

# Effect of different saturated fatty acids as interesterified triacylglycerols on lipid metabolism in rats and hamsters

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*To examine the cholesterolemic nature of long-chain saturated fatty acids in relation to the effect of palm oil, rats and hamsters were fed purified diets containing 7 to 8% interesterified fats in which saturated fatty acids, lauric, myristic, palmitic, and stearic acids were the sole variable. Feeding with a stearic acid fat-containing diet to these animals resulted in lower absorption of dietary fat, greater excretion of fecal neutral steroids, and lower concentration of hepatic cholesterol. The lower concentration of plasma cholesterol in hamsters fed a stearic acid fat-containing diet without cholesterol seemed to be attributed to the lower intestinal absorption of cholesterol rather than the enhanced catabolism of plasma cholesterol. Although not as markedly, palmitic acid also showed effects somewhat similar to those of stearic acid. Thus, saturated fatty acids exerted distinct effects on various parameters of lipid metabolism, and hence, the effect of palm oil should be evaluated at the broad view point. (J. Nutr. Biochem. 6:195–200, 1995.)*

**Keywords:** palm oil; saturated fatty acid; absorption of fatty acids; cholesterol; fecal steroids; catabolism of LDL

## Introduction

Palm oil, which is rich in palmitic acid, has long been considered a typical hypercholesterolemic saturated fat, though it simultaneously contains considerable amounts of oleic and linoleic acids. The classical studies by Keys et al.<sup>1</sup> and Hegsted et al.<sup>2</sup> clarified the contrasting effect of saturated (S) and polyunsaturated (P) fatty acids on the concentration of plasma cholesterol. Although these pioneer studies pointed out the diversity of individual fatty acids in their effect on plasma cholesterol, recent information by other investigators indicates that saturated fatty acids cannot be regarded as a single entity, and different saturated fatty acids appear to affect the plasma cholesterol concentration differently.<sup>3,4</sup>

Bonanome and Grundy<sup>3</sup> showed that stearic acid is not hypercholesterolemic but rather reduces plasma cholesterol in humans, and they suggested that palmitic acid is respon-

sible for the elevating effect of saturated fats. In contrast, Hayes proposed an antihypercholesterolemic propensity of palmitic acid in monkeys.<sup>4</sup> These authors proposed the hypercholesterolemic nature of myristic acid. Recent studies by Hayes et al.<sup>5</sup> have indicated that when the supply of linoleic acid is above the threshold level (approximately 6% total energy), no fatty acids increase the plasma cholesterol level. In accordance with these observations, palm oil has been shown to be less hypercholesterolemic than predicted from its high palmitic acid content.<sup>6,7</sup>

To understand the effects of different fatty acids on cholesterolemia, the use of dietary fats with a definite fatty acid composition is a prerequisite. This may be achieved by mixing different fats appropriately, but it is virtually impossible to prepare fats with an exactly comparable composition. In this context, it is easier to prepare the fats in which one saturated fatty acid is the sole variable by interesterification.

However, the difference in the structure of dietary fat may also be taken into account for their effect on plasma cholesterol. The triacylglycerol structure of palm oil is characterized by a relatively high proportion of the symmetrical triacylglycerol, palmitic-oleic-palmitic structure (30 to

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36%),<sup>8</sup> and this structure may affect the cholesterolemic nature of palmitic acid.<sup>9</sup> However, the comparison of palm oil and randomized palm oil showed no difference in the absorption and in the effect on the serum cholesterol level of rats.<sup>10</sup> Thus, interesterified fats with different saturated fatty acids can be used to examine their effect on plasma cholesterol.

Hence, using the interesterification technique, we prepared dietary fats in which the saturated fatty acids were the sole variable and fed these fats to rats and hamsters to examine the effect of lauric, myristic, palmitic, and stearic acids on the serum cholesterol concentration, fecal steroid excretion, and catabolism of low density lipoprotein (LDL).

