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Compare Effects Of Diets Made With Different Saturated Fatty Acids On Body Weight Regulation And Metabolism In Wister Rats

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COMPARE EFFECTS OF DIETS MADE WITH DIFFERENT SATURATED FATTY ACIDS ON BODY WEIGHT REGULATION AND METABOLISM IN WISTER RATS.

by

DILRUBA FATEMA

THESIS

Submitted to the Graduate School of Wayne State University, Detroit, Michigan in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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Approved by:

__________________________
Advisor

__________________________
Date
DEDICATION

I would like to dedicate my work to my husband Mohammad Saklayen who stood by me at every stage and supported me throughout my studies. Without his help I wouldn’t be where I am now. I would also like to dedicate it to my loving parents Md. Alauddin and Maksuda Khanam who always encouraged me to pursue higher studies. I would also like to mention my two sons Ahmad and Sameen. The time I studied was the time away from them but they understood and cooperated. Ahmad helped me with the tidbits of computers that would take hours for me to learn.
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and two modified groups in Wister rats.
CHAPTER 1

Introduction

Saturated fatty acids

In recent years developing countries have transitioned into consuming foods low in nutrition and there is more and more tendency to eat calorie -dense foods instead of a healthy low fat, high fiber and low calorie based diet (1). As a consequence of these changes in food pattern, a significant increase in the number of people with the metabolic syndrome (which includes glucose intolerance, obesity, hypertension, insulin resistance, type 2 diabetes, dyslipidemia and cardiovascular disease) has been observed worldwide (2). Therefore, it is very important to identify the specific foods, nutrients or eating patterns which are associated with these metabolic abnormalities, so the necessary recommendations can be made to improve the dietary patterns for these people (2). Typically, these individuals have more than one risk factors of metabolic origin but the most predominant of them is obesity (3). Due to the specific effects, some fatty acids have on different metabolic activities, dietary fat composition can contribute to the development of obesity by changing fat oxidation, and thus influence both body weight and composition (3). Obesity-associated low grade chronic inflammation as manifested by macrophage infiltration in white adipose tissues is significantly related to high fat diets in human and animals (4). Lean and obese individuals do not seem to stimulate fat
oxidation in the same way when fed the high fat diets (5). Different types of dietary fats have a major impact in body fat increase (6). The deposition of body fat can increase significantly more with saturated fatty acids (SFAs) when compared to omega -3 and omega -6 rich diets (6).

Earlier study has shown that there have been specific effects observed on plasma cholesterol levels with different individual SFAs (7). Lauric acid (12:0), the predominant fatty acid in coconut oil, is the most potent SFA for increasing TC and LDL cholesterol levels (7). There are two other common SFAs which also raise blood cholesterol levels namely myristic (C14:0) and palmitic acids (C16:0) (7).

Based on their origin, dietary SFAs can be categorized into two groups, animal fats and vegetable oils (8). Palm oil, soybean oil, palm kernel oil, coconut oil available from vegetable origin are high in SFAs (8). In western diets, the most common long-chain SFAs are stearic (C18:0) and palmitic (C16:0) acid which are considered as the main SFAs in human plasma and tissue as well (8). However each SFA is different based on their specific fatty acid profiles (9). Palm oil mainly consists of palmitic acid (C16:0) and oleic acid (18:1n-9) whereas coconut oil is high in lauric acid (C12:0) (9).

According to the recommendation by Institute of Medicine calories from fat intake should be limited to 20% to 35% of total daily calorie intake and the average intake of SFA content should be less than 10% of total energy consumed (10).
Stearic acid is a common fatty acid of all meats and dairy products, therefore the effect of stearic acid on metabolism need to be evaluated (11). Beef tallow generally contains 18% to 19% of stearic acid and is the major source of stearate in US diet (11). Results from previous study indicate that lean beef is no more considered as hypercholesterolemic and need not be taken out when trying to lower the cholesterol levels (11).

All SFAs have their own characteristics, and are different based on their carbon chain lengths which affect their metabolism (12). SFAs contain carbon atoms between 12 and 24 with no double bond (12). Generally short chain fatty acids (SCFAs) contain two, three or four carbon atoms and liver can fast metabolize these SCFAs (12). Medium chain fatty acids (MCFAs) contain six, eight, ten or twelve carbon atoms and these are mostly found in butterfat and tropical oils like coconut oil, but not abundant in foods (12). These MCFAs are absorbed and carried directly to the liver and oxidized for energy (12). Long chain fatty acids (LCFAs) which contain 16 carbon atoms or more and will need more cleavage steps and decarboxylation during metabolism (13). Lard, beef tallow, soy, canola oil, cocoa butter are some of the common LCFAs that are used frequently (13).

SCFAs and MCFAs have been identified as ‘anti-viral’ and ‘anti-bacterial’ and can be protective against viruses and bacteria which cause gastrointestinal damages and heart disease. In contrast LCFAs do not have this protective effect (14).
Body composition and dietary fatty acids

In human body, 80-90% fat is stored in the subcutaneous depots (just under the skin, abdominal, gluteal-femoral) and rest of the 10-20% body fat is stored in the visceral depots (omentum and mesenteric) (15).

