Replacing Carbohydrate with Fat deters the onset and progression of Type II Diabetes Mellitus in male Nile rats

Senior Thesis

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Bumjoon Park, May 2017

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Abstract:

Since the past couple decades, there has been rising concerns about the increase in incidence of Type II Diabetes Mellitus worldwide. However, there has been contradictory evidence regarding how the macronutrients in the diet, such as carbohydrates, fats and proteins, and the quality of the food especially fat, such as the type of fat saturation, affects the onset of diabetes. In order to investigate the relationship between different gradients of carbohydrate and fat, the fat qualities, and the incidence and severity of diabetes in Nile rats, two studies were designed to feed weanling male Nile rats five different diets with graded carbohydrate:fat ratios while keeping protein constant at 20% energy (70:10:20, 60:20:20, 50:30:20, 40:40:20, and 20:60:20 for CHO:fat:PROT). To assess the onset and progression of T2DM, data from body weight, random blood glucose, fasting blood glucose, the oral glucose tolerance test, food intake, water intake and necropsy were collected and analyzed during a 10 week treatment period of 150 male Nile rats. The results indicate that replacing simple CHO with fat reduces T2DM, whereas the type of fat saturation only had minimal effects by comparison. To conclude, simple CHO is the underlying cause of diabetes in Nile rats, and there is a strong genetic component to this tendency. Further work must be conducted to determine which genes regulate this phenomenon.
Introduction

During the past couple decades, there has been a sudden increase in the incidence of Metabolic Syndrome (MetS) and the associated Type 2 diabetes mellitus (T2DM) worldwide [1]. Currently, there are more than 300 million people diagnosed with T2DM globally, and it is expected to be around 550 million by 2030 with the current prevalence rate [1]. T2DM is characterized by either insulin resistance by the cells that utilize glucose or decreased insulin secretion leading to its limited availability for glucose clearance [2]. Due to this abnormality, the cells are unable to effectively access glucose as energy. In that case, the body must rely on fat for energy, leading to ketosis and body wasting away to skin and bones. T2DM not only affects the health of an individual, but it also influences the quality of life and the health system overall. If hyperglycemia in diabetes is left untreated, it could lead to long-term complications such as nerve damage resulting in infections and possibly amputation, retinopathy leading to blindness, kidney damage, and an increased risk of heart attacks and strokes [1, 3].

There has been much contradicting evidence regarding how the macronutrient content (ie. carbohydrate, fat, and protein) and the quality of the food (ie. refined vs. complex carbohydrates, types of fatty acids, etc.) affect diabetes [4, 5, 6]. Some researchers find that the more carbohydrates one consumes, the more likely one is to develop diabetes while others suggest that dietary fats are implicated in diabetes development [6, 7, 8]. Furthermore, there are not many conclusive findings on how different types of fats, such as saturated, monounsaturated, or polyunsaturated, affect the outcome of diabetes. Blood glucose control, body weight, and plasma lipids, all of which are indicators of MetS, are also known to affect the course of disease in the novel animal model for T2DM, the Nile rat (Arvicanthis niloticus).

Blood Glucose

The concentration of blood glucose (BG) is a common and simple measurement used in determining T2DM and MetS status, and the term diabetes means that blood glucose is above normal. It is also well established that the amount of glucose in the blood determines whether insulin is functioning properly or not [9]. Many studies have tried to find whether there is a significant difference in BG among those who follow different diets in order to reduce the incidence of T2DM and MetS. However, not enough research has been done to firmly conclude which different diet compositions cause significant effects in BG that can also be demonstrated to influence the outcome of T2DM. This is partly because the macronutrient matrix and various combinations of CHO:fat:PROT mixes is completely and extremely difficult to control in clinical trials.

Animals studies. One study assigned thirty BALB/cByJ male mice to a high fat diet, high fructose diet, or a standard chow diet [10]. The high fat diet consisted of 45% kcal from fat, the high fructose consisted of standard chow and 10% w/v powdered fructose in drinking water, and the standard chow diet consisted of only the standard rodent chow. The study found no significant difference in the blood glucose levels between the three diets, and did not expand on the reasons as to why no significance was observed.

However, in another study, C57BL6/J mice and male Wistar rats were fed diets rich in Long Chain Fatty Acid (LCFA, mostly 16:0 -18:2), Medium Chain Fatty Acid (MCFA, mostly 8:0 – 14:0), or a standard low fat laboratory chow (71:8:21, CHO:Fat:PROT %energy) [11]. The high fat diets fed to mice consisted of 45% energy from fat, whereas the rat studies contained 59% energy from fat, and the percent energy from carbohydrates and proteins were not specified. They performed an ipGTT (intraperitoneal) after 5 weeks of feeding and overnight fasting. They found that the LCFA diet significantly impaired glucose tolerance compared to the low fat diet (P<0.01), whereas impairment by MCFA diet was milder, also compared to the low fat diet (P<0.05). In rats, they placed euglycemic-hyperinsulinemic clamps and implanted double jugular cannulas to collect plasma samples. They found that plasma glucose was
significantly higher in rats fed LCFA than in rats fed the low fat control diet. In MCFA rats, the blood glucose levels were slightly, but not significantly, higher than the control group. The point is that LCFA were worse than the high carbohydrate diet in these rodent models, which contradicts a review by Feinman [7], where they show and explain 12 reasons why high carbohydrate diet is the main factor in the development of diabetes.

Also, a study with male SDT rats that were assigned to either a high fat diet or a standard diet measured the FBG levels and performed an oral glucose tolerance test at 24 weeks of age [12]. The blood glucose was measured at 0 (FBG), 30, 60, and 120 minutes after orally administering glucose. They found that rats fed high fat diet had a significantly lower FBG compared to the rats fed standard diet (P<0.05). Not only the fasting levels, but the 30 and the 60 minute glucose levels of OGTT were significantly lower in the high fat diet (P<0.01 and P<0.05, respectively), indicating that high fat is not hyperglycemic postprandially compared to the standard diet. The genetic makeup of SDT rats have a spontaneous mutation in the fa gene, which causes hyperphagia that ultimately leads to diabetic conditions, such as hyperglycemia. However, a high-fat diet may have offset the effects of the mutation by an unknown mechanism.

In 2013, male Nile rats were fed either a low carbohydrate diet (10:70:20) or a high carbohydrate diet (70:10:20) for 7 weeks after weaning for 4 weeks [13]. With both diets, the FBG was below 80 mg/dl and showed no significance. However, this study also measured the non-fasting, random blood glucose (RBG). Although FBG did not show any significant differences between diets, RBG was significantly higher for the high carbohydrate diet than the values in the low carbohydrate (ie. high fat) diet (P<0.05). Further studies indicate that FBG is a less sensitive indicator of diabetes status in young Nile rats and does not tend to rise until the animal is severely glucose tolerant.

A study with a guinea pig model found no significant differences in fasting blood glucose (FBG) between animals that were fed a low carbohydrate (high fat) diet and a low-fat diet, suggesting that guinea pigs are not likely to be good models for diet-induced diabetes research [14].

**Human studies.** In humans, a study assigned either a 60% carbohydrate or a 40% carbohydrate diet and measured the FBG in thirty insulin-resistant participants [15]. They found no significant differences in the FBG between those who ate 60% and the 40% energy as carbohydrate. However, other studies observed significant differences in the FBG when diets of varying carbohydrate levels were fed. In one crossover study, healthy 40-75 year old participants were given a high-fat diet (15:80:5), rich in saturated fatty acids (S:M:P of 16:7:1, essential fatty acid deficient), which is strange diet, but was only fed for 24 hours, and a high-carbohydrate diet (60:25:15) with equal ratios of S:M:P for fat. A control, average North American diet (55:25:20 as CHO:fat:PROT) was used (at least 3 days prior to the experiment) to establish a baseline [16]. Participants on the high fat (n=38, 31 male, 7 female) and high carbohydrate diets (n=12, all male) consumed their experimental diets for 24 hours. Each participant was fed the control diet for 72 hours, which included a dietary plan based on the American Heart Association (S:M:P of 1:2.4:1.6 fatty acid ratio) that they had to follow. At the beginning of each test diet day, the FBG was measured. There was a significant rise in the FBG after the 24 hour with SFA hi-fat diet compared to the baseline established by the control diet, but no significance between the SFA diet and high carbohydrate diet, possibly due to the number of participants in high carbohydrate diet being much lower than the high fat diet or SFA and carbohydrates being equally unhealthy.

