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Both dietary 18:2 and 16:0 may be required to improve the serum LDL/HDL cholesterol ratio in normocholesterolemic men

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In a double-blind crossover study, 23 healthy normocholesterolemic male volunteers were fed carefully designed whole food diets enriched by oleic acid (canola, CAN), palmitic acid (palm olein, POL), or an American Heart Association Step 1 fat blend (AHA). Resident males received each diet during three consecutive 4-week periods. The diets supplied approximately 31% energy as fat and <200 mg of cholesterol/day. The percent energy (% en) from each dietary fatty acid was strictly controlled to compare low-16:0, high-18:1 (CAN) or high-16:0, low-18:2 (POL) intake with a balanced intake of each (AHA). The first two diets represented direct exchange of 7% en between 18:1 + 18:2 (CAN) and 16:0 (POL), whereas the main difference between POL and AHA was <4% en exchanged between 16:0 and 18:2. Serum total cholesterol (TC), very low density lipoprotein cholesterol (VLDL-C), and LDL-C were not significantly affected by the three diets despite manipulation of these key fatty acids. However, both CAN (low saturates [SATs], high monounsaturates [MONOs]) and POL (high SATs, low polyunsaturates [POLYs]) depressed HDL-C significantly (−8 mg/dL) relative to the AHA (mod SATs, mod POLYs) diet. Consequently, the AHA diet increased HDL_3-C and lowered the LDL/HDL cholesterol ratio significantly relative to the CAN and POL diets. Neither serum Lp(a), apoA1, nor apoB were affected by diet. These data support the previous observation that in normolipemic humans consuming a moderate fat load (<31% en) low in myristic acid (14:0) and dietary cholesterol, the effect of palmitic acid (16:0) on TC and the LDL/HDL ratio is comparable to that of monounsaturated oleic acid (18:1). Furthermore, a definite intake of POLYs and SATs may be essential for maximizing HDL_3-C under these conditions. (J. Nutr. Biochem. 6:179–187, 1995.)

Keywords: fatty acids; palmitic; oleic; lipoproteins; cholesterol; humans

Introduction

Diets containing a high proportion of saturated fatty acids elevate plasma concentrations of total and low density lipoprotein (LDL) cholesterol.[1–3] Because an increased LDL cholesterol (LDL-C) concentration represents an increased risk for coronary heart disease,[4,5] recommendations have been made to reduce intake of dietary saturated fatty acids and to increase consumption of polyunsaturates (POLYs) and monounsaturates (MONOs).[4,6,7] Concern has been raised that excessive POLYs consumption could be detrimental if it decreases high density lipoprotein (HDL).[8] The alternative possibility, i.e., that saturated fat intake could be suboptimal, has not been considered even though certain saturated fatty acids are known to increase HDL as they expand the LDL pool.[9–12] Ideally, one would like to consume a dietary fat that lowers LDL while enhancing HDL concentration.

Historically, research in both animals and humans indicates that all saturated fatty acids are not equivalent in their cholesterol raising ability.[9,11–15] Indeed, in the original

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studies of Keys[1] and Hegsted[13] stearic acid (18:0) did not appear cholesterolemic, a point recently confirmed in humans consuming diets in which the fatty acid exchanges between natural fats were carefully controlled.[11,16] Nevertheless, the commonly cited equations of Keys[17] and Hegsted[18] that are used to predict the serum cholesterol response to dietary fats, conveniently adopted a single term for "saturates" that encompasses three saturated fatty acids (12:0, 14:0, 16:0), which were assumed for convenience sake to be equally cholesterolemic. Until recently this generally accepted tenet tended to pre-empt efforts to differentiate between individual saturated fatty acids, i.e., 12:0, 14:0, 16:0, 18:0, for their cholesterolemic effects.

Hayes et al.[14] fed three species of nonhuman primates cholesterol-free diets in which the total energy contribution from saturates (SATs), MONOs, and POLYs were kept constant. Using this approach, the substitution of approximately 10% en as 16:0 for 12:0 + 14:0 resulted in significant decreases in both total and LDL-C concentrations. Additional study led to the hypothesis that 16:0 may not raise cholesterol in normolipemic individuals (total cholesterol [TC] < 200 mg/dL) when dietary cholesterol intake is low.[19–21] This hypothesis was confirmed in a tightly controlled human dietary trial in which 5% energy (% en) was exchanged between the same fatty acids in normocholesterolemic subjects consuming diets containing about 30% fat energy and <200 mg/day dietary cholesterol.[9] Compared with the 12:0 + 14:0-rich diet, the 16:0-rich diet significantly reduced both total and LDL-C.

The above results infer that in certain circumstances dietary 16:0 may be neutral, or at least less cholesterolemic than the combination of 12:0 plus 14:0. This relative neutrality of 16:0 was affirmed in normocholesterolemic men and women when a 7% en exchange between 16:0 (from palm olein) and 18:1 (from olive oil) generated identical LDL and HDL values.[10] However, in part because of its predominance in saturated fats and in part because it is typically consumed as animal fat containing cholesterol, 16:0 has long been considered the primary cholesterol-raising fatty acid, especially when compared with 18:1 and 18:2.[3,22] It does appear that dietary 16:0 can modestly increase TC when LDL receptor activity is compromised.[20,21] A healthful alternative to a high saturated fat intake is the American Heart Association (AHA) Step 1 diet, in which total fat is restricted to 30% en and SATs, MONOs, and POLYs each contribute about 10% of total energy. Such diets have proven beneficial in reducing elevated serum cholesterol levels,[23–25] furthering the notion that maximal reduction in saturated fat intake is desirable.

