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Cholesterol vehicle in experimental atherosclerosis. 21. Native and randomized lard and tallow

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Tallow and lard are used as representative animal fats in experimental diets. We have compared their respective atherogenic properties by feeding each fat (14%) as part of a semi-purified diet containing 0.5% cholesterol. The lard containing diet was significantly ($P < 0.001$) more atherogenic than the diet containing tallow. Lard and tallow both contain approximately 24% palmitic acid (16:0) but the palmitic acid is distributed differently in their component triglyceride molecules. Over 90% of 16:0 of lard is at the SN_2 position, whereas only approximately 15% of the 16:0 of tallow is in that position. The fats were randomized so that every component fatty acid was present at each triglyceride carbon to approximately one third of its total concentration. Randomized tallow and lard had 8.5% and 7.6% of their component 16:0 at the SN_2 position, respectively. The atherogenicity of diets containing either randomized lard or tallow was virtually the same and significantly lower than the atherogenicity of the diet containing native lard. This study confirms earlier studies from our laboratory that suggest that the level of 16:0 at the SN_2 position of a triglyceride influences its atherogenicity. (J. Nutr. Biochem. 9:582–585, 1998) © *Elsevier Science Inc. 1998*

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Introduction

In the design of nutritional studies, tallow and lard are used interchangeably as examples of animal fat. However, there are differences in the compositions of these fats that might give different results in studies of dietary effects on various aspects of lipid metabolism. Lard contains more linoleic acid and less stearic acid than tallow. Although the two fats contain similar levels of palmitic acid, they differ in its distribution: over 90% of the palmitic acid of lard is carried at the SN_2 position of the triglyceride, whereas only 15% of the palmitic acid of tallow is carried at the SN_2 position. The studies described herein were performed to determine if these two common animal fats possess different properties with regard to their atherogenic potential and if randomization (rearrangement) might affect these properties.

Methods and materials

Male New Zealand white rabbits (starting weight 2.4–2.5 kg) were fed a semi-purified diet containing 14% of either lard or tallow. The fatty acid compositions of the native and randomized fats in tallow and lard are given in *Table 1* and *Table 2*, respectively. In native tallow 15.5% of the total palmitic acid appears at the SN_2 position, and in randomized tallow 34.3% of the total palmitic acid is found at that position. The percentage of total palmitic acid found at the SN_2 position in lard and randomized lard is 99.4% and 35.3%, respectively. The structures were determined using the method of Laakso and Christie.[1] To forestall possible essential fatty deficiency, the diets (*Table 3*) also contained 1% corn oil. After analysis of the starting fats, cholesterol was added to the diets to bring their cholesterol content up to 0.5%. The diets were prepared to our specifications and pelletted by Dyets, Inc. (Bethlehem, PA USA).

The rabbits were housed individually in a room maintained at

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Table 1 Fatty acids of tallow[1]

Acid	%	% at SN_2 Native	% at SN_2 Randomized
14:0	3.32	1.70	0.93
16:0	24.79	3.84	8.50
16:1w7	2.87	1.55	0.74
17:0	1.30	0.19	0.37
18:0	17.09	2.24	6.17
18:1w9	39.64	19.66	13.12
18:1w7	2.60	0.35	0.90
18:2w6	2.65	1.70	0.76

[1]This table only includes fatty acids occurring at >1% concentration.

constant temperature and humidity and kept on a 12-hour on-off light cycle. They were given food and water ad libitum. After 60 days rabbits were bled under light barbiturate anesthesia and then sacrificed by exsanguination. Plasma total and high density lipoprotein (HDL) cholesterol and triglycerides were determined using appropriate kits (Sigma, St. Louis, MO USA). Plasma lipoproteins were separated by ultracentrifugation[2] and cholesterol content of the various fractions was determined using kits. Livers were weighed and aliquots were extracted with chloroform: methanol 2:1.[3] The lipid extract was analyzed for free and total cholesterol[4] and triglycerides.[5] Aortas were cleaned of adhering tissue and graded visually for severity of atherosclerosis.[6] The native and randomized fats were gifts of the National Live Stock and Meat Board (Chicago, IL USA) (lard) and RJR Nabisco (East Hanover, NJ USA) (tallow). Experimental procedures were approved by the Wistar Institutional Animal Care and Use Committee (IACUC).

Results

The atherogenic effects of lard and tallow were compared in two separate experiments that used eight and ten rabbits per group, respectively. In both groups, 16 of 18 rabbits survived. The results have been combined in *Table 4*. It is evident that the two fats exerted similar results on total cholesterol but the percent of HDL cholesterol was significantly lower in rabbits fed lard. Liver cholesterol was higher in the lard-fed rabbits but levels of liver esterified cholesterol were similar.