## Methods and materials

### Dietary fats

We prepared two kinds of interesterified fats either rich in or low in  $\alpha$ -linolenic acid according to the method of Kuksis.<sup>11</sup> The P/S ratio of these fats was adjusted to 0.4 referring to the profile of dietary fats commonly consumed in the Western countries. The interesterified fat rich in  $\alpha$ -linolenic acid was prepared by using saturated triacylglycerol (either glycerol-trilaurate, -trimyristate, -tripalmitate or -tristearate) with rapeseed oil and soybean oil exhibiting saturated:oleic:linoleic: $\alpha$ -linolenic acid ratios of 5:3:1.4:0.6. The interesterified fat low in  $\alpha$ -linolenic acid was prepared by using saturated triacylglycerol with high oleic safflower oil and soybean oil exhibiting saturated:oleic:linoleic acid ratios of 5:3:2. The fatty acid compositions of these fats are shown in Table 1. We have included a fairly high proportion of  $\alpha$ -linolenic acid as a source of polyunsaturated fatty acids in one preparation in order to determine the possible preferable effect of this type of polyunsaturated fatty acid in the regulation of lipid metabolism.

### Animals

Male Sprague-Dawley rats, 4 weeks old, were given a commercial nonpurified diet (NMF, Oriental Yeast, Tokyo, Japan) and acclimatized for 3 days in an air-conditioned room. The experimental diet was prepared according to the recommendation of the American Institute of Nutrition<sup>12</sup> and contained by g/kg of diet: casein, 200; interesterified fat rich in  $\alpha$ -linolenic acid, 70; vitamin mixture, 10; mineral mixture, 35; choline bitartrate, 2; DL-methionine, 3; cellulose, 50; cholesterol, 2; Na-cholate, 0.5; corn starch, 150; and sucrose 477.5. The animals were fed diets ad libitum for 3 weeks. Feces were collected for the last 2 days.

Male Syrian hamsters, 6 weeks old and housed individually in stainless mesh cages, were given a nonpurified diet (NMF, Oriental yeast) and acclimated for 7 days in an air-conditioned room. All the experimental diets (g/kg) were cholesterol-enriched (2 g/kg) and contained: casein, 200; interesterified fat low in  $\alpha$ -linolenic acid, 80; corn starch, 350; cellulose, 100; wheat bran, 50; mineral mixture, 35; vitamin mixture, 10; DL-methionine, 3; choline bitartrate, 2; inositol, 0.5; sucrose, 169.5 or 167.5.<sup>13</sup> The animals were given free access to the diet for 4 weeks. Feces were collected for 2 days during the last week.

At the end of the feeding period, the animals were deprived of feed for 7 hr (0600 to 1300 hr), and, under light diethyl ether anesthesia, blood was withdrawn from the abdominal aorta.

### Dietary fat absorption

The apparent fat absorption was calculated on the basis of fecal fatty acid analysis.<sup>14</sup>

### Fecal steroid excretion

Fecal neutral and acidic steroids were determined by GLC using 5 $\alpha$ -cholestane and nordeoxycholic acid as the internal standards.

### Catabolism of LDL

Immediately before the LDL turnover study, plasma was harvested from blood of 6-week-old male hamsters fed a nonpurified diet (NMF, Oriental Yeast), and LDL was isolated at a density of 1.020 to 1.055 kg/L. This fraction showed only apolipoprotein B on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The LDL was labeled with <sup>125</sup>I and injected through the femoral vein of hamsters that were fed experimental diets for 4 weeks. A small amount of blood was obtained by heart puncture at 1 min and at 0.5, 1, 4, 8, and 24 hr. The disappearance of the radioactivity was best described by a biexponential curve confirming a two-pool model. The curves were fitted by a least square techniques, and the fractional catabolic rate (FCR) was determined according to the method of Mathews.<sup>15</sup>

## Results

### Fecal excretion of fatty acids

The fecal excretion of fatty acids in rats increased proportionately with a chain length of the saturated fatty acids (in mg/day, lauric acid fat, 32.8  $\pm$  2.5; myristic acid fat, 44.1  $\pm$  6.8; palmitic acid fat, 127  $\pm$  4; and stearic acid fat, 221

**Table 1** Fatty acid composition of interesterified fats

Fatty acid composition	Fat rich in $\alpha$ -linolenic acid				Fat low in $\alpha$ -linolenic acid			
	Lauric acid fat	Myristic acid fat	Palmitic acid fat	Stearic acid fat	Lauric acid fat	Myristic acid fat	Palmitic acid fat	Stearic acid fat
	(mol/100 mol)							
12:0	52.2	0.1	0	0	51.5	0.1	1	1
14:0	0.2	50.1	0	0	0.1	48.6	0.9	0.2
16:0	2.8	2.6	50.8	4.2	2.6	3.1	48.9	5.5
18:0	0.9	0.9	1.7	43.3	1.7	1.7	2.3	40.1
18:1	26.1	27.9	28.6	31.6	26.0	27.2	28.9	31.3
18:2 (n - 6)	12.5	12.5	13.0	14.2	17.4	19.2	18.7	22.7
18:3 (n - 3)	5.3	5.9	5.9	6.6	0.7	0.1	0.3	0.2

References 23 and 24.

