (beef tallow, lard) select more energy from protein and less from carbohydrate than rats fed a diet enriched in ISFAs (HCO group) or in PUFAs (SBO group). This suggested relationship has its limitations because lard and beef tallow only contain about 39% and 50% total SFAs (United States Department of Agriculture, 1979), whereas the MCT and HCO diets used in the present experiment contain about 85% total SFAs. Thus, by including lard or beef tallow, not only does relative composition of SFA chain length vary across groups, total SFA content also varies.

The proposed association between SFA chain length and macronutrient selection is shown in Figure 5.3. The protein and carbohydrate selection behaviour of each group over the 14-day selection period (from Experiment 1) and the expected selection pattern of HSB is shown for comparison (based on a previous experiment using another high LSFA fat source, beef tallow (McGee & Greenwood, 1989)). This figure suggests that the influence of ISFAs (HCO) on protein and carbohydrate
Figure 5.3. Total macronutrient selection over 14-day selection period. Data from HSB group were deleted from this analysis. (HSB) = expected selection based on selection from previous experiment using a high long-chain saturated fat source (beef tallow) (McGee & Greenwood, 1989). Rats consumed one of 4 experimental diets for 14 days followed by selection diets for 14 more days. See Table 5.1 for further description. Data analyzed by ANOVA. Values are mean ± SEM. N = 10/11 per diet fat treatment. Values not sharing a common letter are significantly different (p<0.01). Rats were fed one of four fat sources: medium chain triglyceride oil (MCT; enriched in 8:0 and 10:0), hydrogenated coconut oil (HCO; enriched in 12:0 and 14:0), fully hydrogenated soybean oil (HSB; enriched in ≥16:0), or soybean oil (SBO; enriched in PUFAs).
selection may not be intermediate between the influence of MSFAs (MCT) and LSFAs (beef tallow/expected HSB), which suggests that the connection between SFA chain length and selection may be bell-shaped. In other words, there may be no simple association between increasing SFA chain length and increasing protein intake (or decreasing carbohydrate intake). It is proposed that rats fed shorter and longer chain SFAs select a high protein, low carbohydrate diet, whereas rats fed ISFAs choose the opposite profile of selection.

If indeed the proposed bell-shaped association is correct, then the data would suggest that whole-body fatty acid oxidation and routes of fatty acid absorption are not the prime parameters involved in directing protein and carbohydrate selection. As discussed previously, both rates of fatty acid oxidation and routes of absorption vary directly with SFA chain length (Senior, 1968; Harkins & Sarett, 1968a; Leyton et al., 1987). It was hypothesized that SFA chain length was the mediating factor in protein and carbohydrate selection via a peripheral pathway possibly related to these parameters. Thus, it was expected that there would be a correlation between SFA chain length and protein and carbohydrate selection. This could explain the findings of previous studies (Crane & Greenwood, 1987; McGee & Greenwood, 1989; McGee & Greenwood, 1990a; McGee & Greenwood, 1991; Mullen & Martin, 1990; Mullen & Martin, 1992a; Grossman et al., 1994) which suggest that rats fed a high LSFA diet (beef tallow or lard) choose more protein and less carbohydrate than rats fed a high ISFA diet (coconut oil) or a high PUFA diet (soybean or corn oil). If the hypothesis was correct, rats fed MCT oil (enriched in MSFAs) should consume a low protein, high carbohydrate diet compared to rats fed HCO (enriched in ISFAs), which should in turn choose less protein and more carbohydrate than the HSB group (enriched in LSFAs). In other words, the HCO group would be expected to select somewhere in between MCT and LSFA rats. Furthermore, the SBO (enriched in PUFAs) group would be expected to choose similarly to the shorter fatty acid groups because of their similar rapid rates of oxidation compared to LSFAs.
The SBO and HCO groups chose similarly to what was expected based on previous studies (i.e., a low protein, high carbohydrate diet). In contrast, as mentioned, the MCT animals showed the exact opposite trend of selection of what was anticipated. These animals chose similarly to the selection pattern shown in past studies by rats fed a high LSFA diet (i.e., high protein, low carbohydrate diet compared to animals fed a high PUFA diet). Thus, it appears that the dietary fat effect on selection cannot be explained by relative rates of fatty acid oxidation or by routes of absorption. The observed feeding behaviour of the rats can also not be explained simply by the absolute content of SFAs in the fat source. If this were the mediating factor then rats fed MCT, HCO, or any other high saturated fat diet should choose similarly.

The results of Experiment 1 therefore suggest that there may be no obvious link between routes of fatty acid absorption or rates of whole-body fatty acid oxidation and macronutrient selection. These findings are consistent with those previously reported, which suggested that varying the dietary n-6 to n-3 fatty acid ratio (these fatty acids differ in rates of oxidation (Leyton et al., 1987)) had no affect on macronutrient selection (McGee & Greenwood, 1990a).

In contrast to the general consensus in the literature that rates of whole-body oxidation of SFAs increases with decreasing chain length, some research suggests that such a relationship between SFA chain length and rates of peroxisomal beta-oxidation does not exist. Chance and McIntosh (1994) showed that rates of beta-oxidation of SFAs in peroxisomes isolated from rat liver actually exhibited a bell-shaped association with chain length. The highest rate of oxidation was found with an ISFA (12:0), followed by 16:0, 18:0 and 8:0. C4:0 was not oxidized at all. In other words, the ISFA was oxidized at the highest rate and both the MSFA and the LSFAs were oxidized at slower rates. Research by Lazarow (1978) supports the suggestion that peroxisomal oxidation of both MSFAs and LSFAs is more rapid than the oxidation of ISFAs. This bell-shaped association between SFA chain length and rates of beta-oxidation may be partly explained by noting that the prominent feature of peroxisomal beta-oxidation
appears to be chain-shortening of long-chain fatty acids, rather than complete beta-oxidation (Osmundsen et al., 1991). Thus, very short-chain SFAs are not oxidized at all, whereas, as the SFA chain length increases above 12 carbons there is a decrease in the rate of beta-oxidation since more chain shortening occurs with the longer chain fatty acids.

The bell-shaped association between SFA chain length and peroxisomal oxidation rates is similar to the proposed bell-shaped association between SFA chain length and macronutrient selection that was suggested by the results of Experiment 1 and from those of previous experiments. Thus, although the peroxisomal fatty acid oxidation studies were performed in vitro, it is tempting to speculate that peroxisomal fatty acid oxidation rates may be involved in mediating macronutrient selection. Although peroxisomes are estimated to account for only about 10% of fatty acid oxidation in the liver under normal circumstances (Osmundsen et al., 1991), high fat diets have been shown to significantly increase hepatic peroxisomal beta-oxidation (Neat et al., 1980; Neat et al., 1981; Thomassen et al., 1982; Thomassen et al., 1985). Thus, if the magnitude of action of peroxisomal fatty acid oxidation can be shown to be functionally significant, then this parameter may indeed be important in mediating dietary fat-induced macronutrient selection.

In past studies which suggested that SFA concentration was important in mediating selection, dietary cholesterol and peroxide products were considered as potential variables (McGee & Greenwood, 1990a). Although not specifically tested, the present data suggest that dietary cholesterol and peroxide products are not important in mediating macronutrient selection. Since only vegetable oils were utilized, which are free of dietary cholesterol, and differences in selection patterns were found among the dietary groups, it appears that cholesterol is not important in mediating selection. However, the role of plant sterols can not be ruled out. Since differences in selection were found between diets containing high concentrations of
SFAs, which would have little opportunity to form peroxide products, it also appears that this variable is not important in mediating macronutrient selection.

The selection pattern of rats in the HSB group is not clear as these animals did not uniformly consume the fat source in their diet. However, a notable, but inconclusive finding of Experiment 1 was that the rats in this group chose considerably more carbohydrate than protein (see Table 5.2 and Figure 5.2). Thus, it appears that rats ingesting low levels of fat, although not deficient in linoleic and α-linolenic acid, replace these calories with a surplus of energy from carbohydrate.

In conclusion, the present experiment suggests that SFA chain length may be an important mediator of subsequent protein and carbohydrate selection. It is proposed that rats fed a diet containing a high concentration of MSFAs or a high concentration of LSFAs subsequently select more protein and less carbohydrate than rats fed a diet containing a high concentration of ISFAs. This suggests that rates of whole-body fatty acid oxidation as well as routes of fatty acid absorption may not be primary factors in mediating dietary fat-induced macronutrient selection, but that peroxisomal fatty acid oxidation may be involved. Numerous studies indicate that dietary fat sources influence protein and carbohydrate selection in a highly consistent manner, which suggests that some mechanism precisely mediates this feeding behaviour. The proposed hypothesis suggests that the parameters involved in the mechanism may vary in a bell-shape with SFA chain length. That is, both the shortest (MSFAs) and longest (LSFAs) chain SFAs would be expected to affect some parameter in a similar manner, yet the intermediate length SFAs (ISFAs) would be expected to influence the same parameter differently.
CHAPTER 6

EXPERIMENT 2: Effects of Dietary Saturated Fatty Acid Chain Length on Insulin Concentration, Glucose Tolerance, and Macronutrient Selection
6. EXPERIMENT 2: Effects of Dietary Saturated Fatty Acid Chain Length on Insulin Concentration, Glucose Tolerance, and Macronutrient Selection

6.1 Introduction

Previous research suggests that SFA content is the important component of dietary fat in mediating macronutrient selection (McGee & Greenwood, 1990a). Experiment 1 further suggests that specific SFA chain length is important in mediating selection. In addition, this experiment suggests that rates of whole-body fatty acid oxidation and varying routes of absorption of different fatty acids may not be the primary mechanism that mediates this feeding behaviour. The proposed bell-shaped association between SFA chain length and macronutrient selection suggested by Experiment 1 and previous studies shows a similar pattern to rates of peroxisomal fatty acid oxidation, which opens the possibility that this parameter may be involved in the mechanism. Despite these significant findings, the mechanism that mediates dietary fat-induced macronutrient selection remains unclear.

Experiment 2 was designed to assess the role of insulin sensitivity and glucose tolerance in the mediating mechanism. SFAs have been implicated as mediators of insulin response. High SFA diets have been linked to the impairment of insulin sensitivity and the development of insulin resistance (van Amelsvoort et al., 1986; Field et al., 1989; Storlien et al., 1991; Pan & Berdanier, 1991; Clandinin et al., 1993). Moreover, research by van Amelsvoort et al. (1988) suggests a specific role for SFAs, rather than PUFAs or the SFA:PUFA ratio in regulating insulin response. Relevant to the current hypothesis, the same fat sources that have been shown to influence feeding behaviour also differentially affect insulin concentration. Rats fed a high beef tallow diet (Mullen & Martin, 1992b) or a high lard diet (Kaufman et al., 1994; Dulloo et al., 1995) have a higher fasting insulin concentration than rats fed a high PUFA diet. Both intravenous MCT injection (Lasekan et al., 1992) and MCT diets (Hill et al., 1990; Nakamura, 1994) have also been shown to increase insulin concentration in
comparison to a high PUFA injection or diet. A higher fasting insulin concentration generally indicates a decrease in insulin sensitivity.

One recent study appears to suggest that there may be a correlation between the influence of fatty acids on insulin release and the influence of fatty acids on macronutrient selection. Opara et al. (1994) perifused islets from mouse pancreases with fatty acids of various chain lengths, and found an inhibitory effect on basal insulin secretion with 4:0 and 18:0, no effect with 6:0 and 16:0, and a stepwise increase in insulin secretion from 8:0 to a peak with 12:0, and a lesser influence with 14:0. Thus, the effect of fatty acid stimulated insulin secretion was bell-shaped, with shorter and longer chain SFAs showing minimal influence, and intermediate length SFAs showing the strongest influence.

Although the study by Opara et al. (1994) was performed in vitro on mice, these results can be compared with the selection data from Experiment 1. The influence of SFA chain length on insulin secretion and the proposed relationship between dietary SFA chain length and subsequent carbohydrate selection are both bell-shaped (see Figure 6.1). Dietary fat sources enriched in LSFAs (e.g., beef tallow, lard) and MSFAs (e.g., MCT oil) influence rats to choose a relatively high protein-low carbohydrate diet, and these fatty acids minimally affect insulin secretion in the mouse pancreas. Rats fed fat sources enriched in ISFAs (e.g., hydrogenated coconut oil) consume the opposite pattern of selection and these fatty acids highly stimulate insulin release. The high PUFA sources (e.g., soybean and corn oil) influence selection similarly to the high ISFA sources, and also highly stimulate insulin secretion.

Based on the current literature it appears reasonable that insulin may be a component of the mechanism involved in macronutrient regulation following dietary fat consumption. Research supports the notion that insulin sensitivity responds to differences in dietary fatty acid composition. The same fat sources that influence
**Figure 6.1.** Rationale for experiment 2. Results from Opara et al. (1994) looking at the *in vitro* effect of saturated fatty acid chain length on insulin secretion in mouse pancreases (a) compared to the effect of dietary saturated fatty acid chain length on carbohydrate selection from experiment 1 (MCT and HCO) and from a previous experiment (beef tallow enriched in ≥16:0; McGee & Greenwood, 1989) (b). MCT= medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO= hydrogenated coconut oil (enriched in 12:0 and 14:0).
feeding behaviour also influence insulin concentration. Moreover, consistent with the behavioural literature, dietary SFAs may play a more important role than other components of dietary fat in influencing insulin sensitivity. Finally, since the reason that SFA chain length influences any parameter in a bell-shaped manner is not readily obvious, the data that suggest that SFA chain length influences insulin response in such a manner is relatively unique.

Experiment 2 was designed to examine the relationship between SFA chain length and macronutrient selection (original objective of Experiment 1) and to investigate the hypothesis that dietary fat-induced changes in glucose or insulin concentration, or glucose tolerance, may be involved in regulating macronutrient selection.