The current study design re-examined the putative neutrality of a 16:0-rich diet, comparing it to an 18:1-enriched diet or an AHA Step 1 diet in normolipemic male subjects consuming about 200 mg/day of cholesterol. In addition, appreciating the possible importance of 18:2 on LDL receptor activity[26,27] and the saturated fatty acid contribution to circulating HDL,[10–12,14,19] we examined these relationships to determine whether at some point the intake of SATs or POLYs might prove to be suboptimal. Thus, the design addressed two key questions regarding the LDL/HDL ratio in the same normocholesterolemic individuals consuming whole food diets. First, would maximal displacement of saturated fatty acids (16:0) by monounsaturates (18:1) be more beneficial to total cholesterol and the LDL/HDL ratio than the more balanced ratio between fatty acid classes inherent in the AHA Step 1 diet? Second, is it advisable to maximally substitute MONOs for POLYs to preclude any decrease in HDL that might result from a high POLY intake? The answer to both questions was negative, suggesting that both SATs and POLYs, but not MONOs, contributed to an optimal LDL/HDL ratio in this population. In fact, MONOs appeared quite neutral in these normolipemic individuals consuming a natural diet containing a moderate amount (30% en) of fat.

Materials and Methods

Subjects

Subjects were members of the Royal Malaysian Army undergoing training at the army signal school. On the basis of a questionnaire and a screening examination, 24 volunteers were chosen. They were healthy individuals who were not on any medication affecting lipid metabolism, smoked <7 cigarettes/day and consumed no alcohol. They were advised to maintain their regular pattern of physical exercise which included light drills in the mornings and soccer games in the evenings. On the day of bleeding, subjects were exempted from their morning drills so as not to distort the serum lipid analyses. Approval of the study was obtained from the Human Ethics Committee, Ministry of Health, Government of Malaysia, and written informed consent was obtained from all subjects. Twenty-three subjects successfully completed the study. The entry characteristics (mean ± SD) of the 23 subjects were age 22 ± 4 years (range 19–24), body mass index (BMI) 21.3 ± 1.7 kg/m^2 (range 19.5–24.7), serum total cholesterol (TC) 174 ± 23 mg/dL (range 130–226), serum triglycerides (TG) 88 ± 27 mg/dL (range 51–148), LDL-C 105 ± 46 mmol/L (range 65–135), and high density lipoprotein cholesterol (HDL-C) 45 ± 10 mg/dL (range 30–63).

Experimental design

The study comprised four periods totaling 15 weeks. All volunteers began the study with a 3-weeks control period that represented the habitual diet that the volunteers consumed at their army mess. This habitual diet (27% en as fat) incorporated the typical Malaysian recipes and nutrient content described previously.[9] Subsequently, all volunteers continued to eat this basal diet in which two-thirds of the fat energy was replaced by a blend of fat based on the American Heart Association (AHA) Step 1 diet followed by two fat substitutions in random order: an oleic acid–rich diet based on canola oil (CAN) and a palmitic acid-rich diet based on palm olein (POL). Each dietary test period lasted 4 weeks with a crossover design for the last two fats.

All four diet periods utilized basic Malaysian foods relatively low in fat (27% en). Using a 7-day rotating menu, the selected fats were readily incorporated into the foods as the sole cooking fat via the typical Malaysian stir-fry or deep-fry preparation of food ingredients. Comparison of the fat content of the diets prior to adding the experimental fats indicated that the added oils provided approximately 20% of the total dietary energy (with 31% total energy from fat during the three experimental periods). Subjects were provided with all four meals (breakfast, lunch, dinner and supper), which were prepared fresh each day by a special caterer. Adherence to the present menus and cooking oil allotments was monitored in the kitchen during all meal preparations.

Because the subjects were restricted to the camp during the

entire 15-week experimental period (except Sunday afternoons), they willingly ate all meals. This provided for excellent compliance. They were also monitored to ensure a relatively constant food intake throughout the study, which allowed for a stable BMI as determined by weekly body weight records. A questionnaire at the end of the trial revealed that the subjects were unable to identify the order or source of the dietary fats being consumed.

Composition of fat blends and 7-day rotating menus

The fatty acid composition (from GLC) of the fats fed in the study is shown in *Table 1*. During the habitual diet period, the fat blend used for cooking represented a mixture of palm oil and coconut oil in the approximate ratio of 85 and 15%, respectively, which is a common oil profile of the Malaysian diet. Most of the coconut oil fatty acids are derived from the milk extruded from coconut meat used to flavor foods. Coconut milk was largely excluded in food preparations during the remaining three dietary test periods since our previous study indicated a significant cholesterolemic effect of 12:0 + 14:0 acids on serum total and LDL cholesterol.[9] The AHA blend was derived from soybean oil (50%), palm oil (40%), and canola oil (10%). The oleic acid–rich diet utilized canola oil containing 56% oleic acid, whereas the palmitic acid–rich diet (39% 16:0) was derived from POL, the relatively liquid fraction of palm oil used as cooking oil.

Composition of each diet from the 7-day rotating menus as consumed by the volunteers (*Table 2*) was analyzed as described previously.[9] The intake of total energy, fat, protein, carbohydrate, and cholesterol were not significantly different between dietary periods, but the % en derived from saturated fatty acids did differ such that POL>AHA>CAN. This increment was primarily attributed to 16:0 at 11.2, 8.2, and 3.2% en, respectively. The % en derived from POLYs was significantly lower in the POL diet compared with the AHA and CAN diets, whereas the % en from MONOs was comparable with the AHA and POL diets but significantly higher in the CAN diet. Thus, manipulation of fat provided an experimental design that compared the exchange of dietary 16:0 (POL) with either 18:2 (AHA, about 4% en exchange) or 18:1 + 18:2 (CAN, about 7% en exchange) (*Table 2*).

Laboratory methods

Twenty-milliliter fasting blood samples were collected by venipuncture in 2 × 10 ml Monovette® tubes (#051104, Sarstedt, Numbrecht, Germany) at the end of the 3-week habitual diet and thereafter at the end of each 4-week dietary period. Blood in the first Monovette was allowed to clot for exactly 2 hr at room temperature (~24°C), and serum was prepared for the analysis of TC, TG, HDL-C, as well as the isolation of lipoprotein classes by ultracentrifugation. The second Monovette was placed in a waterbath at 37°C, and blood was allowed to clot for precisely 60 min. Serum from this preparation was used for the analysis of serum fatty acid composition, apolipoproteins, and lipoprotein Lp(a). Serum was isolated following centrifugation at 1200g and 20°C for 15 min.