Even though the ideal amount of body fat is a subjective measure, researchers agree that body fat excess of 22% of body weight for young men and 32% for young women increases disease risks (16). If the excess fat is distributed around the trunk of the body which is known as central or visceral obesity (apple-shaped, mainly found in man), it presents even greater risks for diseases (diabetes, stroke, heart disease, hypertension) than if it is on the lower body (pear-shaped, mainly found in woman) (16). Earlier study has shown that different dietary fat influence the location of fat in the body and there is a strong correlation between central obesity and cardiovascular disease (17).

Previous study has shown that diets rich in polyunsaturated fatty acids (PUFAs) can be helpful in lowering abdominal fat due to a higher rate of oxidation of PUFAs (18). Depending on the need of body, carbohydrates can also be metabolized into fatty acids but the efficiency of the deposition of individual dietary fatty acids is higher when comparing to the deposition of de novo synthesized fatty acids (19).

According to a study by Flatt et al (20), total energy intake and expenditure determine the amount of energy stored in the adipose tissue. However types of
diets also have a great impact on this effect as well. Several metabolic changes are strongly related to high fat diets such as decreased lipolytic activity and decreased leptin concentration (21), obesity and insulin resistance (22).

**Dietary fatty acid intake and lipid metabolism**

Considering the CVD risk factors, it is now a known fact that the ‘type’ of dietary fat has greater impact on CVD risk than the amount (23). The effects of dietary SFA on plasma lipoproteins associated with CVD risk are by elevating LDL-cholesterol concentration (23). It has been shown that using of SFAs instead of PUFA, increases total LDL and HDL cholesterol levels (24).

Another study by Montoy et al (25) examined the effects of individual fatty acids on lipid metabolism. They gave four different types of diets containing SFAs (palm oil), MUFAs (olive oil), n-6 PUFAs fat diet (sunflower diet) and n-3 PUFAs diet (sunflower oil supplemented with fish oil). According to their results, PUFAs and MUFAs produced a better lipid profile compare to SFAs. Furthermore, the effects of SFAs on lipids and lipoproteins can be determined by the amount of PUFAs in the diet (26). LDL cholesterol level is affected by SFAs if the PUFAs intake is below a threshold level (~5% of energy) (26). There are also indications from earlier studies that individual types of SFAs will affect LDL and HDL cholesterol differently (27). If carbohydrate (CHO) is substituted with SFAs of long chain length, there is a little increase in LDL cholesterol levels. Among the SFAs, lauric acid had significantly higher increase in cholesterol concentration compared
to stearic acid (27).

**Leptin**

In energy metabolism as well as lipid and glucose homeostasis, white adipose tissue plays an important role by producing hormones such as leptin and adiponectin to regulate food intake and metabolism (28). In recent years researchers have shown growing interest in obesity genetics because of the identification and sequencing of OB (Lep) gene, which was discovered in 1994 and it is located on chromosome 7 in humans (29). There is a rapid increase in obesity during the last several decades. But only a small portion of this increase can be explained by genetic alterations (30). The majority of this increase is due to other reasons, such as changes in diet, habits and lack of exercise (30).

It is well established that in regulating energy intake and expenditure, leptin is known to reduce food intake by transmitting a satiety signal to the central nervous system and increase metabolism and energy expenditure by increasing thermogenesis (30). Previous study has indicated that there was an increase in leptin level in obese population with insulin resistance and dyslipidemia (30). This increase in serum leptin concentration (hyperleptinemia) has been suggested to be caused by leptin resistance (31). Hypertension, impaired glucose metabolism, and pro-atherogenic condition could be influenced by hyperleptinemia as well (32). Leptin is also responsible for the function of growth and reproduction system (33). Leptin decreases pancreatic beta cell production of insulin and enhances insulin action (34).
Adiponectin

Adiponectin is a protein product from chromosome 3q27. It is secreted by adipocytes and it regulates glucose levels and fatty acid breakdown but the specific signaling pathways that regulate the different metabolic effects of adiponectin are still not identified clearly (35). Adiponectin is a key factor in down regulating various metabolic functions which may cause obesity, type 2 diabetes (T2DM), atherosclerosis and non–alcoholic fatty liver disease (NAFLD) (36). Lack of adiponectin is also an independent risk factor for metabolic syndrome (36). Previous study has shown that in patients with cardiovascular disease, hypertension and obesity, plasma adiponectin concentrations were decreased (37). Earlier study has reported that in mice, insulin resistance can be completely reversed if there is a combination of specific physiological doses of adiponectin and leptin administered at the same time (38).

Insulin resistance and fatty acids

Insulin resistance is a physiological condition when cells do not respond to the normal function of insulin, leading to hyperglycemia which is a significant risk factor for T2DM. Insulin resistance has also been implicated as an independent risk factor for CVD and is associated with other physiological abnormalities (39). Because insulin induces a number of metabolic responses, a defect or deficit in insulin signaling may cause adverse metabolic changes (40). The secretion of insulin by pancreatic beta cells decreases with age for humans and this decrease will cause hyperglycemia faster in individuals with insulin resistance (40).
Previous study has shown that high fat diet causes insulin resistance (41). According to a study by Ikemoto et al (42) the function of insulin could be influenced by individual fatty acid profile of the diet. Based on their study, diets high in different SFAs decrease the function of insulin. The mechanisms for how dietary fat types (quality) influence insulin sensitivity are not adequately and comprehensively understood yet (43). However, it is assumed that the effects of individual dietary fatty acids on this biological function are influenced by the characteristics of cell membranes and their FA components (43). Membrane fluidity and stiffness could change with a small modification of FA composition of the sarcolemma(43). The function of insulin could be influenced by a specific fatty acid like lauric or stearic acid, through changes in cell signaling pathways and differences in insulin receptor binding as well (43).