### 6.2 Experimental design

#### 6.2.1 Design

As in Experiment 1, four different fat sources were used in Experiment 2. To repeat the selection profiles of Experiment 1, MCT, SBO and HCO were again utilized. To more reliably examine the selection pattern of a long-chain saturated fat source, HSB was incorporated into the diet as a fine powder to ensure a homogenous mixture (see Materials and Methods section for details of diet preparation). The total amounts of SFAs remained similar between the MCT, HCO and HSB groups. To investigate the connection between the effect of long-term dietary fat consumption on macronutrient selection and insulin sensitivity, fasting and expected peak insulin concentration were measured and an oral-glucose tolerance test was performed following an over-night fast.

The experimental design is graphically represented in Figure 6.2. The first 28 days of Experiment 2 were identical to Experiment 1 except 2-day fecal output was collected from all animals at the end of both the experimental and selection periods to analyze the extent of fatty acid absorption for each fat source. Plasma insulin and glucose tolerance were measured following the selection period. All rats were
Figure 6.2. Experimental design. The experiment was performed in two phases (20/21 rats in each phase). The second phase began one week after the first phase began. Fat sources: 1) MCT = medium chain triglyceride oil (enriched in 8:0 and 10:0); 2) HCO = hydrogenated coconut oil (enriched in 12:0 and 14:0); 3) HSB = fully hydrogenated soybean oil (enriched in ≥16:0); 4) SBO = soybean oil (enriched in PUFAs).
weighed and food intake was measured every second day throughout the entire experiment. The experiment was performed in two phases. Both phases were identical to each other so that results could be collapsed together, but each phase consisted of only 5 rats per group instead of 10. The second phase of the experiment started one week after the first phase started.

Following the 28 days of feeding (14 days on experimental diets, followed by 14 days on selection diets) rats were re-fed the experimental diets for at least 4 days to stabilize for differences in protein and carbohydrate selection. Following the four days of stabilization feeding, food cups were removed as rats were fasted over-night for an oral glucose tolerance test the next morning. This was repeated for four rats (one from each dietary group) each day until blood was collected from all 40 rats. Rats were unanaesthetized and minimally restrained during blood collection. However, xylocaine, a topical anesthetic, was applied to the tip of the tail to reduce stress to the animal. About 10 min after the xylocaine was applied, one small clip to the tail (about 1 mm) was made with a pair of sharp scissors and blood was immediately collected as the fasting blood sample. After 5 minutes, rats were given an oral dose of 1.5 g glucose/kg body weight as a 25% glucose solution in normal saline (0.9% NaCl) via gavage. Blood samples were collected by removing the scab from the end of the tail at -5, 10, 15, 20, 30, 45, 60, 90, 120, and 150 min following the glucose load. All samples were collected in eppendorf vials containing heparin and NaF and centrifuged to obtain plasma. At -5 and 15 min, 300 μL of blood were collected in order to obtain enough plasma to analyze both plasma glucose and plasma insulin values at the fasting and expected peak times. The expected peak time has been established previously (Williamson et al., 1996). At all other times, 100 μL of blood were collected for plasma glucose analysis only.

The amount of total blood collected from each rat was 1.4 mL (10 samples were collected for glucose analysis but only fasting and peak samples were collected for insulin analysis).
Post-mortem plasma insulin values were also determined. Blood was collected from the trunk of the animals following decapitation (rats were anesthetized with CO₂ prior to decapitation) approximately 210 min after the glucose load (i.e., 1 hour after the last blood sample was collected).

6.2.2 Design rationale

Because the blood collection procedure requires an extended time period for each animal, the experiment was performed in two phases to avoid a possible confounding variable (different rats being fed for much longer periods than others before blood collection).

Rats that have not been handled on a regular basis tend to become stressed when they are handled. Since blood collection requires extensive handling of the animals, all rats were handled every second day throughout the entire experiment instead of every fourth day (as in Experiment 1) so that the blood collection procedure would be less stressful.

To examine the influence of the fat sources on insulin concentration and glucose tolerance, the blood collection procedure would ideally be performed at the end of the initial 14-day experimental period. This would make it possible to determine the state of the animals after 14 days of adapting to the various fat sources, and immediately prior to selection. However, because of the possible stress to the animals involved, and the possible influence that this may have on their subsequent behaviour during the selection period, the blood collection procedure was performed following the selection period. Rats were put back onto experimental diets after the selection period for a minimum of 4 days to stabilize for the differences in protein and carbohydrate selection which occurred during the selection period. Since type and amount of fat were constant throughout the entire experiment, the effect of fat source alone on these parameters could be determined.

To minimize pain and stress to the animals, which can influence blood glucose and insulin concentration (Gartner et al., 1980; Joint working group on refinement,
1993), rats were unanaesthetized (anaesthetic can influence blood characteristics) and minimally restrained during blood collection.

The amount of total blood collected from each rat was 1.4 mL. To avoid the risk of putting the rats into shock, the maximum amount of blood that can safely be collected is 10% of the total volume in the animal (Joint working group on refinement, 1993). Rats contain approximately 50 mL blood per 1 kg. Since the rats in this experiment weighed about 300 g at the time of blood collection, their total blood volume was about 15 mL (10% of this is 1.5 mL). Because of this limitation, 10 samples were collected for glucose analysis but only fasting and peak samples were collected for insulin analysis.

6.3 Results

6.3.1 Body weight

Initial body weights did not differ among any of the four dietary groups (Table 6.1). Following the 14-day experimental period, rats in the HCO and SBO groups weighed more than MCT and HSB rats (P<0.0002) (Table 6.1). This initial decrease in growth rate in rats fed MCT was anticipated from previous results (Furuse et al., 1992; Experiment 1 from the present thesis). Over the 14-day selection period, however, changes in body weight did not differ among any of the four dietary groups (Table 6.1) (covariate analysis was performed to adjust for differences in body weight at the start of the selection period (end of experimental period)).

6.3.2 Total food intake

Over the 14-day experimental period and over the 14-day selection period (Table 6.2), rats in the MCT, HCO, and SBO groups did not differ with respect to total food intake. Over both feeding periods, rats fed the HSB diet consumed more food than rats fed the other three diets (P<0.0001) (Table 6.2).
Table 6.1 Body Weight Data for Rats Fed Various Diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Initial</th>
<th>End of Experimental</th>
<th>End of Selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT</td>
<td>84.9 ± 2.1a</td>
<td>198.0 ± 3.1a</td>
<td>293.9 ± 4.8a</td>
</tr>
<tr>
<td>HCO</td>
<td>85.5 ± 1.7a</td>
<td>214.5 ± 3.4b</td>
<td>327.4 ± 7.0a</td>
</tr>
<tr>
<td>HSB</td>
<td>85.4 ± 1.7a</td>
<td>201.0 ± 2.5a</td>
<td>303.2 ± 3.2a</td>
</tr>
<tr>
<td>SBO</td>
<td>84.6 ± 1.6a</td>
<td>214.1 ± 3.0b</td>
<td>323.5 ± 6.6a</td>
</tr>
</tbody>
</table>

1. Rats consumed experimental diets (24% protein energy, 36% carbohydrate, 40% fat) for 14 days followed by selection diets for 14 more days. Rats selected from a high protein diet (55% protein, 5% carbohydrate) and a high carbohydrate diet (55% carbohydrate, 5% protein) both containing the same dietary fat as in experimental period. All diets were isoenergetic. Data analyzed by ANOVA. Values are mean ± SEM. N = 10 or 11/diet fat treatment. Values not sharing a common superscripted letter are significantly different (p<0.0002).

2. MCT=medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO=hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB=fully hydrogenated soybean oil (enriched in ≥16:0); SBO=soybean oil (enriched in PUFAs)

3. No significant differences result of covariate analysis performed to adjust for differences in body weight at start of selection period.
Table 6.2 Total Food Intake Over Experimental and Selection Periods

<table>
<thead>
<tr>
<th>Diet²</th>
<th>Intake/14 days³ experimental (g)</th>
<th>Intake/14 days³ selection (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT</td>
<td>240.3 ± 3.5³</td>
<td>293.1 ± 5.7³</td>
</tr>
<tr>
<td>HCO</td>
<td>254.5 ± 5.7³</td>
<td>330.5 ± 9.6³</td>
</tr>
<tr>
<td>HSB</td>
<td>307.4 ± 6.5³</td>
<td>419.0 ± 18.3³</td>
</tr>
<tr>
<td>SBO</td>
<td>255.6 ± 5.1³</td>
<td>329.0 ± 6.7³</td>
</tr>
</tbody>
</table>

¹ Rats consumed experimental diets for 14 days followed by selection diets for 14 more days. See Table 6.1 for further description. Data analyzed by ANOVA. Values are mean ± SEM. N = 10 or 11/diet fat treatment.

² MCT=medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO=hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB=fully hydrogenated soybean oil (enriched in ≥16:0); SBO=soybean oil (enriched in PUFAs)

³ Values not sharing a common superscripted letter are significantly different (p<0.0001).
6.3.3 Comparison of total food intake to body weight gain

A comparison of total food intake and total body weight gain over the 14-day selection period is shown in Figure 6.3. Rats in the HSB group consumed more total food than animals in each of the other three dietary groups (P<0.0001), yet these rats did not gain more weight than the animals in the other dietary groups. This observation was surprising since all diets were isocaloric.

6.3.4 Fecal excretion

Fecal samples were collected over a 2-day period at the end of the experimental period. The 2-day fecal excretion (dry weight) for the HSB rats was greater than the excretion for the HCO and SBO rats (Figure 6.4), which was in turn greater than the excretion for the MCT animals (P<0.0001). The lower fecal weight excreted by MCT animals may be partly explained by noting that the MCT experimental diet contained only 75.0 g/kg dietary fibre rather than 88.5 g/kg (content in HCO, HSB and SBO diets; Table 4.1). This difference was necessary in order to make all diets isocaloric, since MCT oil only contributes 8.3 kcal/g.

6.3.5 Fecal fat

Feces were analyzed for fat content in order to determine the source of the greater fecal excretion in the HSB group. The feces excreted by HSB animals contained a higher percentage of fat than the feces excreted by HCO animals (Table 6.3). In turn, the feces excreted by HCO animals contained a higher percentage of fat than the feces excreted by MCT and SBO animals (P < 0.0001).

Almost 60% of the dietary fat consumed by HSB animals was excreted in the feces, and therefore not absorbed by these animals (Table 6.4). HCO rats excreted less dietary fat in their feces than HSB rats (P<0.0001), but more than MCT or SBO animals (P<0.0001), which did not significantly differ from each other.

Since the MCT, HCO and HSB diets were supplemented with flaxseed and safflower oil to eliminate the possibility of a linoleic or α-linolenic deficiency, the total
Figure 6.3. Total body weight gain (a) and total food intake (b) over 14-day selection period. Rats consumed one of 4 experimental diets for 14 days followed by selection diets for 14 more days. See Table 6.1 for further description. Rats were fed one of four fat sources: medium chain triglyceride oil (MCT; enriched in 8:0 and 10:0), hydrogenated coconut oil (HCO; enriched in 12:0 and 14:0), fully hydrogenated soybean oil (HSB; enriched in >16:0), or soybean oil (SBO; enriched in PUFAs). Data analyzed by ANOVA. For (a), data were analyzed using covariate analysis to adjust for differences in body weight at start of selection period. Values are mean ± SEM. N = 10 or 11/diet fat treatment. Values not sharing a common letter are significantly different (p < 0.0001).
Figure 6.4. 2-Day dried fecal excretion at end of experimental period. Feces were collected on day 14 of the experimental period which represents total fecal output over days 13 and 14. Rats consumed one of 4 experimental diets for 14 days followed by selection diets for 14 more days. See Table 6.1 for further description. Medium chain triglyceride oil (MCT; enriched in 8:0 and 10:0), hydrogenated coconut oil (HCO; enriched in 12:0 and 14:0), fully hydrogenated soybean oil (HSB; enriched in ≥16:0), soybean oil = SBO (enriched in PUFAs). Data analyzed by ANOVA. Values are mean ± SEM. N = 10 or 11/diet fat treatment. Values not sharing a common letter are significantly different (p<0.0001).
<table>
<thead>
<tr>
<th>Diet</th>
<th>Fat component in feces (% by weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT</td>
<td>3.71 ± 0.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCO</td>
<td>9.08 ± 0.60&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HSB</td>
<td>45.02 ± 0.46&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBO</td>
<td>5.58 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> All values based on analysis of 2-day dried fecal excretion at end of 14-day experimental period. Experimental diets consisted of 24% protein energy, 36% carbohydrate, 40% fat. Data analyzed by ANOVA. Values are mean ± SEM. N = 10 or 11/diet fat treatment. Values not sharing a common superscripted letter are significantly different (p<0.0001).

<sup>2</sup> MCT = medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO = hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB = fully hydrogenated soybean oil (enriched in ≥16:0); SBO = soybean oil (enriched in PUFAs)
Table 6.4 Percent of Total Dietary Fat and Percent of Each Dietary Fat Component Excreted in Feces

<table>
<thead>
<tr>
<th>Diet</th>
<th>% of total dietary fat excreted in feces</th>
<th>Dietary fat component</th>
<th>% of each fat component excreted in feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT</td>
<td>1.52 ± 0.20(^a)</td>
<td>MCT component</td>
<td>1.27 ± 0.24(^a)</td>
</tr>
<tr>
<td>HCO</td>
<td>5.09 ± 0.32(^b)</td>
<td>HCO component</td>
<td>5.49 ± 0.38(^b)</td>
</tr>
<tr>
<td>HSB</td>
<td>58.48 ± 1.09(^c)</td>
<td>HSB component</td>
<td>69.09 ± 12.39(^c)</td>
</tr>
<tr>
<td>SBO</td>
<td>2.99 ± 0.37(^*)</td>
<td>SBO component</td>
<td>2.99 ± 0.37(^*)</td>
</tr>
</tbody>
</table>

\(^1\) All values based on analysis of 2-day dried fecal excretion at end of 14-day experimental period. Experimental diets consisted of 24% protein energy, 36% carbohydrate, 40% fat. Data analyzed by ANOVA. Values are mean ± SEM. N = 10 or 11/diet fat treatment. Values not sharing a common superscripted letter are significantly different (p<0.0001).

