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The Effect of Dietary Beef Tallow and Palm Oil on Development of Atherosclerosis in the ApoE-Deficient Mouse

Final Report

To The

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And

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Background

Atherosclerosis is the main cause of cardiovascular diseases, which are the leading cause of morbidity and mortality in Western societies. Both genetic and environmental factors are involved in the development of atherosclerosis. There is a clear indication that diet is a major environmental influence on the development of atherosclerotic lesions. Among dietary factors, dietary fat has received intense scrutiny resulting in a general impression that oils rich in polyunsaturated fatty acids lower serum cholesterol and saturated fats present an increased risk for elevated cholesterol and cardiovascular disease. However, a review of the literature suggests that not all saturated fats affect blood lipids the same and that tallow, which is high in stearic acid, may actually be hypocholesterolemic.

Although plasma cholesterol concentration has long been recognized as a risk factor for atherosclerosis, it does not provide a direct measurement of the presence or progression of the disease. The apolipoprotein E knockout mouse is an animal model developed for studying atherosclerosis. The animals develop severe hypercholesterolemia and atherosclerotic lesions. The lesions are similar in appearance and distribution to those observed in humans fed a Western-type diet.

Objective

The objectives of the study were to examine the influence of fat type on the progression of atherosclerosis in the ApoE-deficient mouse, measure blood lipids, and examine markers of oxidative stress and inflammation.

Experimental Design

Animals and diets. Five-week old male C57BL/6J ApoE-deficient mice (Jackson Laboratory, Bar Harbor, ME) were divided into 4 dietary groups with 12 animals per group. The AIN-93G diet was modified to include 15% fat in all diets. AIN-93G with 15% soybean oil with or without 0.15% cholesterol served as the positive and negative controls, respectively. The saturated fats tested were palm oil and tallow. They were fed at 14% with soybean oil added at 1% to provide essential fatty acids for growing animals.

The diets were fed ad libitum for 20 weeks. Animal body weights were measured at the beginning of the study, after 15 weeks and at the end of the study. A 24 hour urine collection was taken during week 17.

Lipids. Blood was collected from non-fasted animals at 4 and 15 weeks by tail-nick. At the end of study, serum and a section of liver tissue was collected from fasting animals. Plasma, serum, and liver cholesterol concentrations were determined enzymatically. Serum triglycerides were determined by use of a commercial kit (Sigma Diagnostics, TRO100).

Arterial Preparation for Visualization of Atherosclerotic Lesions. Preparation of the arteries was done following the method of Dr. Eitzman at the University of Michigan. The arterial tree was stained with oil red O to visualize lipids, pinned onto a pan with

black wax and photographed. Image Pro Plus software was used to quantitate the area of the arterial tree, aortic arch, and atherosclerotic lesion in the respective areas. Lesion areas of the aortic arch or arterial tree were expressed as a percentage of the respective areas.

Urinary Thiobarbituric Acid Reactive Substances (TBARS) and Amyloid P (SAP). Total oxidative stress and inflammation was estimated by measurement of urinary TBARS and serum amyloid protein (SAP) respectively.

Inflammation was estimated by measurement of serum amyloid protein (SAP) by enzyme-linked immunosorbent assay, as per the method of Dr. John Belcher's laboratory, University of Minnesota.

Statistical Analysis. Data was analyzed using SAS with analysis of variance by diet. The Student-Newman-Keuls multiple range test was used to determine differences as a function of diet.

Results

Numerous studies have indicated that oils rich in polyunsaturated fatty acids, such as soybean oil, lower serum cholesterol relative to fats high in saturated fats. It can be seen in Table 1 that the presence cholesterol in the diet was more of a determining factor in plasma and serum cholesterol concentrations than fat saturation in the ApoE mouse model. It should also be noted that contrary to the cholesterol response in rat model, tallow in the diet statistically increased plasma and serum cholesterol over palm oil. There was no effect of fat saturation or tallow on serum triglyceride concentration or liver cholesterol concentration (**Tables 1 and 2**).

Table 1. Effect Of Fat Type On Plasma And Serum Lipid Levels In The ApoE-Deficient Mouse

Diet	Plasma Cholesterol[1] 4 weeks mg/dL	Plasma Cholesterol[1] 15 weeks mg/dL	Fasting Serum Cholesterol[1] 20 weeks mg/dL	Fasting Serum Triglyceride[1] 20 weeks mg/dL
Negative Control	422 ± 20 c	474 ± 31 c	391 ± 24 d	67.0 ± 6.0 a
Positive Control	972 ± 57 b	955 ± 48 ab	497 ± 30 c	32.9 ± 2.0 b
Palm Oil	966 ± 23 b	900 ± 51 b	674 ± 46 b	44.5 ± 2.7 b
Tallow	1248 ± 45 a	1085 ± 51 a	789 ± 36 a	40.9 ± 3.0 b

[1]Values are means ± SEM per diet group. Values in a column that do not share the same letter are significantly different ($p<.05$) using the Student-Newman-Keuls test.

