Differing sources of dietary fat alter the character of metabolic syndrome induced in the C57BL/6 mouse.

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INTRODUCTION

Certain conditions of metabolic syndrome—obesity, insulin resistance, dyslipidemia, central adiposity, impaired fasting glucose, low HDL-cholesterol, and elevated triglycerides—can be effectively studied in mice using a high-fat diet to induce these symptoms. Typically, particularly over much of the past decade, certain "DIO" (diet-induced obesity) purified ingredient diets have been used for this purpose, utilizing almost exclusively porcine lard as the fat source, with total dietary fat contributing ≥60% of the total energy (kcal). The particular type and source of dietary fat, however, can significantly influence the onset and extent of obesity and diabetes; levels of glucose, cholesterol, insulin, and triglycerides; and the locations of body fat deposits.

MATERIALS & METHODS

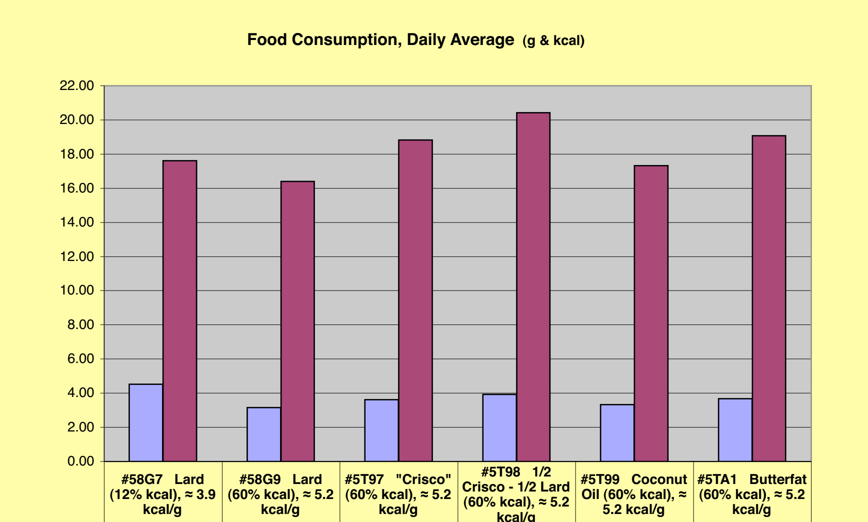
Male "Black 6" mice (C57BL/6NCrlBR) (Charles River Labs), at 9 weeks of age and approximately 22-23 gm body weight were placed on high-fat purified diets in one of 6 groups (N=7-9), individually-housed (PreClinOmics, Inc.). For 100 days, they were fed a low-fat (12% kcal fat) control diet (TestDiet® #58G7), and a series of high-fat (60% kcal) diets, identical but for fat source: lard (TestDiet® #58G9), hydrogenated vegetable oil "Crisco" (TestDiet® #5T97), ½ lard-½ Crisco (TestDiet® #5T98), coconut oil (TestDiet® #5T99), and butterfat (TestDiet® #5TA1).

Body weights were recorded at onset and at one-week intervals thereafter. Food consumption was monitored for 3 animals of each group for two 7-day intervals, one early in the study, one late, to determine average daily consumption. Blood glucose, cholesterol, triglycerides, and insulin levels were measured at days 1, 43, 85, and 101. Oral glucose tolerance tests were conducted at Day 99, over three hours at 30-minute intervals. Distribution of body fat deposits was determined by necropsy.