\(^2\) MCT=medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO=hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB=fully hydrogenated soybean oil (enriched in ≥16:0); SBO=soybean oil (enriched in PUFAs)

Fat components in each diet:
- MCT = 85.01% MCT, 13.22% Safflower oil, 1.77% flaxseed oil;
- HCO = 83.95% HCO, 14.13% Safflower oil, 1.92% flaxseed oil;
- HSB = 83.95% HSB, 14.16% Safflower oil, 1.89% flaxseed oil;
- SBO = 100.00% SBO.

\(^3\) % of total dietary fat consumed that was excreted in the feces and therefore not absorbed

\(^4\) % of each specific fat source that was excreted in the feces and therefore not absorbed, assuming that the safflower-flaxseed oil component of each diet was absorbed to the same extent as SBO.
fecal fat content does not show the percentage of each actual fat source that was absorbed. Therefore, the specific absorption of each fat component, assuming that the flaxseed-safflower oil mixture was absorbed to the same extent as SBO is also shown in Table 6.4. This assumption is reasonable since flaxseed oil, safflower oil and SBO all contain similar concentrations of SFAs and unsaturated fatty acids. These data show that almost 70% of the HSB component of the HSB diet was not absorbed by the rats fed this fat source. The HCO component of the HCO diet was excreted less than the HSB component (P<0.0001), but more than the MCT or SBO components (P<0.0001), which did not significantly differ from each other.

Taken together, the fecal fat analysis suggests that most of the dietary HSB was not absorbed by the animals that ingested this fat source and therefore passed into the feces. Furthermore, HCO was absorbed significantly less than MCT or SBO.

6.3.6 Effect of fecal fat on absorbed energy

The present data suggest that a huge percentage of HSB was not absorbed, as well as a significant component of each of the other dietary fat sources. Clearly, the nonabsorbable fats did not contribute any energy to the various diets. Thus, although all of the diets were designed to be isocaloric (4.274 kcal/g diet), the animals in different dietary groups did not actually absorb diets of the same caloric density.

The apparent total energy absorption for each diet group over both the experimental and selection periods when calculated assuming that all diets were isocaloric (4.274 kcal/g diet) and with adjusted values based on fecal fat loss are shown in Table 6.5. (Since fecal content of protein and carbohydrate were not determined, the actual absorption of each diet is not known, however, only a very minimal amount of nonabsorption of these macronutrients would be expected and no differences among groups would be expected. The apparent absorption of diets based on loss of dietary fat energy to feces will be referred to as, “absorption”, throughout the thesis). In order to re-calculate the absorbed caloric density of each diet (adjusted for loss of fat energy to feces), all nonabsorbed fats (ingested and excreted in feces) were
Table 6.5  Total Caloric Absorption During Experimental and Selection Periods Adjusted for Fecal Fat Excretion

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total Caloric Intake (4.274 kcal/g diet)</th>
<th>Total Caloric Absorption (4.274 kcal/g diet)</th>
<th>Adj. for Loss of Calories from Fecal Fat Excretion</th>
<th>Adj. for Loss of Calories from Fecal Fat Excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT</td>
<td>1027.0 ± 15.1^a</td>
<td>1020.8 ± 15.0^a</td>
<td>1252.8 ± 24.5^a</td>
<td>1245.2 ± 24.4^a</td>
</tr>
<tr>
<td>HCO</td>
<td>1087.9 ± 24.5^a</td>
<td>1065.8 ± 24.0^a</td>
<td>1412.4 ± 41.2^a</td>
<td>1383.6 ± 40.3^a</td>
</tr>
<tr>
<td>HSB</td>
<td>1314.0 ± 27.6^b</td>
<td>1006.5 ± 21.1^a</td>
<td>1790.8 ± 78.1^b</td>
<td>1371.8 ± 59.8^*</td>
</tr>
<tr>
<td>SBO</td>
<td>1092.6 ± 22.0^*</td>
<td>1079.5 ± 21.7^*</td>
<td>1406.3 ± 28.8^*</td>
<td>1389.5 ± 28.4^*</td>
</tr>
</tbody>
</table>

^1 Rats consumed experimental diets for 14 days followed by selection diets for 14 more days. See Table 6.1 for further description. Data analyzed by ANOVA.

Values are mean ± SEM. N = 10 or 11/diet fat treatment. Values not sharing a common superscripted letter are significantly different (p<0.0001).

^2 MCT=medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO=hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB=fully hydrogenated soybean oil (enriched in ≥16:0); SBO=soybean oil (enriched in PUFAs)

^3 Adjusted kcal/g diet values based on 2-day dried fecal fat excretion: MCT = 4.248; HCO = 4.187; HSB = 3.274; SBO = 4.223
given the Atwater value of 9 kcal/g except MCT, which was given a value of 8.3 kcal/g (Harkins & Sarett, 1968b). The adjusted absorbed caloric densities of each diet were calculated as follows: MCT = 4.248 kcal/g diet; HCO = 4.187; HSB = 3.274; and SBO = 4.223.

The greater intake of total food by HSB animals (and apparent total calories) over both the experimental and selection periods appears to be completely explained by fecal fat loss (see Table 6.5). That is, when the non-absorbed dietary fat components are considered, it appears that over both dietary periods, animals in all groups absorbed the same number of total calories. Thus, the observation that HSB animals did not gain more weight than the animals in the other dietary groups, even though they consumed more total food, can be explained by the fact that they did not absorb more energy. HSB animals had to consume more total food simply to get the same amount of energy as animals fed the other diets.

6.3.7 Macronutrient selection

6.3.7.1 Analysis to directly compare data to Experiment 1

To compare the present results directly to Experiment 1, data were expressed as percent of energy selected from protein and carbohydrate assuming all diets were isocaloric.

Over the selection period, MCT animals selected more energy as protein and less as carbohydrate than animals in each of the other three dietary groups (P<0.02) (Table 6.6). The macronutrient selection results for rats in the MCT, HCO and SBO groups are consistent with the results of Experiment 1 (compare Table 5.2, Experiment 1 to Table 6.6).

6.3.7.2 Analysis considering dietary fat absorption

In previous macronutrient selection experiments, relative protein and carbohydrate selection results have been expressed as a percentage of total energy consumed. However, in those studies, it had been assumed that all animals consumed isocaloric diets (containing identical levels of dietary fat), whereas this is clearly not the case in the present experiment. In previous studies, as well as the present
Table 6.6 Total Food Intake and Macronutrient Selection During Selection Period

<table>
<thead>
<tr>
<th>Diet</th>
<th>Intake/14 days (g)</th>
<th>% of Energy from Protein</th>
<th>% of Energy from Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT</td>
<td>293.1 ± 5.7a</td>
<td>39.2 ± 3.7a</td>
<td>19.8 ± 3.8a</td>
</tr>
<tr>
<td>HCO</td>
<td>330.5 ± 9.6a</td>
<td>27.3 ± 3.5b</td>
<td>32.0 ± 3.6b</td>
</tr>
<tr>
<td>HSB</td>
<td>419.0 ± 18.3b</td>
<td>23.8 ± 4.8b</td>
<td>35.6 ± 5.0b</td>
</tr>
<tr>
<td>SBO</td>
<td>329.0 ± 6.7a</td>
<td>23.2 ± 3.8b</td>
<td>36.2 ± 3.8b</td>
</tr>
</tbody>
</table>

1 Rats consumed experimental diets for 14 days followed by selection diets for 14 more days. See Table 6.1 for further description. Data analyzed by ANOVA. Values are mean ± SEM. N = 10 or 11/diet fat treatment.

2 MCT=medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO=hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB=fully hydrogenated soybean oil (enriched in ≥16:0); SBO=soybean oil (enriched in PUFAs).

3 Values not sharing a common superscripted letter are significantly different (p<0.0001).

4 Values not sharing a common superscripted letter are significantly different (p<0.02).
experiment, all selection diets were designed to contain 40% of calories from fat and animals had the choice between protein and carbohydrate to make up the remaining 60% of their caloric intake. However, in the present experiment, based on fecal fat excretion, HSB animals actually absorbed only 21.7% of their calories from fat; MCT rats, 39.6%; HCO rats, 38.8%; and SBO rats, 39.3%. Thus, an increase in the selection of protein or carbohydrate in a diet that contains less absorbable fat (e.g., HSB rats) may be the result of a general increase in calories from these macronutrients rather than a specific preference for protein or carbohydrate.

The selection data were analyzed in two ways: 1) To normalize for the differences in absorbed dietary fat calories (and consequently, protein and carbohydrate calories) across dietary groups, the relative preference for protein and carbohydrate was expressed as a percentage of total protein and carbohydrate consumed (total non-fat energy consumed). 2) Data were also analyzed in terms of absorbed energy selected from protein and carbohydrate.

6.3.7.3 Macronutrient selection over 14-day selection period

Over the selection period MCT animals selected relatively more protein (expressed as a percentage of total non-fat energy consumed) and less carbohydrate than rats in the other three dietary groups (P<0.02) (Table 6.7). The selection behaviour among the other three dietary groups did not significantly differ.

Over the selection period, the percent of absorbed energy selected from protein did not significantly differ among groups (Table 6.8). MCT rats selected less energy as carbohydrate than HSB and SBO rats (P<0.002). The carbohydrate intake of the HCO animals did not significantly differ from any of the other three dietary groups. The table also shows that total energy absorbed did not differ among groups, which indicates that the differences in feeding behaviour are specific to protein and carbohydrate selection.
Table 6.7. Macronutrient Selection During Selection Period

<table>
<thead>
<tr>
<th>Diet</th>
<th>% of Energy from Protein as a % of total non-fat energy consumed&lt;sup&gt;3&lt;/sup&gt;</th>
<th>% of Energy from Carbohydrate as a % of total non-fat energy consumed&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT</td>
<td>66.5 ± 6.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.5 ± 6.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCO</td>
<td>46.1 ± 6.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.9 ± 6.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HSB</td>
<td>40.2 ± 8.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59.8 ± 8.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBO</td>
<td>39.1 ± 6.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.9 ± 6.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Rats consumed experimental diets for 14 days followed by selection diets for 14 more days. See Table 6.1 for further description. Data analyzed by ANOVA. Values are mean ± SEM. N = 10 or 11/diet fat treatment. Values not sharing a common superscripted letter are significantly different (p<0.02).

<sup>2</sup> MCT = medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO = hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB = fully hydrogenated soybean oil (enriched in ≥16:0); SBO = soybean oil (enriched in PUFAs)

<sup>3</sup> % of energy from protein = (protein intake (kcal)/protein intake (kcal) + carbohydrate intake (kcal)) * 100

<sup>4</sup> % of energy from carbohydrate = (carbohydrate intake (kcal)/protein intake (kcal) + carbohydrate intake (kcal)) * 100
Table 6.8 Total Caloric Absorption and Macronutrient Selection During Selection Period Adjusted for Loss of Calories from Fecal Fat Excretion

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total caloric absorption/14 days selection adjusted for loss of calories from fecal fat excretion</th>
<th>% of Absorbed Energy Selected from Protein</th>
<th>% of Absorbed Energy Selected from Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT</td>
<td>1245.2 ± 24.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.4 ± 3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.9 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCO</td>
<td>1383.6 ± 40.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.9 ± 3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.6 ± 3.6&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>HSB</td>
<td>1371.8 ± 59.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.0 ± 6.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.5 ± 6.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBO</td>
<td>1389.5 ± 28.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.5 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.7 ± 3.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1 Rats consumed experimental diets for 14 days followed by selection diets for 14 more days. See Table 6.1 for further description. Data analyzed by ANOVA. Values are mean ± SEM. N = 10 or 11/diet fat treatment. Values not sharing a common superscripted letter are significantly different (p<0.002).

2 MCT=medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO=hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB=fully hydrogenated soybean oil (enriched in ≥16:0); SBO=soybean oil (enriched in PUFAs)

3 Adjusted kcal/g diet values based on 2-day dried fecal fat excretion: MCT = 4.248; HCO = 4.187; HSB = 3.274; SBO = 4.223
6.3.7.4 Day-to-day macronutrient selection

The relative protein and carbohydrate selection patterns of each dietary group for each 2-day period over the 14-day selection period are shown in Figure 6.5. Data are expressed as a percentage of total non-fat energy consumed. Relative protein and carbohydrate intake did not differ among any of the dietary groups for any of the 2-day periods up to day 6 of selection. However, for each 2-day period from day 8 onwards, MCT rats selected more protein and less carbohydrate than rats in the other three dietary groups (day 8, 10 and 12: $P<0.01$; day 14: $P<0.0004$). The protein and carbohydrate selection patterns of SBO, HCO, and HSB rats did not differ from day 8 onwards.