In summary, there are many controversies on how different composition of diet causes significant changes, if any at all, in BG. The answer to this question is not obvious and even confusing, due to all the contradictory conclusions. Therefore, this study looks into this issue using the Nile rat model of T2DM.
**Body Weight**

There is an ongoing debate on whether certain diets with different macronutrient ratios (carbohydrates:fat:protein) affects body weight, at least in growing animals, as opposed to adult humans where it is much more difficult to assess because they are through growing.

**Animal studies.** In animal models, several studies did not find significant differences in body weight based on the kind of diet fed. In one study mentioned earlier, they randomly assigned and fed 8 week old male C57BL6/J mice and male Wistar rats a high fat diet rich in long chain fatty acids (LCFA), high fat diet rich in medium chain fatty acids (MCFA), or a low fat diet for 5 weeks [11]. Each high fat diet fed to mice consisted of 45% calories as fat and each high fat diet fed to rats consisted of 59% energy as fat. With the low fat control diet, both mice and rats’ diets consisted of 71:8:21, CHO:Fat:Protein. In the C56BL6/J mice, no significant difference was noted in body mass between different diets. In contrast, the male Wistar rats fed the high fat diet rich in MCFA had a significantly lower body weight compared to rats fed the diet rich in LCFA (P<0.01). Likewise, in guinea pigs, they found no significant difference in the body weight between low-carbohydrate (high fat) diet (LCD, 10:60:30) and low-fat diet (LFD, 55:20:25) [10]. They assigned twenty 18 mo old male Hartley guinea pigs either LCD or LFD. Although they changed the carbohydrate-fat ratio, they did not keep protein ratios consistent. This could have played a role in the lack of significance regarding body weights.

Despite studies that do not show significant changes in body weight due to differences in macronutrient ratios, others have shown changes in body weight as the CHO:fat ratio of the diet was altered. They found that mice fed a high fat diet gained more weight as the level of dietary carbohydrates increased [17]. They fed C57BL/6 mice either a normal chow diet (65:12:23) or a high fat diet (which consisted of 58% calories from fat and 0.1%, 5%, 10%, or 25.5% carbohydrates balanced with protein). This means that either the carbohydrate or the protein was a significant determinant of weight gain. However, another study found that the body weight in male Spontaneously Diabetic Torii (SDT) rats fed a high fat diet (18:62:20) were significantly higher than weight in rats fed a standard diet (61:14:25) [12]. This indicates that fat plays a greater role in weight gain than carbohydrates in these rats.

**Human studies.** Not only in animal models, but in adult humans, research does not show consistent effects of diet composition on body weight loss. One study assigned insulin-resistant subjects to a high-CHO diet (60:25:15, n=30) or a mod-CHO diet (40:45:15, n=27) [15]. The amount of calories from protein remained consistent at 15%, and both diets were calorie restricted at 750 kcal/day. After 16 weeks of eating their respective diets, both groups significantly lost weight compared to their initial body weight. However, no significant difference in weight loss was noted between the two diets. One problem was there was no control for the low-calorie intake. Another study conducted a meta-analysis of randomized controlled clinical trials with 2,788 subjects on the effects of low carbohydrate diets (≤45% energy from carbohydrates, unspecified percent energy of other macromolecules) versus low fat diets (≤30% energy from fats, unspecified percent energy of carbohydrates and proteins), and found no significant differences between the body weights of the two diets after 6 to 24 months of study duration [18]. This contradicts the point where they state that weight loss on low carbohydrate ketogenic diet is generally better than low fat diets [7].

In humans, they studied the effects of a low calorie diet (LCD, unspecified ratio of carbohydrate:fat:protein) versus a low carbohydrate ketogenic diet (LCKD, goal of 80 kcal/d carbohydrate consumption) on diabetic and non-diabetic participants [19]. Twenty-four diabetic participants (and 119 non-diabetic controls) were assigned to the low calorie diet (n=143), and 78 diabetic subjects (and 142 non-diabetic participants) were assigned to the ketogenic diet (n=220). The low carbohydrate diet induced a much greater weight loss after 24 weeks than the low calorie diet. The participants received a list of recommended and restricted foods to follow the low-carbohydrate ketogenic
diet. For the low calorie diet, participants were limited to a 2200-calorie diet with guidelines to follow. At the end of 24 weeks, the LCKD resulted in a body weight decrease of around 12% in both diabetic and non-diabetic participants, while the body weight decrease was much lower (7% and 5% in diabetic and non-diabetic participants, respectively) in LCD.

The point is that, even with all these researches, no one can definitively conclude whether high fat or low fat diet causes any significant changes in the body weight. Therefore, this research identifies whether significant changes occur in the body weight of Nile rats depending on the type of diet fed.

Plasma Lipids

Studies have shown that insulin resistance and T2DM are associated with plasma lipid abnormalities [20]. These include increase in triglyceride and LDL-C, and reduced HDL-C [21]. However, there are discrepancies regarding how certain ratios of macronutrients affect the plasma lipid profile.

Animal studies. In one study, C57BL/6 mice were fed standard chow or a high fat diet (58% fat) with different ranges of carbohydrates (0.1%, 5%, 10%, and 25.5% energy complemented with protein) [17]. Plasma triglyceride increased in all high-fat diets, regardless of the amount of carbohydrates. In male Nile rats, which were assigned either a high carbohydrate diet (70:10:20) or a low carbohydrate diet (10:70:20), they found that the plasma triglyceride and total cholesterol were significantly higher (P<0.05) in the high carbohydrate diet than the low carbohydrate diet as diabetes developed [13].

In another study, they measured triglycerides (TG) of Wistar rats that were fed either standard laboratory chow, a high fat diet rich in LCFA, or a high fat diet rich in MCFA [11]. The plasma triglyceride was significantly higher in LCFA rats compared to the control diet, and MCFA diet only had a small increase compared to control. The plasma triglyceride concentration in the low-fat control diet was 7.2 ± 0.1 mmol/L, while the MCFA resulted in 7.5 ± 0.2 mmol/L. However, with the LCFA diet, the plasma triglyceride spiked up to 8.1 ± 0.2 mmol/L, which is significantly higher than the control, but not significant compared to MCFA, which was intermediate.

Human studies. Although they reported significant elevations in TG with high fat diets in rats, other studies found different results in humans during meta-analysis of 23 randomized controlled trials that compared low carbohydrate diets with low fat diets [11, 18]. Compared to the low fat diets, the low carbohydrate diets induced significantly lower serum triglyceride, but not as great an effect on reduction in total cholesterol as the low fat diets. This is most likely due to the design differences that tested rats versus humans, and potentially shows the weakness in using rats, more specifically Wistar rats, as an animal model for this type of research.

Furthermore, in humans, the plasma TG response to a low carbohydrate ketogenic diet was compared to baseline TG before the diet and found no significant change in TG or TC between the pre- and post-ketogenic diets [22]. A similar study was conducted with a low carbohydrate ketogenic diet and low calorie diet in subjects with Type II Diabetes as specified before, but according to their figure, both diabetic and non-diabetic subjects fed the low carbohydrate ketogenic diet had significant decreases (P<0.0001) in triglyceride and total cholesterol compared to the initial values prior to the 24 week study [19].

In humans, they randomly assigned insulin resistant subjects to either a high fat diet (40:45:15) or high carbohydrate diet (60:25:15)[15]. Similar to the Nile rat study, they found that after 16 weeks the triglycerides were significantly lower on high fat, but in this case, the high carbohydrate diet did not alter TG [13]. They found in another study a similar result with overweight and obese men who were assigned a ketogenic diet after a baseline diet [23]. The high fat ketogenic diet was (5:80:15), while the normal
baseline diet was (50:35:15). They found that after 4 weeks, the plasma triglyceride tended to decrease, after the ketogenic diet but not significantly.