Isolation of serum lipoproteins was performed using a Beckman SW 41 rotor in a Beckman LM-70 ultracentrifuge according to a slightly modified method of Terpstra et al.[28] as previously described.[29] All isolation procedures were started within 4 hr of the blood collection.

Cholesterol and TG concentrations in each of these lipoprotein fractions and in serum were determined on an autoanalyzer (Express 550, Corning, Corning, NY), using commercial enzymatic kits (Gilford Diagnostis, UK). Recovery rates of the lipoproteins were calculated as the sum of both cholesterol and TG contents of the fractions and expressed as a percentage of the serum TC or TG.

As an index of compliance, the fatty acid composition of serum was measured in each subject at the end of all four dietary periods. One-half a milliliter of serum was used for the extraction of total lipids from serum according to the Bligh and Dyer method.[30] The extracted lipids were dried under a stream of nitrogen and then converted to their methyl esters using BF_3/MeOH. A Perkin Elmer AutoSystem gas chromatogram (Perkin-Elmer Corporation, Norwalk, CT) fitted with a capillary column and temperature programmed from 180 to 240°C at 4°C/min was used for the analysis.

Serum Lp(a) was measured by a 1-step sandwich Elisa using monospecific polyclonal anti-apo(a) antibodies (Immunozym Lp(a), Immuno GMBH, Germany). Diluted serum samples were incubated with the conjugate consisting of a monospecific and monovalent anti-apo(a) antibody coupled with peroxidase. Nonspecific serum components and unbound conjugates were removed by washing, followed by a second incubation during which the enzyme reaction took place. The Lp(a) concentration in the sample was measured at 450 nm by means of an ELISA reader, and the Lp(a) concentration was quantitated by using a reference curve obtained in the same test run.

On their habitual (baseline) diet for the 3-week run-in period, volunteers consumed 11.5 ± 1.3 MJ of energy per day with fat

Table 1 Fatty acid composition of fat blends incorporated into diets

Fatty acid	AHA blend 50% soybean oil 40% palm oil 10% canola	Oleic acid–rich 100% canola oil (CAN)	Palmitic acid–rich 100% palm olein (POL)
		(g/100 g of fatty acids)	
Myristic-14:0	0.2	ND	0.8
Palmitic-16:0	25.7	5.6	38.9
Stearic-18:0	4.1	1.8	4.0
Oleic-18:1 (n-9)	37.4	56.0	45.0
Linoleic-18:2 (n-6)	29.3	25.8	10.9
Linoleic-18:3 (n-3)	3.3	9.8	0.4
Erucic-22:1 (n-9)	ND	0.5	ND
Total SATs	30.0	7.4	43.7
Total MONOs	37.4	56.5	45.0
Total POLYs	32.6	35.6	11.3

AHA, American Heart Association; ND, not detected; SATs, saturated fatty acids; MONOs, monounsaturated fatty acids; POLYs, polyunsaturated fatty acids.

Table 2 Daily nutrient intake during the four dietary periods analyzed from double portions of 7-day menus

Nutrient	Dietary period			
	Baseline	AHA	CAN	POL
Energy				
MJ/day	11.5 ± 1.3	11.9 ± 1.4	12.0 ± 1.8	12.0 ± 1.7
Kcal/day	2714 ± 307	2808 ± 330	2832 ± 425	2832 ± 401
Protein				
% en	15.8 ± 1.4	16.3 ± 1.9	16.0 ± 2.3	16.1 ± 2.0
g/day	107 ± 10	114 ± 14	113 ± 17	114 ± 15
Carbohydrates				
% en	55.2 ± 2.8	52.4 ± 3.0	52.3 ± 3.1	52.1 ± 3.6
g/day	375 ± 19	368 ± 22	370 ± 23	369 ± 27
Fat				
% en	29.0 ± 1.7	31.3 ± 1.6	31.7 ± 1.9	31.8 ± 2.1
g/day	87.5 ± 4.2	97.7 ± 5.6	99.7 ± 5.2	100.1 ± 4.9
Saturated FA (% en)				
Total	12.2 ± 0.4	10.1 ± 0.8[a,c]	6.0 ± 0.7[a,b]	13 ± 1.3[b,c]
12:0	0.8 ± 0.3	ND	ND	ND
14:0	1.3 ± 0.2	0.3 ± 0.2	0.5 ± 0.3	0.5 ± 0.3
16:0	8.6 ± 0.4	8.2 ± 0.5[a,c]	3.9 ± 0.5[a,b]	11.2 ± 0.8[b,c]
18:0	1.3 ± 0.1	1.5 ± 0.2	1.2 ± 0.2	1.4 ± 0.2
Monounsaturated FA (% en)				
Total	12.0 ± 0.4	12.9 ± 1.0[a]	17.5 ± 1.3[a]	14.3 ± 1.2
16:1 (n-9)	0.4 ± 0.1	0.8 ± 0.2	0.4 ± 0.1	1.1 ± 0.1
18:1 (n-9)	11.3 ± 0.5	11.8 ± 0.9[a]	16.9 ± 1.0[a,b]	12.8 ± 1.2[b]
Polyunsaturated FA (% en)				
Total	3.8 ± 0.2	8.3 ± 0.7[b]	7.7 ± 0.5[a]	4.1 ± 0.4[a,b]
18:2 (n-6)	3.1 ± 0.2	7.3 ± 0.6[b]	6.4 ± 0.4[a]	3.5 ± 0.2[a,b]
18:3 (n-3)	0.7 ± 0.2	1.0 ± 0.2[a]	1.3 ± 0.2[a]	0.6 ± 0.1
P/S ratio	0.31 ± 0.01	0.82 ± 0.02	1.28 ± 0.03	0.40 ± 0.02
Cholesterol (mg/day)	188 ± 21	201 ± 17	194 ± 16	197 ± 23

Values are means ± SD; $n = 7$. P/S rato, polyunsaturated/saturated fatty acid ratio. ND, not detected.
[a,b,c]Values with common superscript differ significantly ($P < 0.05$).

contributing 29.0 ± 1.7% en of which 12.2 ± 0.4, 12.0 ± 0.4, and 3.8 ± 0.2% en (means ± SD; $n = 7$) were derived from the SATs, MONOs, and POLYs, respectively. Dietary cholesterol intake was 188 ± 32 mg/day. The baseline BMI remained stable at 21.1 ± 1.8 kg/m^2 (range 19.0–24.8).

