

Effects of dietary monounsaturated fatty acids on plasma lipids in humans

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Abstract In order to test whether monounsaturated fatty acids (MUFA, M) would lower plasma cholesterol and/or triglycerides (TG), 14 young men were studied in a metabolism ward. They were given two experimental diets with the same P/S ratio (P: polyunsaturated; S:saturated fatty acids) but different P + M/S ratios in composition of dietary fatty acids. Six men were in experiment I. In two groups of three, each group was given either the high or the low P + M/S ratio diet for 6 weeks. In experiment II, two groups of four men were subjected to a 3-week cross-over feeding trial (total of 6 weeks). We found in the cross-over study that when the dietary P/S ratio was fixed at 1.0, those subjects given a diet with the higher P + M/S ratio of 4.5 showed significantly higher plasma total TG, very low density lipoprotein (VLDL)-TG, low density lipoprotein (LDL)-TG, and LDL-cholesterol than when given a diet with a lower P + M/S ratio of 1.5. Plasma total cholesterol, apoprotein A-I, and apoprotein B-100 were not significantly different between the two dietary periods. Plasma fatty acid patterns showed a significantly higher plasma MUFA level during the period of a higher P + M/S diet. ■ In contrast to some recent reports, our studies indicate that a large amount of dietary MUFA may raise some fractions of plasma lipids in humans. —Chang, N. W., and P. C. Huang. Effects of dietary monounsaturated fatty acids on plasma lipids in humans. *J. Lipid Res.* 1990. 31: 2141–2147.

Supplementary key words very low density lipoprotein • polyunsaturated and saturated fatty acids • triglycerides • apoA-I • apoB-100

Epidemiological studies show that diets rich in saturated fatty acids (SFA) and cholesterol seem to increase the risk of coronary heart disease (CHD) (1, 2). A high plasma LDL-C level is now widely recognized as a major risk factor of CHD, whereas a high level of HDL is considered a negative risk factor of CHD (3–5). During the last three decades many investigators have reported that polyunsaturated fatty acids (PUFA) will decrease and SFA will increase plasma total cholesterol and LDL-C (6–10). But monounsaturated fatty acids (MUFA), such as oleic acid, are considered to have no effect on plasma lipids (11). Therefore, for the purpose of predicting the cholesterolemic effect of dietary fat, fatty acid composition is frequently expressed in terms of a PUFA to SFA (P/S) ratio. Diets with higher P/S ratios decrease the plasma LDL-C (10, 12, 13), HDL-C (6, 7, 10, 13–16), and

VLDL-C (6, 10, 12, 15, 17) when compared to the diets with lower P/S ratios.

Very recently, MUFA-rich diets were reported to decrease plasma total cholesterol and LDL-C without decreasing HDL-C in humans (14, 18, 19), and thus the role of MUFA in lipid metabolism has received renewed attention. However, Peifer et al. (20) and Beynen (21) report that MUFA might increase plasma and liver total cholesterol levels in hypercholesterolemic rats and rabbits. Huang and Chang (22) also observed that, when the dietary P/S ratio was fixed at 1.0 and the diet was supplemented with 1% cholesterol, those rats given a diet with a PUFA + MUFA/SFA (P + M/S) ratio of 5.7 showed significantly higher plasma total cholesterol, triglyceride, LDL-C, and liver total cholesterol than those given a diet with a P + M/S ratio of 1.4. They also observed that, when either PUFA or SFA was fixed at a constant level, rats given a diet of a higher M/S ratio showed significantly higher plasma cholesterol levels (N. W. Chang and P. C. Huang, unpublished data). It seems that the cholesterolemic effect of MUFA on plasma lipid levels is still uncertain.

In the present study, apparently healthy young men were enrolled in a study to determine the effects of dietary MUFA on plasma lipids. The experimental diets contained fat with a P/S ratio of 1.0 but different P + M/S ratios of 1.5 or 4.5.