The percent of absorbed energy (i.e. corrected for fecal fat excretion) selected as protein and as carbohydrate for each dietary group for each 2-day period of the 14-day selection period are shown in Figure 6.6. Protein intake did not differ among groups until day 8 of selection. From day 8 to day 12 MCT rats selected more protein energy than SBO rats (day 8: $P<0.03$; day 10: $P<0.04$; day 12: $P<0.05$) and HSB and HCO animals did not differ significantly from any of the groups. On day 14, MCT rats selected more protein energy than any of the other dietary groups ($P<0.009$). HSB rats consumed more carbohydrate energy than all three of the other dietary groups on day 2 of selection ($P<0.006$). On days 4 and 6, HSB rats consumed more carbohydrate energy than MCT rats ($P<0.007$) and HCO and SBO groups did not differ from any of the other groups. From day 8 to 14, MCT rats consumed less carbohydrate energy than any of the other dietary groups (day 8 and 10: $P<0.003$; day 12: $P<0.002$; day 14: $P<0.0001$).
Figure 6.5. Percent of energy chosen as protein and as carbohydrate as a percentage of total non-fat energy consumed for each 2-day period during the 14-day selection period. Rats consumed one of 4 experimental diets for 14 days followed by selection diets for 14 more days. See Table 6.1 for further description. Data analyzed by ANOVA. Values are mean ± SEM. N = 10/11 per diet fat treatment. Values not sharing a common letter are significantly different (p < 0.01). Rats were fed one of four fat sources: medium chain triglyceride oil (MCT; enriched in 8:0 and 10:0), hydrogenated coconut oil (HCO; enriched in 12:0 and 14:0), fully hydrogenated soybean oil (HSB; enriched in ≥16:0), or soybean oil (SBO; enriched in PUFAs).
Figure 6.6. Percent of absorbed energy selected as protein and as carbohydrate for each 2-day period during the 14-day selection period, adjusted for loss of fat calories in feces. Rats consumed one of 4 experimental diets for 14 days followed by selection diets for 14 more days. See Table 6.1 for further description. Data analyzed by ANOVA. Values are mean ± SEM. N = 10/11 per diet fat treatment. Values not sharing a common letter are significantly different (p < 0.05). Rats were fed one of four fat sources: medium chain triglyceride oil (MCT; enriched in 8:0 and 10:0), hydrogenated coconut oil (HCO; enriched in 12:0 and 14:0), fully hydrogenated soybean oil (HSB; enriched in ≥16:0), or soybean oil (SBO; enriched in PUFAs).
6.3.7.5 Macronutrient selection over last 8 days of selection period

As shown in Figures 6.5 and 6.6, selection patterns appeared to be more stable from day 8 of selection to day 14. Therefore, selection data were also analyzed over this 8 day period. These data represent 8 days of selection (days 7 through 14 inclusive) since the measurement of intake on each day represents 2 days of selection (e.g., the values on day 8 represent selection over days 7 and 8).

Through days 7 to 14, MCT animals selected relatively more protein and less carbohydrate as a percentage of total non-fat energy consumed than animals in the other three dietary groups (P<0.005) (Table 6.9).

Selection data through days 7 to 14 expressed as the percentages of absorbed energy chosen from each macronutrient are shown in Table 6.10. MCT rats selected more protein energy than SBO rats (P<0.02) and the protein selection of HCO and HSB rats did not differ from any of the other groups. MCT rats selected less carbohydrate energy than rats in all of the other three dietary groups (P<0.0009). Differences in feeding behaviour can be attributed to macronutrient selection since total caloric absorption per gram body weight gain did not differ across groups. The absorption-per-gram-body-weight-gain-data are presented to normalize for any differences in total caloric absorption due to differences in body weights across groups on day 6 of selection (represents body weight at beginning of day 7).
Table 6.9 Macronutrient Selection Over Days 7 to 14 of Selection Period

<table>
<thead>
<tr>
<th>Diet</th>
<th>% of Energy from Protein as a % of total non-fat energy consumed</th>
<th>% of Energy from Carbohydrate as a % of total non-fat energy consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT</td>
<td>72.7 ± 6.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.3 ± 6.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCO</td>
<td>46.3 ± 6.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.7 ± 6.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HSB</td>
<td>42.7 ± 8.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.3 ± 8.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBO</td>
<td>39.5 ± 6.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.5 ± 6.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1 Rats consumed experimental diets for 14 days followed by selection diets for 14 more days. See Table 6.1 for further description. Data represent cumulative intake and selection over days 7 to 14. Data analyzed by ANOVA. Values are mean ± SEM. N = 10 or 11/diet fat treatment. Values not sharing a common superscripted letter are significantly different (p<0.005).

2 MCT = medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO = hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB = fully hydrogenated soybean oil (enriched in 16:0); SBO = soybean oil (enriched in PUFAs)

3 % of energy from protein = (protein intake (kcal)/protein intake (kcal) + carbohydrate intake (kcal)) * 100

4 % of energy from carbohydrate = (carbohydrate intake (kcal)/protein intake (kcal) + carbohydrate intake (kcal)) * 100
Table 6.10 Cumulative Caloric Absorption and Macronutrient Selection Over Days 7 to 14 of Selection Period - Adjusted for Loss of Calories from Fecal Fat Excretion

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total caloric absorption over days 7 to 14 per gram body weight gain</th>
<th>% of Absorbed Energy Selected from Protein</th>
<th>% of Absorbed Energy Selected from Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT</td>
<td>13.1 ± 0.4a</td>
<td>43.0 ± 3.7a</td>
<td>16.2 ± 3.8a</td>
</tr>
<tr>
<td>HCO</td>
<td>12.9 ± 0.4a</td>
<td>28.0 ± 3.6ab</td>
<td>32.5 ± 3.7b</td>
</tr>
<tr>
<td>HSB</td>
<td>13.7 ± 0.8a</td>
<td>32.9 ± 6.3ab</td>
<td>44.5 ± 6.5b</td>
</tr>
<tr>
<td>SBO</td>
<td>13.1 ± 0.7a</td>
<td>23.7 ± 4.0b</td>
<td>36.4 ± 4.0b</td>
</tr>
</tbody>
</table>

1 Rats consumed experimental diets for 14 days followed by selection diets for 14 more days. See Table 6.1 for further description. Data represent cumulative intake and selection over days 7 to 14. Data analyzed by ANOVA. Values are mean ± SEM. N = 10 or 11/diet fat treatment.

2 MCT=medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO=hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB=fully hydrogenated soybean oil (enriched in ≥16:0); SBO=soybean oil (enriched in PUFAs)

3 Adjusted kcal/g diet values based on 2-day dried fecal fat excretion: MCT = 4.248; HCO = 4.187; HSB = 3.274; SBO = 4.223

4 Values not sharing a common superscripted letter are significantly different (p<0.02).

5 Values not sharing a common superscripted letter are significantly different (p<0.0009).
6.3.8 Plasma glucose

Following an over-night fast, an oral glucose tolerance test was performed on each rat in order to assess glucose tolerance responses to long-term consumption of each of the four dietary fat sources. The results of the oral glucose tolerance test are presented in Figure 6.7. No significant differences in plasma glucose concentration were found among the four dietary groups at any individual time point when analyzed as absolute concentration (Figure 6.7) or as percent of basal glucose (data not shown). No significant differences in areas under the glucose tolerance curves, which represents mean glucose excursions, were found among the four dietary groups (data not shown).

6.3.9 Plasma insulin

Only plasma samples from 34 rats were used for all insulin analyses because the samples collected from the remaining animals were highly haemolyzed, and were therefore discarded. Haemolysis can alter the results of the radioimmunoassay of insulin (Brodal, 1971).

Fasting and expected peak insulin concentrations were measured in order to assess insulin sensitivity. No significant differences in fasting or 15 min plasma insulin concentrations were found among the four dietary groups when analyzed as absolute concentration (Table 6.1) or as percent of basal insulin (data not shown). Although no significant differences were found, fasting insulin concentrations are shown in Figure 6.8, so that the trends can be clearly seen. MCT rats tended to have higher fasting insulin values, followed by HSB, SBO and HCO animals. Post-mortem plasma insulin concentrations were also measured. MCT rats had higher post-mortem insulin concentrations than rats from any of the other three dietary groups (P<0.04) (Table 6.1).

6.3.10 Other measures of insulin sensitivity

The plasma glucose:insulin ratio was determined as another method of assessing insulin sensitivity (Caro, 1991). A lower ratio represents decreased insulin sensitivity
(increased insulin resistance). No significant differences among the four dietary groups were found when fasting and 15 min plasma glucose to plasma insulin ratios were analyzed (data not shown).

The fasting insulin resistance index (FIRI) was also determined (normalized to an expected glucose of 5 mmol/L and insulin of 200 pmol/L to give a reference range centred around unity). FIRI = fasting glucose (mmol/L) x fasting insulin (pmol/L) / 1000 (Duncan et al., 1995). A higher FIRI represents a relative decrease in insulin sensitivity (increase in resistance). Again, no significant differences were found among the four dietary groups (data not shown).
Figure 6.7. Oral glucose tolerance test. Rats consumed one of four diets each containing 40% of calories from fat for at least 32 days. MCT = medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO = hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB = fully hydrogenated soybean oil (enriched in ≥16:0); SBO = soybean oil (enriched in PUFAs). Rats were fasted overnight and then given an oral glucose dose (1.5g/kg body weight). Blood samples were collected from a small clip at the end of the tail at -5, 10, 15, 20, 30, 45, 60, 90, 120 and 150 min following the glucose load. Values are mean ± SEM. N=10/11 per diet fat treatment.
Table 6.11 Plasma Insulin Concentrations

<table>
<thead>
<tr>
<th>Diet</th>
<th>Fasting</th>
<th>15 min</th>
<th>Post-mortem</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT</td>
<td>245.0 ± 34.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>580.7 ± 104.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>343.5 ± 48.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCO</td>
<td>178.7 ± 14.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>689.7 ± 62.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>245.3 ± 21.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HSB</td>
<td>213.0 ± 19.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>583.5 ± 137.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>221.3 ± 24.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBO</td>
<td>189.0 ± 16.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>690.9 ± 154.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>205.3 ± 37.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1 Rats consumed one of 4 diet fat treatments each containing 40% of calories from fat for at least 32 days. Rats were fasted overnight and then given an oral glucose dose (1.5g/kg body weight). Blood samples were collected for insulin analysis from a small clip at the end of the tail at -5 and 15 min following the glucose load. Post-mortem blood was collected for insulin analysis from the trunk following decapitation. Data analyzed by ANOVA. Values are mean ± SEM. N=10/11 per diet fat treatment.

2 MCT=medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO=hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB=fully hydrogenated soybean oil (enriched in ≥16:0); SBO=soybean oil (enriched in PUFAs)

3 Values not sharing a common superscripted letter are significantly different (p< 0.04).
Figure 6.8. Fasting plasma insulin concentration. Rats consumed one of four diets each containing 40% of calories from fat for at least 32 days. MCT=medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO=hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB=fully hydrogenated soybean oil (enriched in ≥16:0); SBO=soybean oil (enriched in PUFAs). Rats were fasted overnight and then given an oral glucose dose (1.5g/kg body weight). Blood samples were collected from a small clip at the end of the tail at -5, 10, 15, 20, 30, 45, 60, 90, 120 and 150 min following the glucose load. Values are mean ± SEM. N=10/11 per diet fat treatment. Values are not significantly different.
6.3.1 Regression analysis

To determine if insulin, glucose, or insulin sensitivity correlated with macronutrient selection, regression analysis was performed. The data used for macronutrient selection was from day 7 through 14 of the selection period. This period represents stable selection patterns for each dietary group. The energy selected from protein and carbohydrate was expressed in two ways: 1) as a percentage of total non-fat energy consumed from day 7 through 14 and 2) as the percent of absorbed energy selected from day 7 through 14. Regressions comparing insulin and glucose measures to protein and carbohydrate selection are shown in Table 6.12.

Fasting insulin concentrations were positively correlated with protein intake and negatively correlated with carbohydrate intake when expressed as a percentage of total non-fat energy consumed (protein and carbohydrate: \( r^2 = 0.179, P<0.013; \) Figure 6.9) and when expressed as the percent of absorbed energy selected (protein: \( r^2 = 0.186, P<0.011; \) carbohydrate: \( r^2 = 0.159, P<0.019; \) Figure 6.10). Post-mortem insulin concentrations were also positively correlated with percent of energy chosen as protein and negatively correlated with carbohydrate when expressed as a percentage of total non-fat energy consumed \( (r^2 = 0.180, P<0.011) \) and when expressed as the percent of absorbed energy selected (protein: \( r^2 = 0.130, P<0.034; \) carbohydrate: \( r^2 = 0.175, P<0.012) \). The fasting plasma glucose:insulin ratio was negatively correlated with percent of energy chosen as protein and positively correlated with carbohydrate when expressed as a percentage of total non-fat energy consumed \( (r^2 = 0.119, P<0.046) \). However, when expressed as the percent of absorbed energy selected, protein intake \( (r^2 = 0.143, P<0.027) \), but not carbohydrate intake was significantly correlated with the fasting glucose:insulin ratio. There was a trend for FIRI to increase with percent of energy chosen as protein and decrease with percent of energy chosen as carbohydrate with both methods of analysis, but these trends were not significant.
Table 6.12  Linear Regressions of Protein and Carbohydrate Selected Over Days 7 to 14 of Selection with Plasma Glucose, Plasma Insulin and Insulin Sensitivity measures.1,2

<table>
<thead>
<tr>
<th>Variables3</th>
<th>% of total non-fat energy consumed</th>
<th>% of absorbed energy selected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% protein</td>
<td>% carbohydrate</td>
</tr>
<tr>
<td></td>
<td>$r^2$</td>
<td>p-value</td>
</tr>
<tr>
<td>0 gluc</td>
<td>0.004</td>
<td>0.695</td>
</tr>
<tr>
<td>10 gluc</td>
<td>0.020</td>
<td>0.400</td>
</tr>
<tr>
<td>15 gluc</td>
<td>0.000</td>
<td>0.951</td>
</tr>
<tr>
<td>20 gluc</td>
<td>0.002</td>
<td>0.803</td>
</tr>
<tr>
<td>30 gluc</td>
<td>0.020</td>
<td>0.388</td>
</tr>
<tr>
<td>45 gluc</td>
<td>0.020</td>
<td>0.386</td>
</tr>
<tr>
<td>60 gluc</td>
<td>0.004</td>
<td>0.691</td>
</tr>
<tr>
<td>90 gluc</td>
<td>0.001</td>
<td>0.847</td>
</tr>
<tr>
<td>120 gluc</td>
<td>0.028</td>
<td>0.300</td>
</tr>
<tr>
<td>150 gluc</td>
<td>0.015</td>
<td>0.450</td>
</tr>
<tr>
<td>0 ins</td>
<td>0.179</td>
<td>0.013</td>
</tr>
<tr>
<td>15 ins</td>
<td>0.002</td>
<td>0.797</td>
</tr>
<tr>
<td>p.m. ins</td>
<td>0.180</td>
<td>0.011</td>
</tr>
<tr>
<td>AUC</td>
<td>0.022</td>
<td>0.387</td>
</tr>
<tr>
<td>0 gluc:ins</td>
<td>0.119</td>
<td>0.046</td>
</tr>
<tr>
<td>15 gluc:ins</td>
<td>0.001</td>
<td>0.856</td>
</tr>
<tr>
<td>FIRI</td>
<td>0.086</td>
<td>0.091</td>
</tr>
</tbody>
</table>

1 Rats consumed one of 4 diet fat treatments each containing 40% of calories from fat for at least 32 days. Rats were fasted overnight and then given an oral glucose dose (1.5g/kg body weight). Blood samples were collected from a small clip at the end of the tail at -5, 10, 15, 20, 30, 45, 60, 90, 120 and 150 min following the glucose load. Post-mortem blood was collected for insulin analysis from the trunk following decapitation. Data for individual rats (N=34 to 40). The strength of the correlation is expressed as the coefficient of determination ($r^2$).