**Coconut oil**

Coconut oil is an edible oil, extracted from the kernel of mature coconuts and it has a long shelf life for up to two years in some cases, because of its low oxidation rate(44). It contains 91% saturated fat of which 60% is lauric acid (C: 12) (45). Regular coconut oil is not hydrogenated but when it is used as an ingredient in processed foods (like candy, cookie etc.), it is partially hydrogenated to increase its melting point for better shelf life (46). That’s when coconut oil becomes ‘bad’ food because partial hydrogenation produces trans fatty acids. For this reason researchers became concerned about the consumption of high amount of coconut oil (46). Earlier study (47) demonstrated that lauric acid raises
blood cholesterol levels markedly which led to the belief that tropical oils contribute to CVD. Much of this increase in blood cholesterol levels are due to increase in LDL and HDL cholesterol. However, it also decreases the total cholesterol and HDL ratio so lauric acid may causes CVD through pathways not related to HDL levels (47). By increasing concentrations of cholesterol and phospholipids, coconut oil causes hyperlipidemia (48). Another study in rabbits observed that the rabbits fed with isocaloric coconut oil had symptoms of atherosclerosis and metabolic syndrome without any obvious weight gain compared to the rabbits that were given only standard chow diet (49).

**Beef tallow**

Tallow is an animal fat processed from suet and currently in United States, most of the tallow comes from edible/inedible rendering industries, meat packing and poultry industries (50). Basically inedible tallow is used as a supplement for animal feed and also used in soap, lubricants etc (50). It contains mostly triglycerides (fat), consisting of mainly stearic (C18:0) and oleic (C18:1n-9) acids (51). For meat industries, beef tallow is one of the most important by-products (51). For high heat cooking this is one of the best fats because of its high smoking point (400°-450° F) and can avoid generating free radicals (52). Oxidation causes free radicals that damage tissues, cells and organs in the body (52).

Previous study has shown that beef tallow can protect animals from metastatic breast tumors (53). Based on their report, the function of conjugated linoleic acid (CLA) was not affected by other fatty acids (palmitic, oleic and
stearic) in beef tallow. There was a clear indication that dietary beef tallow, even though contains only little amount of CLA, is capable of increasing the efficiency and efficacy of dietary CLA and decreasing the incidence of tumorigenesis (53).

Finally, according to a study by German et al (54), for individual SFAs intakes (lauric, stearic, palmitic, myristic etc.), there is no safe limit that can be recommended for individuals who are from different background and maintaining different lifestyle. The significance of different type of fatty acids in different individuals is not well studied. An extensive research should be conducted for such determination taking into account the variance of individual differences.
CHAPTER 2

Objectives of this study

The objectives of this study were to assess:

1) The effects of both the quality (coconut oil & beef tallow) and quantity (low & high) of dietary fat on weight gain/loss in Wister rats.

2) The blood profiles and metabolism of different types and quantity of dietary fat in Wister rats.
CHAPTER 3

Methods

Study design

This study was based on a 2x2 factorial design to investigate the effects of both the quality (coconut oil and beef tallow) and quantity (high and low) of dietary fat.

Animals

Forty two male Wister rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN) at eight weeks of age for this study. They were housed one per polycarbonate cage under standard laboratory conditions (room temperature, 22\(^0\)-24\(^0\) C, relative humidity, 50-52%, light dark cycle 12 : 12 hour). The animals were 80-100 grams on arrival and they were provided food and water ad libitum. After one week of adaptation, rats were randomly divided into four groups and received their respective diets: control low fat (CLF, n=10), control high fat (CHF, n=10), modified low fat (MLF, n=11), and modified high fat (MHF, n=11). Animal care and handling were according to federal guidelines and under the administration of Division of Laboratory Animal Resources (DLAR), Wayne State University, and according to Animal Investigation Committee (AIC) approved protocol.

Diet

Rats were fed modified AIN -93M purified rodent diet as control low fat
(CLF, 40gm fat/kg with 30gm/kg from coconut oil and 10gm/kg soybean oil). High fat diet (CHF) contained 240gm/kg fat with 230gm/kg coconut oil, and 10gm/kg soybean oil. The modified low fat (MLF, 40gm/kg) contained 30gm/kg modified beef tallow, 10gm/kg soybean oil and modified high fat (MHF, 240gm/kg) with 230gm/kg modified beef tallow, 10gm/kg soybean oil. All rats were fed their respective diets for ten weeks. All diets were commercially prepared in pellet form by Dyets, Inc. (Bethlehem, PA). The composition of the diets are listed in Table 1, and the fatty acid composition of the fats used is listed in Table 2.