MATERIALS AND METHODS

Subjects

Fourteen healthy normolipidemic young men, recruited from the student body of the College of Medicine, National Taiwan University, were studied in a metabolic

Abbreviations: PUFA, P, polyunsaturated fatty acids; MUFA, M, monounsaturated fatty acids; SFA, S, saturated fatty acids; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; apoA-I, apolipoprotein A-I; apoB-100, apolipoprotein B-100; C, cholesterol; TG, triglyceride; PL, phospholipid; HPLC, high performance liquid chromatography.

ward. Their ages ranged from 20 to 26 years. Their mean body weight was 63 ± 6 kg (mean \pm SD). At the beginning of the experiment, the subjects had plasma total cholesterol levels of less than 200 mg/dl, and their triglycerides (TG) were all below 100 mg/dl (Table 1). None had symptoms or a recent history of cardiovascular disease, nor any diseases of the gastrointestinal tract, kidney, or endocrine system. The protocol and purpose of the study was explained to the subjects, and all subjects gave informed consent.

Diets and feeding trials

Before each of the two experiments, the subjects were on ordinary diets of their own choice. Two test diets (Table 2) were used in both experiments I and II. Both diet 1 and diet 2 had a P/S ratio of 1.0, but diet 1 had a P + M/S ratio of 1.5 and diet 2 had a P + M/S ratio of 4.5 (Table 3). Fatty acid patterns of these mixed oils (soybean oil and coconut oil or soybean oil and olive oil) were analyzed by high-performance liquid chromatography (HPLC) (23) to verify the correct P/S and P + M/S ratios. Dietary fat supplied 30% of the total calories in both diets. Meals of a 5-day cycle menu for each subject were cooked and consumed under the supervision of a dietitian. It was also verified by analysis that the composition of the cooking oil did not change during the food preparation. To avoid the interference of fish oil, the cycle menu did not include fish.

In experiment I, there were three subjects in each of the groups and the two groups consumed either diet 1 or diet 2 for 6 weeks, respectively. Cholesterol intake was about 304 mg/day during the first 4 weeks and was in-

creased to about 634 mg/day during the last 2 weeks to see whether the higher cholesterol intake would result in a more visible difference in plasma lipid levels between the two dietary groups. In experiment II, four of the eight subjects were given diet 1 and the other four subjects were given diet 2, both for 3 weeks. Then the test diets were exchanged for another 3 weeks (a 3-week cross-over feeding trial for a total 6-week experimental period). Body weight was measured daily before breakfast, and caloric intake (about 43 kcal/kg per day) was adjusted to maintain a stable weight.

Blood sampling and lipid analyses

Fasting blood samples were obtained on day 0, 7, 14, 21, 28, 35, and 42. Each sample was analyzed for: 1) total cholesterol (C), TG, and phospholipid (PL); 2) C, TG, and PL of VLDL, LDL, and HDL fractions; and 3) plasma total lipid, apoA-I, apoB-100 levels and plasma fatty acid patterns. VLDL, LDL, and HDL were obtained by ultracentrifugation at densities of 1.006, 1.063, and 1.210 g/ml, respectively. Plasma and lipoprotein cholesterol and TG were determined by commercial kit enzymatic methods (Boehringer Mannheim Co.). Phospholipid was determined by using an enzyme kit (BioMerieux Co.). Plasma apoA-I and apoB-100 were measured by a turbidimetric immunoassay using Beckman Co. kits. Plasma total lipid was determined by the method of Frings and Donn (24). Plasma lipid was extracted with chloroform-methanol 2:1 mixture according to the Folch, Lees, and Sloane Stanley method (25). The fatty acid pattern was analyzed by the method of Miwa and Yamamoto using HPLC (23).

TABLE 1. Characteristics of 14 male subjects on entry

Subject ^a	Age	Body Weight	Height	Plasma Lipids		
				Total Cholesterol	Triglyceride	Phospholipid
	<i>yr</i>	<i>kg</i>	<i>cm</i>		<i>mg/dl</i>	
1	23	75	172	170.6	69.0	166.9
2	22	70	169	171.6	53.2	133.3
3	20	60	173	134.0	67.1	149.2
4	25	53	163	195.4	96.8	166.9
5	22	68	172	129.9	65.3	152.7
6	22	59	164	146.1	50.5	143.4
7	24	72	170	134.8	74.5	140.9
8	22	68	182	182.5	70.0	178.1
9	21	58	168	172.5	60.8	187.8
10	26	57	167	156.7	60.8	175.4
11	22	63	170	192.4	73.1	190.3
12	23	62	173	140.3	75.3	161.7
13	22	56	164	116.0	93.0	144.1
14	24	59	165	149.0	61.0	164.2
Mean	22.7	62.9	169.4	156.6	69.3	161.1
\pm SD	1.6	6.7	5.0	24.7	13.1	17.8

^aSubjects 1 to 6 participated in experiment I and subjects 7 to 14 participated in experiment II.