TestDiet Formula >	#58G7	#58G9	#5T97	#5T98	#5T99	#5TA1
Predominant Fat Source >	Lard	Lard	Crisco (hydrogenated vegetable oil)	1/2 Crisco - 1/2 Lard	Coconut Oil, hydrogenated	Butterfat
	"12% kcal fat" Low-Fat Control	"60% kcal fat"	"60% kcal fat"	"60% kcal fat"	"60% kcal fat"	"60% kcal fat"
Protein %	18.0%	24.2%	24.2%	24.2%	24.2%	24.2%
Carbohydrate %	67.0%	27.8%	27.8%	27.8%	27.8%	27.8%
Fat %	5.2%	34.7%	34.7%	34.7%	34.7%	33.4%
Fiber %	4.1%	5.5%	5.5%	5.5%	5.5%	5.5%
Energy, calories	3.87 kcal/gm	5.21 kcal/gm	5.21 kcal/gm	5.21 kcal/gm	5.21 kcal/gm	5.21 kcal/gm
from Protein %	18.6%	18.6%	18.6%	18.6%	18.6%	18.6%
from Carbohydrate %	69.4%	21.4%	21.4%	21.4%	21.4%	21.4%
from Fat %	12.0%	59.9%	59.9%	59.9%	59.9%	59.9%
Ingredients (%)						
Casein, Vitamin-Free	19.8700	26.7600	26.7600	26.7600	26.7600	26.7600
DL-Methionine	0.1419	0.1911	0.1911	0.1911	0.1911	0.1911
L-Cystine						
Dextrin	48.6172	18.9872	18.9872	18.9872	18.9872	18.9872
Maltodextrin						
Sucrose	16.5580	6.3715	6.3715	6.3715	6.3715	6.3715
Cellulose (*insoluble* fiber)	1.4193	1.9114	1.9114	1.9114	1.9114	1.9114
Inulin (*soluble* fiber)	1.4193	1.9114	1.9114	1.9114	1.9114	1.9114
Corn Oil	4.7310	6.3715	6.3715	6.3715	6.3715	6.3715
Soybean Oil						
Butterfat (anhydrous milk fat)						28.3150
Coconut Oil					28.3150	
"Crisco"			28.3150	14.1575		
Lard	0.4257	28.3150		14.1575		
RP Mineral Mix #10 (25.8% fiber)	4.7310	6.3715	6.3715	6.3715	6.3715	6.3715
Choline Chloride	0.1892	0.2548	0.2548	0.2548	0.2548	0.2548
RP Vitamin Mix (95% sucrose)	1.8924	2.5486	2.5486	2.5486	2.5486	2.5486
Food Color	0.0050	0.0060	0.0060	0.0060	0.0060	0.0060
Total	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**

FOOD CONSUMPTION

Average daily consumption of the high fat diets ranged from a low of 3.15 g/16.40 kcal (#58G9, Lard) to a high of 3.93 g/20.42 kcal (#5T98, Crisco-Lard), for an average of 3.54 g/18.4 kcal per diet for all the high-fat diets. In contrast, the animals on the low-fat control diet (#58G7) ate far more food (4.51 g) to consume 17.6 kcal, within 5% of the average caloric intake of the high-fat groups.