To put it briefly, there is not enough research to confidently conclude how diet composition affects plasma lipid profiles. Hence, this research uses the Nile rat model to provide concrete evidence from carefully designed diets of the effects of diet on plasma lipids.

**Fat Quality**

There are many different fatty acids that the general population consumes everyday. Fats are characterized by the number of double bonds in the carbon chain: none (saturated), one (monounsaturated), or multiple (polyunsaturated). Most bonds are in the cis-position, but there are some with trans-positions (trans-fat) when fats are modified artificially for product stability [24]. Furthermore, the saturated fatty acids can be grouped based on their length: the most common ones are long-chain fatty acids (≥12:0-18:0) and less frequently found are medium chain fatty acids (10:0 and 8:0) [25]. Although most people associate high intakes of fat with bad health, they do not consider the quality of the fat (ie. the total fatty acid profile and mixture of LCFAs in the entire diet) that are frequently linked to health risks [26]. Both the degree of saturation and chain length are important in considering the health effects of fats [27, 28].

They performed a large prospective cohort study on the effects of fat on CHD using 130,000 healthy women and men by assessing diet intake with a questionnaire every 4 years for 24 to 30 years [29]. Substituting SFA with PUFA was associated with 25% lower risk of coronary heart disease (CHD). Additionally, substituting SFA with unsaturated fats (MUFA and PUFA) was associated with significantly reduced risk of CHD (17%). However, substitution of SFA with trans-fat or refined carbohydrates did not alter the risk of CHD. A separate experiment performed two prospective longitudinal cohort studies on 73,147 healthy women and 42,653 healthy men and also arrived at similar conclusions [30]. Isocalorically replacing 1% of energy from 16:0 SFA with other healthier dietary macromolecules (such as PUFA, whole grains, or plant proteins) resulted in significantly lower risk of CHD. When 16:0 SFA was replaced with PUFA, whole-grain carbohydrates, and plant proteins, the hazard ratios of CHD were 0.88 (P=0.002), 0.90 (P=0.01), and 0.89 (P=0.01), respectively. After pooling 12:0 – 18:0 SFA and isocalorically replacing 1% of energy with PUFA, whole-grain carbohydrates, and plant proteins, the hazard ratios were 0.92 (P<0.001), 0.94 (P=0.08), and 0.93 (P<0.001), respectively. Thus, they concluded that replacing 1% of daily energy intake from SFA (12:0-18:0) with other healthier alternatives resulted in 6-8% reduced risk of CHD, while replacing 16:0 SFA with healthy alternatives resulted in 10-12% reduced risk of CHD.

A report by the World Health Organization in 2016 reviewed the effects of different fatty acids on plasma lipid levels in healthy adults [24]. By replacing SFA with other macromolecules (PUFA, MUFA, and carbohydrates), they found that TC, LDL, and HDL levels decreased significantly. However, with TG levels, it tended to decrease significantly, except for when SFA was replaced with carbohydrates where it significantly increased. The point is that carbohydrate energy results in an exceptionally strong risk for CHD, and from the diabetes perspective, on T2DM as well [13].

Therefore, not only the composition of the diet, but also the quality of fat seems to matters. A second study on Nile rats will keep the diet ratios consistent with the other study, but use a fat quality similar to what the regular American consumes to gain further insight on how the quality of fat affects the health outcome in the Nile rat model of T2DM.
Methods

Study Design

This study was divided into two experiments and was designed to answer the following questions: how do different ratios of carbohydrates to fat in a diet affect the onset of type II diabetes in Nile rats? How does different dietary fat quality affect the development of T2DM in Nile rats?

In order to investigate the relationship between different levels of carbohydrate versus fat, the quality of saturation-unsaturation, and the incidence of diabetes in Nile rats, two studies (NR Study 150 and 151) were designed to feed weanling male Nile rats five different diets with graded carbohydrate:fat:protein ratios while keeping protein constant at 20% energy (e.g. 70:10:20, 60:20:20, 50:30:20, 40:40:20, and 20:60:20). These macronutrient ratios between the two studies were kept exactly the same, but the fat content between the two studies was manipulated to determine the effect that fat quality has on diabetes in Nile rats. One fat (Margarine A, Study 150) was rich in readily-metabolizable medium chain fatty acids (MCFA rich in 8:0 to 14:0) with a 1:1:1 ratio between saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids (S:M:P ratio). The results were compared to the second study (as well as many previous studies) that fed a Western fat blend (Margarine B, Study 151), designed as the average American fat blend, with long chain fatty acids (LCFA) that had a SFA, MUFA, and PUFA ratio of 9:8:3, or 3:1.7:1 as S:M:P. To assess the onset and progression of T2DM, data from body weight, random blood glucose, fasting blood glucose, the oral glucose tolerance test, food intake, water intake and necropsy were collected and analyzed for a 10wk treatment period.

The hypotheses were:
1) that the Nile rats fed diets higher in fat and lower in carbohydrate will have lower incidence and less severe Type II Diabetes Mellitus (T2DM), and
2) the Nile rats fed MCFA (8:0 – 14:0) with a 1:1:1 ratio for S:M:P in Marg A would develop less diabetes compared to the rats in the second study (Marg B) and previous studies that were fed fats similar to the average American fat blend, which contained LCFA (16:0, 18:0) with a S:M:P ratio of 9:8:3.

Based on previous studies, we suspected that the RBG, which is a medium-sensitive indicator of diabetes in Nile rats and the most reliable predictor of diabetes pathology found at necropsy, would be more severe in Nile rats that consumed diets higher in carbohydrate, ie. providing a greater glycemic load [31].

Animals and Diet

138 male Nile rats (Arvicanthis niloticus) from the Foster Biomedical Research breeding colony were separated at weaning (3wk old, average body weight of 31.6 g) and were fed an experimental diet for one week before food intake was recorded to allow the rats to get accustomed to the diet. All Nile rats were placed in individual small sized cages (5 inches) in an air-conditioned room with a 12-hour light cycle, temperatures of 68-72°F, and humidity of 40-60%. In experiment 1, 69 rats were divided into five dietary groups (70:10:20, 60:20:20, 50:30:20, 40:40:20, and 20:60:20) with fat based on Marg A with 1:1:1 ratio for S:M:P. The second experiment had similar diet groups, except the fat quality used in these diets were based on animal fats with LCFA and S:M:P ratios of 9:8:3. All the Nile rats were wild-type meaning that they were randomly bred and not a result of inbreeding.
Body weight and Body Weight Gain per Day

After 6 weeks on the experiment, the rats were fasted overnight for 16 hours and their fasting body weight (FBW), fasting blood glucose (FBG) and 30 minute OGTT were measured. On a non-feeding day at 6 weeks in the study, usually on Thursdays, their random body weight (RBW) and random blood glucose (RBG) measurements also were recorded. At 10 weeks, the Nile rats were tested again for FBW, FBG, 30’ OGTT and 60’ OGTT after overnight fasting. On a non-feeding day, RBW and RBG were measured again. At the end of the study, the Nile rats were fasted overnight, euthanized and necropsied the following morning after their FBW and FBG were recorded. Organs (liver, kidney, cecum) and major fat pads (epididymal, perirenal-retroperitoneal, and interscapular brown fat) were weighed and blood was collected by cardiopuncture for analysis of plasma total cholesterol (TC) and triglycerids (TG). The cecum fecal content, liver, and kidney were harvested.

The body weight of each animal was measured in the form of fasting body weight (FBW) and random body weight (RBW). At 6 weeks and at 10 weeks, the rats were fasted overnight and their body weight was measured the following morning before beginning OGTTs. At non-feeding days, their body weight was measured before the blood glucose values were measured. In order to take the body weights of the Nile rats, they were placed inside a cylindrical tube, which was set on an Ohaus Scout Pro SP2001 balance that was tared with the tube beforehand.

The body weight gained per day was calculated by taking the difference between the Nile rats terminal body weight and initial body weight and dividing by the number of days the rats were on the study.

Food Intake, Food Efficiency, and Water Intake

All rats were fed for 10wks with fresh food provided every Mon, Wed, and Fri, while measuring food intake during those days. Their caloric intake was calculated by taking their food intake in g/d and converted to kcal/d based on the kcal/g of diet.