TABLE 2. Composition of test diets

	Diet 1	Diet 2
Energy intake (kcal)	2580	2580
Protein (energy %)	15	15
Carbohydrate (energy %)	55	55
Fat (energy %)	30	30
Fat sources (energy %)		
Fat in foods ^a	7.3	7.3
Cooking oil		
Soybean oil	15.0	1.8
Olive oil		20.9
Coconut oil	7.7	

Dietary cholesterol intake was 304 mg/day by analysis. In experiment I, supplemental cholesterol, 330 mg in powder form, was added into egg yolk during the 5th and the 6th week. Energy and nutrient intake figures are for a 60-kg man.

^aFat (in energy %) derived from the following foods: egg, 2.0; chicken, 0.4; pork, 0.6; soybean products, 2.2; toast, 0.6; defatted milk powder, 0.1; rice, 0.8; vegetables, 0.3; and fruits, 0.3.

Apparent fat digestibility

In experiment II, the subjects' fecal specimens of the last 3 days in each dietary period were collected and analyzed for fat content and the apparent fat digestibility was calculated.

Data compilation and statistical analyses

When the cross-over experiment indicated a significant dietary effect, a paired *t*-test, the two-period change-over design test (26), and the Mann-Whitney test (27) were applied to evaluate the level of statistical significance. All statistical inferences were based on a two-tailed test.

TABLE 3. Fatty acid composition of mixed dietary fat

Fatty Acids	Diet 1 ^a	Diet 2 ^a
	%	
6:0	0.1	
8:0	1.6	
10:0	1.6	
12:0	12.6	
14:0	5.9	0.3
16:0	12.8	13.3
18:0	4.4	4.3
Subtotal	39.0	17.9
16:1	0.6	0.8
18:1	19.5	61.2
Subtotal	20.1	62.0
18:2	33.9	15.7
18:3	4.3	1.4
20:4	0.5	0.5
22:6	0.3	0.3
Subtotal	39.0	17.9
P/S ratio	1.0	1.0
P + M/S ratio	1.5	4.5

^aCooking oil in diet 1: 92.1% soybean oil + 7.9% coconut oil; in diet 2: 33.9% soybean oil + 66.1% olive oil.

RESULTS

The subjects reported no gastrointestinal disturbances during the experimental period. Their body weights did not change significantly throughout the study period.

Experiment I

The plasma lipid changes with time are shown in Fig. 1. At the end of week 4, the average plasma TG level increased by 63.4% on diet 2 and increased by 26.2% on diet 1 as compared with the respective baseline data. However, the difference between the mean increases was not significant when tested by the Mann-Whitney test, probably because of the small size of the experimental groups. Similar results were obtained for VLDL-TG, VLDL-C, and VLDL-PL. The plasma total cholesterol and phospholipid levels changed only slightly. The increased cholesterol intake (634 mg/day) during the 5th and 6th week did not affect the plasma cholesterol, TG, and phospholipid levels in either dietary group.

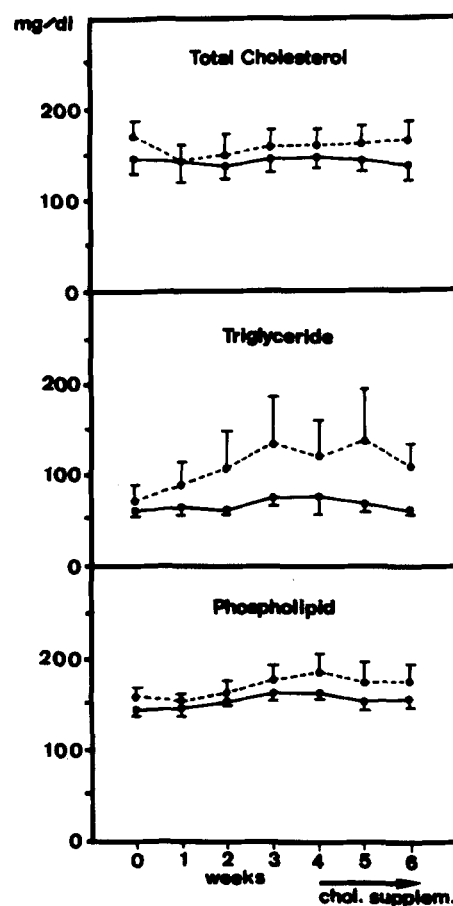


Fig. 1. Plasma lipid levels of subjects throughout the time course study in experiment I. (—) Diet 1 (P + M/S = 1.5) group, n = 3; (---) Diet 2 (P + M/S = 4.5) group, n = 3. The vertical line indicates SD.