Procedure

After one week of adaptation period all forty two rats were separated randomly into 4 groups. The groups were as follows: CLF (n=10), CHF (n=10), MLF (n=11) and MHF (n=11). Food intake and body weight were measured twice a week. Left-over foods were discarded and fresh food offered at each weighing. All foods were refrigerated at 4°C until use.

Sacrifice procedure

At the end of the 10 week feeding period, all rats were fasted overnight. The next morning, these rats were sacrificed by briefly exposing to carbon dioxide and then they were decapitated. Trunk blood was immediately collected in a tube containing 0.15% EDTA, vortexed and centrifuged at 1000xg for 20 min at 4°C. Plasma was collected and stored at -20°C for later biochemical analyses. Ten rats were killed each day.
The carcasses were eviscerated and the liver was removed and visible fat in the abdominal cavity was carefully collected, weighed and recorded as internal fat. Rest of the carcass was labeled and frozen for later body composition analysis.

**Biochemical Assays**

**Blood parameters**

 Plasma glucose, cholesterol, triglyceride were measured using kits purchased from Pointe Scientific INC (Canton, MI). Plasma leptin and insulin levels were determined by radioimmunoassay (RIA) techniques. The Rat Leptin RIA kit was purchased from Linco Research (St. Charles, Missouri). Insulin was measured by using a RIA kit purchased from ICN Pharmaceuticals, Inc. (Costa Mesa, CA). Insulin sensitivity was evaluated by the Homeostasis Model Assessment (HOMA) (55). Plasma adiponectin levels were assayed using a mouse adiponectin RIA kit purchased from Linco Research (St. Charles, Missouri).

**Body composition analysis**

 Body composition analyses were completed on shaved and eviscerated carcasses according with the procedure described by Jen et al (56). All of the carcasses were autoclaved and homogenized. Then triplicate homogenate samples were taken to measure the lipid and water content. Total lipid content in the carcass was measured by the methods of Folch et al (57) and this lipid was
considered as the subcutaneous fat. The total fat content in each rat was determined by adding the internal fat and subcutaneous fat together.

**Statistical analysis**

Mean and standard error of the mean were calculated for every variable in this study. Factorial analysis of variance (ANOVA) was used to assess differences in body weight and food intake, plasma glucose, insulin, leptin, adiponectin, cholesterol, triglyceride and total fat content. Significance was set at p<0.05. All calculation/analysis were performed using the SPSS version 22 (IBM SPSS Inc. Armonk, NY). Post-hoc comparisons (multiple comparison test) were performed when a significant effect was found to identify the specific groups that contributed to the difference.
CHAPTER 4

Results

Body weight

Figure 1 shows the body weights of the four different groups of rats in grams. Increased body weight were observed throughout the experiment. MHF gained significantly lower than the CLF, CHF and MLF groups.

Sacrifice weight

At sacrifice both quality (Coconut (C): 527±46g, Modified beef tallow (M): 472±62g, p< 0.0001) and quantity (Low fat (L): 521±41g, High fat (H): 475± 68g, p<0.002) contributed the body weight differences. A significant interaction was also observed (p<0.003). Fat quantity did not make a difference for rats fed the control diets (528±44g, 527±46g). However, rats fed the modified high fat diet (427±46g) weigh significantly less than those fed the modified low fat (516±40g) diet.

Body composition

Figure 2 demonstrates the body fat as percent of total body weight observed in the 4 groups. Figure 3 demonstrates the total fat content observed in the 4 groups. Our data analysis indicated that both the quality (Control: 7.70 ±2.2% vs Modified: 6.41±2.2%, p<0.016) and the quantity (L: 7.79 ±1.66%, H: 6.26±2.53%, p=0.009) influenced to the body fat percent. There was also a
significant interaction (p= 0.012) observed which indicates modified fat reduced
body fat percent when the fat consumption was high as demonstrated by the fact
that MHF (4.7±1.0%) had significantly less body fat percent than the other 3
groups.

**Energy intake**

Figure 4 displays the total calorie intake among the four groups based on
their respective diets. No significant differences (p>.05) were observed among the
4 groups.

**Blood Cholesterol Levels**

Table 3 shows the plasma cholesterol levels observed in our study.
Regardless of the quality and quantity of the oils used in the diets, there were no
significant differences (p>.05) observed among the four groups.

**Blood Triglyceride Levels**

Triglyceride concentrations with different fatty acids were presented in
Table 3. The amount of fat (L: 198±29 mg/dl, H: 189±62mg/dl) in the diet did not
change the total TG levels. However modified fat significantly reduced TG levels
compared to coconut oil (coconut oil: 234±18 mg/dl, modified fat: 157±36mg/dl,
p<0.0001). The significant interaction (p<0.0001) indicates that MHF group had
significantly lower TG than MLF group, but this effect was not observed in the
cocoanut oil groups.
Glucose

As shown in Table 3, Coconut and modified groups had similar glucose levels and therefore no significant difference (p>0.05) was observed regard to fasting plasma glucose. The amount of fat in the diets did not alter the blood glucose levels either.