**Table 2** Apparent absorption rate of different saturated fatty acid fats in rats and hamsters\*

Group	Rats†		Hamsters‡	
	Cholesterol-containing diet	Cholesterol-free diet	Cholesterol-free diet	Cholesterol-containing diet
		(%)		
Lauric acid fat	97.5 ± 0.2 <sup>c</sup>	97.2 ± 0.2 <sup>b</sup>		97.4 ± 0.1 <sup>c</sup>
Myristic acid fat	96.4 ± 0.5 <sup>c</sup>	96.8 ± 0.2 <sup>b</sup>		97.5 ± 0.1 <sup>c</sup>
Palmitic acid fat	90.3 ± 1.1 <sup>b</sup>	96.2 ± 0.2 <sup>b</sup>		96.1 ± 0.1 <sup>b</sup>
Stearic acid fat	84.0 ± 1.6 <sup>a</sup>	89.6 ± 0.7 <sup>a</sup>		93.3 ± 0.5 <sup>a</sup>

\*Values within a diet group with different superscript letters are significantly differently at  $P < 0.05$ .  
 †,‡Six animals per groups.<sup>23,24</sup>

± 16). Consequently, the apparent absorption rate of dietary fat was lowest in the stearic acid fat and highest in the lauric and myristic acid fats, the palmitic acid fat being intermediate (Table 2). In feces of the stearic and palmitic acid fat groups, these fatty acids occupied approximately 80% of the total fatty acids excreted, whereas the proportion of myristic and lauric acid was approximately 40 and 8% in rats fed the corresponding fats, respectively.

The fecal excretion of fatty acids in hamsters tended to increase proportionately with a chain length of the saturated fatty acids, though not as prominently as was observed in rats (in mg/day, lauric acid fat, 26.5 ± 1.1; myristic acid fat, 27.0 ± 1.1; palmitic acid fat, 34.0 ± 1.3; and stearic acid fat, 90.7 ± 7.7 in a cholesterol-free diet, and lauric acid fat, 20.9 ± 0.6; myristic acid fat, 19.4 ± 1.0; palmitic acid fat, 30.5 ± 1.2; and stearic acid fat, 49.1 ± 4.9 in a cholesterol-added diet). Thus, the apparent absorption rate of dietary fat was lowest in the stearic acid fat and highest in the lauric and myristic acid fat, the palmitic acid fat being intermediate (Table 2). The addition of cholesterol to the diet resulted in a slightly, but significantly, higher absorption rate of dietary fat ( $P < 0.05$  by two-way ANOVA). In the stearic and palmitic acid fats, these fatty acids occupied 40 to 70% of the total fatty acids excreted, whereas the proportion of myristic and lauric acid was 4 to 20% in rats fed these acids (in percentage, lauric acid fat, 4.1; myristic acid fat, 9.2; palmitic acid fat, 39.7; and stearic acid fat, 63.9, in a cholesterol free diet, and lauric acid fat, 8.6; myristic acid fat, 19.5; palmitic acid fat, 61.0; and stearic acid fat, 67.0, in a cholesterol-added diet).

**Fecal steroid excretion**

Figure 1 shows the effects of dietary fats on fecal excretion of steroids measured by GLC.<sup>16</sup> Although no difference was observed in fecal weight on the dry basis (data not shown), there was a significant increase in fecal neutral but not acidic steroids in rats fed stearic acid fat compared with those fed other triacylglycerols.