To test the effects of the randomized fats, four groups of eight rabbits each were used. All the lard fed rabbits survived but one rabbit in each of the tallow groups was lost. Total cholesterol levels were not significantly different among the four groups (*Table 5*). The rabbits fed lard exhibited significantly more severe atherosclerosis than those fed tallow (*Table 6*). Randomization of tallow led to somewhat more palmitic acid in the SN_2 position and increased the severity of atherosclerosis, whereas randomization of lard caused a large reduction in the amount of palmitic acid at the SN_2 position and a significant decrease in the severity of atherosclerosis. As in the two previous experiments (*Table 4*), tallow was significantly less atherogenic than lard but randomization of the fats (*Table 6*) rendered them of equal atherogenic potential. Atherogenicity of randomized tallow was 7% more severe than tallow and that of randomized lard was 52% less severe than lard.

Our earlier studies[7,8] have shown that the presence of significant levels of palmitic acid at the SN_2 position of a fat increases its atherogenicity. In the first two experiments in this study (*Table 4*), rabbits fed lard exhibited significantly more severe atherosclerosis than did those fed tallow. In those experiments the cholesterol levels were similar regardless of the type of dietary fat. In both experiments the

Table 2 Fatty acids of lard[1]

Fatty acid	%	% at SN_2 Native	% at SN_2 Randomized
14:0	1.27	0.90	0.42
16:0	21.41	21.28	7.56
16:1w7	2.26	1.12	0.69
18:0	10.83	1.22	3.90
18:1w9	38.95	4.87	12.74
18:1w7	3.24	0.42	1.09
18:2w6	15.54	2.33	4.65

[1]This table only includes fatty acids occurring at >1% concentration.

Table 3 Diet composition

Ingredient	g/100 g	% Calories
Casein	25.0	25.2
DL-methionine	0.3	
Starch	20.2	20.4
Sucrose	20.1	20.3
Cellulose	14.0	
Lard or tallow	14.0	31.8
Corn oil	1.0	2.3
Mineral mix	4.0	
Vitamin mix	1.0	
Choline bitartrate	0.2	
Cholesterol	0.2–0.3[1]	

[1]Average of amount added to lard or tallow to bring cholesterol content of diet to 0.5%.

Table 4 Influence of lard or tallow on atherogenesis in rabbits (summary of 2 experiments)

	Group: Lard	Group: Tallow
Weight gain (g)	−19 ± 64	−84 ± 78
Liver weight (g)	80.1 ± 4.36	70.1 ± 4.17
Liver as % body wt	2.89 ± 0.13	2.52 ± 0.13
Plasma lipids (μM/L)		
Total cholesterol	17.12 ± 0.62	15.52 ± 1.24
% HDL-cholesterol	6.4 ± 0.6	9.2 ± 0.8 (a)
Triglycerides	3.75 ± 0.70	2.86 ± 0.35
Liver lipids (mM/100 g)		
Total cholesterol	8.07 ± 1.09	5.15 ± 0.62 (b)
% Ester	85.6 ± 1.62	83.7 ± 1.92
Triglycerides	2.23 ± 0.25	2.43 ± 0.50
Atherosclerosis		
Aortic arch	1.44 ± 0.19	0.69 ± 0.16 (a)
Thoracic aorta	1.06 ± 0.18	0.41 ± 0.13 (a)

Rabbits fed semi-purified diets containing 14% test fat and 0.5% cholesterol for 2 months. Aortas graded visually on a 0–4 scale.[6]
a) $p < 0.01$; b) $p < 0.05$

Table 5 Lipids of plasma lipoproteins in rabbits fed native or randomized lard or tallow

	Group			
	Lard	Rand. lard	Tallow	Rand. tallow
Plasma lipids (μM/L)				
Cholesterol				
Total	23.95 ± 4.76	21.57 ± 3.96	30.44 ± 4.03	30.75 ± 4.27
VLDL	9.83 ± 1.50	10.19 ± 1.86	11.02 ± 2.72	11.30 ± 2.15
IDL	8.55 ± 3.85	9.18 ± 2.46	12.49 ± 3.05	17.20 ± 2.64
LDL	4.22 ± 1.50	1.71 ± 0.13	2.51 ± 1.09	1.66 ± 0.57
HDL	0.62 ± 0.10	0.44 ± 0.03a	0.54 ± 0.10	0.65 ± 0.08a
Triglycerides				
Total	1.98 ± 0.58	0.66 ± 0.06a	1.63 ± 0.32	2.52 ± 0.49a
VLDL	0.46 ± 0.16	0.15 ± 0.06	0.32 ± 0.07	0.68 ± 0.25
IDL	0.77 ± 0.28	0.18 ± 0.02a	0.69 ± 0.34	1.24 ± 0.42a
LDL	0.52 ± 0.16a	0.17 ± 0.02ab	0.52 ± 0.16b	0.25 ± 0.07
HDL	0.26 ± 0.03ab	0.17 ± 0.01acd	0.26 ± 0.03ce	0.36 ± 0.01bde

Rabbits fed semi-purified diets containing 14% test fat and 0.5% cholesterol for 2 months.
In any horizontal row values bearing same letter are significantly different $p < 0.05$.

rabbits showed a slight net weight loss. In the third study the rabbits gained weight and their cholesterol levels were higher but there was no difference in atherosclerosis among the dietary groups. *Table 7* summarizes the distribution of severity of atherosclerosis seen in the three experiments in which native tallow and lard were fed.