Insulin

The effects of the four diets on insulin were shown in Table 3. There were significant differences (p=.026) observed between the coconut oil (51.9±8.0μU/ml) and modified fat (44±10.8μU/ml) groups. The amount of fat in the diets did not change the blood insulin levels. Insulin sensitivity was evaluated by Homeostasis Model Assessment (HOMA). The data showed that even though modified fat reduced the HOMA (CLF:35.9±7.1, CHF:33.1±6.4, MLF:29.9±6.0, MHF:32.2±16.5) the difference failed to reach significance. Neither the quality nor the quantity of the diet fats affected the insulin sensitivity. There was no interaction between quality and quantity of fat identified.

Leptin

Table 3 presents the plasma leptin concentration observed in the 4 groups. The modified fat significantly reduce (p=0.032) the leptin levels compare to coconut oil. The amount of fat in the diets did not alter blood leptin levels. There was a significant interaction (p=0.009) observed indicating that modified fat reduced leptin when the fat consumption was high, but would not affect leptin
levels when the fat consumption was in the normal range. Since the MHF had significantly lower body fat content, the leptin per gram of body fat was calculated. There was no difference among the four groups in leptin/body fat content. Therefore, the lower leptin level in the MHF group was due to the reduced body fat content, not due to changes in leptin sensitivity.

**Adiponectin**

Table 3 displays the change in plasma adiponectin levels among the four diet groups. From our results, it is clear that the type of fat (quality) influenced the adiponectin concentrations. The modified groups (8.81±2.14ng/ml) had significantly increased (p=0.03) adiponectin levels compared to Coconut groups (7.41±1.9ng/ml). The amount of fat did not contribute to the differences among the groups.
CHAPTER 5

Discussion

This study was conducted to determine the influence of different saturated fatty acids on body weight regulation and metabolism in Wister rats. Male Wister rats were fed diets made with either coconut oil (high in lauric acid 12:0) or a modified beef tallow (enriched with stearic acid 18:0) ad-lib for ten weeks. The results of our study demonstrated that the modified groups weighed significantly less than the Coconut groups. Percent body fat was shown to be lower in modified groups than the Coconut groups. We also observed that modified groups had significantly less triglyceride and insulin levels compared to Coconut groups. There were no differences observed with regard to energy intake, cholesterol and glucose levels. Modified group had significantly high levels of adiponectin and lower leptin than the Coconut groups.

The role of SFAs as the main cause of cardiovascular disease has not been established beyond all reasonable doubt. Different saturated fats have different effects on human body. Results from our study demonstrated that MHF did not have an impact on plasma cholesterol even when fed at a high amount. An earlier study (58) showed that stearic acid (high in beef tallow) has a unique property of not raising plasma cholesterol concentration. The “neutral” or hypocholesterolemic effect of dietary stearic acid was confirmed by many earlier studies (58, 11). Our data support previous findings. It is not clear if any adverse
effects on blood cholesterol levels will be observed when even higher amount of this modified fat is consumed. Further research with higher amount of stearic acid or longer period of feeding is warranted.

The mechanisms as to why stearic acid as a SAF doesn’t raise blood cholesterols are still not clearly delineated. One of the possible explanations would be that stearic acid makes changes in the bile acid metabolism and reduce the production of hydrophobic secondary bile acids (59). Cholesterol is hydrophobic by nature. As a result of decreased bile hydrophobicity, the solubility of cholesterol is also reduced and thus reduces the absorption of cholesterol. Guerciolini et al (60) also indicated that if there is an imbalance between the fat and bile ratio, fat globules remain intact and cannot be emulsified inside the intestine. These fat globules are not absorbed at all and will pass through for fecal excretion. Imaizumi et al (61) did a study on hamsters and reported that the excretion of fecal fatty acids were significantly increased by feeding cholesterol free diets made with stearic acid (18:0) compared to diets made with lauric (12:0) acid. They also stated that because of the low absorption of stearic acid, it failed to raise plasma cholesterol levels. Feces were not collected in our study so we cannot infer any conclusion in this aspect. But we can relate the use of modified beef tallow with enriched stearic acid in our study which reflects no significant differences in cholesterol profile among the four diet groups (Table 3). We also speculate that poor absorption of stearic acid compare to lauric acid is the mechanism behind our findings. More specific studies are needed to confirm
these mechanisms in the future.

The Coconut SFA used in this study is coconut oil which is high in lauric acid content. Although lauric acid is a medium chain fatty acid, previous study has classified lauric acid as a cholesterol raising saturated fatty acid (62), as it increases both LDL and HDL cholesterol concentration at the same time. Coconut oil also contain myristic acid (14:0) and palmitic acid (16:0) which have been identified as potential cholesterol raising fatty acids. Another study by Denke and Grundy (63) reported a strong correlation between LDL cholesterol and total cholesterol concentration based on the cholesterol raising SFAs (lauric and palmitic). But in the present study, when we compared coconut oil (lauric acid) and modified beef tallow (stearic acid), we did not observe any adverse effects of lauric acid on plasma cholesterol concentration. The reason for this discrepancy of current study and previous study deserves further investigation.

A different study (64) showed that shortly after the absorption of beef tallow, stearic acid desaturates into oleic acid which is a monounsaturated fatty acid and does not raise cholesterol. On the other hand, for this type of desaturation, lauric acid (coconut oil) is not a good substrate. Based on the different chain length and degree of saturation point, each dietary fatty acid undergoes different metabolic pathways. After absorbed directly into the portal vein, MCFAs are then rapidly transported to the liver for next step which is “beta-oxidation”. Using this mechanism MCFA increases diet–induced thermogenesis. In contrast, LCFAs are absorbed via the intestinal lymphatic ducts
and then chylomicrons transport these LCFAs through the thoracic duct into the systemic circulation (65). These differences in the metabolic pathways influence the low absorption of stearic acid compared to the rapid absorption of lauric acid. These may partially explain the different cholesterol raising effects of beef tallow and coconut oil.