Food efficiency was calculated by dividing their body weight gained per day (g/d) by their daily caloric intake (kcal/d) and multiplying by 1000. Greater food efficiency indicated that the rats gained more weight per calorie consumed.

Representative water intake, a good clinical measure of diabetes, was recorded by weighing the water bottle before and after the 9th week of study.

Blood Glucose

At 6 weeks and 10 weeks, random blood glucose (RBG) and fasting blood glucose (FBG) measurements were taken while the Nile rats were under anesthesia (50% O₂/CO₂) either on non-feeding days or after fasting overnight for 16 hours. The blood was obtained by lancet puncture at the lateral tail vein and using a Contour Blood Glucose Meter (Bayer Co., Elhart, IN, USA).

Oral Glucose Tolerance Test (OGTT)

Rats were fasted for 16 hours overnight. After recording their body weight and tail vein blood glucose, they were administered 1.75 g/mL dextrose solution (10.5 g of dextrose in 6 mL of water) through oral
gavage and blood glucose was assessed after 30 minutes at 6 weeks and 30 minutes plus 60 minutes at 10 weeks. The body weight was multiplied by 2.25 to determine exactly how much glucose solution had to be administered per rat in uL.

**Organ Weights and Body Length**

The liver, kidney, cecum, and the major fat pads (epididymal, perirenal-retroperitoneal, and interscapular brown) were weighed and expressed as a percentage of their body weight. This was calculated by dividing the weight of the organ (g) by the terminal body weight (g) and multiplying by 100. After exsanguinations and removal of all the organs except for the brain, the carcass weight was measured, which is a lean body mass estimate for muscle and skeleton.

Body length was measured from the base of the tail to the snout of the rat in centimeters using a standard cm ruler.

**Plasma TC and TG**

Plasma TC and TG were determined spectrophotometrically using infinity kits (Thermo Fisher Scientific Inc., Middletown, VA, USA; TG ref # TR22421, TC ref # TR13421).

**Statistical Analysis**

The Statistical Package for the Social Sciences (SPSS) was used to analyze statistical significances. The One-Way Anova was used for the study, which was run with Fischer’s Least Significant Difference (LSD) test. Tukey’s Post Hoc analysis was used to look for significant patterns in the data. A p-value of <0.05 was considered to have statistical significance. Graphs and tables were generated using Microsoft Excel and Word 2007.
Results

Nile rat Study 150 and Study 151

These studies utilized three different measures of blood glucose in order to establish the degree and incidence of diabetes in these rats. The oral glucose tolerance test (OGTT) used the 30 minute glucose with a cut-off of 175 mg/dL to distinguish between resistant or susceptible rats in terms of their diabetes. A second measure was the random blood glucose (RBG) with the cutoff at <75> mg/dL to designate resistant or susceptible rats in terms of diabetes progression. The final measure of blood glucose was the fasting blood glucose (FBG) with a cut-off of 60 mg/dL. Of the three estimates, OGTT was the most sensitive, RBG was mid-sensitive, and FBG was the least sensitive for diagnosing diabetes clinically in these 10wk experiments. The reason for using these values and their set points for estimating their sensitivity is outlined below along with the relative values for these three measures.

Study 150 (Marg A, S:M:P of 1:1:1)

Resistant vs susceptible rats. Study 150 utilized Marg A for fat, which is based on Palm Kernel Oil (PKO) to generate a balance between S:M:P of1:1:1, during the 10wk study (see exact composition in Table 1). The diets ranged from the highest CHO intake (70% calories) to the lowest CHO at 20% calories while replacing CHO calories with fat as Marg A across diets. Using RBG as the main index for the percent incidence of diabetes, the incidence was highest for Diet 196 (60:20:20) at 64%, and was lowest for the lowest CHO intake with Diet 199, which was 20:60:20 and induced a 21% incidence. The percent incidence was defined by rats separated as susceptible with RBG greater than 75 mg/dl compared to resistant rats with less than 75 mg/dL. The various types of data, from body weight to blood lipids, were then sorted according to the resistance or susceptibility among rats for each diet group in order to gain an understanding whether dietary CHO or fat was exerting the most influence over the diabetes induced.

Body Weight For the 10wk body weight values across diets, the susceptible rats for the middle three diets (60:20:20, 50:30:20, and 40:40:20) had gained considerably more weight than the resistant rats fed those 3 diets, indicating an apparent genetic aspect of susceptibility. The highest carbohydrate intake 70:10:20 and the lowest carbohydrate intake at 20:60:20 indicated that the susceptible rats either did not gain more weight (70:10:20, likely due to ketosis from hiCHO) or even gained less weight (20:60:20) compared to the resistant rats. It is noteworthy that the initial body weights at three weeks of age for Marg A were similar for all diets and both subcategories of rats. Both resistant and susceptible rats weighed a little over 30 g at the beginning of the study and unlike Study 151 (see below and AmFat Blend in Marg B), there was no prediction of which rats were going to become diabetic based on their initial weight.

Food Intake, Caloric Intake, Food Efficiency and Body Weight Gain Per Day The parameters of food intake and caloric intake are interesting because they indicate that the diabetic susceptible rats consumed more grams and ate significantly more calories for the first three diets with higher carbohydrate intakes. However, these values for the last two diets with higher fat in the susceptible rats were similar to, or even less than, caloric intakes observed for the resistant rats fed the higher fat intakes. Notice that rats fed the higher fat diets also tended to account for the higher calorie content of those diets by simply eating
less food, and thus adjusted their caloric intake to normal. This made the caloric intake for the resistant rats across all the diets appear about the same, i.e., between 34 and 35 kcal per day despite great differences in caloric density of the diets.

In terms of food efficiency, the resistant rats revealed an increasing trend in efficiency (weight gain per calorie) as they consumed diets with higher fat content, whereas the susceptible rats revealed no predictable pattern in efficiency and varied at random depending on which diet they were eating.

Body weight gain per day also was highest among the susceptible rats for the middle diets, and resistant rats tended to increase gain per day as the fat increased, a point reflected in their food efficiency.

**Random Blood Glucose, Oral Glucose Tolerance Test, and Water Intake** Random blood glucose after 10 wks was significantly greater in the diabetic (susceptible) rats than the non-diabetic (resistant) rats (by definition and according to predetermined selection based on the 75 mg/dl cutoff), and the highest RBGs were associated with higher carbohydrate diets and dropped off stepwise as fat increased from 30 to 40 to 60% energy. In other words, the RBG seemed to follow the amount of CHO in the diet, not fat intake. Note that the RBG in resistant rats remained about 60mg/dl for all diet groups, indicating that value was low and stable in the absence of diabetes, independent of the CHO-fat diet ratio.

The oral glucose tolerance test (OGTT) reflected the random blood glucose value. After 10 wks, the 30’ OGTT (typically when the glucose peaks in the assay) was again higher for the first three diets having the most carbohydrate and decreased as fat increased from 20% to 40% to 60% of energy among the susceptible rats. The resistant rats all had similar, normal values in the 165-178 mg/dl range. Again, the implication is that CHO, not fat in the diet, was the main driver behind elevated blood glucose in diabetic rats.

Water intake, as an index for kidney failure during advancing T2DM in susceptible rats, was reflective of the random blood glucose so that the worst diabetes as determined by RBG also revealed a 3-to 4-fold increase in water intake and polyuria, especially among rats fed the three greatest intakes of carbohydrate. The last two diets with carbohydrate at only 40 or 20% of calories ended up with normal water intakes, with no significant difference between the susceptible and resistant rats, suggesting that even water loss and kidney diuresis were more related to hiCHO diabetes than hiFat diabetes.

**Organ Weights and Body Length** Organ weights followed the storyline for diabetes measured by random blood glucose, such that liver weights increased significantly in diabetic (susceptible) rats and were relatively constant at 3.7 to 4.2% of body weight, except for the highest fat group, where the susceptible animals had normal liver weights.

Kidney weights, on the other hand, were only raised by the highest carbohydrate diet at 70% carbohydrate, showing about a 30% increase in weight. Otherwise, kidney weights were stable across diets and there was no significant difference noted between kidneys for rats resistant or susceptible to diabetes.