2 % protein and carbohydrate energy = % energy selected over days 7 to 14 of the Selection period

3 # gluc = time of plasma glucose sample after oral glucose dose (min);
# ins = time of plasma insulin sample after oral glucose dose (min);
p.m. ins = post-mortem plasma insulin; AUC = area under the plasma glucose tolerance curve (0 to 150 min); 0 gluc:ins = fasting plasma glucose to fasting plasma insulin ratio; 15 gluc:ins = 15 min plasma glucose to 15 min plasma insulin ratio; FIRI = fasting insulin resistance index (Duncan et al, 1995)
Figure 6.9. Linear regressions of two measures of insulin sensitivity with percent of energy selected as protein or as carbohydrate over days 7 to 14 of selection period expressed as a percentage of total non-fat energy consumed. Both plots suggest that decreased insulin sensitivity (increased insulin resistance) positively correlates with increased protein intake and negatively correlates with carbohydrate intake. Fasting plasma insulin vs. percent protein or carbohydrate energy ($r^2 = 0.18$, $p<0.013$) (a, c). Fasting plasma glucose: insulin ratio vs. percent protein or carbohydrate energy ($r^2 = 0.12$, $p<0.046$) (b, d). Rats consumed one of four diets each containing 40% of calories from fat for at least 32 days. MCT=medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO=hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB=fully hydrogenated soybean oil (enriched in >16:0); SBO=soybean oil (enriched in PUFAs). Rats were fasted overnight and then given an oral glucose dose (1.5g/kg body weight). Blood samples were collected from a small clip at the end of the tail following the glucose load. Values are for individual rats. $N=34$. 
Figure 6.10. Linear regressions of two measures of insulin sensitivity with percent of absorbed energy selected as protein or as carbohydrate over days 7 to 14 of selection period adjusted for loss of fat calories to feces. Both plots suggest that decreased insulin sensitivity (increased insulin resistance) positively correlates with increased protein and negatively correlates carbohydrate intake (except d, n.s). Fasting plasma insulin vs. percent protein ($r^2 = 0.19$, $p<0.011$)(a) or carbohydrate energy ($r^2 = 0.16$, $p<0.010$)(c). Fasting plasma glucose to plasma insulin ratio vs. percent protein ($r^2 = 0.14$, $p<0.027$)(b) or carbohydrate energy ($r^2 = 0.11$, $p>0.05$, n.s) (d). Rats consumed one of four diets each containing 40% of calories from fat for at least 32 days. MCT=medium chain triglyceride oil (enriched in 8:0 and 10:0); HCO=hydrogenated coconut oil (enriched in 12:0 and 14:0); HSB=fully hydrogenated soybean oil (enriched in >16:0); SBO=soybean oil (enriched in PUFAs). Rats were fasted overnight and then given an oral glucose dose (1.5g/kg body weight). Blood samples were collected from a small clip at the end of the tail following the glucose load. Values are for individual rats. N=34.
To determine if the associations between insulin and glucose measures and selection could be better represented by a non-linear curve, second and third order regression analyses were performed. Analyses for the correlations between protein and carbohydrate selection and fasting insulin, post-mortem insulin, fasting glucose:insulin ratio and FIRI were determined. Although the $r^2$ values of the higher order curves were greater than the $r^2$ values for the linear (first order) regression lines, $P$ values showed that the associations were not significant (data not shown). Thus, the only significant associations were seen with the linear regression lines.

Regressions were also analyzed to compare insulin sensitivity measures to each other (Table 6.13). Highly significant correlations were found between three measures of insulin sensitivity (fasting insulin, fasting glucose:insulin ratio, and FIRI). Fasting insulin was negatively correlated with the fasting glucose:insulin ratio ($r^2 = 0.54$, $P<0.0001$), fasting insulin was positively correlated with FIRI ($r^2 = 0.83$, $P<0.0001$) and the glucose:insulin ratio was negatively correlated with FIRI ($r^2 = 0.20$, $P<0.0086$).

Although the possibility of finding significant correlations by chance increases with multiple comparisons, fasting insulin, the fasting glucose:insulin ratio and FIRI are all measures of insulin sensitivity. Therefore, it is unlikely that these significant correlations are due to chance.

Taken together, the significant linear correlations suggest that a decrease in insulin sensitivity (increase in insulin resistance) leads to an increase in preference for protein (and a corresponding decrease in carbohydrate preference). The data further suggest that insulin sensitivity contributes to the regulation of both the percent of absorbed energy selected from protein and carbohydrate and to the regulation of the relative intakes of each of these macronutrients.
Table 6.13 Linear Regressions Comparing Methods of Insulin Sensitivity Determination

<table>
<thead>
<tr>
<th>Variable (^2)</th>
<th>Variable (^2)</th>
<th>(r^2)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ins</td>
<td>0 gluc:ins</td>
<td>0.541</td>
<td>0.0001</td>
</tr>
<tr>
<td>0 ins</td>
<td>FIRI</td>
<td>0.832</td>
<td>0.0001</td>
</tr>
<tr>
<td>0 gluc:ins</td>
<td>FIRI</td>
<td>0.197</td>
<td>0.0086</td>
</tr>
</tbody>
</table>

\(^1\) Rats consumed one of 4 diet fat treatments each containing 40% of calories from fat for at least 32 days. Rats were fasted overnight and then given an oral glucose dose (1.5g/kg body weight). Blood samples were collected from a small clip at the end of the tail following the glucose load. Data from individual rats (N=34).

The strength of the correlation is expressed as the coefficient of determination (\(r^2\)).

\(^2\) 0 ins = fasting plasma insulin; 0 gluc:ins = fasting plasma glucose to fasting plasma insulin ratio; FIRI = fasting insulin resistance index (Duncan et al, 1995).
6.4 Discussion

One objective of this experiment was to further clarify the relationship between SFA chain length and macronutrient selection patterns of rats. The present data demonstrate that the selection patterns for the MCT, HCO, and SBO dietary groups are highly reproducible (compare Table 5.2 with Table 6.6) in that MCT-fed rats continued to select more protein and less carbohydrate than rats fed either HCO or SBO diets. However, HSB was insufficiently absorbed by rats fed this fat, and as a result, the specific relationship between SFA chain length and macronutrient selection could still not be determined. HSB appears to be an anomalous fat source that acts more like a low caloric substance or fat substitute than a normal dietary fat. Although the lack of absorption of HSB make the data difficult to interpret, the difference in selection between the MCT and HCO groups suggests that SFA chain length may be involved in mediating dietary fat-induced macronutrient selection.

A second objective was to explore possible mechanisms mediating dietary fat-induced alterations in feeding behaviour. Although glucose and insulin concentration did not differ among diet groups, measures of insulin sensitivity significantly correlated with protein and carbohydrate selection. These results suggest that a decrease in insulin sensitivity leads to an increase in protein and a decrease in carbohydrate intake.

6.4.1 Macronutrient selection

As in Experiment 1, rats fed MCT selected more protein energy and less carbohydrate energy over the 14-day selection period than HCO and SBO regardless of the method of data calculation (i.e. correction for non-absorbed fat). As discussed in Experiment 1 (see section 5.4), one possible explanation is that there may be something unique about MCT that is important in mediating selection. Results from this experiment can not rule out this explanation. Nevertheless, since the most prominent difference between MCT and HCO is SFA chain length composition, and
total SFA content of these two diets did not differ, SFA chain length may be involved in mediating selection.

Rats fed the HSB diet chose significantly less protein energy and more carbohydrate energy (expressed as the percentage of total non-fat energy consumed) than MCT rats and their macronutrient selection pattern did not differ from that of animals in the HCO and SBO groups (see Table 6.7). These observations indicate that the consumption of HSB leads to a selection pattern that is not consistent with the pattern produced by other LSFA sources (e.g., lard, beef tallow). A number of studies have consistently shown that animals fed these other LSFA sources select more protein and less carbohydrate than animals fed diets enriched in PUFAs (soybean and corn oil) (Crane & Greenwood, 1987; McGee & Greenwood, 1989; McGee & Greenwood, 1990a; Mullen & Martin, 1990; McGee & Greenwood, 1991; Mullen & Martin, 1992a; Grossman et al., 1994), whereas in the present experiment, HSB rats chose similarly to SBO rats. Although these findings seem contradictory, the HSB fat source was not fully absorbed and as a result, the effect of LSFAs on macronutrient selection could not be determined as it had been intended. Hence, although the problem with HSB in Experiment 1 (i.e., animals not uniformly consuming the fat) was corrected in Experiment 2 (i.e., by grinding HSB into a fine powder), the relationship between SFA chain length and macronutrient selection could not be established.

The feeding behaviour of HSB animals may be indicative of the macronutrient selection pattern of animals fed a low fat diet rather than a high LSFA diet. Consequently, the selection behaviour of HSB animals cannot be fairly compared with that of lard and beef tallow-fed animals of previous studies. In addition, HSB cannot be fairly compared with MCT and HCO as intended. The results obtained with HSB animals are discussed further in the next section (6.4.2).

In support of the results presented in Experiment 1, day-to-day selection behaviour did not differ among dietary groups until day 8 of selection (see Figure 6.5). The differences continued through to day 14, and therefore suggest that an adaptation
period is necessary before the dietary fat sources fully influence the animals' feeding behaviour. A comparison of Figure 5.2 from Experiment 1 and Figure 6.5 from Experiment 2 shows that the shape of the selection curves (which represent relative protein and carbohydrate selection as a percentage of total non-fat energy consumed) are remarkably similar, and thus indicate a high level of reproducibility.

Since selection behaviour from day 7 through 14 appeared more stable, and more reflective of the full influence of dietary fat on selection, macronutrient intake from day 7 to day 14 of selection was analyzed. These data show the same differences in significance across groups as over the entire 14-day period, but the differences are more pronounced (compare Table 6.7 with Table 6.9).

6.4.2 Fully hydrogenated soybean oil

In several macronutrient selection studies that have previously been reported, no significant differences in total energy intake over the selection period were observed when all diets were isocaloric containing identical amounts of energy from fat (Crane & Greenwood, 1987; McGee & Greenwood, 1989; McGee & Greenwood, 1990a; McGee & Greenwood, 1991). Although all diets in the present experiment were designed to be isocaloric and contain 40% of energy from fat, the HSB rats consumed a significantly greater amount of total food than the rats from the other dietary groups, yet body weight gain did not differ (see Figure 6.3). This surprising observation can be explained by the fact that the fat content in the HSB diet was not fully absorbed. The animals in the HSB group excreted almost three times the weight in feces as the rats in each of the other three dietary groups (see Figure 6.4). Fecal analysis confirmed that almost 70% of the fully hydrogenated soybean oil component of the HSB diet was not absorbed (see Table 6.4). Hence, HSB acted more like a low caloric substance or fat substitute than a normal dietary fat source. In fact, assuming that the absorbed component of HSB contributed the Atwater value of 9 kcal/g, the HSB fat source only contributed 2.8 kcal/g. As a result, rats in the HSB group had to eat more food in order to obtain the same amount of energy as the other animals and grow normally. Indeed,
when the loss of dietary fat to feces was considered, total energy absorbed over both the experimental and selection periods did not differ among any of the four dietary groups (see Table 6.5). Thus, animals fed HSB were able to completely compensate for the lower absorbable-energy-density of their diets. (These values actually represent apparent energy absorbed since fecal content of protein and carbohydrate were not determined. However, only a very minimal amount of nonabsorption of these macronutrients would be expected and no differences among groups would be expected. Apparent absorption of diets based on fecal fat content has been referred to as, "absorption", throughout the thesis).

The explanation for the lack of absorption of HSB in this study is supported by some research that suggests that tristearate (a triglyceride containing three stearic acids) is poorly digested and absorbed in animals (Mattson, 1959; Bergstedt et al., 1990; Bergstedt et al., 1991; Kamei et al., 1995). Although tristearate is a rare phenomenon in natural fat sources, 84% of HSB is composed of stearic acid (18:0; analyzed by gas chromatography), which suggests that a large component of this fat must be made up of tristearate. Furthermore, one recent study suggests that a highly hydrogenated soybean oil fed at only 50 g/kg diet (as opposed to 190 g/kg in the present experiment) was also poorly absorbed (Kamei et al, 1995). Thus, this fat is poorly absorbed at both high and low fat levels.

Rats in the HSB group selected more carbohydrate energy than protein energy. Furthermore, they selected similarly to SBO animals, which is in contrast to the results of numerous studies which suggest that rats fed diets enriched in LSFA (beef tallow or lard) select differently from rats fed diets enriched in PUFAs (soybean and corn oil) (Crane & Greenwood, 1987; McGee & Greenwood, 1989; McGee & Greenwood, 1990a; Mullen & Martin, 1990; McGee & Greenwood, 1991; Mullen & Martin, 1992a; Grossman et al., 1994). However, the feeding behaviour of HSB animals may be indicative of the behaviour that would be expected following the consumption of a low fat diet rather than a high LSFA diet. The present findings suggest that rats
ingesting low levels of fat, although not deficient in EFAs, replace these calories with a surplus of energy from carbohydrate.