From our results it is clear that modified groups had significantly lower level of blood triglyceride than that of the coconut oil groups. Modified beef tallow further lowered the blood TG levels when the fat content in the diet was high. In present study, we did not examine the mechanisms that produced these differences of triglyceride levels among the groups. But a possible explanation could be found from a related study by Hu et al (66) who used sunflower oil and beef tallow in their experiment and found similar results of reduced triglyceride levels by decreasing the chylomicrons size in the diet containing beef tallow. Lower digestibility of beef fat could be attributed as a possible explanation of this lower production of chylomicrons (66). A study by Mattson (67) also indicated that absorbability of the various triacylglycerol of stearic acids was directly related to the concentration of stearic acid in the sn-2 position. When stearic acid is esterified at the sn-2 position, it is well absorbed. But if it is esterified at the sn-1 or the sn-3 position, triacylglycerol is released as free stearic acid and poorly absorbed. Thus by altering the position of the stearic acids in the triglyceride molecule, the absorption levels will be significantly changed. In beef tallow, the stearic acids are more likely in the sn-1 and sn-2 position. This may have lowered
the absorption of beef tallow and support the findings reported by Imaizumi et al (61) indicating that stearic acid increased fecal sterol excretion. In a separate study Grundy (68) showed that hamsters that were fed stearic acid instead of palmitic acid had significantly enhanced activity of hepatic LDL receptor and reduced concentration of plasma cholesterol. In our present study we did not measure the LDL receptor activity but our results suggest that the low levels of triglyceride were observed due to the influence of modified beef tallow that was given in a high fat diet. On the other hand, lauric acid absorbed rapidly and contributed to the increased triglyceride concentration. Rapid clearance of these particles might be another prominent factor as well (65).

An important finding in our study was that modified high fat groups weighed significantly less (Figure 1) than the Coconut groups. It is well established that medium chain fatty acids are easily oxidized compared to long chain fatty acids, leads to greater energy expenditure, results in less bodyweight and decreased size of fat depots (69). Our results have shown that rats fed with a fat diet made with modified fat (beef tallow) did not gain as much as the coconut oil group weight. Logically we would expect that because of the influence of long chain fatty acids (here stearic acid) modified groups will gain much more. But our results did not show that. Thus the only possible explanation for this result could be attributed to the reduced absorption of stearic acid in the beef tallow.

The difference in body fat % was also examined (Figure 3) in our study. Based on the results modified high fat group had significantly lower body fat %
than the other 3 groups. There are couple of possibilities that may influence the body fat %. Stearic acid might be oxidized efficiently and did not contribute to increase body fat % in MFH group but we did not measure fat oxidation so we cannot conclude on that aspect. We have also measured energy intake to find out if there is any relation between body weight and food intake. Our results showed that there were no significant differences among the four diet groups regarding energy intake (Figure 4). It is well known that if the concentration of leptin increases, it sends a signal to reduce the food intake and increase the energy expenditure (70). But in our study leptin did not influence the change in body weight. Reduced leptin levels that we observed in modified groups is due to reduced body fat. A relative decrease in leptin levels would be expected to lead moderate weight gain but we did not observe this in MHF group. Thus it is possible that stearic acid is poorly absorbed and most of the fat was excreted through feces and thus contributed to lose more % fat when the fat content was high in modified group.

High fat diets cause insulin resistance and certain fatty acids differ on their adverse effects on insulin actions (41). Insulin secretion is increased by long chain fatty acids with a high degree of saturation. Different fatty acids can alter some of the activities of cell membranes which play a major role in metabolism. A smaller change in fatty acid composition can make a strong modification in the function of cell membrane. Efficient signal function through these cell membranes depends on the specific position and orientation of fatty acid composition of cell
membrane (43). Thus specific fatty acid can modify membrane fluidity and stiffness and play a key role by controlling the function of insulin signaling. Based on our results (Table 3), Modified group had significantly lower insulin but similar glucose levels compared to Coconut groups, and implies that modified fat did not have any adverse effects on insulin function. We did not observed any change in insulin sensitivity among the groups. If we had prolonged our study we would have observed a significant difference in insulin sensitivity. Further studies are needed to derive a definite conclusion concerning different SFAs and insulin sensitivity.

Table 3 refers that leptin concentration were significantly lower in MHF compared to all other 3 groups. No difference between the four groups was observed when leptin is expressed in per unit body fat. Thus the change in leptin levels was not due to changes in leptin sensitivity, but rather the reduction in body fat content.