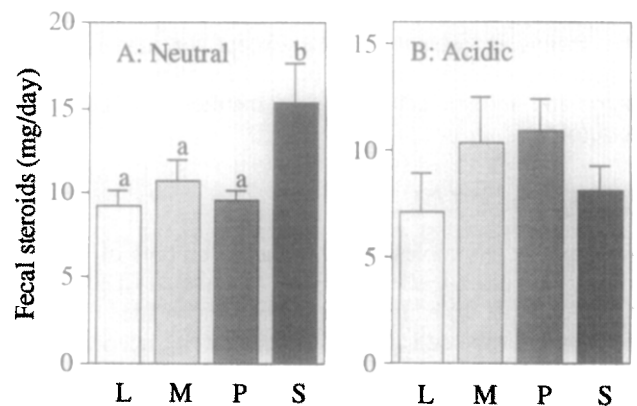
Figure 2 shows the fecal steroid excretion in hamsters. The cholesterol-containing diet reduced fecal weight compared with the cholesterol-free diet ( $P < 0.05$  by two-way ANOVA; data not shown). Fecal neutral steroid excretion was higher in hamsters fed the cholesterol-containing diet than in those fed the cholesterol-free diet, but dietary cholesterol did not affect fecal acidic steroid excretion. There

was a significantly greater excretion of fecal neutral steroids in the stearic acid group irrespective of dietary cholesterol, whereas excretion of acidic steroids was lower in this group than in the palmitic acid fat group in the cholesterol-free diet. However, the difference was not seen when the cholesterol-added diets were fed. Among the four types of dietary fats, fecal neutral steroid excretion was the lowest in two myristic acid fat groups, both in cholesterol-free and cholesterol-added diets.

**Serum and liver lipid**

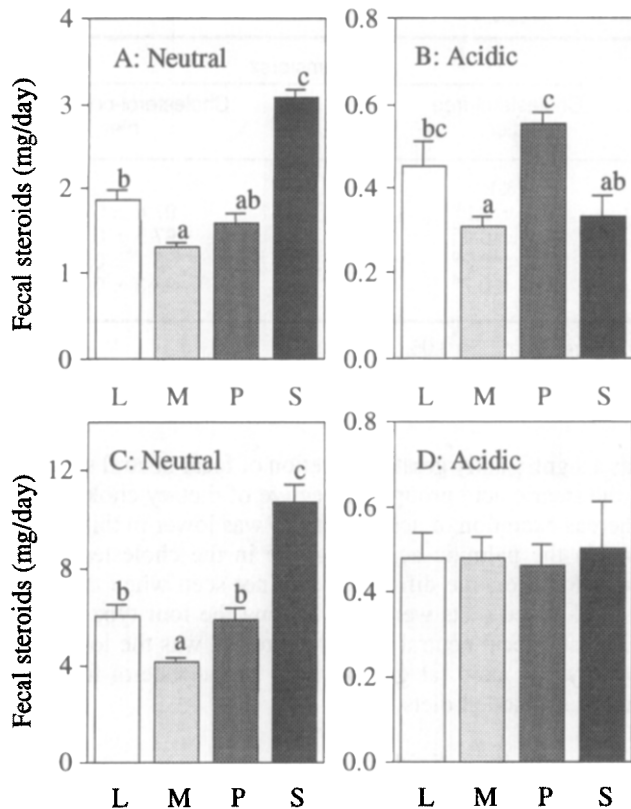
Dietary fat did not affect significantly the concentrations of plasma cholesterol and triacylglycerol, although the latter tended to be lower in rats fed lauric or myristic acid fats (Figure 3). The concentration of liver triacylglycerol decreased proportionately with a chain length of dietary saturated fatty acids, being highest in the lauric acid fat group and lowest in the stearic acid fat group. The concentration of liver cholesterol also was lowest in the stearic acid fat group.