Cecum weights followed the pattern found in kidney in the sense that the diabetic rats consuming the highest carbohydrate had the greatest cecum weight, whereas all the other cecum weights from the different diet groups, both resistant and susceptible, were similar and normal at about 1.1% of body size.
Adipose tissue on the other hand reflected both diabetic status and dietary carbohydrate intake, which were related as pointed out earlier. The epididymal fat was slightly reduced in the susceptible animals fed the most carbohydrate at 70% and 60% energy from carbohydrate, likely reflecting early ketosis and fat catabolism, which was also reflected in water intake. Diets with only 50% and 40% energy from carbohydrate tended to leave susceptible rats with a higher percentage of epididymal fat. Perirenal fat had a similar profile in that 70:10:20 diet richest in CHO reflected the most severe diabetes evidenced by random blood glucose and water intake showing that ketosis was probably in effect, whereas the susceptible animals were actually showing less amount of fat in the various fat pools at the highest and lowest CHO intakes. This is also reflected in total fat where 70:10:20 gave rise to the least amount of total fat, especially in susceptible rats with ketosis and fat burning. Interestingly, the greatest amount of fat was found in susceptible rats consuming (40:40:20), which has an energy profile close to US diet profile.

At the same time the body length data showed that all the animals were growing linearly at the same rate, a little over 13 cm body length at the end of the study.

Plasma TC and TG Plasma cholesterol and plasma lipids in general indicated a significant effect of diet on the diabetic lipid profile, and in the case of total cholesterol, an interaction between diet and diabetes. For total cholesterol, the pattern for the first three high-carbohydrate diets was similar in that TC was increased in the susceptible rats, but rather stable in the resistant rats. Triglycerides were more remarkable, becoming elevated in susceptible rats across all diets, except for the last high-fat diet, which contained the least amount of carbohydrates. Triglycerides were essentially unchanged between resistant or susceptible rats for Marg A.

Study 151 (Marg B, S:M:P of 3:1.7:1)

Study 151 utilized margarine B (Average American Fat Blend based on butter and tallow, S:M:P of 45:40:15) as the main fat sources, but followed the same design as Study 150, where a ‘healthy blend of 1:1:1’ as Smart Balance made with PKO was the fat source. For margarine B, note that it provided a P/S ratio of only 0.33, which is slightly lower (more saturated) than the average American P/S ratio of 0.40. On the other hand, the SMP ratio for margarine A was 33:34:33 with a P/S ratio of 1.00 as found in Smart Balance.

Body weight The body weight changes for Margarine B were a little more interesting than Margarine A in that a significant increase in body weight was found in susceptible rats compared to the resistant rats across diets, with the exception of the middle at 50:30:20% energy. Also, it’s interesting that the initial body weights at 3wks of age, when the rats were first introduced to their diet, were already heavier in susceptible rats that eventually became diabetic. At the point of entry and assignment to diet groups, we had no knowledge which rats were going to be diabetic, so it was prophetic that given margarine B, the rats that weighed slightly more at weaning were actually the ones that became diabetic when fed Marg B, unlike the story with margarine A, where there was no predictability based on their initial body weight. This suggests that Marg B, but not Marg A, helped bigger rats at weaning develop diabetes during growth, even though overall it appeared that Marg A induced a slightly greater incidence of diabetes with more severe outcomes in the mid-range of CHO intake (60:20, 50:30, and 40:40 for CHO:fat). This remains somewhat of a puzzle, but likely reflects the less amount of polyunsaturated fat in Marge B.
Food intake, caloric intake, Food Efficiency and Body Weight Gain Per Day Food intake followed the body weight pattern, where the susceptible rats ate more food and consumed more calories, especially at the higher carbohydrate intakes, but this tended to disappear (40:40) and actually reverse (20:60) at the 2 higher-fat intakes.

In terms of food efficiency, there was essentially no difference between resistant and susceptible rats, except at the highest fat intake, where food efficiency became greater for rats that developed diabetes, while the middle diet (50:30:20) tended to be less food efficient in the susceptible group compared to the resistant counterpart.

Similar to food efficiency, with body weight gain per day the susceptible animals revealed the greatest weight gain per day, except for the middle diet (50:30), which showed a drop in body weight gain per day in the susceptible group.

Random Blood Glucose, OGTT, and Water Intake Random blood glucose after 10 weeks was again obviously higher in the diabetic animals by definition, but the greatest increases observed were in 70:10:20 and 50:30:20 groups, with less remarkable increases at the two highest fat intakes, ie. the two lowest carbohydrate intakes. As with Margarine A, all of the resistant rats showed essentially the same RBG at about 60 mg/dL. It is noteworthy that the middle diet (50:30:20) once again had the highest RBG, or essentially the most severe diabetes out of all the diet groups.

For the 30’OGTT, the glucose value was in the 240 to 320 mg/dL range for all susceptible subgroups, and the resistant rats all tended to be less than 175 mg/dL as one might predict. Water intake was not as dramatically increased by hiCHO as it was with Margarine A, but it was still most apparent for the highest intake of carbohydrate and much less apparent across the other groups.

Organ Weights and Body Length The liver weight increased in the susceptible rats of all groups except for 40:40:20, where it was normal and not significantly changed between resist and suscept rats. The kidney weight was not appreciably affected by diet or diabetes status in a predictable manner, and no significance was recorded for diets or diabetes. Cecum weight was similar across all rats, and there was no significant effect in terms of diet. For adipose pools, the perirenal and brown fat were increased significantly in susceptible rats, especially again for 40:40:20. Total fat followed the same pattern as the perirenal and brown fat with the most dramatic effect in 40:40:20, suggesting that diet had some combination of carbs, fat and protein that accentuated body fat accumulation for both margarine B and margarine A. It is noteworthy that the middle diet at 50:30:20 revealed no significant difference between resistant and susceptible groups, especially the brown fat, which was slightly lower than in the resistant counterpart.

Contrary to margarine A, the body length of rats in the susceptible group was significantly greater across all the diets compared to the resistant rats, which was similar to body weight pattern. Thus Marg B, but not Marg A, seemed to stimulate overall growth in susceptible rats or it may have slightly depressed axial bone growth in resistant rats, as the resistant groups were consistent across the board at about 13.1 cm, which was slightly low compared to all rats fed Marg A in Study 150.

Plasma TC and TG Plasma lipids revealed a significant effect due to diabetes once again with susceptible rats having higher total cholesterol for all groups except for 40:40:20, where it was unchanged
between resistant and susceptible rats. Triglyceride showed the same effect of increasing triglycerides among the susceptible rats across the board, making triglycerides slightly more sensitive to the diabetes than the plasma cholesterol, and somewhat more consistently than Marg A in Study 150. In terms of severity, the susceptible groups of the first three hiCHO diets were greater than the last two hiFat diets, which were similar to each other. It is noteworthy that the TG values of the middle 50:30:20 were most severely elevated compared to all the other diets, similar to the RBG story.

**Discussion.**

The main focus of these studies was to determine the relative importance of CHO versus fat on the outcome of T2DM in the male Nile rat during the 10wks from weaning to maturity when they are most vulnerable to their 'spontaneous' diabetes onset. Earlier studies [13, 31, 32] had demonstrated the importance of hiCHO diets for accelerating the diabetes outcome, but the impact of replacing CHO with fat was relatively unexplored in the model. This was deemed critical because many studies in conventional rodents such as the C57BL mouse or STD rat were used to emphasize the role of fat for inducing the diabetes. But many studies have questioned the use of such models, especially since they do NOT respond to hiCHO diets, unlike the human situation [7] or the Nile rat [13, 31], both of which are most sensitive to CHO, not fat. In addition to the basic comparison between CHO and fat energy in the diabetes process, a second layer of questioning compared two types of fat, Marg A that had a balanced blend of fats and fatty acids to provide an equal dose of S:M:P with SATs coming from MCFA as PKO, and Marg B which was based on butter, tallow, soybean oil and lard as largely representative of animal fats designed to mimic the average American Fat blend.

Several differences appeared to exist between responses to margarine A and margarine B.