TABLE 4. Plasma lipid contents of subjects after a 3-week dietary period in experiment II

	Diet 1 (n = 8)	Diet 2 (n = 8)
	mg/dl	
Total cholesterol	151.3 ± 21.7	155.0 ± 21.9
Total triglyceride	68.7 ± 9.6 ^a	82.7 ± 7.9 ^a
Total phospholipid	166.9 ± 14.9	172.0 ± 18.0
Total lipid	389.9 ± 39.8 ^b	417.4 ± 31.5 ^b
VLDL-C	10.8 ± 3.0	12.3 ± 3.2
VLDL-TG	44.7 ± 10.4 ^c	56.2 ± 9.9 ^c
VLDL-PL	14.6 ± 1.9	17.3 ± 2.7
LDL-C	83.6 ± 16.8 ^d	89.5 ± 18.7 ^d
LDL-TG	14.4 ± 3.2 ^e	16.8 ± 4.4 ^e
LDL-PL	53.4 ± 8.3	53.9 ± 10.0
HDL-C	54.0 ± 10.6	49.8 ± 6.8
HDL-TG	7.7 ± 2.5	8.6 ± 2.8
HDL-PL	82.8 ± 11.5	84.4 ± 10.2

Matched superscripts (a-e) indicate that there is a significant difference between the mean values ($P < 0.05$) both by paired *t*-test and the two-period change-over design test. The P/S ratio was 1 in both diets. The P + M/S ratios were 1.5 in Diet 1 and 4.5 in Diet 2. Values given as mean ± SD.

Experiment II

This feeding trial was a 3-week cross-over trial for a total of 6 weeks. Whether the diet-order (diet 1 → diet 2 or diet 2 → diet 1) affected the plasma lipid levels was first analyzed as described by Grizzle (26), and it was found that the carry-over effect was negligible. Therefore, either the diet 1 or diet 2 data of the first 3-week period were pooled with respective data of the second 3-week period.

Levels of plasma total cholesterol, TG, PL, and total lipid of individual subjects at the end of the two dietary periods are shown in Table 4 and Fig. 2. We found that when the dietary P/S ratio was fixed at 1.0, those subjects given the diet with a higher P + M/S ratio of 4.5 (diet 2) showed significantly higher plasma TG and total lipids

than those subjects given the diet with a lower P + M/S ratio of 1.5 (diet 1) ($P < 0.05$, both by the paired *t*-test and a two-period change-over design test (26). However, no significant difference was observed between the two dietary groups in the plasma levels of total cholesterol and PL.

Table 4 and Fig. 3 also show that when given diet 2 for 3 weeks, the subjects had significantly higher plasma VLDL-TG and LDL-TG, and LDL-C levels than when diet 1 was given ($P < 0.05$).

The two dietary groups did not significantly differ in the lipid contents of HDL fractions. In accord with this result, the amounts of plasma apoA-I and apoB-100 were also not significantly different between the groups (Table 5).

When the subjects were on diet 2 for 3 weeks, plasma fatty acid patterns showed greater proportions for 18:1, 18:0, 16:0, and 16:1 fatty acids than when subjects were on diet 1 (Fig. 4). The difference between the two 18:1 peaks was most visible ($P < 0.05$).

The apparent fat digestibility levels of the test diets in the subjects were found to be all above 94%, and there was no significant difference between the two diets (diet 1: 97.1 ± 1.2%; diet 2: 96.2 ± 1.5%).

DISCUSSION

This study examined the effects of dietary MUFA on plasma lipids in humans using a commercial olive oil rich in oleic acid. Similar studies were performed recently by Mattson and Grundy (14), Grundy (18), and Bonanome and Grundy (28) in a metabolic ward using liquid formula diets containing high-oleic acid safflower oil. Even though the P/S ratio of their test diets was not described, it can be assumed to be rather low, and the dietary fat provided about 40% of the total calories. The P/S ratio of an average diet in Taiwan is about 1.0, and its dietary fat pro-