The lipid data in hamsters are shown in Figures 4 and 5 for cholesterol-free diets and cholesterol-added diets, respectively. The fat rich in stearic acid caused a significantly lower concentration of plasma cholesterol compared with other fats when diets were free of cholesterol, although such



**Figure 1** Effects of different saturated fatty acid fats on fecal excretion of neutral (A) and acidic (B) steroids in rats fed cholesterol-added diets. L, lauric acid; M, myristic acid; P, palmitic acid; S, stearic acid. <sup>ab</sup>Values with different letters are significantly different at  $P < 0.05$ .<sup>23</sup>

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**Figure 2** Effects of different saturated fatty acid fats on fecal excretion of neutral (A, C) and acidic (B, D) steroids in hamsters fed cholesterol-free (A, B) and cholesterol-added (C, D) diets. L, lauric acid; M, myristic acid; P, palmitic acid; S, stearic acid. <sup>abc</sup>Values with different letters are significantly different at  $P < 0.05$ .<sup>24</sup>

a hypocholesterolemic effect of stearic acid fat disappeared when cholesterol was added to the diets. Dietary fat did not affect the concentration of plasma triacylglycerol irrespective of dietary cholesterol. Feeding stearic acid fat markedly lowered the concentration of liver cholesterol irrespective of dietary cholesterol. In hamsters fed cholesterol-free diets, palmitic acid fat also lowered the liver cholesterol concentration compared with myristic and lauric acid fats. The liver triacylglycerol concentration was comparable among the groups in respective diets except for a significantly higher concentration in palmitic acid fat compared with the lauric and myristic acid fats when the diet was free of cholesterol.

### Catabolism of LDL

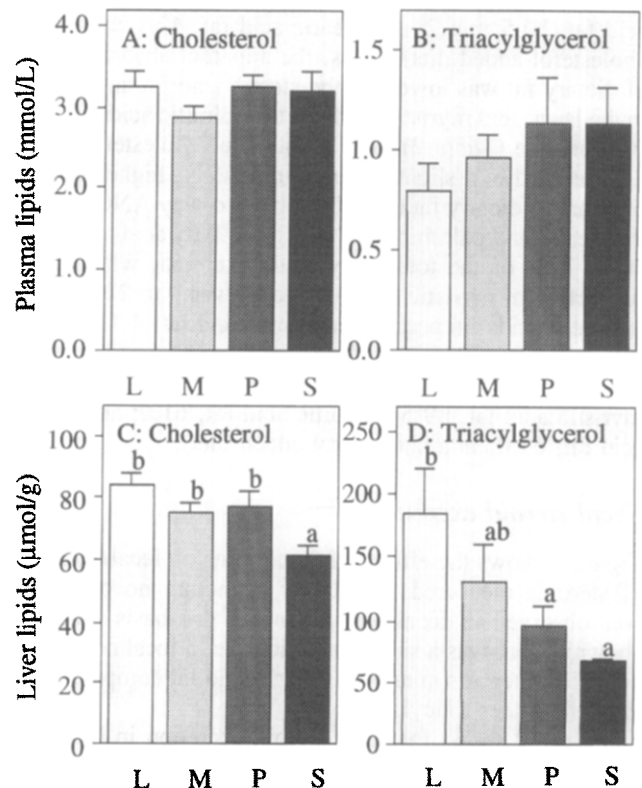
In hamsters fed cholesterol-free diets, the type of fats previously fed did not affect FCR of <sup>125</sup>I-labeled LDL (Figure 6). In contrast, in hamsters fed cholesterol-added diets, the FCR of <sup>125</sup>I-labeled LDL was highest in the stearic acid fat group. However, dietary cholesterol-induced suppression of the FCR was not observed. Since the LDL pool size seemed to be similar among the cholesterol-fed groups, the catabolic rate (FCR × plasma cholesterol concentration) of LDL seemed to be highest in the animals fed a stearic acid fat diet. This observation is in agreement with Woollett et al.<sup>17</sup> who reported that glycerol tristearate compared with

triacylglycerols containing either palmitic, myristic or lauric acids suppressed the reduction of relative hepatic receptor activity when hamsters were fed cholesterol-containing diets. In contrast to hamsters fed cholesterol-added diets, the catabolic rate seemed to be lowest in stearic acid fat when cholesterol-free diets were fed.