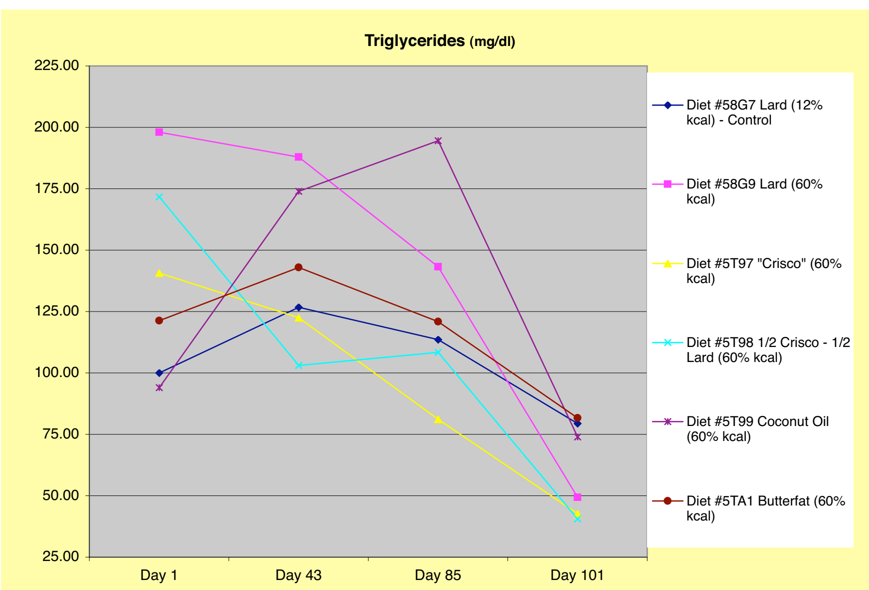


	#58G7 Lard (12% kcal), ≈ 3.9 kcal/g	#58G9 Lard (60% kcal), ≈ 5.2 kcal/g	#5T97 "Crisco" (60% kcal), ≈ 5.2 kcal/g	#5T98 1/2 Crisco - 1/2 Lard (60% kcal), ≈ 5.2 kcal/g	#5T99 Coconut Oil (60% kcal), ≈ 5.2 kcal/g	#5TA1 Butterfat (60% kcal), ≈ 5.2 kcal/g
Grams	4.51	3.15	3.62	3.93	3.33	3.67
Calories	17.61	16.40	18.82	20.42	17.33	19.08

TRIGLYCERIDES

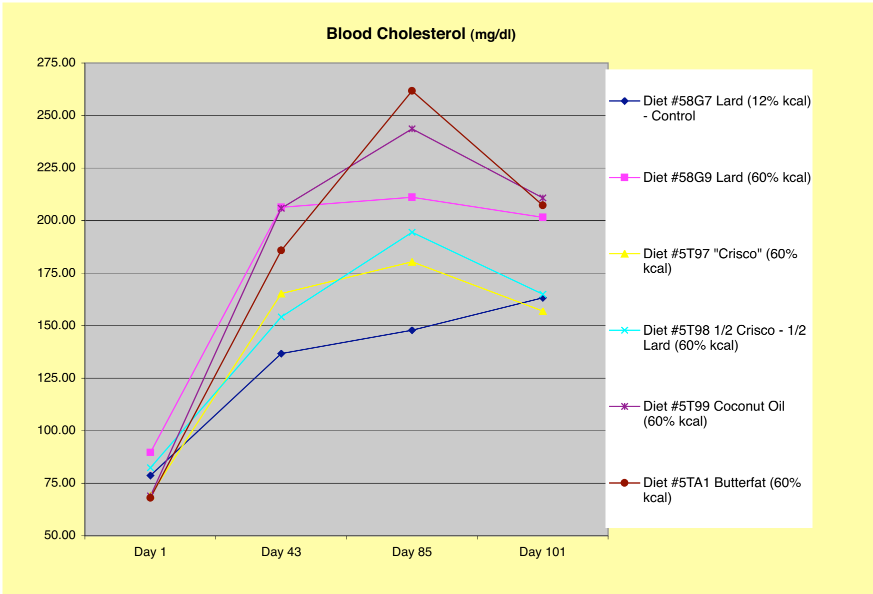
Over the course of the study, triglyceride levels varied considerably from diet to diet.
At the end of the study, Day 101, two high-fat diet groups and the coconut oil diet (#5T99) group all had triglyceride counts clustered at approximately the same level (#58G7 = 79.4 mg/dl; #5TA1 = 81.7 mg/dl; #5T99 =73.8 mg/dl). The other three diets clustered at a lower point (#58G9 = 49.3 mg/dl; #5T97 = 42.8 mg/dl; #5T98 = 40.5 mg/dl). These Day 101 data are suspect, however, because they follow only three days after fasting for the OGTT.

Data at Day 85 may be more representative and pertinent to determine the relative effect of the different fat sources on triglyceride concentrations. These data show the low-fat control (#58G7) at 113.6 mg/dl, very close to the butterfat diet (#5TA1) at 120.8 mg/dl. The highest level by far was found in the coconut oil diet (#5T99) at 194.5 mg/dl. Of the other three, the triglyceride levels increased as the Crisco was replaced by lard (#5T97, Crisco = 81.1 mg/dl; #5T98, lard-Crisco = 108.4 mg/dl; and, #58G9, lard = 143.2 mg/dl).