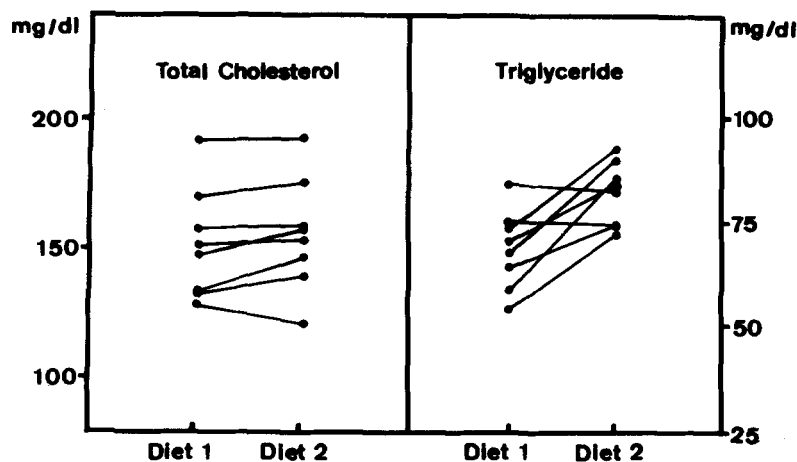


Fig. 2. Plasma levels of total cholesterol and triglyceride after a 3-week dietary period in experiment II. Number of subjects: diet 1 (P + M/S = 1.5) period, n = 8; diet 2 (P + M/S = 4.5) period, n = 8.

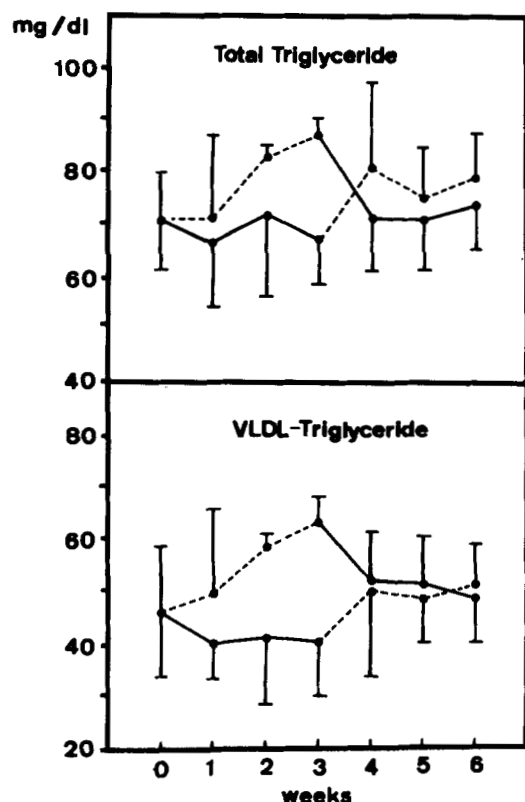


Fig. 3. Changes of plasma total triglyceride and VLDL-TG levels of subjects in the cross-over study (experiment II). (—) Diet 1 (P + M/S = 1.5) group; (---) Diet 2 (P + M/S = 4.5) group. Each point represents the mean value of four subjects. The vertical line indicates SD.

vides about 30% of the total calories. Because of the compositional difference between the ordinary American and Taiwanese diets, and also because the effect of MUFA might be better studied with the P/S ratio of the test diets kept constant, the present studies were conducted.

It is noteworthy that the effect of dietary fat on plasma lipids is not only related to the P/S ratio but also related to the MUFA. The MUFA-rich diets were reported to decrease plasma total cholesterol and LDL-C, but did not decrease HDL-C in hypercholesterolemic patients (14, 18, 29) and healthy men (19). Sirtori et al. (30) reported that an olive oil diet did not reduce plasma total cholesterol as

TABLE 5. Plasma apoA-I and apoB-100 levels of subjects after a 3-week dietary period in experiment II

	Diet 1 (n = 8)	Diet 2 (n = 8)
	mg/dl	
ApoA-I	111.8 ± 11.6	108.7 ± 16.6
ApoB-100	55.5 ± 11.7	54.9 ± 9.4

The P/S ratio was 1 in both diets. The P + M/S ratios were 1.5 in Diet 1 and 4.5 in Diet 2. Values given as mean ± SD.