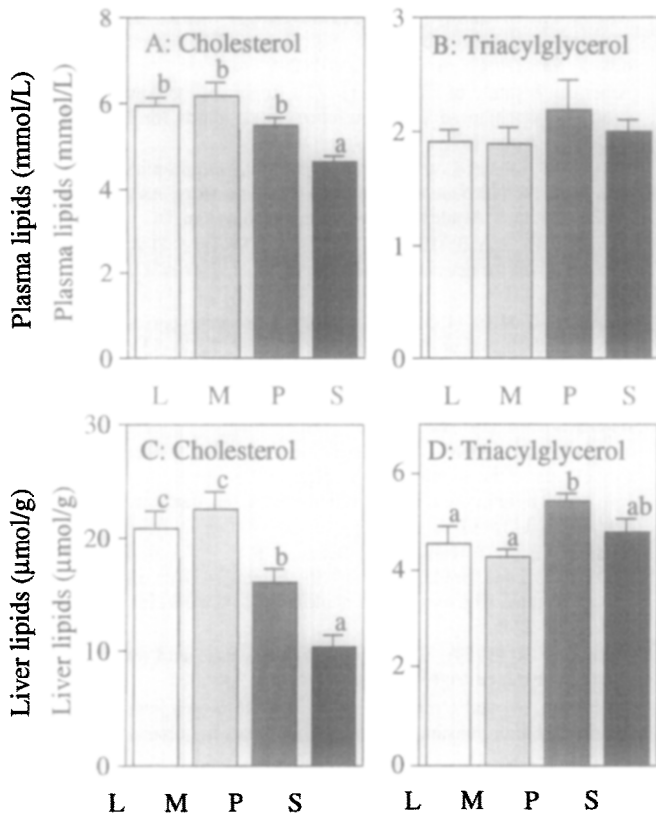
## Discussion

Fat absorption has been reported to increase as fatty acid chain length was decreased when homogeneous saturated triacylglycerols of 12- to 18-carbon chains were fed to rats.<sup>18</sup> Lesser fat absorption or digestion may account for the lesser cholesterol absorption observed with glycerol-tristearate or -tripalmitate diets, since unabsorbed or undigested stearate or palmitate may reduce the availability of cholesterol to the jejunum mucosa.<sup>18</sup> Such saturated fatty acids may also precipitate calcium or magnesium ions which are important in micellization. Mattson<sup>19</sup> reported that the absorbability of the homogeneous saturated triacylglycerols is inversely proportional to the amount of glycerol-tristearate in the diet. However, other workers have not confirmed a low rate of stearic acid absorption in humans<sup>20</sup> and in rats.<sup>21</sup> Since our interesterified fat contained 10 to 15% of a homogeneous saturated type of triacylglycerol, absorption of dietary fat was studied in rats and hamsters.

These studies with rats and hamsters showed that stearic



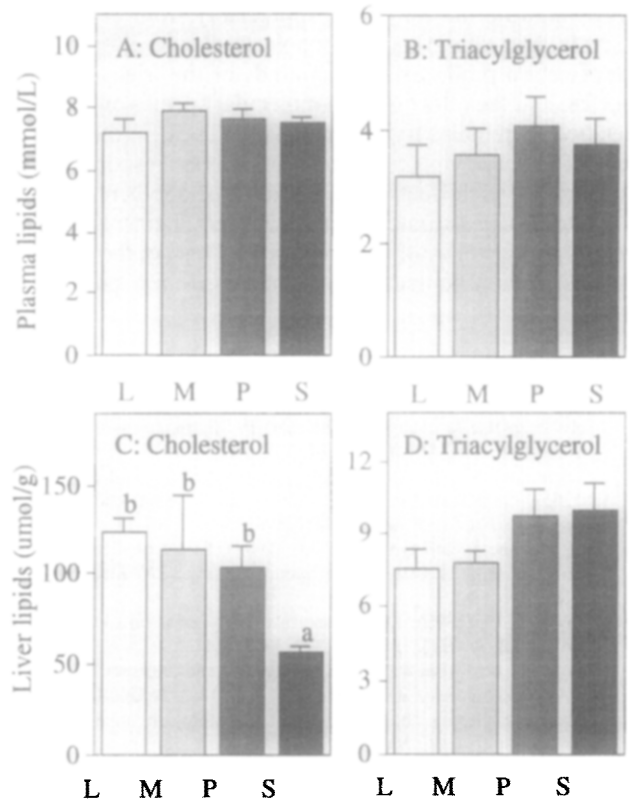
**Figure 3** Effects of different saturated fatty acid fats on plasma (A, B) and liver (C, D) lipid concentrations in rats fed cholesterol-added diets. L, lauric acid; M, myristic acid; P, palmitic acid; S, stearic acid. <sup>ab</sup>Values with different letters are significantly different at  $P < 0.05$ .<sup>23</sup>