CHOLESTEROL

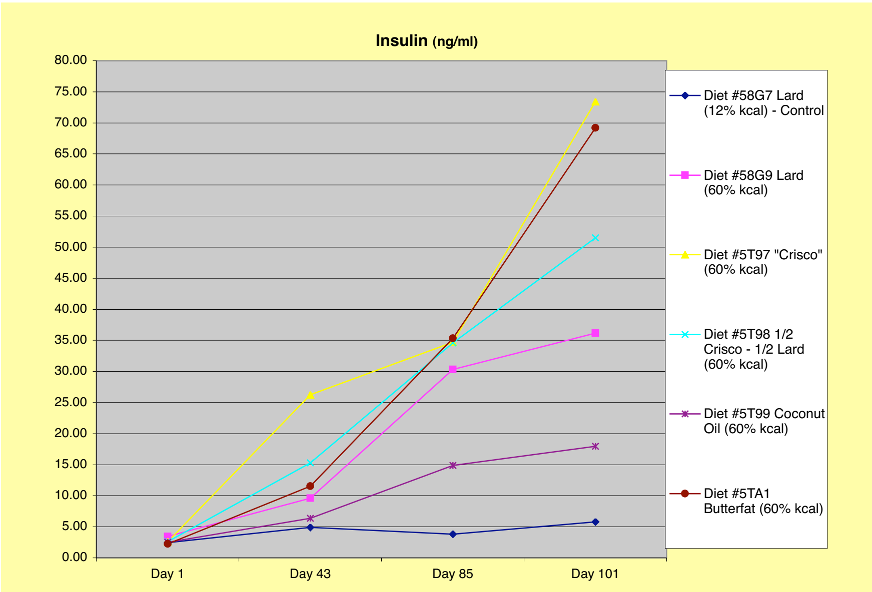
Blood cholesterol levels for all groups were measured within a range of 65-90 mg/dl at the onset. Levels of all groups increased by Day 43, with the low-fat control (#58G7 = 136.8 mg/dl) being the lowest and the high-fat lard (#58G9 = 205.9 mg/dl) and coconut oil (#5T99 = 205.9 mg/dl) being the highest. By the end of the study, Day 101, three high-fat diet groups, butterfat, lard; and coconut oil all had cholesterol counts clustered at approximately the same level. The two other high-fat diet groups, lard-Crisco and Crisco, had cholesterol counts at virtually the same level as the low-fat control. These Day 101 data are suspect, however, because they follow only three days after fasting for the OGTT.



Data at Day 85 may be more representative and pertinent to determine the relative effect of the different fat sources on triglyceride concentrations. These data show the low-fat control diet (#58G7) at 147.8 mg/dl. The butterfat diet (#5TA1 = 261.7 mg/dl) and coconut oil diet (#5T99 = 243.6 mg/dl) had the highest levels. Of the other three, the cholesterol levels increased as the Crisco was replaced by lard (#5T97, Crisco = 180.4 mg/dl; #5T98, lard-Crisco = 194.5 mg/dl; and, #58G9, lard = 211.1 mg/dl).