The effect of long-term feeding of a very low or fat-free diet on subsequent protein and carbohydrate selection has not been systematically tested in rats. However, Mullen and Martin (1990) showed that rats fed 5% or 20% (wt/wt) beef tallow diets for a very acute period subsequently chose more carbohydrate and less protein than rats fed a 34% (wt/wt) beef tallow diet. Moreover, the lower fat-fed rats actually ate more carbohydrate and the same amount of protein in their initial diets compared to the high beef tallow fed rats, yet, the low fat-fed rats subsequently selected more carbohydrate. In other words, the low fat-fed animals subsequently chose more carbohydrate than the high fat-fed animals even though the low fat diets contained more carbohydrate. Since an initially high carbohydrate diet may be expected to lead to lower subsequent carbohydrate intake, these data suggest that the low fat nature of the diet may actually be even more important in influencing subsequent carbohydrate selection than the amount of carbohydrate in the diet. Further evidence which suggests that a low-fat diet leads to an increase in carbohydrate consumption comes from studies employing the use of a noncaloric fat substitute. Adult men (Rolls et al., 1992) and children (Birch et al., 1993) consuming olestra (noncaloric fat substitute) subsequently choose more carbohydrate compared to subjects consuming fat, without a reduction in total energy intake. Considering these findings, it would be interesting to systematically investigate the effects of a very low fat diet on protein and carbohydrate selection in future experiments.

6.4.3 Macronutrient selection after adjustment for loss of dietary fat calories to feces due to differences in the absorption of dietary fat sources

The HCO fat component was absorbed significantly less than the MCT or SBO components (see Table 6.4). Although over 94% of the fat was absorbed (not nearly as pronounced as the nonabsorption of HSB), these data nonetheless suggest that HCO contributed significantly less energy to the HCO diet than SBO or MCT oil contributed
to those respective diets. As a result of the different extents of absorption of the fat sources, all macronutrient selection data were re-analyzed to determine the percent of absorbed energy consumed from each macronutrient by each dietary group. In interpreting these data it must be considered that although all diets were designed to contain 60% non-fat energy, because of the differences in dietary fat absorption, HSB animals actually absorbed 79.3% of energy from protein and carbohydrate and HCO, MCT and SBO rats absorbed 61.2%, 60.4% and 60.7% non-fat energy respectively. The macronutrient selection results presented above as a percent of total non-fat energy consumed are normalized for this discrepancy and suggest that animals regulate their relative protein and carbohydrate consumption dependent on fat source consumed. In contrast, the following discussion explores the regulation of the percentage of absorbed protein and carbohydrate energy selected, rather than relative consumption.

When expressed as percentages of absorbed energy (i.e., including absorbed fat energy) selected as protein and carbohydrate, the selection profiles across the MCT, HCO and SBO groups are almost the same as when the data were presented as a percentage of total non-fat energy (i.e., excluding absorbed fat energy) consumed since only minor differences in absorption were evident across these groups. Thus, these data indicate that when expressed as the percentage of absorbed energy selected, MCT rats still consumed more protein and less carbohydrate than animals fed HCO or SBO (see Figure 6.6). The major differences across groups are the result of differences with HSB animals because they absorbed much more total non-fat energy than the other groups. Thus, relative to the other groups, both HSB protein and carbohydrate energy consumption are inflated when expressed as the percentages of absorbed energy. This is evident by comparing Figure 6.5 with Figure 6.6 which show selection profiles over each 2-day period during selection. The overall and day 7 to 14 selection profiles of each group, expressed as the percent of absorbed energy selected, are shown in Tables 6.8 and 6.10. Since total caloric absorption over the selection period did not differ across groups (Table 6.8) and total caloric absorption per gram body weight gain over
days 7 to 14 did not differ across groups (Table 6.10), differences in feeding behaviour can be attributed to macronutrient selection.

These data are difficult to interpret since the differences across groups are obscured by the high content of both protein and carbohydrate that HSB animals consumed. These data raise the question of whether or not rats regulate their relative consumption of protein and carbohydrate or regulate absolute percentages of each macronutrient. HSB animals selected about 40% of their total non-fat calories from protein and 60% from carbohydrate (Table 6.7) and actually absorbed 31% of their energy from protein and about 47% from carbohydrate (Table 6.8). It is not clear whether these animals exhibited a drive to maintain the 40:60 ratio of protein to carbohydrate intake or whether it was important for the animals to specifically absorb 31% of energy from protein or specifically absorb 47% from carbohydrate and simply make up the difference with the other macronutrient. The present experiment was not designed to answer this question.

Several lines of evidence suggest that rats regulate protein consumption quite precisely when experimental conditions are constant, whereas the evidence for the precise regulation of carbohydrate is not as complete (see Anderson, 1994 for review). The argument in favour of a specific regulation for protein consumption versus carbohydrate consumption partly lies with the assumption that amino acids are essential, whereas beyond very small quantities, there is no evidence for a carbohydrate requirement. Nevertheless, Mullen and Martin (1992a) have suggested that dietary SFA-induced macronutrient selection behaviour is driven by an avoidance of carbohydrate rather than by a preference for protein. In contrast to the view that animals only control the ingestion of one of these macronutrients, independently of the other, it is also possible that the protein:carbohydrate ratio is regulated. Kim et al. (1991) suggested that individual rats may select a specific protein:carbohydrate ratio in order to maintain an optimal, "hedonistic level" of brain serotonin.
Despite the lack of agreement on the driving force behind macronutrient selection, the one thing that does seem clear is that within a constant set of experimental conditions, rats select macronutrients with remarkable consistency. Dependent on the mechanism behind the driving force, animals may exhibit more than one strategy of feeding behaviour. For example, animals may select to maintain an adequate absolute level of protein or carbohydrate in order to avoid deficiency, but beyond this point, an optimal ratio of protein to carbohydrate may be important. In the present experiments, it is clear that the ingestion of different dietary fat sources influence animals to select various amounts of protein and carbohydrate. It is not clear whether dietary fat influences animals to select for a particular ratio of protein to carbohydrate or an absolute level or protein or carbohydrate. Furthermore, it is possible that fat sources of varying fatty acid compositions affect macronutrient selection differently. That is, the consumption of a specific fat source may influence animals to select a minimum level of protein or carbohydrate, whereas the consumption of another fat source may influence an animal to select a specific ratio of protein to carbohydrate. Elucidating the mechanism involved in the mediation of dietary fat-induced macronutrient selection may increase our understanding of the physiological "strategy" behind the drive to select specific macronutrients.

6.4.4 Role of glucose and insulin in mediating dietary fat-induced macronutrient selection

In order to investigate a possible mechanism that may be involved in the mediation of macronutrient selection by dietary fatty acid chain length, glucose and insulin responses to the experimental diets were determined. The rationale for this investigation was based on evidence which suggests that dietary SFAs can influence insulin response (van Amelsvoort et al., 1988; Storlien et al., 1991; Clandinin et al., 1993). In addition, further documentation suggests that SFA stimulated insulin secretion may be mediated by chain length in a non-linear manner (Opara et al., 1994) similar to how dietary SFA chain length appears to mediate macronutrient selection.
The results from Experiment 2 support the hypothesis and suggest that the effect of dietary fat on insulin sensitivity may play a role in mediating macronutrient selection. Using fasting insulin and the fasting glucose:insulin ratio as predictors of insulin sensitivity (a high fasting insulin concentration and a low fasting glucose:insulin ratio are indicative of a decrease in insulin sensitivity), the \( r^2 \) values suggest that insulin sensitivity accounts for about 11 to 19% of the variation in protein and carbohydrate selection, and is significant (see Figures 6.9 and 6.10 and Table 6.12). Moreover, it appears that both the percent of absorbed energy selected as protein and carbohydrate, as well as the relative proportions of each macronutrient are predicted by insulin sensitivity. A decrease in insulin sensitivity (increase in insulin resistance) is associated with an increase in preference for protein and a corresponding decrease in preference for carbohydrate. Although a cause-and-effect relationship cannot be confirmed from any correlation, these findings indicate a possible connection that can be tested in future studies. However, since insulin sensitivity was found to account for a relatively small portion of the variability in selection and no significant diet effect on insulin sensitivity was found, other factors are also likely to play important roles. Nevertheless, these findings are significant and increase our understanding of the mechanism involved in mediating dietary fat-induced behavioural alterations.

Since no significant differences were found among diet groups with respect to any of the glucose or insulin measurements, the contribution of diet versus genetics in influencing insulin sensitivity cannot be clearly determined. However, there was a trend for dietary groups to cluster with respect to the measures of insulin sensitivity. The MCT rats (which consumed more protein and less carbohydrate than rats in the other three diet groups) tended to have the highest fasting insulin concentration and the lowest fasting glucose:insulin ratio (Figure 6.8 and Figure 6.9) which indicates that this diet impaired insulin sensitivity more than the other diets. Furthermore, an inbred strain of rats was utilized (low genetic variability) and the only variable that differed
among diet groups was fat source. It therefore appears that the dietary fat source consumed contributed to the observed changes in insulin sensitivity. This implies that insulin sensitivity may be important in mediating dietary fat-induced changes in macronutrient selection.

The present findings, which indicate a connection between decreased insulin sensitivity and increased protein and decreased carbohydrate intake, are consistent with some other reports that have investigated feeding behaviour in diabetic rats. When given the opportunity to self-select, diabetic rats have been shown to choose higher intakes of protein than normal rats (Woodger et al., 1979; Peng & Evenson, 1979; Bartness & Rowland, 1983). By selecting this high protein, low carbohydrate diet, these diabetic animals are able to improve their hyperglycemic state (Bartness & Rowland, 1983). This behaviour has also been shown to reduce secondary changes associated with diabetes, such as lenticular cataracts and levels of sorbitol and fructose in peripheral nerves (Baxter & Schofield, 1980 cited in Bartness & Rowland, 1983).

Since a decrease in insulin sensitivity may be indicative of a pre-diabetic state, the macronutrient selection pattern seen in the present study may be a beneficial response to this state.

In order for insulin to influence any behaviour, including macronutrient selection, some central process must be affected either directly or indirectly. Recent evidence suggests that circulating insulin can stimulate glucose uptake by the brain via the insulin-sensitive glucose transporter, GLUT 4 (Livingstone et al., 1995). GLUT 4 has been detected in the pituitary, the hypothalamus, the medulla (Brant et al., 1993) and the cerebellum (Rayner et al., 1994). Hence, alterations in insulin-stimulated glucose transport in some brain region may ultimately lead to behavioural changes. Central glucose levels have been shown to influence a number of behavioural parameters, including learning and memory, possibly by interacting with central neuromodulators and neurotransmitters (Lee et al., 1988; Gold & Stone, 1988; Wenk, 1989). Thus, one possible explanation for the present findings is that dietary fat
influences peripheral insulin response, which in turn stimulates glucose uptake by the brain, ultimately leading to alterations in behaviour.

Another way in which circulating insulin concentrations could affect subsequent behaviour is by impacting on insulin concentration in the CSF. CSF concentrations of insulin have been shown to be responsive to peripheral insulin (Plata-Salaman, 1991). Furthermore, CSF insulin has been shown to be involved in regulating food intake (Plata-Salaman, 1991). Alternatively, locally synthesized (i.e., in the CSF) insulin may be influenced by peripheral events, which could also ultimately impact on behaviour.

The present study enhances our understanding of the influence that dietary fat has on macronutrient selection. The future challenge will be to further understand the ways in which the central nervous system responds to dietary changes. It will be important to determine whether the brain receives information about feeding directly and/or indirectly via peripheral pathways. Possibly, a number of integrated factors including a role of the central nervous system and periphery will be shown to be important in mediating dietary fat-induced behavioural alterations.
CHAPTER 7

GENERAL DISCUSSION
7. GENERAL DISCUSSION

7.1 Introduction

The experiments discussed in the present thesis were designed to test the hypothesis that dietary SFA chain length mediates macronutrient selection in post-developmental rats and to explore possible mechanisms which may be involved in mediating this feeding behaviour.

The following discussion is broken down into a number of parts in order to briefly summarize the experimental results of the present thesis and examine the future challenges of continuing research.

7.2 Macronutrient selection

7.2.1 Discussion of experimental results

Four dietary fat sources were utilized in the present experiments in order to attempt to determine the role of SFA chain length in mediating macronutrient selection in post-weanling rats following the long-term consumption of diets containing 40% fat energy (comparable to present North American consumption patterns) and adequate levels of EFAs. The MCT, HCO and HSB diets all contained approximately the same concentration of total SFAs (about 85%; see Table 4.4) but differed with respect to relative composition of different chain length SFAs. MCT, HCO and HSB were enriched in MSFAs, ISFAs and LSFAs, respectively. Thus, by comparing the macronutrient selection behaviour of animals fed these three diets, the role of SFA chain length, independent of total SFA content could be determined. SBO (low in SFAs, high in PUFAs) was used as a fourth dietary fat source for comparison to previous research.

Although the design of the experiments was sound, HSB was not uniformly consumed by the animals in Experiment 1 and was shown to be poorly absorbed by rats when it was consumed (Experiment 2). Due to these unanticipated findings, the
relationship between increasing SFA chain length and protein and carbohydrate selection could not be determined from the experiments described in this thesis.

Both experiments indicate that the protein and carbohydrate selection profiles of animals fed MCT (enriched in MSFAs), HCO (enriched in ISFAs) and SBO (enriched in PUFAs) diets are highly reproducible. Specifically, rats fed a diet enriched in MSFAs selected a relatively higher proportion of energy from protein and less from carbohydrate than rats fed a diet enriched in either ISFAs or in PUFAs. The observations that differences in macronutrient selection across these diet groups occurs in the absence of differences in total energy consumption, and that the differences are not evident until after 6 days of selection feeding, are also reproducible. These findings indicate that differences in feeding behaviour due to dietary fat type are specific for macronutrient selection and that a period of adaptation to selection diets appears to be necessary before the full influence of fat type on macronutrient selection is evident.