Serum apolipoproteins, apoA 1 and apoB were determined on a clinical autoanalyzer (Express 550, Corning) using immunoprecipitin kits (Atlantic Antibody, Stillwater, MN) provided with an antibody monospecific for human apolipoproteins. Serum samples were preincubated and them mixed with the antiserum resulting in the formation of an insoluble antigen–antibody complex. The absorbance of the solution was measured at 340 nm. A calibration curve was generated by assaying a series of standards with known concentrations, and serum values for apoA1 or apoB were obtained by interpolation from their respective calibration curves.

Statistical analysis

Results are presented as means ± SD, which were calculated after the habitual diet and at the end of the three dietary periods. For statistical analysis, mean differences among the three fat treatments were analyzed by paired *t*-test coupled to the Bonferoni correction. Statistical significance was defined at $P < 0.05$. Since no period effect was noted in this study, we concluded that the diet sequence had no influence on the outcome of the trial.

Results

Throughout the study the volunteers maintained a constant body weight that was reflected in a stable BMI. Since the volunteers were confined to the army base during the study period (except Sunday afternoons) and ate all meals provided, compliance was excellent. Judging from the dietary composition of the 7-day rotating menus as eaten by the volunteers (*Table 2*), it was also apparent that the desired fatty acid exchanges during our dietary intervention had been achieved.

Serum TC and LDL-C (*Table 3*) were unaffected by the different dietary fatty acid manipulations, despite significant shifts in % en derived from POLYs, MONOs, and SATs incorporated in the three dietary fats. Serum HDL-C was, however, significantly elevated (17%) by the AHA diet in comparison with either the CAN or POL diets, both of which produced identical levels of HDL-C after the 4-week dietary comparisons. The elevation in HDL-C by the AHA diet reflected a significant increase in HDL_3-C, whereas HDL_2-C was unaffected by these diets. As a result of the modulation in lipoproteins, the AHA diet resulted in a significant 14% reduction in the LDL-C/HDL-C ratio compared with the other two diet periods. In comparison with the AHA diet, the CAN diet significantly elevated triglycerides (TG) by 22%. No significant differences were noted between POL and CAN for any serum lipid parameter measured in this study. The diets exerted minimal effect on apolipoproteins (apoA 1 and apoB) and consequently the ratio of apoA 1/apoB was unaffected.

Serum Lp(a) level following the 3-week baseline (means

Table 3 Serum and lipoprotein lipids following dietary treatments

Criterion	Baseline	Diets AHA	CAN	POL
		(μmol/L)		
Serum TC	4.46 ± 0.64	4.51 ± 0.61	4.44 ± 0.67	4.54 ± 0.62
VLDL-C	ND	0.33 ± 0.10	0.38 ± 0.13	0.36 ± 0.10
LDL-C	2.64 ± 0.51	2.41 ± 0.46	2.44 ± 0.05	2.56 ± 0.49
HDL-C	1.18 ± 0.26	1.44 ± 0.18[a,b]	1.23 ± 0.31[a]	1.23 ± 0.28
HDL_2-C	ND	0.44 ± 0.13	0.13 ± 0.13	0.44 ± 0.10
HDL_3-C	ND	0.90 ± 0.15	0.77 ± 0.10	0.72 ± 0.08
LDL/HDL ratio	2.24	1.68 ± 0.40[a,c]	2.15 ± 0.94[c]	2.20 ± 0.70[a]
Serum TG	0.94 ± 0.36	0.73 ± 0.25	0.94 ± 0.34	0.85 ± 0.31
Serum apolipoproteins g/L				
Apo A1	ND	1.33 ± 0.17	1.31 ± 0.20	1.31 ± 0.14
ApoB	ND	0.89 ± 0.18	0.86 ± 0.24	0.86 ± 0.18
Apo B/A1 ratio	ND	0.68 ± 0.18	0.67 ± 0.22	0.67 ± 0.22
Lp(a)	30 ± 18	31 ± 20	30 ± 22	31 ± 20

Values are means ± SD; n = 23. ND, not detected.
[a,b]Means with common superscript differ significantly by repeated measures ANOVA, $P < 0.01$.
[c,d]Means with common superscript differ significantly, $P < 0.05$.

± SD; mg/dL) was 30 ± 18 mg/dL (mean ± SD), and no dietary fat-induced modification of the Lp(a) level was observed in these volunteers (*Table 3*).

Fatty acid profiles from total serum lipids analyzed at the end of each dietary period (*Table 4*) revealed that three saturated fatty acids (12:0, 14:0, 16:0) were statistically (but not clinically) elevated in subjects consuming the palm olein or canola oil diets, whereas percentages of serum linoleic acid (18:2) and stearic acid (18:0) were significantly elevated following consumption of the AHA diet. Serum oleic acid, on the other hand, was not affected by any dietary treatment.