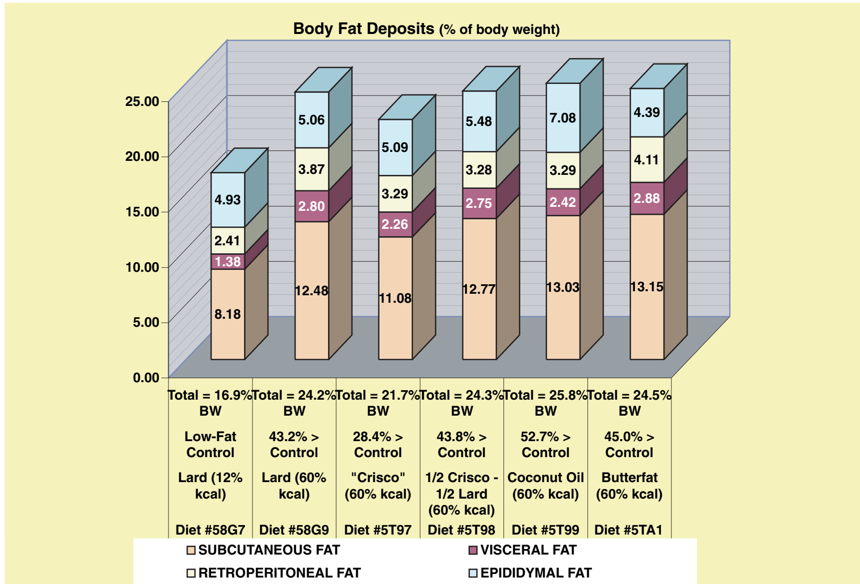
INSULIN

Dramatic differences were demonstrated in the ultimate (Day 101) insulin levels of the various groups. As expected, the lowest insulin levels were found in the low-fat control group (#58G7 = 5.8 ng/dl). The coconut oil diet group was next (#5T99 = 18.0 ng/dl), and the butterfat diet group had the penultimate levels (#5TA1 = 69.2 ng/dl). Of the other three, the insulin levels increased as the lard was replaced by Crisco (#58G9, lard = 36.2 ng/dl; #5T98, 1/2 Crisco-1/2 lard = 51.5 ng/dl; #5T97, Crisco = 69.2 ng/dl).



FAT DEPOSIT

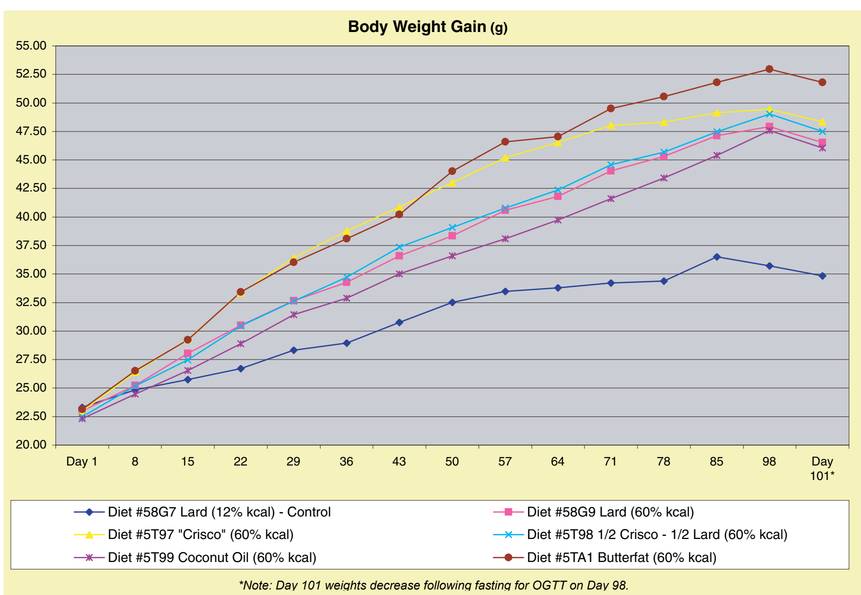
Necropsy determined total body deposition in various areas: and its fat the distribution of fat by deposit area: subcutaneous, visceral, retroperitoneal, and epididymal. Total body fat was 16.9% in the low-fat control group (#58G7). Of the high-fat diets, the Crisco diet (#5T97) produced the lowest body fat (21.7%, 28.4%> control), and the coconut oil diet (#5T99) produced the highest (25.8% bw, 52.7>control). The mid-range was almost identical: lard (#58G7) = 24.2% bw; lard-Crisco (#5T98) = 24.3%; and, butterfat (#5TA1) = 24.5%.



The only apparent significant differences in fat distribution were found in the epididymal fat deposits, the largest being coconut oil (#5T99 = 7.08% bw), 29% larger than the next largest, lard-Crisco (#5T98 = 5.48% bw). This lard-Crisco diet, the lard diet (#58G9 = 5.06% bw), and the Crisco diet (#5T97 = 5.09% bw) occupied the mid-range. The smallest epidiymal deposit was found not in the low-fat control diet (#58G7 = 4.93% bw), but in the butterfat diet group (#5TA1 = 4.39% bw).