Adiponectin is categorized as a special form of anti-inflammatory adipokine which controls insulin sensitivity (71). A relationship has been identified between cardiovascular risk factors and insulin resistance with hypoadiponectinaemia due to obesity (72). In our study, the type of fatty acid (quality) had a marked impact on adiponectin levels. Modified fat had significantly high level of adiponectin compared to Coconut groups. This difference may be explained due to the type of fatty acid that we used in our study. Earlier study by Tomas et al (73) reported that adiponectin decreases weight gain by increasing fatty acid oxidation in muscle via
AMPK activation through Adipo R1 and acetyl-CoA carboxylase inhibition. Another study by Yamauchi et al (74) also reported that adiponectin is unique in itself because it lowers triglyceride, body weight and blood glucose levels and improve insulin sensitivity which also support our findings. According to them adiponectin increases the acyl CoA oxidase, uncoupling protein -2 in muscle, and increase gene expression of fat- combustion-related substances like CD36 and through these mechanisms adiponectin improves blood parameters. These mechanisms may also explain our results. But comprehensive studies are needed to get a better understanding of the mechanisms of adiponectin function and confirm our findings.

Finally, modified beef tallow that we used in our current study proved to have a "neutral" effect on plasma cholesterol profile and this modified beef tallow is different from most of the other saturated fats Future studies should aim to identify the additional benefits of beef tallow that can be used for long term weight management and glucose management. A large sample size and prolonged duration of feeding would have decreased variability and might have increased the potential of detecting other metabolic differences. Each specific saturated fatty acid has different biosynthetic pathways. Specific enzymes that metabolize these different types of fatty acids can also vary significantly. It is very clear that the ‘type’ of fat (quality) matters more than the amount of fat (quantity) when comparing the effects of lauric acid and stearic acid. More research is needed to further delineate the mechanisms that stearic acid exerts its beneficial health
effect.
Figure 1: Body weight of the four rat groups (Mean ± SEM)

Note: Asterisk (*) indicates MHF is significantly different (P <0.05) when compared to CLF, CHF, MLF. There is no difference noted among the MLF, CHF and CLF groups.
Figure 2: Body fat as percent of total body weight of the four rat groups (Mean ± SEM)

Note: Asterisk (*) indicates MHF is significantly different (P <0.05) when compared to CLF, CHF, MLF. There is no difference noted among the MLF, CHF and CLF groups.
Figure 3: Total body fat content of the four rat groups (Mean ± SEM)

Note: Asterisk (*) indicates MHF is significantly different (P <0.05) when compared to CLF, CHF, MLF. There is no difference noted among the MLF, CHF and CLF groups.
Figure 4: Energy intake of the four diet groups (Mean ± SEM)
Table 1: Composition of the diets used in this study

Modified AIN-93M purified Rodent Diet with Low fat, High fat, and Modified Low, Modified High Fat

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control (low fat)</th>
<th>Control (high fat)</th>
<th>Modified Beef Tallow (low fat)</th>
<th>Modified Beef Tallow (high fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram/kg</td>
<td></td>
<td>Gram/kg</td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>140</td>
<td>200</td>
<td>140</td>
<td>360</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>465.392</td>
<td>141.52</td>
<td>465.392</td>
<td>46.395</td>
</tr>
<tr>
<td>Dyetrose</td>
<td>155</td>
<td>97.37</td>
<td>155</td>
<td>40</td>
</tr>
<tr>
<td>Maltose Dextrin</td>
<td>100</td>
<td>80</td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>65</td>
<td>50</td>
<td>65</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Coconut Oil</td>
<td>30</td>
<td>230</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Modified Beef Tallow</td>
<td>-</td>
<td>-</td>
<td>30</td>
<td>230</td>
</tr>
<tr>
<td>Salt mix #210050</td>
<td>35</td>
<td>45.5</td>
<td>35</td>
<td>90</td>
</tr>
<tr>
<td>Vitamin Mix #310025</td>
<td>10</td>
<td>13</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>Choline Bitartrate</td>
<td>2.5</td>
<td>3.25</td>
<td>2.5</td>
<td>3.25</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>1.8</td>
<td>3.9</td>
<td>1.8</td>
<td>3.9</td>
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<tr>
<td>Vitamin E (500 IU/g)</td>
<td>0.3</td>
<td>0.39</td>
<td>0.3</td>
<td>0.39</td>
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<tr>
<td>TBHQ</td>
<td>0.008</td>
<td>0.07</td>
<td>0.008</td>
<td>0.065</td>
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</table>

Source: Dyets inc-2508 Easton A Bethlehem, Pennsylvania 18017
### Table 2: Fatty acid composition in coconut oil and modified beef tallow

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Coconut Oil</th>
<th>Modified Beef Tallow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric (12:0)</td>
<td>48.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Myristic (14:0)</td>
<td>17.6</td>
<td>3.3</td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
<td>8.4</td>
<td>25.5</td>
</tr>
<tr>
<td>Palmitoleic (16:1)</td>
<td>-</td>
<td>3.4</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>2.5</td>
<td>21.6</td>
</tr>
<tr>
<td>Oleic (18:1)</td>
<td>6.5</td>
<td>38.7</td>
</tr>
<tr>
<td>Linoleic (18:2)</td>
<td>1.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Lenolenic (18:3)</td>
<td>-</td>
<td>0.6</td>
</tr>
<tr>
<td>Arachidic (20:0)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Arachidonic (20:4)</td>
<td>-</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Source: Chow, CK. Fatty acids in foods and their health implication, 3rd edition.