Discussion

Dietary 16:0, 18:1 and 18:2 on TC

The objective of this study was to evaluate the relative benefit of replacing either dietary saturates (16:0 in palm olein) or polyenes (18:2 in the AHA blend) with dietary monoenes (from canola oil) in the Malaysian diet. The focus was on serum lipids and lipoproteins in normolipemic subjects consuming their typical moderate-fat (30% en) diet. One of the striking features of these data was the constant TC value across all diet manipulations. Only subtle shifts in the LDL/HDL ratio or TG values coupled with changes in serum fatty acid profiles gave any indication that the dietary fat had been altered. In general, these results agree with the original observation in Trappist monks[31] and a previous Malaysian study,[10] as well as newer human data from India,[32] that found TC and LDL-C (as well as HDL-C) were unchanged in normolipemic men and women consuming diets enriched with either palm olein, olive oil, or peanut oil, where 16:0 was exchanged for 1:1 for 18:1 up to 13% en[31] and 7% en[10] or up to 4% en for 18:2.[32] These data support the modeling demonstrated in normocholesterolemic cebus monkeys, also at 31% en from fat, where 16:0

Table 4 Serum fatty acid composition following dietary treatments

Fatty acid	Habitual	Diets AHA	CAN	POL
		(percent distribution)		
12:0	2.3 ± 0.6	0.6 ± 0.3[a,b]	1.5 ± 0.5[a]	1.5 ± 0.[b]
14:0	1.5 ± 0.2	0.7 ± 0.2[a,b]	1.0 ± 0.3[a]	1.1 ± 0.4[b]
16:0	25.0 ± 2.5	23.1 ± 2.3[a,b]	24.4 ± 2.9[a]	25.5 ± 2.2[b]
16:1(n-7)	2.7 ± 0.4	2.2 ± 0.2	2.7 ± 0.6	2.0 ± 0.1
18:0	5.4 ± 0.6	5.0 ± 0.8[a,b]	4.4 ± 0.6[a]	4.3 ± 0.5[b]
18:1(n-9)	33.3 ± 1.6	34.9 ± 2.00	35.7 ± 2.0	35.7 ± 1.9
18:2(n-6)	22.1 ± 1.3	24.1 ± 1.3[a,b]	22.7 ± 1.4[b,c]	20.7 ± 1.3[a,c]
18:3(n-3)	4.0 ± 0.3	3.7 ± 0.03	3.9 ± 0.2	3.6 ± 0.3
20:4(n-6)	1.9 ± 0.5	1.48 ± 0.5	1.5 ± 0.2	1.7 ± 0.2
24:0	0.2 ± 0.4	0.61 ± 0.5	0.5 ± 0.2	0.9 ± 0.4

Values are means ± SD; n = 23.
[a,b,c]Means among test diets with common superscript are significantly different ($P < 0.05$).

and 18:1 also were found to exert equivalent effects on plasma lipids.[19,21] Collectively the results imply that in normolipemic individuals who typically consume a relatively low-fat diet (<30% en), it is extremely unlikely that exchange between natural fats rich in 16:0 or 18:1, or even modest exchange with 18:2, will have an appreciable effect on TC during consumption of whole food diets.

On the other hand, in a previous human study,[9] we demonstrated that the selective substitution of dietary 16:0 for 12:0 + 14:0 in a comparable diet containing 3% en as 18:2 and 30% en resulted in significantly lower TC and LDL-C in 15/17 young men. A similar finding was reported in 10/15 Danish men when 5% en as 12:0 + 14:0 (palm kernel oil) was exchanged for 16:0 (palm oil) in a high-fat diet (40% en).[11] Thus, in these two studies 25/32 subjects experienced substantial decreases in LDL-C and TC when fed 16:0-rich fats, the differential response possibly depending on a relatively lower total fat intake (30% en) and individual metabolic circumstances at intervention. These data not only confirm individual responsiveness to saturated fat[3] but variability in response to the type of saturate and suggest that 16:0 is typically less cholesterolemic than 12:0 + 14:0 in most normocholesterolemic subjects consuming naturally occurring triglycerides low in cholesterol. Based on these facts and further modeling of the fatty acid impact on lipoprotein metabolism,[20] we hypothesized that 16:0 would be equivalent to 18:1 (and even 18:2) provided that: (1) the test subjects were normocholesterolemic (<200 mg/dL); (2) dietary cholesterol intake was <300 mg/day; (3) the POLY content of the diets did not exceed 20% en, where depression of HDL-C might be a factor; and (4) the exchange between fatty acids occurred above the critical "threshold" for 18:2 intake (see related "threshold" discussion in Ref. 33).

The present results support this hypothesis, but interpretation is complicated by the apparent relationship between the dietary 18:2/16:0 ratio and HDL-C. The 17% increase in HDL-C during the AHA diet appeared to reflect the fatty acid intake, although a similar ratio between these fatty acids in other human studies have not yielded the same elevation in HDL.[32,34,35] A shift in dietary POLYs from approximately 4 to 8% en coupled with at least 10% en from dietary SATs in this 30% fat en diet generated the most desirable lipoprotein cholesterol profile in these volunteers. The result was an HDL-C level surprisingly superior to the 18:1-rich CAN (low SATs) or 16:0-rich POL (low POLYs) diets. Further evidence of the effect was a simultaneous enhancement of the LDL/HDL cholesterol ratio.

Dietary fatty acids and HDL-C

The impact of specific dietary fatty acids on HDL-C is not clearly defined for any species, presumably because the issue is complicated at the very least by the total fat intake, the P/S ratio, the level of dietary cholesterol, as well as species differences in HDL metabolism. On the one hand, very high POLY diets (>20% en) have been reported to decrease human[22,36] and monkey[19] HDL-C in high-fat diets during short-term feeding trials, probably reflecting reduced VLDL secretion and depressed apoA1 synthesis.[19,36] For example, Mattson and Grundy[22] found a greater decrease in HDL-C with a diet contributing 31% en from POLYs than a MONO-rich diet (4% en as POLYs). On the other hand, Mensink and Katan[23] and Berry et al.[37] observed no significant decrease in HDL-C when comparing a high-POLY with a high-MONO diet, but their diets contained reasonable POLY loads (4 to 12% en). Thus, the concern over high POLYs would seem unwarranted within practical POLY intakes associated with high-fat, Western diets where saturates are typically >10% en. The situation may change when the POLYs/SATs ratio is appreciably greater than 1.0, especially if total fat intake is low (<30% en) and the study is short-term.[34,38]