The selection data could be interpreted to suggest that something unique about MCT may be mediating selection (such as the high glycerol content of MCT; see section 5.4 for discussion). However, the differences in the relative composition of various chain length SFAs between the MCT and HCO diets in the absence of a difference in total SFA content (see Table 4.4 for fatty acid composition of diets), suggest that SFA chain length may be important in mediating macronutrient selection. If only total SFA content was important, then these two diet groups would be expected to select protein and carbohydrate similarly. Further supporting the suggestion that SFA chain length may be important are the data that indicate that SFAs are important in mediating macronutrient selection (McGee & Greenwood, 1990a). Nevertheless, more research is needed to clearly determine the role of SFA chain length in mediating macronutrient selection.

The results obtained with animals fed HSB suggest that this fat is poorly absorbed by rats. The macronutrient selection profile observed in these animals
appears to be indicative of the profile of selection that is seen following the consumption of a low-fat diet. The data suggest that following a low-fat diet, animals select more carbohydrate than protein and select relative proportions of protein and carbohydrate similar to animals fed a high PUFA (SBO) diet or a high ISFA (HCO) diet. Moreover, rats are able to fully compensate for the lack of fat calories and actually consume the same amount of absorbable energy as animals fed high-fat diets. The present findings indicate that HSB only contributes 2.8 kcal/g rather than the Atwater value of 9 kcal/g that approximates the caloric contribution of most dietary fat sources. Thus, HSB appears to behave more like a low-caloric substance or fat substitute than a normal dietary fat.

Fecal analysis of HCO-fed animals suggests that this fat is absorbed less efficiently than MCT or SBO. Thus, a value slightly less than 9 kcal/g would be more accurate in calculating the caloric contribution of HCO. Like HSB-fed animals, HCO-fed animals were able to fully compensate calorically for the lesser concentration of fat energy in their diets.

The present findings add support to the growing body of research which indicates that variations in dietary fatty acid composition, in the absence of EFA deficiency, in post-developmental animals, and at levels similar to those consumed by humans, can profoundly influence numerous behaviours. The wide variation of behaviours that can be influenced by normal variations in dietary fat, including learning, memory, pain sensitivity, thermoregulation, and feeding behaviour suggest that the impact of dietary fat on the central nervous system is widespread and functionally significant. More research will hopefully help determine the important mechanisms involved and allow us to understand the relevance of these data to the human.

7.2.2 Proposed relationship between SFA chain length and macronutrient selection

Previous research has suggested that SFA content is the specific component of dietary fat that is important in mediating both macronutrient selection (McGee &
Greenwood, 1990a) and cognitive behaviour (Greenwood & Winocur, 1996). However, since only fat sources enriched in LSFAs (e.g., lard and beef tallow) had been used previously, the influence of specific chain length SFAs on behaviour had apparently not been previously examined. The present results suggest that SFA chain length may be important in mediating dietary fat-induced macronutrient selection, but the specific relationship between chain length and selection remains undefined.

The hypothesis presented here (discussed in section 5.4) based on the results of the present experiments, combined with the results of previous research, is that the association between SFA chain length and macronutrient selection may be bell-shaped. That is, it is proposed that rats fed diets enriched in MSFAs and LSFAs select more energy as protein and less as carbohydrate than rats fed diets enriched in ISFAs.

The proposed bell-shaped association between dietary SFA chain length and macronutrient selection suggests that whole-body fatty acid oxidation and routes of fatty acid absorption may not mediate selection in any direct way, since current research suggests that these parameters vary linearly with SFA chain length (Senior, 1968; Leyton et al., 1987). One exception in the current literature is that peroxisomal fatty acid oxidation does appear to vary in a bell-shape with SFA length (Chance & McIntosh, 1994). Thus, although peroxisomal fatty acid oxidation has only been shown to account for a relatively small proportion of whole-body oxidation (Osmundsen et al., 1991), this parameter may be involved in the mechanism that mediates dietary fat-induced macronutrient selection.

7.2.3 Discussion of previously considered confounding variables

One final area of discussion involves the role of dietary cholesterol and peroxide products as potential variables in mediating macronutrient selection. In previous studies which have suggested that SFA concentration was the important component of dietary fat in mediating selection, these components were considered confounding variables since they would be expected to vary concurrently with SFA concentration. The results described in the present thesis suggest that these variables are not
important in regulating selection. In order to control for dietary cholesterol, all fat sources used in the present experiments were vegetable oils, which are free of cholesterol. To control for an influence of peroxide products, three diets containing very high levels of SFAs were used since these diets would not be very susceptible to peroxidation. Thus, the fact that differences in macronutrient selection across dietary groups were evident in the absence of dietary cholesterol and peroxide products, implies that these factors are likely not important in mediating selection.

7.3 Role of insulin sensitivity in mediating dietary fat-induced macronutrient selection

Despite the body of literature that suggests that dietary SFA content can influence a number of behaviours, the mechanism mediating these behaviours is unclear. Attempts to isolate a key mechanism have been numerous. Although brain membrane fatty acids are responsive to dietary fat, they do not appear to mediate selection (McGee & Greenwood, 1990b). The data are mixed with regards to the role of serotonin, and the data implicating a peripheral influence of fatty acid oxidation or the vagus nerve are inconsistent with regards to the time periods needed to influence these parameters and the time periods needed to influence selection. As a result, the possibility that the periphery is involved in mediating macronutrient selection was investigated by determining the role of the influence of dietary fat on insulin sensitivity. Most likely, no one factor will ever be shown to be solely responsible for the dietary fat effect on behaviour, and both peripheral and central systems will ultimately be shown to be involved in the mediating mechanism.

The present results indicate that the effect of dietary fat on insulin sensitivity may be involved in mediating dietary fat-induced macronutrient selection. Significant associations between insulin sensitivity and protein and carbohydrate selection suggest that a decrease in insulin sensitivity leads to a preference for a higher protein and lower carbohydrate diet. The contribution of insulin sensitivity in mediating
macronutrient selection may be small since no differences in glucose tolerance or insulin sensitivity were observed across dietary groups and only 11 to 19% of the variation in selection could be explained by levels of insulin sensitivity. Further studies will elucidate the extent of the role of insulin sensitivity and other factors in mediating dietary fat-induced macronutrient selection.

The present findings are consistent with the hypothesis that dietary fat-induced changes in insulin sensitivity mediates macronutrient selection via its affect on brain uptake and utilization of glucose. The present results, as well as others, suggest that dietary fat type can influence peripheral insulin response. In turn, the recent data which suggest that the insulin-sensitive glucose transporter, GLUT 4, is present in the brain, indicates that such changes in peripheral insulin concentrations could conceivably impact on brain glucose concentrations. Finally, numerous studies suggest that changes in brain glucose concentration can influence several behaviours. Thus, although the present study only represents the beginning of the link, further research will help determine whether or not the connection between dietary fat-induced changes in insulin sensitivity can ultimately affect behavioural alterations via changes in brain uptake and utilization of glucose.

7.4 Future work

7.4.1 Design considerations for comparing dietary saturated fatty acid chain lengths

The data described in this thesis combined with previous research suggest that rats fed diets enriched in MSFAs and LSFAs might select a higher protein and a lower carbohydrate diet than rats fed diets enriched in ISFAs. The experiments discussed here were designed to determine the influence of SFA chain length on macronutrient selection. Three fat sources, which each contain high levels of total SFAs, but differ in their relative concentrations of different chain length SFAs were utilized: medium-chain triglyceride oil (MCT) which is enriched in MSFAs (8:0 and 10:0), hydrogenated coconut oil (HCO) which is enriched in ISFAs (12:0 and 14:0) and fully
hydrogenated soybean oil (HSB) which is enriched in LSFAs (16:0 and 18:0). Flaxseed and safflower oil were blended with these fat sources in order to obtain three isocaloric diets, containing 40% of calories from fat, adequate levels of EFAs, similar concentrations of total SFAs (84 or 85% of dietary fat content), and different relative compositions of different chain length SFAs. Thus, the design allowed three diets to be used which were virtually identical except for the relative composition of different chain length SFAs. However, as noted above, because the HSB fat source was not fully absorbed, the selection behaviour of animals in this group was more indicative of the behaviour following a low-fat diet than a high LSFA diet and could not be used to described the behaviour of animals absorbing high levels of LSFA.

As a result of the lack of absorption of HSB, only the influences of MSFAs and ISFAs on macronutrient selection are clear from the present experiments. Although numerous studies have repeatedly shown the consistent selection profile of animals fed other fat sources enriched in LSFAs, including lard, beef tallow and hydrogenated corn oil (Crane & Greenwood, 1987; McGee & Greenwood, 1989; McGee & Greenwood, 1990a; Mullen & Martin, 1990; McGee & Greenwood, 1991; Mullen & Martin, 1991; Mullen & Martin, 1992a; Grossman et al., 1994), another study is necessary in order to conclusively show the effects of MSFAs, ISFAs and LSFAs within one set of experimental conditions.

The problem with the present experiment was with the LSFA fat source (HSB). Hence, the easiest way to more reliably compare the effects of MSFAs, ISFAs and LSFAs would be to replace HSB with another fat source that is enriched in LSFAs. However, there are difficulties with this approach. Over 97% of HSB is made up of LSFAs and total SFA content makes up over 99% of this fat. In contrast, lard and beef tallow contain about 37% and 44% LSFAs respectively (United States Department of Agriculture, 1979). Total SFA content of lard and beef tallow are about 39% and 50% respectively. HSB was used in order to make the MCT, HCO (which each contain very high levels of total SFAs) and HSB diets all contain over 84% total SFAs, so that
the specific effects of different chain length SFAs could be examined. If beef tallow or lard was used in place of HSB, this could not be accomplished. In other words, because a beef tallow or lard diet would contain less total SFAs, the MCT, HCO and beef tallow diets would differ in both their relative content of chain length SFAs and in their relative contents of total SFAs. Hence, total SFA content would be a confounding variable.

Another approach to the problem of comparing beef tallow to MCT and HCO would be to dilute MCT and HCO with other fat sources so that they would contain the same content of total SFAs as beef tallow. This approach is reasonable because all three diets would contain the same content of total SFAs and only differ in the relative contents of different chain length SFAs. However, since the total content of MSFAs and ISFAs in MCT and HCO respectively would be greatly decreased, the effects that were seen with these fat sources in the present experiments may not be the same. A final problem with using beef tallow or lard in place of HSB is that by introducing an animal fat the confounding variable, dietary cholesterol, is added to the picture. One way of getting around this problem would be to add dietary cholesterol in similar proportions to each diet.

Based on all of the noted difficulties with designing such an experiment, the reasons for attempting to use HSB are readily apparent. Unless a different fat source can be found that contains a very high level of both total SFAs and LSFAs, and is absorbed efficiently, the two methods noted above may be the best way to clearly understand the influence of SFA chain length on macronutrient selection or any other behaviour within one experiment. Of course, it is rare, if not impossible to design an experiment with no confounding variables. Thus, by comparing the results of such studies which approximate ideal conditions to those of other studies such as the ones presented here, the data will become more complete.

7.4.2 Role of peroxisomal fatty acid oxidation in mediating macronutrient selection
The observations from both experiments combined with data from previous literature suggest that SFA chain length may vary in a bell-shape with macronutrient selection. Both LSFAs and MSFAs appear to influence animals to select more protein and less carbohydrate than animals fed ISFAs. Thus, if this proposed bell-shaped relationship is true, the influence of SFA chain length on the parameter or parameters that mediate macronutrient selection may also be expected to vary in a bell-shape. This was one of the reasons for investigating insulin response, which has been shown to vary in a bell-shape with SFA chain length (Opara et al., 1994). As mentioned in the discussion of the experiments, another parameter which has been shown to vary in a bell-shape with SFA chain length is peroxisomal fatty acid oxidation. Thus, if the role of peroxisomal fatty acid oxidation can be shown to be functionally significant, its role in mediating dietary fat-induced macronutrient selection deserves attention. Furthermore, any other parameter that can be shown to be influenced by SFA chain length in a similar bell-shape manner also warrants further investigation.

7.4.3 Further research to determine the role of insulin sensitivity in mediating dietary fat-induced macronutrient selection

The results of the present experiment indicate that insulin sensitivity is significantly associated with a subsequent increase in protein and a decrease in carbohydrate intake. The studies conducted in the experiments discussed here were designed to determine if a correlation exists between SFA chain length and measures of insulin sensitivity and glucose tolerance, and whether or not these parameters in turn correlate with macronutrient selection. Now that a correlation has been established, further research would be warranted to solidify this association. Firstly, the measurements to analyze insulin sensitivity should be conducted immediately following the 14-day experimental period, since this is the time immediately prior to selection. However, it would be necessary to use one set of animals to analyze insulin measurements and another set to analyze macronutrient selection so that selection behaviour would not be affected by blood collection. In the present experiments the
insulin analyses were performed following the selection period and an additional 4-day stabilization period so that one set of animals could be used and so that a number of other problems could be avoided (see section 6.2 Experimental Design for an explanation of the procedure). However, now that a significant correlation between insulin concentration and macronutrient selection has been found, such an experiment could be justified.

A second consideration that would be important in solidifying the present conclusions would be to use a more precise method of analyzing insulin sensitivity. Again, until now, such an invasive procedure was not warranted. The Bergman minimal model method (Bergman et al., 1985) has been cited often as a standard method of accurately calculating an insulin sensitivity index. This method requires the application of a frequently sampled intravenous glucose tolerance test, which requires blood collection at numerous time points in order to determine both glucose and insulin concentrations. In order to perform such a procedure on rats, surgery to attach indwelling catheters to the animals would be necessary.

If a correlation between insulin sensitivity and macronutrient selection becomes more clear and can be reliably reproduced, the next step would be to determine causality. One way of examining this would be to attempt to normalize insulin sensitivity across dietary fat groups, and determine if the dietary fat effect on macronutrient selection is still evident. If the normalizing of insulin sensitivity eliminates the effect of dietary fat on selection then the conclusion that insulin sensitivity may indeed be involved in the mediating mechanism could then be made.