Table 2. Effect Of Fat Type On Liver Cholesterol Concentration In The ApoE-Deficient Mouse

Diet	Liver Cholesterol[1] mg/g
Negative Control	5.8 ± 0.63 b
Positive Control	18.1 ± 2.1 a
Palm Oil	15.6 ± 1.2 a
Tallow	16.6 ± 2.2 a

[1]Values are means ± SEM.
Values that do not share the same letter are significantly different ($p<.05$) using Student-Newman-Keuls Test.

Excretion of urinary TBARs is an indication of lipid oxidation within the animal. The TBAR results in **Table 3** suggest that consumption of the saturated fat reduced lipid oxidation. Resistance to oxidation of plasma lipids with beef tallow has also been shown by Dr Csallany's group in our Department.

It is now widely believed that inflammation is associated with atherosclerosis. SAP the mouse homologue to C-reactive protein, a well accepted marker of inflammation. Numerically, the SAP concentration in the positive control group was greater than in the negative control, with the SAP concentration for tallow and palm oil falling in between (**Table 3**). However, there were no statistically significant differences among the group, due to high variability in the positive control group.

Table 3. Effect of Diet on Markers of Oxidative Stress and Inflammation in the ApoE Deficient Mouse.

Diet	Urinary T-BARS[1] µg/24-hours	Serum Amyloid Protein[2] µg/ml
Negative Control	0.583 ± 0.084 b	50.8 ± 6.0
Positive Control	1.052 ± 0.182 a	92.7 ± 20.4
Palm Oil	0.432 ± 0.049 b	76.9 ± 10.1
Tallow	0.531 ± 0.092 b	73.19 ± 7.18

[1]Values are means ± SEM, n=3-4. Values in a column that do not share the same letter are significantly different (P <.05).
[2] Values are means ± SEM per group, n=8-10.

Cardiovascular disease is due to the development of atherosclerotic lesions. The ApoE deficient mouse is genetically modified to be deficient in apoprotein E, which is a constituent of all lipoproteins except LDL. ApoE functions as a ligand for receptors that remove chylomicron and VLDL remnants from the circulation. As seen in **Table 1** above, plasma cholesterol becomes greatly elevated when the animals are fed cholesterol. Inclusion of beef tallow as the fat source caused the greatest elevation.

The atherosclerotic lesion results show a somewhat different pattern (**Table 4**). As expected, the animals fed cholesterol had more atherosclerosis than those fed the cholesterol-free diet. However, animals fed beef tallow did not develop the most atherosclerosis, in spite of having the highest serum cholesterol concentration. The % of area with lesions was not statistically different than the palm oil-fed animals. Both palm oil and tallow feeding resulted in more lesion development than soybean oil.

Table 4. Atherosclerotic Lesion Area in the Aortic Arch and Arterial Trees of ApoE-Deficient Mice fed the Experimental Diets for 20 Weeks

Diet	% Area of Aortic Arch with Lesions [1]	% Area of Arterial Tree with Lesions [1]
Negative Control	1.95 ± 0.58 c	1.16 ± 0.3 c
Positive Control	19.6 ± 2.7 b	8.02 ± 0.8 b
Palm Oil	25.5 ± 2.6 ab	13.2 ± 0.98 a
Tallow	28.8 ± 2.1 a	12.41 ± 0.84 a

[1]Values are means ± SEM per diet group. Values represent the percent lesion area per area measured using Image Pro Plus software.
Values in a column that do not share the same letter are significantly different ($p<.05$) using Student-Newman-Keuls Test.

Summary

Previous studies in our laboratory in rats have demonstrated that tallow feeding reduced liver cholesterol concentrations and decreased dietary cholesterol absorption, while having no effect on plasma cholesterol. Given that plasma cholesterol in rats in resistant to change, the lack of a plasma cholesterol lowering effect was not unexpected. The effects of tallow feeding on serum cholesterol in humans have consistently shown either a reduction or no effect. Thus, the finding of a serum cholesterol elevation with tallow in the present study was unexpected.

Given the previous body of knowledge that tallow, in most studies, reduces plasm cholesterol, and that plasma cholesterol is now established as a causal factor in development of atherosclerosis, it was hypothesized that tallow would reduce atherosclerosis in this animal model, the apoE-deficient mouse. This model, developed in 1994, is becoming widely used to study dietary effects on atherosclerosis. However, our results indicated increased atherosclerosis in mice fed tallow or palm oil. Whether this result translates directly to humans is uncertain. Although the current prevailing wisdom is that it does, one cannot exclude the possibility that for investigations of the effect of fat type on atherosclerosis, the model may not be appropriate.

To conclude, our hypothesis that tallow would reduce atherosclerosis in an animal model was not supported. Indeed, our results indicate an acceleration of atherosclerosis. Because of the unexpected nature of these findings, we are currently conducting an experiment of the effect of tallow feeding on cholesterol concentrations in another animal model, the hamster, in order to better understand the effects of dietary tallow on cholesterol metabolism.