By way of example, Warburg et al.[34] recently fed diets similar to ours for 3–4 weeks except subjects were switched from a 40% en high-SAT diet (20% en as SATs, 4% en as POLYs) to either a 30% en, high-MONO diet (16% en as MONOs) or a 30% en, high-POLY diet (10% en as POLYs), with both fat-reduced diets causing a significant (−9 mg/dL) reduction in HDL-C. Thus, similar to a previous study[38] but unlike our current data, HDL-C was equally depressed at POLY intakes of 4% en or 10% en during fat-reduced diets, suggesting that the removal of SATs (from 20 to 10% en, primarily in the form of 16:0) to achieve fat-reduction, accounted for the decrease in HDL-C (as well as LDL-C) more than any shift in the 18:2/16:0 ratio. However, replacement of dietary saturated fat by carbohydrate (down to 25% en from fat) actually increased HDL-C in obese patients when caloric restriction and weight loss were also instituted,[39] and no lasting deleterious effect on HDL was found when either high-carbohydrate diets (or the simple replacement of SATs by POLYs) were fed for a sufficiently long period (6 months to 4 years) to allow for metabolic equilibration to the altered fat intake.[40,41] Populations having low HDL-C in the context of very low TC, e.g., the Tarahumara Indians,[42] presumably reflects their subsistence on a high-carbohydrate, very low-fat diet (<20% en) where saturated fat intake (along with 18:2) ironically may be too low to sustain HDL. For example, saturates represented only 2% en in the Tarahumara diet containing 12% en from fat.[42]

Thus, careful scrutiny of the literature supports the rather surprising implication that the optimal HDL/LDL ratio, at least in normolipemic individuals consuming moderate-fat diets, may depend on an appropriate balance between SATs and POLYs, with excess MONOs appearing neutral, or even detrimental, to this relationship. For example, in several human reports HDL increased during consumption of palm oil[29,43,44] or when 14:0-rich fats represented a major portion of fat calories,[9–12] but was severely depressed by high 18:0 intake.[11] Similar enhancement of the HDL/LDL ratio was found in cebus monkeys only when a high dietary 16:0 intake was coupled with adequate 18:2.[14,19] In regard to MONOs, epidemiological evaluation of over 8,500 subjects in a cardiovascular study revealed a negative correlation between 18:1 intake and HDL-C, questioning the efficacy of dietary 18:1 for maintaining HDL in a long-term diet,[45] or even short-term in a reduced-fat diet.[34] The present data suggest that high 18:1 intake in a low-to-moderate fat diet may displace too much 16:0 and/or 18:2 and result in lower HDL-C.

As stated above and elsewhere,[20,33] a high intake of

12:0, 14:0, or 16:0 may not enhance the HDL/LDL ratio unless 18:2 intake is adequate, especially in situations where total fat intake is 30% en or less. Thus, a positive SAT effect may depend on concomitant enhancement of LDL_r activity by 18:2.[26,27,46] This threshold concept for maximal up-regulation of LDL_r activity by 18:2 includes the notion that 18:2 intake below threshold would limit LDL_r activity and removal of LDL and VLDL remnants, thereby depressing HDL production which depends, in part, on adequate VLDL catabolism.[47] The 3.5% en as 18:2 in the present POL diet did not appear to be below threshold for TC or LDL-C since these values were not significantly reduced by increasing 18:2 intake to 8% en, even though 3.5% en was not sufficient for enhancing the HDL-C pool in the present situation.

Further work is needed to determine whether this relationship is real or simply a transient observation associated with the limited duration of this study, whether it depends on a specific 18:2/SAT fatty acid ratio or even an absolute intake of certain fatty acids, and whether the overall fat load (% en) impacts the relationship.

Conditional aspect of 16:0 cholesterolemia

Although the commonly applied multiple regression equations of Keys[17] and Hegsted[18] imply that the saturated fatty acids (12:0, 14:0, and 16:0) are equally cholesterolemic, the best equation of Hegsted et al.[13] originally indicated that dietary 14:0 was four times more cholesterolemic than 16:0 in humans, a difference independently confirmed by recent meta-analysis.[48] Re-analysis of the Hegsted data, in combination with data from monkeys[20,21] and gerbils,[15] suggests that both dietary cholesterol intake and the initial LDL receptor "setpoint" may have contributed to the cholesterolemic effect of 16:0 described by Hegsted.[13] Applying either the Keys or Hegsted equations to our present data predicted cholesterol values during the 16:0-rich diet that were approximately 13 mg/dL higher than those actually observed. However, if 16:0 was considered neutral, this discrepancy disappeared. Thus, similar to the situation in hamsters[46] and cebus monkeys,[21] the cholesterol-raising aspect of 16:0 in humans may pertain only when hepatic LDL_r activity is reduced, especially by dietary cholesterol intake,[20,21] and when the VLDL production rate is high.[49,50]

Another potential shortcoming of the Keys–Hegsted regressions is that they do not incorporate a nonlinear relationship observed between serum cholesterol and increasing increments of dietary 18:2.[20] Nor do they make allowance for the differences in the putative LDL_r "setpoint" reflected in LDL-C differences between individuals or populations at the time of intervention. These two aspects of the problem may be interrelated[20] (also see discussion in Ref. 33). The present diets provided <8% en as 18:2 with 30% en as fat and minimal dietary cholesterol, and they were fed to a normolipemic population whose LDL receptor activity was presumably minimally compromised at the time of intervention.