Discussion

In the three experiments in which the atherogenic effects of lard and tallow were compared, lard was seen to be significantly ($P < 0.001$) more atherogenic. An appreciable number of the rabbits fed tallow exhibited no atherosclerosis in the thoracic aorta, which might be due to the presence of a rather high level of stearic acid (17%). Earlier studies have shown cocoa butter[9] or fats designed to contain high levels of stearic acid[10] to be less atherogenic than might be expected from their level of saturation.

Randomization of lard decreased the amount of palmitic acid at the SN_2 position by 64% and decreased severity of atherosclerosis by approximately 50%. Randomization of tallow increased the palmitic acid at SN_2 by 120% and increased atherogenicity by 7%. It should be pointed out that the level of palmitic acid present in the SN_2 position of randomized tallow is still 60% lower than that in native lard. Randomized tallow and lard had similar levels of palmitic acid at the SN_2 position (8.50% and 7.56%, respectively) and exhibited similar levels of severity of atherosclerosis (*Table 6*). In an earlier study involving the use of higher levels of dietary cholesterol and less fat, randomized lard was shown to be 10% less atherogenic than native lard.[11]

Rabbits fed randomized lard had higher levels of liver cholesterol than did the other groups and showed significantly higher weight gain. Relative liver weights were elevated in the animals fed the randomized fats. The plasma low density lipoprotein (LDL)/HDL cholesterol ratios of the rabbits fed the randomized fats were approximately 44% lower than those of rabbits fed the native fats.

The mechanism underlying these observations remains moot. Small, dense LDL particles have been shown to be less atherogenic than larger and lighter ones[12] but randomization does not appear to influence LDL particle size.[8] Redgrave et al.[13] reported that the structure of a triglyceride influenced its metabolism. The presence of saturated fatty

Table 6 Necropsy data on rabbits fed native or randomized tallow and lard

	Group			
	Lard	Rand. lard	Tallow	Rand. tallow
Weight gain, g	488 ± 126a	900 ± 83abc	516 ± 104b	321 ± 189c
Liver weight, g	72.9 ± 4.0a	99.3 ± 4.5abc	81.6 ± 3.5b	77.7 ± 6.0c
Liver as % body wt.	2.57 ± 0.11ab	3.05 ± 0.14a	2.84 ± 0.19	3.09 ± 0.21b
Liver lipids (mM/100 g)				
Total cholesterol	8.83 ± 1.64	8.64 ± 1.15a	9.76 ± 1.46	13.11 ± 1.31a
% Ester	74.8 ± 3.00	76.7 ± 1.91	73.9 ± 2.95	76.3 ± 2.14
Triglyceride	1.10 ± 0.11	0.99 ± 0.16	1.42 ± 0.18	1.42 ± 0.20
Atherosclerosis				
Aortic arch	2.69 ± 0.28ab	1.50 ± 0.28a	1.29 ± 0.24b	1.50 ± 0.53
Thoracic aorta	1.75 ± 0.28abc	0.69 ± 0.19a	0.79 ± 0.28b	0.79 ± 0.28c

Rabbits fed semi-purified diets containing 14% test fat and 0.5% cholesterol. Aortas graded visually on a 0–4 scale.[6]
In any horizontal row values bearing same letter are significantly different $p < 0.05$.

Table 7 Severity of atherosclerosis in rabbits fed tallow or lard (3 experiments)

	Tallow (23/26)		Lard (24/26)	
Grade	Arch	Thoracic	Arch	Thoracic
4.0	0	0	0	0
3.5	0	0	3	1
3.0	0	0	2	0
2.5	1	0	3	1
2.0	1	1	4	4
1.5	5	1	4	6
1.0	4	7	5	5
0.5	8	3	3	6
0.0	4	11	0	1
Avg.	0.87 ± 0.14a	0.52 ± 0.12b	1.85 ± 0.20a	1.29 ± 0.16b

a)Tallow versus lard $p < 0.001$.
b) Tallow versus lard $p < 0.001$.
Aortas were graded visually on a 0–4 scale.[6]

acids at the SN_2 position of a triglyceride slowed its removal from the circulation. Fats containing palmitic acid at the SN_2 position are more completely absorbed than other fats.[14–16] Thus, the combination of more efficient absorption and less active removal from the circulation of some fats may result in increased exposure of the aortic tissue to these fats, thus increasing the risk of atherosclerosis even when blood levels of cholesterol and triglycerides do not vary.

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