**Figure 4** Effects of different saturated fatty acid fats on plasma (A, B) and liver (C, D) lipid concentrations in hamsters fed cholesterol-free diets. L, lauric acid; M, myristic acid; P, palmitic acid; S, stearic acid. <sup>ab</sup>Values with different letters are significantly different at  $P < 0.05$ .<sup>24</sup>

acid fat compared with lauric, myristic, and palmitic acid fats lowered the absorption of dietary fat and increased fecal excretion of neutral steroids. The markedly low concentration of hepatic cholesterol in the stearic acid fat group, therefore, could be attributable to the reduction of intestinal absorption of cholesterol. The lower concentration of plasma cholesterol in hamsters fed a stearic acid fat diet without cholesterol also could be attributable to the reduced intestinal absorption of cholesterol rather than enhanced catabolism of plasmic cholesterol.  $\alpha$ -Linolenic acid in the diet appeared to have no significant influence on the specific effect of stearic acid fat.

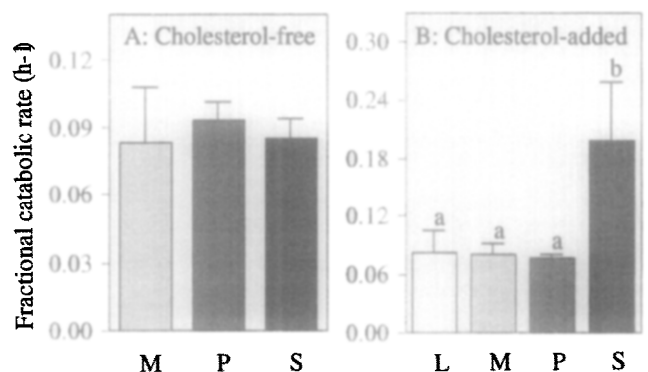
As compared with stearic acid fat, other saturated fatty acids also showed some distinct effects on dietary fat absorption, fecal excretion of neutral and acidic steroids, and hepatic cholesterol concentration, although the magnitude was rather marginal. In the cholesterol-added diet, the absorption of palmitic acid fats was lower compared with lauric and myristic acid fats both in rats and hamsters. In the cholesterol-free diet, hamsters fed myristic acid fats showed lower fecal excretion of neutral and acidic steroids compared with those fed palmitic or lauric acid fats. Fecal neutral steroid excretion in hamsters fed myristic acid fat was the lowest when the diet was added with cholesterol, although such difference was not observed in rats. In addition, the liver cholesterol concentration was also lower in hamsters fed palmitic acid fat compared with lauric and



**Figure 5** Effects of different saturated fatty acid fats on plasma (A, B) and liver (C, D) lipid concentrations in hamsters fed cholesterol-added diets. L, lauric acid; M, myristic acid; P, palmitic acid; S, stearic acid. <sup>abc</sup>Values with different letters are significantly different at  $P < 0.05$ .<sup>24</sup>

myristic acid fats when given cholesterol-free diets. Thus, in addition to stearic acid, palmitic acid may also have favorable effects on lipid metabolism, although to a lesser extent than stearic acid.

In human study with interesterified fats, McGandy et al.<sup>22</sup> obtained results that conflicted with studies using naturally occurring fat sources. Especially relevant was their finding that, unlike the previous study by Hegsted et al.,<sup>2</sup>



**Figure 6** Effects of different saturated fatty acid fats on fractional catabolic rate of LDL in hamsters fed cholesterol-free (A) and cholesterol-added (B) diets. L, lauric acid; M, myristic acid; P, palmitic acid; S, stearic acid. <sup>abc</sup>Values with different letters are significantly different at  $P < 0.05$ .<sup>24</sup>

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stearic acid fat did not differ appreciably from palmitic or myristic acid fats in its hypercholesterolemic effect. Interesterification produces various kinds of the triacylglycerol species, and the effect of each molecular species on the lipid metabolism remains to be determined. Thus, in the case of palm oil the triacylglycerol structure may be responsible for its characteristic effect on cholesterol metabolism.<sup>10</sup>

In summary, saturated fatty acids exert distinct effects on various aspects of lipid metabolism, and hence, the effect of palm oil should be evaluated at the broad view point.

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