That 16:0-rich fat can raise plasma cholesterol levels, including HDL-C, in hypercholesterolemic subjects is apparent in the data of Nestel et al.[43] When moderately cholesterolemic subjects (225 mg/dL) were fed whole food diets (35% en as fat) enriched in 18:1, 16:0, or *trans*-18:1 (elaidic acid), the 18:1-rich diet resulted in significantly lower LDL-C and HDL-C compared with 16:0. Their findings regarding 16:0 contrast with our results as well as the original comparison between olive oil and palm oil in Trappist monks,[31] and an exchange between palm olein (16:0) and peanut oil (18:1) in Benedictine nuns,[51] but concur with certain other human studies evaluating 16:0-rich palm oil in subjects with initially elevated cholesterol levels,[3,22,52] or even normal entry values.[12] However, most such studies provided fat nearer to 40% en and incorporated modified triglycerides as sources of certain fatty acids, both of which may account for the discrepant results. Evaluation of the various aspects of the situation[19] has suggested that exchange of 16:0 tends not to be more cholesterolemic than 18:1, or occasionally even 18:2 as in the current study, when the exchange is between natural triglycerides fed at ≤30% en, the plasma cholesterol levels are normal (<200 mg/dL), and the intake of 18:2 is above the threshold requirement for the conditions involved. The conclusion that 16:0 can be "conditionally cholesterolemic" is not incompatible with our current findings, even though all the circumstances required to elicit its cholesterolemic potential are not apparent at this time. Two obvious possibilities that await future investigation include the significance of total fat load, e.g., 40 versus 30% en, and the structure of triglyceride molecular species incorporating the 16:0.[53]

Lp(a) and dietary fat

The serum Lp(a) was assessed because it may be influenced by dietary fatty acids. Hornstra et al.[54] demonstrated that replacement of habitual fat in the Dutch diet by 16:0-rich palm oil significantly decreased serum Lp(a) levels. It was suggested that the beneficial decrease in Lp(a) was due either to a component present in palm oil or displacement of a factor in the fat of the habitual Dutch diet, e.g., *trans* fatty acids. Subsequently, Mensink et al.[55] gathered data from three dietary trials to suggest that *trans* monounsaturated fatty acids elevate Lp(a) in comparison to other commonly occurring dietary fatty acids. This was confirmed by Nestel et al.[43] who found that diets rich in 16:0, 18:1 or a habitual Australian diet hardly affected Lp(a), whereas *trans*-18:1 acid caused a significant 30% increase. In our study Lp(a) was unaffected by the fatty acid exchanges, supporting the idea that manipulation of common dietary fatty acids, including 16:0, 18:0, 18:1, and 18:2, will not affect Lp(a) unless they displace dietary *trans* fatty acids (minimally present in the Malaysian diet).

References

1 Keys, A., Anderson, J.T., and Grande, F. (1957). Prediction of serum cholesterol response of man to changes in fats in the diet. *Lancet* **2**, 959–966

2 Pownall, H.J., Shepherd, J., Mantulin, W.W., Sklar, L.A., Gotton, A.U., Jr. (1980). Effect of saturated and polyunsaturated fat diets on the composition and structure of human low density lipoproteins. *Atherosclerosis* **36**, 299–314

3 Grundy, S.M., and Vega, G.L. (1986). Plasma cholesterol responsiveness to saturated fatty acids. *Am. J. Clin. Nutr.* **47**, 822–824