CRC Press, Boca Raton, FL. 2008
Table 3: Blood parameters of two Coconut groups and two modified groups of Wister rats (Mean±SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>CLF</th>
<th>CHF</th>
<th>MLF</th>
<th>MHF</th>
<th>P=</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type</td>
<td>Amount</td>
<td>Interact</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>152±33</td>
<td>160±24</td>
<td>156±49</td>
<td>195±57</td>
<td>ns</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>222±12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>245±14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>176±20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>137±38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0001</td>
</tr>
<tr>
<td>GL (mg/dl)</td>
<td>141±13</td>
<td>128±14</td>
<td>134±19</td>
<td>140±25</td>
<td>ns</td>
</tr>
<tr>
<td>INS (µU/ml)</td>
<td>51.5±8.7</td>
<td>52.2±7.7</td>
<td>44.8±7.3</td>
<td>44.2±14.7</td>
<td>0.026</td>
</tr>
<tr>
<td>INS Sen</td>
<td>35.9±7.1</td>
<td>33.1±6.4</td>
<td>29.9±6.0</td>
<td>32.3±16.5</td>
<td>ns</td>
</tr>
<tr>
<td>LEPTIN (ng/ml)</td>
<td>9.4±5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11±6.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.2±4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1±5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.032</td>
</tr>
<tr>
<td>ADIPONECTIN (ng/ml)</td>
<td>7±1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7±2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8±8.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10±2.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values with different superscript (a,b,c) in each row are significantly different from each other (p<.05) or (p<0.01).
CHAPTER 6
Conclusion

Due to the interactions between genes, unhealthy dietary intakes, lack of physical activities and other environmental factors, obesity and diabetes have become major threats to health in our society now. In USA obesity has become the second leading cause, after smoking, of death which could be prevented (75). Since different fatty acids have different metabolic activities, dietary fat composition can contribute to the development of obesity and other metabolic abnormalities by changing fat absorption, oxidation and/or excretion thus influence both body weight and composition. HF diet intake has been repeatedly shown to play significant role in obesity. Therefor the review of different types of fatty acids, their effects on our body weight and regulation has become a major issue these days. Previous studies mostly focused on the effects of dietary SFAs which generally increase blood cholesterol concentration but it has become important now to investigate further regarding the effects of specific fatty acids in a broader health context.

Modified beef tallow, a fat rich in stearic acid, has no adverse effect on plasma cholesterol and glucose levels and also does not increase triglyceride levels. Modified beef tallow that we used in the present study does have impact on weight gain. This modified beef tallow has beneficial effects when the fat content is high. Conforming to previous findings, it is an established fact that beef tallow is no longer considered hypercholesterolaemic and does not have any
deleterious effect on blood profile when it was major fat source in a high fat diet. Beef tallow may increase blood adiponectin levels, thus make it an ideal fat source for individuals with insulin resistance or pre-diabetes.
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reverse insulin resistance associated with both lipopoatrophy and obesity.


ABSTRACT

COMPARE EFFECTS OF DIETS MADE WITH DIFFERENT SATURATED FATTY ACIDS ON BODY WEIGHT REGULATION AND METABOLISM IN WISTER RATS.

by

DILRUBA FATEMA

May 2014

Advisor: Dr. K. L. Catherine Jen

Major: Nutrition and Food Science

Degree: Master of Science

Animal fats are known to cause elevated blood total and low-density-lipoprotein (LDL) cholesterol levels in humans and animals. This study was designed to investigate the effects of both the quality (coconut oil and a modified beef tallow) and quantity (low and high) of dietary fat in body weight regulation and alteration of blood parameters in an animal model. Forty two male Wister rats were randomly divided into 4 groups: Coconut Low Fat (CLF, n=10), Coconut High Fat (CHF, n=10), Modified Low Fat (MLF, n=11), and Modified High Fat (MLF, n=11). They were fed modified AIN-93M purified rodent diet with low fat (40gm fat/kg, 30gm/kg from coconut oil, 10gm/kg soybean oil), high fat (240gm/kg, 230gm/kg coconut oil, 10gm/kg soybean oil), modified low fat (40gm/kg, 30gm/kg modified beef tallow, 10gm/kg soybean oil) and modified high fat (MHF, 240gm/kg, 230gm/kg modified beef tallow, 10gm/kg soybean oil).
230gm/kg modified beef tallow, 10gm/kg soybean oil) for ten weeks. Body weight, body fat content, blood lipid contents and hormones related to obesity and insulin sensitivity were determined. MHF group (427±46g) had significantly lower body weight than the other 3 groups. Body fat percent was significantly lower in modified groups (6.41±2.2%) compared to Coconut groups (7.70±2.2%). Our results showed no significant differences in energy intake among the 4 diet groups (p>.05). No significant difference was observed among the groups with respect to total cholesterol and glucose levels. Modified fat (44.5±10.8 µU/ml) had significantly decreased insulin levels than the coconut oil (51.9±8.0 µU/ml). MHF had significantly lower leptin than CLF, CHF and MLF groups. Adiponectin levels were significantly increased by the modified fat (8.81±2.14ng/ml). In conclusion, this modified beef fat had the least adverse effects compared with coconut oil and was not hypercholesterolaemic, and did not induce obesity when it was given in a high fat diet.
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