First, margarine A was a saturated fat based on PKO with a 1:1:1 ratio between S:M:P. The P/S ratio was 1.0. Margarine B was also a saturated fat-based mixture based on animal saturated fats (fatty acids) but had a ratio of 45:40:15 S:M:P, or 3:2.7:1, and a P/S ratio of 0.33, similar to current US ratio. Surprisingly, these two fat blends had somewhat similar outcomes in terms of overall type II diabetes in the Nile rat, suggesting that the type of fat had minimal impact on the diabetes compared to the level of CHO.

Nonetheless, one might expect the P/S ratio of 1.0 to be the best product as margarine A, but in fact, several aspects of margarine A were even slightly worse than margarine B. It is not clear why that should be, but several points are noteworthy:

1. There was a tendency (not significant) for the 10-week % incidence of type II diabetes to be higher with margarine A than with margarine B.
2. The RBG tended to be higher with margarine A, at least for the three highest carbohydrate diets, which suggests that it acted more like a CHO than a fat, since the Nile rat is known to be CHO-sensitive in terms of diabetes.
3. The 30-min OGTT for both ‘resistant’ and ‘susceptible’ rats were trending higher with margarine B, but no effect of the CHO:fat ratio was apparent, suggesting that the postprandial period of food...
absorption, including glucose, may have been greater and more stressful metabolically for Marg B.

4. But a lower CHO:fat ratio, i.e. higher dietary fat, did appear to decrease the RBG and the % incidence of T2DM across both margarine A and B diets at all levels, meaning a high-CHO intake was definitely bad, but hi-Fat not so bad.

5. All margarine A rats had a FBG at 10 weeks that were similar to each other and normal at <60 mg/dl, except perhaps for the highest fat intakes, where the FBG started to rise.

6. These results concur with Bolsinger [31] which demonstrated that a high glycemic index food and overall high glycemic dietary load (hiCHO from simple CHO) was the single best predictor of type II diabetes in the model and in humans, emphasizing that CHO appears more critical than fat in this regard [7]. Other experiments from the lab concur, because adding dietary soluble fibers as a source of complex CHO that ferments in the large bowel, effectively block the diabetes. [Results Unpublished]

Thirdly, with margarine B in Study 151 at three weeks old, when rats were first assigned to diet at weaning, those that ultimately became diabetic as ‘susceptible’ were already heavier than the ‘resistant’ rats when weaned, which suggests that a low P/S ratio diet (0.33) had more potential to induce type II diabetes in rats already gaining weight faster during growth, possibly due to eating more especially with the hiCHO diets and that those rats were already genetically prone to eat more calories when fed Marg B, for whatever reason. Margarine A, by contrast, did not show that effect except possibly for the 50:30:20 diet.

Fourthly, it is interesting that the peak % incidence of diabetes for Marg B based on RBG was basically at 50% energy from CHO calories, whereas with Marg A it was 60-50-70% energy as CHO. It is of interest that this CHO-fat combo is similar to the current US diet profile of 55:35:15, including Marg B as the fat prototype. It would have been nice to test a Smart Balance Marg C with a one-to-one S:M:P ratio where the saturated fatty acids were derived from 16C and 18C saturated fatty acids, as opposed to the 12C and 14C fatty acids emphasized by the present fats in Marg A and to lesser extent in Marg B (eg. butter, although the other SFAs were long chain).

A fifth point, and not surprising, was that margarine B with its low P/S ratio of 0.33, had a higher total cholesterol and total plasma triglyceride in the ‘susceptible’ rats compared to the ‘resistant’ rats fed Marg A. This was even true for the ‘resistant’ rats fed Marg B, as well. Many studies in humans and animal models demonstrate the importance of the P/S ratio on regulating TC, TG, and the LDL/HDL ratio in the blood, with higher P/S ratios producing more favorable results in this respect [28]. With the present study we now have a suggestion that there is a link to diabetes as well.

The results presented in Figure 1a (Bd Wt vs caloric intake) are interesting because they point to the fact that rats consuming Marg A (lesser extent Marb B) tended to have a wide range in caloric intake that was dictated in part by the level of carbohydrate consumed. Note that the very lowest carbohydrate diet (20:60:20), in other words the highest fat, produced the lowest caloric intake per day of about 32, whereas the highest CHO diet at 70% energy from carbohydrate (70:10:20) produced almost a 30% greater caloric intake at 41 kcal per day, which suggests that something about hiCHO as simple sugars, somehow caused deregulation of food intake so that rats ate too much. Marg B showed the same effect, but not so extreme, ie. the hiCHO led to more calories consumed while the hiFat produced the lowest
intake. A similar story may be present in SDT rats that had their leptin receptor gene mutated by introducing a *fa* allele, which is a genetic factor widely known to induce insulin resistance or obesity, causing their inability to control their appetite [12][33]. Accordingly, simple sugars at high doses may affect a similar gene in the Nile rats that led them to overeat. The other three diets were intermediate in their carbohydrate percent calories at 40, 50 or 60% energy and they were also intermediate in terms of caloric intake ranging from 34 kcal per day to approximately 40 kcal per day for Marg A. In addition, all three of those diets increased body weight relative to the two extremes in carbohydrate intake. These three intermediate carbohydrate diets also ended up with the most body weight expressed as % total fat, ranging from 7.5 to 8.8% of body weight compared to 5.1 and 5.6% of body weight for the outlier carbohydrate intakes. So it seems that something about the CHO:fat ratio affects food intake adversely and can lead to greater fat deposition that seems linked to diabetes, probably from the high glucose intake, with a secondary fat effect on glucose utilization that predisposes to hyperglycemia and T2DM.

In essence, this suggests that there was some sort of interaction between moderate carbohydrate intake and increasing fat percent of calories from 20 to 40% of energy that was hazardous for the ‘susceptible’ rats. By contrast, the inset in figure 1A (and expanded version in Fig 2A) indicate that the resistant rats maintained a very tight reign over caloric intake when consuming Marg A, from a little less than 34 kcal to about 35 kcal /day (and for Marg B between 32 to 34 kcals/day). As a consequence, the resistant rats did not overeat and did not gain excess weight, and most importantly did NOT get T2DM. The nonlinear regression coefficient for figure 1A was very high at r=0.91 for the susceptible rats, indicating a strong nonlinear relationship between caloric intake and final body weight, whereas the correlation for the ‘resistant’ rats fed Marg A (inset in red, and expanded in Fig 2A) was not highly correlated with r= 0.48, indicating there was only a weak relationship between caloric intake and final body weight for those resistant rats fed Marg A. The overall implication is that susceptible rats fed Marg A (and to a lesser extent Marg B) somehow lost control over their caloric intake due to their genetic makeup regulating food intake, which may have resulted from damage initiated by hyperglycemia, which, in turn, caused them to consume more food, and thus more glucose, and thereby develop more diabetes than the resistant rats in a vicious circle that feeds on itself. In Marg B, this also seemed to be further amplified in the young weanling rats that weighed the most when weaned because they gained the most weight and developed the most diabetes over the next 10wks on diet.