WEIGHT GAIN

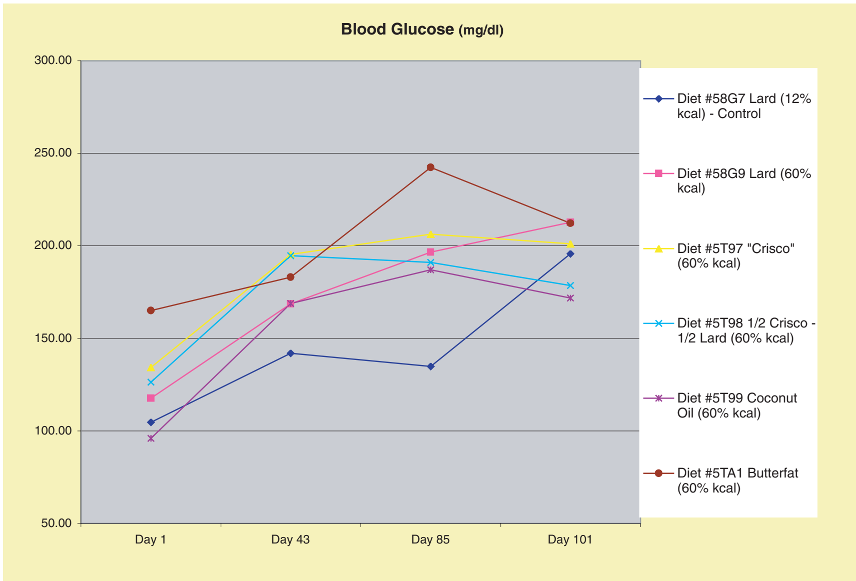
All groups started at the same approximate 22-23 gm. Weight gains were analyzed based on Day 98 data (rather than Day 101, since all groups experienced a weight decrease following fasting on Day 98 for the OGTT.)



The "control" group had the lowest weight (#58G7 = 35.7g, 98 days). Obesity was most pronounced in the Crisco (#5T97 = 49.4ng) and butterfat groups (#5TA1 = 53.0 g). The Crisco (#5T97 = 49.4 g) and lard-Crisco (#5T98 = 49.0 g) groups had virtually identical weight-gains, while the lard (#58G7 = 47.9 g) and coconut oil (#5T99 = 47.6 g) groups gained the least of the high-fat groups.

GLUCOSE

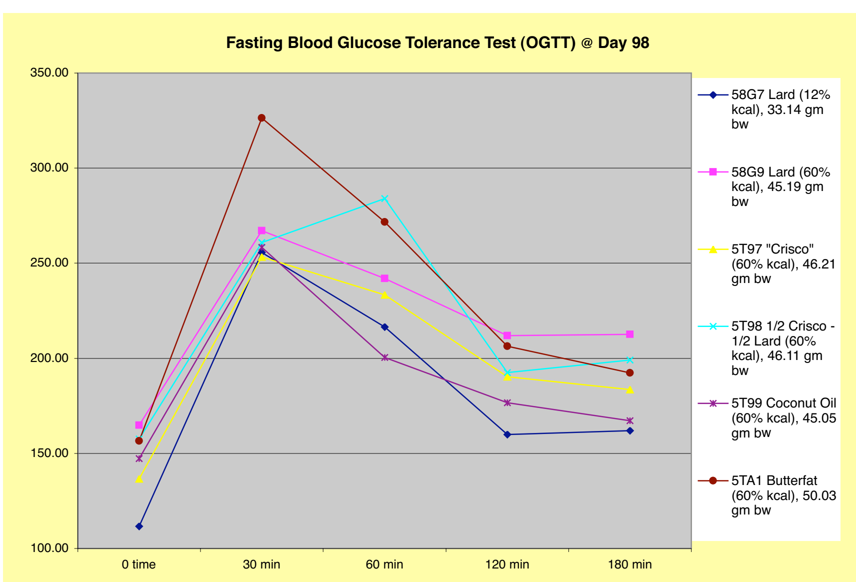
Differences in blood glucose levels are best observed at Day 85. (The Day 101 data are distorted by the fasting glucose tolerance test conducted on Day 98.)