a corn oil diet did in high risk patients. Becker et al. (31) and, more recently, Mensink et al. (32) also found that a MUFA-rich diet did not affect plasma total cholesterol, TG, LDL-C, and HDL-C in normolipidemic humans as compared with an SFA-rich diet. In this study, we could not observe the hypocholesterolemic effect of a high MUFA diet in humans as reported by Grundy and his associates (14, 18, 28). In contrast, we observed that a high oleic acid diet raised the plasma total TG, VLDL-TG, LDL-TG, and LDL-C. Grundy suggested that a diet rich in MUFA (MUFA, 28%; SFA, 4 energy %) appeared to be at least as effective in lowering plasma cholesterol as a diet low in fat (MUFA, 6.7%; SFA, 6.7 energy %) and high in carbohydrates. But the low content of SFA rather than the high content of MUFA in the diet might be responsible in decreasing plasma cholesterol levels. Mensink and Katan (19) reported that an olive oil-rich diet (SFA, 9.8%; MUFA, 24%; PUFA, 5.1 energy %) caused a fall in plasma cholesterol when compared to the Western diet (SFA, 20%; MUFA 12.4%; PUFA, 4.1 energy %). It might also be the effect of a low SFA contents rather than the high MUFA content of the olive oil diet, when compared to the

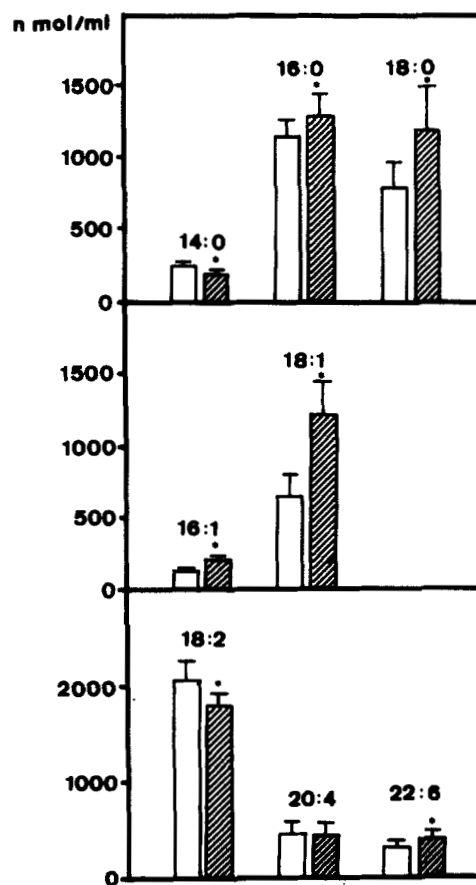


Fig. 4. Plasma fatty acid pattern of subjects after the 3-week dietary period in experiment II. (□) Diet 1: P + M/S = 1.5 (n = 8) (▨) Diet 2: P + M/S = 4.5 (n = 8). *Significantly different from diet 1 period by the paired *t*-test ($P < 0.05$). Results are shown as mean ± SD.

Western diet which induced higher plasma cholesterol levels.

The increase in plasma total TG, VLDL-TG, and LDL-TG perhaps reflected mobilization of a larger amount of VLDL particles from the liver to the blood under conditions of high MUFA intake. Nevertheless, the change in plasma triglyceride levels probably has no biological importance. The differences may relate more to the presence of 15.9% C-6 to C-12 fatty acids in the low MUFA diet; but, as was reported by other investigators (14, 18, 19, 31, 32), the high MUFA diet did not affect the levels of plasma HDL fractions.

The plasma fatty acid pattern was clearly related to the composition of dietary fat. When the subjects were on the MUFA-rich diet, the plasma fatty acid pattern showed greater proportions for 18:1, 18:0, 16:0, and 16:1 fatty acids.

It is interesting to note that MUFA-rich diets were found to increase liver total cholesterol levels in rats (20, 21, 33), rabbits (21), and hamsters (34) as compared with SFA-rich diets. The same result was also obtained in our rat experiments (22) in which the high P + M/S ratio (5.7) diets produced significantly higher concentrations of liver cholesterol than did diets containing a low P + M/S ratio of 1.4 when the P/S ratio was fixed at 1.0. Spady and Dietschy (34) reported that even though hepatic cholesterol levels were higher in olive oil-fed hamsters, as compared to animals fed coconut oil, the rate of receptor-mediated hepatic catabolism of LDL was not changed. The present study indicates that a large amount of dietary MUFA may raise plasma lipids, especially triglycerides. It is not known whether this effect is harmful, but the possibility that MUFA may also increase liver cholesterol in humans may need consideration. ■

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