If indeed a causal relationship between dietary fat-induced changes in insulin sensitivity and macronutrient selection can be found, the next issue would be to determine how such changes in insulin sensitivity are perceived by the brain such that behaviour could ultimately be altered. At this point a number of hypotheses could be examined. As noted in the discussion, one hypothesis is that peripheral changes in insulin response could impact on brain uptake and utilization of glucose, possibly via
the insulin-sensitive glucose transporter, GLUT 4. Another hypothesis is that changes in circulating insulin concentration could impact on CSF insulin concentration, which could affect behaviour. To examine these hypotheses, insulin sensitivity and brain levels of GLUT 4 and CSF insulin could be measured following the long-term feeding of diets containing various fat sources.

Of course, there are numerous other possibilities of examining this area of research, yet, future investigations will hopefully help determine more specifically the mechanism involved in mediating dietary fat-induced macronutrient selection. A further challenge will be to determine the similarities and differences among the mechanisms involved in mediating the influence of dietary fat on a widespread set of behaviours.

7.4.4 Challenge to elucidate a mediating mechanism

The importance of the specific content of dietary SFAs in influencing macronutrient selection has recently been extended to cognitive behaviour (Greenwood & Winocur, 1996). Furthermore, within the realm of cognitive behaviours, the performance of rats fed high SFA diets has been shown to be impaired on a wide set of learning and memory tasks (Coscina et al., 1986; Greenwood & Winocur, 1990; Winocur & Greenwood, 1993; Greenwood & Winocur, 1996). This research suggests that dietary SFAs and now it appears, specific chain length SFAs, have a general influence on the central nervous system that is not localized to one region of the brain. It therefore appears that whatever mediating mechanism is involved, there is likely a common element linking the SFA effect, and more specifically, the SFA chain length effect on macronutrient selection with an effect on cognition. It is conceivable that this mechanism may also be responsible for mediating thermoregulation, pain sensitivity (which have been shown to be influenced by dietary fat composition (Yehuda et al., 1986; Yehuda & Carasso, 1987)) and a wider, yet untested set of behaviours. Thus, the challenge for future researchers will be to
elucidate a plausible mechanism that is not only specifically influenced by SFA chain length but that mediates several behaviours as well.

7.4.5 Fully hydrogenated soybean oil

The observation that fully hydrogenated soybean oil (HSB) was not fully absorbed by rats was completely accidental as HSB was used for its qualities as a dietary vegetable fat source which contains very high levels of LSFAs. Of course, such serendipitous observations are often the most interesting, and indeed, these findings lead to numerous questions worthy of further investigation.

The data presented here suggest that HSB only contributes about 2.8 kcal/g rather than the Atwater value of 9 kcal/g that approximates the caloric contribution of most dietary fat sources. Thus, HSB appears to behave more like a low-caloric substance or fat substitute than a normal dietary fat. An important next step would be to determine if similar effects occur when this fat is consumed by humans. HSB is extremely hard at room temperature but can be ground into a fine powder or blended with other fats in order to incorporate it into foods. The possibility of using HSB as a fat substitute may be of clinical significance for a number of reasons. Obesity and certain body compositions have been shown to play a role in promoting heart disease, stroke, hypertension, and diabetes (Colditz & Wolf, 1996). Reducing the proportion of calories obtained from dietary fat can contribute to controlling weight and body fat distribution. A fat substitute may help promote such a reduction in dietary fat intake.

One non-caloric fat substance that is currently being used in the United States is olestra (formerly known as sucrose polyester). Olestra is a mixture of hexa-, hepta-, and octa-esters formed from the reaction of fatty acids with sucrose (Bergholz, 1992), and contributes no calories to the diet (Mattson & Nolen, 1972, cited in Bergholz, 1992), looks and tastes like a normal dietary fat source, and can be readily incorporated into foods. However, olestra has also been shown to impair the absorption of vitamin A (Mattson et al., 1979; Sletten et al., 1985) and E (Fallat et al., 1976) and lead to an increase in soft stools in some subjects (Bergholz, 1992). By examining HSB further,
it will be possible to determine if such side effects are also evident when HSB is consumed. HSB may taste better, may be cheaper to manufacture, or may be easier to incorporate into foods.

Another extremely important reason to study the effects of HSB on humans is that HSB is already used in some foods that are currently being consumed by Canadians. In contrast, olestra took more than 20 years to be approved by the Food and Drug Administration (FDA) in the United States and has still not been approved in Canada. Since HSB was manufactured for its texture, it appears to have been assumed that it would behave like any other dietary fat. Thus, it has not been considered as anything but a normal fat and has therefore been spared the scrutiny to which olestra has been subjected. Hence, products containing HSB that are currently being ingested by consumers may actually be low-fat products, and may therefore be more desirable than presently realized. Alternatively, HSB may carry side effects with it, of which we are currently unaware. Studies similar to those that have been performed with olestra may be warranted, including the influence of HSB on vitamin absorption, toxicity, fecal output and cholesterol levels.

Although it was not designed for these purposes, Experiment 2 actually represents a good complete initial study for determining the influence of HSB as a fat substitute on feeding behaviour. In other words, if it was suspected that HSB may behave like a fat substitute, and we wanted to design a study to examine its effects on feeding behaviour, we might have designed the study exactly like Experiment 2. Three diets, containing three different fat sources, each containing 40% of calories from fat, were compared to another diet that contained HSB instead of an absorbable fat source. All other nutrients were identical across dietary groups. The results showed total caloric intake across groups did not differ as HSB animals compensated for their less calorically dense diet by consuming more carbohydrate than protein, and gained weight normally, compared to rats in the other three groups. These results are consistent with those of studies examining olestra which suggest that both adult men (Rolls et al.,
1992) and children (Birch et al., 1993) consuming olestra in place of an absorbable fat, subsequently choose more carbohydrate compared to subjects consuming fat, without a reduction in total energy intake. Therefore, the findings of Experiment 2 suggest that at least in rats, HSB appears to behave very similarly to a fat substitute such as olestra.

As a result of the push by industry to market olestra as a safe product, numerous studies have been completed to determine its safety for human consumption (Bergholz, 1992). However, the influence of olestra or any other fat substitute on higher processes has not been examined. Thus, it would be interesting to compare the effects of olestra, HSB, as well as other fat substitutes, such as caprenin (provides about 5 kcal/g (Peters et al., 1991, cited in Webb et al., 1993)), with both low-fat and high-fat diets on feeding behaviour, as well as other more complex behaviours such as learning and memory. In light of the current research which suggests that both levels and type of dietary fat can impact on a number of behaviours, such research with fat substitutes would be very important in order to understand the safety of these products.

7.5 Relevance to humans

Scientific research is important in order to increase our general body of knowledge. Yet, if research never relates to any aspect of human life, it is difficult to justify investing numerous resources solely for the sake of knowledge. Of course, the impact of research on humans is not always readily obvious, and we must consider that at some point in the future, the knowledge that we gain now may become more relevant. In this regard, if intentions are clear, and methods are sound, most research can be justified. Nevertheless, in order to not lose focus, it is important to try to understand the importance of all research in relation to ourselves.

The broad goals of the research presented in this thesis are to help determine the influence of dietary fat type on brain function in healthy individuals that consume nutritionally adequate diets, and to understand the mechanisms involved. Feeding behaviour is one behaviour that has been shown to be influenced by dietary fat.
Furthermore, relative to some higher cognitive behaviours, it is easier to examine, and to this point the feeding behaviour results have directly mirrored the cognitive behaviour results. For instance, SFA content has been shown to be important in mediating macronutrient selection (McGee & Greenwood, 1990a), as well as a number of learning and memory skills (Greenwood & Winocur, 1996). Thus, we have investigated the influence of various fat sources on macronutrient selection in order to help understand how the central nervous system of healthy, cognitively developed animals responds to such changes. Ultimately, we would like to extend these findings to humans. However, because of the numerous uncontrollable variables that are always present in human studies, it is important to first establish clear associations between diet and behaviour in animals.

The influence of dietary fat type on behaviour in healthy individuals is clearly of great importance to our well-being and progression. In many cultures, dietary habits constantly change, often in tandem with social and economic changes as well as with increased public knowledge. Thus, it is important to understand the significance of such changes on both the development of disease and on higher developmental processes. Yet, despite the growing body of literature that supports the notion that normal variations in diet can profoundly influence behavioural parameters, dietary recommendations have only focused on preventing nutrient deficiency and more recently on improving physical well-being. With the increasing attraction to the cheap, high-fat foods that fast food chains can provide, more and more countries and cultures are rapidly changing their diets. Fast food has replaced cafeteria food at some schools across the United States, and this trend will only continue, as education boards attempt to limit costs. Since schools often represent the primary setting in which children and adolescents develop cognitively, it is important to understand the impact of their diets during this period of growth on cognitive development and subsequent behaviour. In addition, it is extremely important to thoroughly understand the impact of life-long dietary habits on cognitive development. Hence, in order to help individuals reach
their maximum potentials, we must consider the role of diet beyond its influence on nutrient deficiency and disease prevention when determining dietary recommendations for all age groups, genders, and other subpopulations.

The specific relevance of feeding behaviour in humans may relate to the prevention and treatment of various disorders. For instance, the regulation of total food intake is important for the treatment of eating disorders such as obesity and anorexia nervosa. Understanding the regulation of specific protein and carbohydrate intake is more important for managing renal and liver disease. Of course, in comparison to animals, the diet of humans may be influenced more by social, economic, and psychological factors than by physiological drive. Nonetheless, at least one study suggests that humans do indeed show a physiological drive to regulate macronutrient consumption (Wade et al., 1981). Monozygotic identical twins reared apart have been shown to consume similar concentrations of both protein and carbohydrate in their diets, whereas dizygotic twins raised in different environments consumed less similar diets. In other words, pre-programmed genetic information appears to be at least as important as environmental factors in determining feeding behaviour.

The observations obtained with HSB and MCT diets in the present studies may have clinical significance. Although the reason for using HSB and MCT oil as dietary fat sources in the present studies was to examine the effects of SFA chain length on feeding behaviour, the lack of absorption of HSB and the significant influence that MCT oil had on macronutrient selection may have further implications. The relevance of HSB to humans is discussed in the previous section (7.4.5). If HSB is not fully absorbed in humans as in rats, its use as a fat substitute could conceivably help people maintain healthy weights and decrease body fat composition. MCT oil has been prescribed in the treatment of adults, children, and newborns with disorders of lipid digestion, lipid absorption, and lipid transport, as well as a number of other conditions (Bach & Babayan, 1982). Some of these include cystic fibrosis, celiac disease,
Crohn’s disease, enteritis, chylothorax, gallbladder disease, and obesity. If the data can be extended to humans, it may be of interest to caregivers who provide MCT oil as part of the nutritional management of an illness, that patients may tend to crave protein over carbohydrate. Since patient compliance to nutritional recommendations is often a major obstacle to effective treatment, this may partly explain why. That is, patients who receive MCT oil may have a difficult time adhering to protein and carbohydrate recommendations if their natural preferences are not consistent with such guidelines.

The reason for examining the influence of different types of dietary fat on insulin sensitivity and glucose tolerance was to help determine the mechanism involved in dietary fat-induced macronutrient selection. In relationship to humans, however, the role of different fat types in chronically altering insulin sensitivity may be important in helping us make dietary recommendations for diabetic patients, whose insulin responses are impaired. The data described here are not clear in this regard. Although no effect of diet was observed with respect to measures of insulin sensitivity or glucose tolerance when rats consumed diets containing MCT, HCO or SBO for 32 days as 40% of dietary calories, or a diet containing 22% of calories from fat (due to lack of absorption of fat in HSB diet), the association between measures of insulin sensitivity and macronutrient selection does suggest that dietary fat type may influence insulin sensitivity. The trend of the data suggest that MCT oil impairs insulin sensitivity the most, followed by HSB, SBO and HCO. Clearly, more research is needed in this area, but caregivers must also realize that monitoring both type and amount of dietary fat intake in diabetic individuals may be extremely important.

The data compiled from the present studies lead to several avenues of future research and ultimate ramifications if the data can be extended to humans. The most important broad recommendation that can be made in relationship to studying the human diet, is that we must realize that even in healthy individuals, not only total dietary fat content, but specific fatty acid composition can profoundly influence the brain in a manner that is functionally significant. More research in this area should
help us ultimately determine dietary recommendations such that each individual can not only decrease their chances of physical and mental illness, but reach their maximum cognitive potential throughout their lives.
CHAPTER 8

CONCLUSIONS
8. CONCLUSIONS

The purpose of this thesis was to determine the influence of dietary SFA chain length on macronutrient selection in post-developmental rats and to explore possible mechanisms which may be involved in mediating this feeding behaviour. The conclusions that can be made from the present experiments are:

1. Rats fed diets enriched in MSFAs (MCT) select a higher proportion of energy from protein and less from carbohydrate than rats fed diets enriched in ISFAs (HCO), in the absence of a difference in total energy consumption. These data suggest that dietary SFA chain length may influence protein and carbohydrate selection, independent of total SFA content, in post-developmental rats following the long-term consumption of EFA sufficient, 40% fat (percent of total dietary energy) diets in a reproducible manner.

2. The relationship between increasing or decreasing SFA chain length and macronutrient selection could not be determined from the present data, due to the lack of absorption HSB (enriched in LSFAs).

3. The present data suggest that a decrease in insulin sensitivity leads to an increase in protein and a decrease in carbohydrate intake. Insulin sensitivity can account for 11 to 19% of the variation in selection behaviour.

4. Fully hydrogenated soybean oil is not fully absorbed by rats and only contributes about 2.8 kcal/g. Thus, this fat source appears to be an anomalous fat source that may be more similar to a low caloric substance or fat substitute than a normal dietary fat.

5. The feeding behaviour of the rats fed HSB suggests that rats fed a low-fat (relatively low energy-dense) diet completely compensate by consuming enough food to obtain the same amount of energy as rats fed higher fat (relatively high
energy-dense) diets. These results suggest that these animals accomplish this by consuming a relatively high carbohydrate-low protein diet.
CHAPTER 9

REFERENCES
9. REFERENCES