At Day 85, the low-fat control group (#58G7) had a glucose level 134.9 mg/dl. The glucose levels of four of the high-fat diet groups all clustered relatively close together (#5T99, coconut oil = 187.1 mg/dl; #5T98, Crisco-lard = 191.0 mg/dl; #58G9, lard = 196.7 mg/dl; and #5T97, Crisco = 206.3 mg/dl). The group on the butterfat diet (#5TA1) had considerably higher blood glucose levels at 242.3 mg/dl.

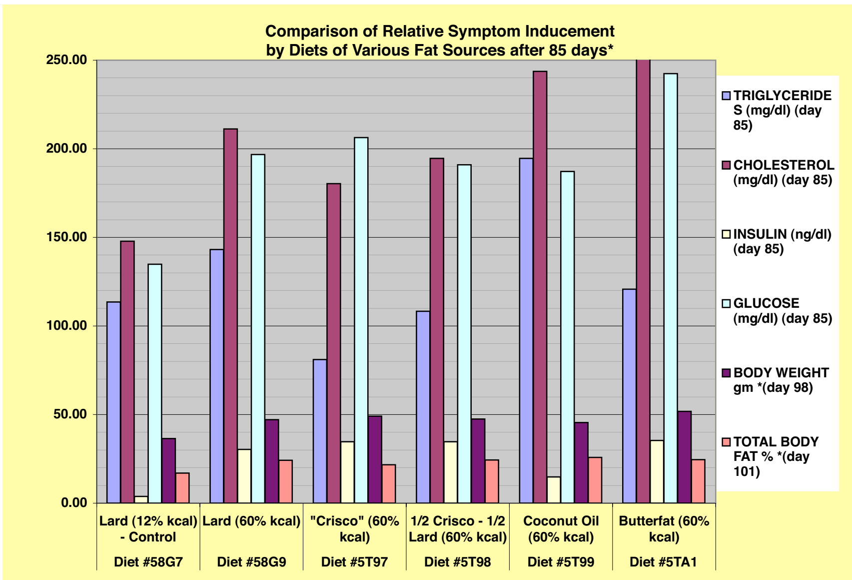
ORAL GLUCOSE TOLERANCE TEST (OGTT)

When fasted at 98 days for the OGGT, the fasting glucose levels of the high-fat diets were higher than of those on the control diet.



COMPARISON OF RELATIVE SYMPTOM INDUCEMENT BY VARYING FATS

Data at Day 85 were used to compare the various diets (data at the end of the study, Day 101, may have been distorted by the fasting for the OGTT at Day 98). For body weight, data at Day 98 (before fasting) were used; and, of course necropsy data of body fat was at Day 101. Data indicate that there are distinct differences in the phenotype induced, particularly certain characteristics pertinent to metabolic syndrome, depending on the particular source of fat in the diet.



In particular, not only the amount of total body fat, but also the location of fat deposits varies depending on the dietary fat source.

CONCLUSION

The results demonstrate that different fat sources have dramatically different effects on development of metabolic syndrome factors.

Hydrogenated vegetable oil (Crisco) appears to produce the greatest level of insulin resistance, while butterfat has the greatest sustained effect on weight gain and glucose levels. Coconut oil has the least effect on these three factors; yet for triglycerides it had the most impact, and for cholesterol the second-highest impact. Butterfat produced the highest cholesterol levels, Crisco the lowest. For triglyceride development, the data suggest that additional study is warranted to determine if Crisco may have some mitigating effect and if the source (type) of fat may be more significant a factor than the amount of the fat.

In the past, the approach to inducing obesity, diabetes, and other diseases has been to use an "in-stock" or "one-size-fits-all" high-fat diet. This study now demonstrates that the most efficacious approach is to produce custom diets, varying the source and combination of fats in order to engineer the precise inducement of a particular metabolic syndrome model, with a specific symptomatic profile.

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