Conclusion

The implication of these studies is that simple CHO is the underlying cause of diabetes in Nile rats, and that there is a strong genetic component to this tendency. The type and amount of fat play a role in this scenario, but the exact connection to regulation of food intake is not apparent at this time. Further work must be conducted to determine why this should be and specifically identify which genes are regulating this interesting phenomenon that plays out between resistant and susceptible rats in terms of their diabetes.
References


Table 1A. NR diet composition (Study 150, MargA)

<table>
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<th>INGREDIENT</th>
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<th>196</th>
<th>197</th>
<th>198</th>
<th>199</th>
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<td>(Fat/Prot %E ratio)</td>
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<td>1.5</td>
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<td>4.5</td>
<td>4.8</td>
<td>5.5</td>
</tr>
<tr>
<td>GL/2000kcal</td>
<td>293</td>
<td>252</td>
<td>210</td>
<td>167</td>
<td>84</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>g/Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
</tr>
<tr>
<td>Lactalbumin</td>
</tr>
<tr>
<td>Dextrose</td>
</tr>
<tr>
<td>Cornstarch</td>
</tr>
</tbody>
</table>

| Fat: Margarine A (80% fat) | 55 (44 fat) | 119 (95 fat) | 185 (148 fat) | 267 (214 fat) | 461 (369 fat) |
| Mineral mix (Ausman - Hayes) | 44  | 47  | 49  | 53  | 61  |
| Vitamin mix (Hayes - Cathcart) | 11  | 12  | 12  | 13  | 15  |
| Choline chloride    | 3    | 3    | 3    | 3    | 3    |
| Cholesterol         | 0.6  | 0.6  | 0.6  | 0.6  | 0.6  |

*60g cornstarch added to 800mL water to form gel
Marg A that is pre-made from tub has 20% water content (house-made is 100% fat)
Marg A composition: Palm Kernel Oil 31%, Soybean Oil 33%, Canola Oil 36%
Table 1B. NR diet composition (Study 151, MargB)

<table>
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<tr>
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<th>Diet</th>
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<tr>
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<tr>
<td>g/Kg</td>
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<tr>
<td>Casein</td>
<td>100</td>
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<tr>
<td>Lactalbumin</td>
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<td>Dextrose</td>
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<td>Cornstarch (+60 gel)</td>
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<tr>
<td>Min. mix (Ausman - Hayes)</td>
<td>44</td>
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<tr>
<td>Mineral mix (Ausman - Hayes)</td>
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<tr>
<td>Vitamin mix (Hayes - Cathcart)</td>
<td>11</td>
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<tr>
<td>Choline chloride</td>
<td>3</td>
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<tr>
<td>Cholesterol</td>
<td>0.6 (0.65)**</td>
</tr>
</tbody>
</table>

*60g cornstarch added to 800mL water to form gel

**Total cholesterol in margarine B, including animal fats (total amount, g/kg diet)

Marg B composition: Butter 21% (from fat), Tallow 46%, Lard 15%, Soybean oil 18%
<table>
<thead>
<tr>
<th>Fatty acid %</th>
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<th>151</th>
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<tr>
<td>4:0+6:0</td>
<td>0.1</td>
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</tr>
<tr>
<td>8:0</td>
<td>1.0</td>
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<td>10:0</td>
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<td>12:0</td>
<td>14.9</td>
<td>0.7</td>
</tr>
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<td>14:0</td>
<td>5.1</td>
<td>4.0</td>
</tr>
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<td>16:0</td>
<td>7.5</td>
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<td>13.8</td>
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<tr>
<td>16:1</td>
<td>0.0</td>
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<tr>
<td>18:1</td>
<td>34.0</td>
<td>36.3</td>
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<td>18:2</td>
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</tr>
<tr>
<td>18:3</td>
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</tr>
<tr>
<td>Total Sats</td>
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<td>44.7</td>
</tr>
<tr>
<td>Total Monos</td>
<td>34.1</td>
<td>40.2</td>
</tr>
<tr>
<td>Total Polys</td>
<td>33.5</td>
<td>14.8</td>
</tr>
</tbody>
</table>

P/S (18:2+18:3/8:0-18:0) | 1.00 | 0.35 |
S:M:P in diet            | 33:34:33 | 45:40:15 |

PUFA % dietary energy

(70:10:20) | 3.4 | 1.5 |
(60:20:20) | 6.7 | 3.0 |
(50:30:20) | 10.1 | 4.4 |
(40:40:20) | 13.4 | 5.9 |
(20:60:20) | 20.1 | 8.9 |

*MargA = PKO, SBO, Canola (31:33:36)
**MargB (AvAmFatBlend) = Butter, Tallow, Lard, Soybean oil (21:46:15:18)
Table 3A. Diabetic assessment of 3 wk old male Nile rats fed diets with different CHO:fat (Marg. A) ratios and Glycemic Loads for 10 wks. Rats subdivided into Resistant or Susceptible to T2DM (NR Study 150)

<table>
<thead>
<tr>
<th>INGREDIENT</th>
<th>CHO:Fat:Prot %E</th>
<th>Diet</th>
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<td></td>
<td>(Fat/Prot %E ratio)</td>
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<td></td>
<td>kcal/g</td>
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<tr>
<td></td>
<td>7.3±0.1</td>
<td>0.7±0.1</td>
<td>0.92±0.14</td>
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<td></td>
<td>20:60:20</td>
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Blood glucose measures

<table>
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<tr>
<th>OGGT &lt;175&gt; T2DM (% incidence)</th>
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<th>n=13</th>
<th>n=14</th>
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<tbody>
<tr>
<td>RBG &lt;75&gt; T2DM (% incidence)</td>
<td>6/8 (57%)</td>
<td>2/12 (86%)</td>
<td>6/7 (54%)</td>
<td>6/8 (57%)</td>
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<tr>
<td>FBG &lt;60&gt; T2DM (% incidence)</td>
<td>12/2 (14%)</td>
<td>10/4 (29%)</td>
<td>9/4 (31%)</td>
<td>7/7 (50%)</td>
</tr>
<tr>
<td>RR (95% CI), RBG</td>
<td>1.00</td>
<td>1.50 (0.73, 3.08)</td>
<td>1.08 (0.46, 2.50)</td>
<td>0.83 (0.33, 2.11)</td>
</tr>
</tbody>
</table>

Average% incidence

| 33% | 48% | 41% | 43% | 26% |

Marg. A fat composition: 33% Palm Kernel Oil, 36% Soybean Oil, 31% Canola Oil

* Significant (P < 0.05) interaction term for diet x diabetes class (RBG <75 mg/dl) revealed by two-way ANOVA.
* Significant (P < 0.05) effect of diet (Gload) by two-way ANOVA.
* Significant (P < 0.05) decrease due to diabetes (RBG >75 mg/dl) by two-way ANOVA.
* Significant (P < 0.05) increase due to diabetes (RBG >75 mg/dl) by two-way ANOVA.

Blood pressure, ML 1/31/17

**S:M:P ratio (33:34:33)**

Significant (P < 0.05) decrease due to diabetes (RBG >75 mg/dl) by two-way ANOVA.
Table 3B. Diabetic assessment of 3 wk old male Nile rats fed diets with different CHO:fat (Marg. B) ratios and Glycemic Loads for 10 wks. Rats subdivided into Resistant or Susceptible to T2DM (NR Study 151)

<table>
<thead>
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<th>INGREDIENT</th>
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<th>201</th>
<th>202</th>
<th>203</th>
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<th>20:60:20</th>
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<tr>
<td></td>
<td>(0.5)</td>
<td>(1.0)</td>
<td>(1.5)</td>
<td>(2.0)</td>
<td>(3.0)</td>
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<tr>
<td>kcal/g</td>
<td></td>
<td>4.0</td>
<td>4.2</td>
<td>4.5</td>
<td>4.8</td>
<td>5.5</td>
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<tr>
<td>GL/2000kcal</td>
<td></td>
<td>293</td>
<td>252</td>
<td>210</td>
<td>167</td>
<td>84</td>
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</table>

Blood glucose measures

<table>
<thead>
<tr>
<th>n=14</th>
<th>n=14</th>
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<th>n=13</th>
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<tbody>
<tr>
<td>OGTT &lt;175&gt; T2DM (% incidence)</td>
<td>10/4 (29%)</td>
<td>7/7 (50%)</td>
<td>6/8 (57%)</td>
<td>8/5 (38%)</td>
</tr>
<tr>
<td>RBB &lt;75&gt; T2DM (% incidence)</td>
<td>10/4 (29%)</td>
<td>7/7 (50%)</td>
<td>6/8 (57%)</td>
<td>8/5 (38%)</td>
</tr>
<tr>
<td>FBG &lt;60&gt; T2DM (% incidence)</td>
<td>12/3 (44%)</td>
<td>9/5 (36%)</td>
<td>10/3 (23%)</td>
<td>10/4 (29%)</td>
</tr>
<tr>
<td>RR (95% CI), RBB</td>
<td>0.90 (0.12, 2.46)</td>
<td>0.75 (0.20, 2.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average% incidence</td>
<td>24%</td>
<td>4%</td>
<td>14%</td>
<td>25%</td>
</tr>
</tbody>
</table>

**Significant (P < 0.05) interaction term for diet x diabetes class (RBG <75 mg/dl>) revealed by two-way ANOVA.
**Significant (P < 0.05) effect of diet (GLoad) by two-way ANOVA.
**Significant (P < 0.05) increase due to diabetes (RBG <75 mg/dl) by two-way ANOVA.
**Significant (P < 0.05) decrease due to diabetes (RBG >75 mg/dl) by two-way ANOVA.

```
<table>
<thead>
<tr>
<th>RBG &gt;75 mg/dl T2DM (% incidence)</th>
<th>resist</th>
<th>suscept</th>
<th>resist</th>
<th>suscept</th>
<th>resist</th>
<th>suscept</th>
<th>resist</th>
<th>suscept</th>
<th>resist</th>
<th>suscept</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=10</td>
<td>n=4</td>
<td>n=10</td>
<td>n=4</td>
<td>n=7</td>
<td>n=11</td>
<td>n=2</td>
<td>n=11</td>
<td>n=3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial (3wk of age)²</td>
<td>31±4</td>
<td>34±5</td>
<td>32±4</td>
<td>32±4</td>
<td>31±3</td>
<td>33±4</td>
<td>30±4</td>
<td>37±0</td>
<td>30±6</td>
<td>37±4</td>
</tr>
<tr>
<td>After 3 wks³</td>
<td>68±6</td>
<td>74±5</td>
<td>69±7</td>
<td>74±3</td>
<td>70±6</td>
<td>73±9</td>
<td>70±9</td>
<td>77±5</td>
<td>64±10</td>
<td>77±15</td>
</tr>
<tr>
<td>After 6 wks³</td>
<td>82±8</td>
<td>93±3</td>
<td>85±9</td>
<td>93±6</td>
<td>87±8</td>
<td>88±10</td>
<td>83±11</td>
<td>93±10</td>
<td>80±9</td>
<td>98±13</td>
</tr>
<tr>
<td>After 10 wks³</td>
<td>95±10</td>
<td>107±6</td>
<td>96±11</td>
<td>109±7</td>
<td>103±10</td>
<td>103±10</td>
<td>97±15</td>
<td>109±11</td>
<td>92±11</td>
<td>108±21</td>
</tr>
</tbody>
</table>

Food intake

<table>
<thead>
<tr>
<th>kg/d ⁴ ⁵</th>
<th>kcal/d ⁴ ⁵</th>
<th>g/d ⁴ ⁵</th>
<th>kcal/g ⁴ ⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1±1.2</td>
<td>7.6±0.6</td>
<td>8.5±0.3</td>
<td>7.5±0.6</td>
</tr>
<tr>
<td>3.4±3.7</td>
<td>3.2±2.1</td>
<td>3.6±1.6</td>
<td>3.4±3.3</td>
</tr>
<tr>
<td>26±3</td>
<td>26±6</td>
<td>27±3</td>
<td>28±3</td>
</tr>
<tr>
<td>34±14</td>
<td>77±60</td>
<td>29±9</td>
<td>30±11</td>
</tr>
</tbody>
</table>

Food efficiency (g BW gained/1000 kcal)

| After 6 wks⁶ | 64±13 | 199±188 | 68±20 | 88±35 | 61±13 | 82±33 | 65±18 | 59±5 | 60±9 | 125±135 |
| After 10 wks⁷ | 63±9 | 262±132 | 57±8  | 160±96| 58±7 | 275±192| 62±6 | 89±6 | 58±9 | 151±121|

OGTT (8G mg/dl)

| After 6 wks | 44±13 | 78±49  | 53±11 | 59±21 | 54±18 | 54±8 | 44±15 | 74±22 | 56±26 | 62±5  |
| After 10 wks | 121±48 | 316±165 | 163±257 | 180±60 | 282±122 | 151±60 | 240±126 | 172±37 | 283±188 | 60±135 | 370±233 |

| Liver³ ² ⁴ ⁵ | 3.4±0.3 | 4.0±1.1 | 3.3±0.2 | 3.7±0.5 | 3.2±0.2 | 4.0±0.7 | 3.3±0.3 | 3.1±0.2 | 3.5±0.3 | 3.9±0.8 |
| Kidney     | 0.9±0.7 | 0.8±0.2 | 0.7±0.1 | 0.8±0.2 | 0.7±0.0 | 0.8±0.2 | 0.6±0.1 | 0.7±0.0 | 0.8±0.1 | 0.8±0.1 |
| Cecum      | 1.1±0.2 | 1.2±0.4 | 1.1±0.1 | 1.0±0.2 | 1.0±0.1 | 1.1±0.2 | 1.0±0.2 | 1.1±0.2 | 1.1±0.2 | 1.2±0.6 |
| Adipose    | 2.7±0.6 | 2.6±0.4 | 2.6±0.6 | 2.5±0.1 | 2.8±0.4 | 3.0±1.0 | 2.6±0.7 | 3.2±0.0 | 2.5±0.8 | 3.5±1.1 |
| Epididymal | 1.5±0.5 | 1.9±0.5 | 1.6±0.5 | 1.9±0.2 | 1.7±0.4 | 1.8±0.3 | 1.5±0.3 | 2.4±0.1 | 1.3±0.4 | 2.0±0.5 |
| Perineal³  | 1.9±0.6 | 2.5±0.7 | 1.9±0.4 | 2.6±0.3 | 2.4±0.6 | 2.1±0.5 | 2.0±0.6 | 2.7±0.3 | 1.6±0.5 | 2.5±0.9 |
| Brown fat³ | 6.1±1.5 | 7.0±1.5 | 6.1±1.3 | 6.9±0.3 | 6.9±1.3 | 6.9±1.1 | 6.1±1.3 | 8.4±0.3 | 5.4±1.4 | 8.0±0.8 |
| Total fat³ | 75±2   | 72±1   | 75±2   | 74±1   | 74±1   | 74±3   | 75±2   | 74±1   | 75±2   | 72±1   |

Body length (cm)³ ² ⁴ ⁵

| 13.1±0.9 | 13.6±0.6 | 13.1±0.3 | 13.5±0.1 | 13.1±0.2 | 13.4±0.4 | 13.2±0.6 | 14.4±0.2 | 13.1±0.4 | 13.5±0.5 |

Values are mean±SD (n=2-11) Fat fed as Marg B. Western Fat Blend

*Marg. B fat composition: 21% Butter, 46% Tallow, 15% Lard, 18% Soybean Oil
**L/M/P ratio (45:40:15)
Figure 1A

Body Weight vs. Caloric Intake for Susceptible Rats fed Marg. A

Figure 2A

Body Weight vs. Caloric Intake for Susceptible Rats fed Marg. B
Figure 1B
Body Weight vs. Caloric Intake for Resistant Rats fed Marg. A

TC: 95
Tfat%BW: 7.2
RBG: 60
20:60:20

TC: 91
Tfat%BW: 6.3
RBG: 61
50:30:20

TC: 102
Tfat%BW: 6.9
RBG: 62
40:40:20

TC: 96
Tfat%BW: 6.2
RBG: 63
60:20:20

r = 0.485

Figure 2B
Body Weight vs. Caloric Intake for Resistant Rats fed Marg. B

TC: 113
Tfat%BW: 6.9%
RBG: 58
50:30:20

TC: 99
Tfat%BW: 6.1%
RBG: 62
40:40:20

TC: 60:20:20

TC: 113
Tfat%BW: 6.1%
RBG: 57
60:20:20

TC: 116
Tfat%BW: 6.1%
RBG: 63
70:10:20

TC: 116
Tfat%BW: 5.4%
RBG: 58
20:60:20

r = 0.984
Figure 3A
Total Cholesterol vs. % en Carbs in Nile Rats fed Marg A

- r = 0.907
- r = 0.816

Figure 3B
Total Cholesterol vs. % en Carbs in Nile Rats fed Marg B

- r = 0.846
- r = 0.170
Figure 4A  RBG vs % en Carb in Nile Rats fed Marg A

Figure 4B  RBG vs % en Carb in Nile Rats fed Marg B
Figure 5A  
30' OGTT vs. % en Carb in Nile Rats fed Marg. A

Figure 5B  
30' OGTT vs. % en Carb in Nile Rats fed Marg. B