4 Blackburn, H. In *Hypercholesterolemia and Atherosclerosis: Patho-*

genesis and Prevention. (D. Steinberg and J.M. Olefsky eds.) p. 53–98, Churchill Livingstone, New York
5 Steinberg, D., and Witztum, J.L. (1990). Lipoproteins and atherogenesis. Current concepts. *JAMA* **264,** 3047–3052
6 American Heart Association. (1990). The cholesterol facts. A summary of the evidence relating dietary fats, serum cholesterol, and coronary heart disease. A joint statement by the American Heart Association and the National Heart, Lung and Blood Institute. *Circulation* **81,** 1721–1733
7 AMA Council of Scientific Affairs. (1983). Dietary and pharmacological therapy for lipid risk factors: a council statement. *JAMA* **250,** 1873–1879
8 Grundy, S.M., and Denke, M.A. (1990). Dietary influences on serum lipids and lipoproteins. *J. Lipid Res.* **31,** 1149–1172
9 Sundram, K., Hayes, K.C., and Siru, O.H. (1994). Dietary palmitic acid lowers serum cholesterol relative to a lauric-myristic acid combination in normolipemic humans. *Am. J. Clin. Nutr.* **59,** 841–846
10 Ng, T.K.W., Hayes, K.C., deWitt, G.F., Jegathesan, M., Satgunasingham, N. Ong, A.S.H., and Tan, D.T.S. (1992). Palmitic and oleic acids exert similar effects on serum lipid profiles in normocholesterolemic men and women. *J. Am. Coll. Nutr.* **11,** 383–390
11 Tholstrup, T., Marckmann, P., Jespersen, J., and Sandstrom, B. (1994). Fat high in stearic acid favorably affects blood lipids and factor VII coagulant activity in comparison with fats high in palmitic acid or high in myristic and lauric acids. *Am. J. Clin. Nutr.* **59,** 371–379
12 Zock, P.L., de Vries, J., and Katan, M.B. (1994). Impact of myristic acid versus palmitic acid on serum lipid and lipoprotein levels in healthy women and men. *Arterioscler. Thromb.* **14,** 567–575
13 Hegsted, D.M., McGandy, R.B., Meyers, M.L., and Stare, F.J. (1965). Quantitative effects of dietary fat on serum cholesterol in man. *Am. J. Clin. Nutr.* **27,** 281–295
14 Hayes, K.C., Pronczuk, A., Lindsey, S., and Diersen-Schede, D. (1991). Dietary saturated fatty acids (12:0, 14:0, 16:0) differ in their impact on plasma cholesterol and lipoproteins in nonhuman primates. *Am. J. Clin. Nutr.* **53,** 491–498
15 Pronczuk, A., Khosla, P., and Hayes, K.C. (1994). Dietary myristic, palmitic and linoleic acids modulate cholesterolemia in gerbils. *FASEB J.* **8,** 1191–1200
16 Kris-Etherton, P.M., Derr, J., Mitchell, D.C., Mustard, V.A., Russel, M.E., McDonnell, E.T., and Salabsky, D. (1993). The role of fatty acid saturation on plasma lipoproteins and apolipoproteins: 1. Effects of whole food diets high in cocoa butter, oleic oil, soybean oil, dairy butter and milk chocolate on the plasma lipids of young men. *Metabolism* **42,** 121–129
17 Keys, A., Anderson, J.T., and Grande, F. (1965). Serum cholesterol response to changes in the diet IV. Particular saturated fatty acids in the diet. *Metabolism* **14,** 776–787
18 Hegsted, D.M. (1991). Dietary fatty acids, serum cholesterol and coronary heart disease. In *Health Effects of Dietary Fatty Acids*. (G.J. Nelson, ed.), p. 50–68, American Oil Chemist's Society, Champaign, IL.
19 Khosla, P., and Hayes, K.C. (1992). Comparison between the effects of dietary saturated (16:0), monounsaturated (18:1), and polyunsaturated (18:2) fatty acids on plasma lipoprotein metabolism in cebus and rhesus monkeys fed cholesterol free diets. *Am. J. Clin. Nutr.* **55,** 51–62
20 Hayes, K.C., and Khosla, P. (1992). Dietary fatty acid thresholds and cholesterolemia. *FASEB J.* **6,** 2600–2607
21 Khosla, P., and Hayes, K.C. (1993). Dietary palmitic acid raises plasma LDL cholesterol relative to oleic acid only at a high intake of cholesterol. *Biochim. Biophys. Acta* **1210,** 13–22
22 Mattson, F.H., and Grundy, S.M. (1985). Comparison of effects of dietary saturated, monounsaturated and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *J. Lipid Res.* **26,** 194–202
23 Mensink, R.P., and Katan, M.B. (1989). Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. *N. Engl. J. Med.* **321,** 436–441
24 Ginsberg, H.N., Barr, S.L., Gilbert, A., Karmally, W., Deckelbaum, R., Kaplan, K., Ramakrishnan, R., Holleran, S., and Dell, R.B. (1990). Reduction of plasma cholesterol levels in normal men on an American Heart Association Step 1 diet or a Step 1 diet with added monounsaturated fat. *N. Engl. J. Med.* **322,** 575–579
25 Barr, S.L., Ramakrishnan, R., and Ginsberg, H. (1992). Reducing total dietary fat without reducing saturated fatty acids does not significantly lower plasma cholesterol concentrations in normal males. *Am. J. Clin. Nutr.* **55,** 675–681
26 Nicolosi, R.J., Stucchi, A.F., and Kowala, M.C. (1990). Effect of dietary fat saturation and cholesterol on LDL composition and metabolism. In vivo studies of receptor and nonreceptor mediated catabolism of LDL in cebus monkeys. *Arteriosclerosis* **10,** 119–128
27 Fernandez, M.L., and McNamara, D.J. (1989). Dietary fat-mediated changes in hepatic apo B/E receptor in the guinea pig: effect of polyunsaturated, monounsaturated, and saturated fat. *Metabolism* **38,** 1094–1102
28 Terpstra, A.H.M., Woodward, C.J.H., and Sanchez-Maniz, F.J. (1981). Improved techniques for the separation of serum lipoproteins by density gradient ultracentrifugation: visualization by prestaining and rapid separation of serum lipoproteins from small volumes of serum. *Ann. Biochem.* **111,** 149–157
29 Sundram, K., Hornstra, G., Houwelingen, A.C.V., and Kester, A.D.M. (1992). Replacement of dietary fat with palm oil: effect on human serum lipids, lipoproteins and apolipoproteins. *Br. J. Nutr.* **68,** 677–692
30 Bligh, E.G., and Dyer, W.J. (1959). A rapid method for total lipid extraction and purification. *Can. J. Biochim. Physiol.* **37,** 911–917
31 Vergroesen, A.J., and Gottenbos, J.J. (1975). The role of fats in human nutrition: an introduction: In *The Role of Fats in Human Nutrition*. (A.J. Vergroesen, ed.), p. 1–44, Academic Press, New York
32 Ghafoorunissa, S., and Reddy, V. (1993). Effects of palm olein on blood lipids and platelet aggregation in healthy Indian subjects. XV International Congress on Nutrition, Adelaide, Australia, p. 174 (Abstract)
33 Hayes, K.C., Pronczuk, A., and Khosla, P. (1995). A rationale for plasma cholesterol modulation by dietary fatty acids: modeling the human response in animals. *J. Nutr. Biochem.* **6,** 188–194
34 Wahrburg, U., Martin, H., Sandkamp, M., Schulte, A., and Assman, G. (1992). Comparative effects of a recommended lipid lowering diet vs a diet rich in monounsaturated fatty acids on serum lipid profiles in healthy young adults. *Am. J. Clin. Nutr.* **56,** 678–683
35 Sarkkinen, E.S., Uusitupa, M.I.J., Pietinen, P., Aro, A., Ahola, I., Penttila, I., Kervinen, K., and Antero, Y.A. (1994). Long-term effects of three fat-modified diets in hypercholesterolemic subjects. *Atherosclerosis* **105,** 9–23
36 Shepherd, J., Packard, C.J., and Patsch, J.R. (1978). Effects of dietary polyunsaturated and saturated fat on the properties of high density lipoproteins and the metabolism of apolipoproteins. A-I. *J. Clin. Invest.* **60,** 1582–1591
37 Berry, E.M., Eisenberg, S., Haratz, D., Friedlander, Y., Norman, Y., Kaufman, N.A., and Stein, Y. Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins-the Jerusalem Nutrition Study: high MUFAs vs high PUFAs. *Am. J. Clin. Nutr.* **53,** 899–907
38 Kuusi, T., Ehnholm, C., Huttunen, K., Kostianinen, P., Pietinen, U., Leino, U., Uusitalo, U., Nikkari, T., Iacono, J.M., and Puska, P. (1985). Concentration and composition of serum lipoproteins during a low-fat diet at two levels of polyunsaturated fat. *J. Lipid Res.* **26,** 360–367
39 Leenen, R., van der Kooy, K.,Meyboom, S., Seidell, J.C., Deurenberg, P., and Weststrate, J.A. (1993). Relative effects of weight loss and dietary fat modification on serum lipid levels in the dietary treatment of obesity. *J. Lipid Res.* **34,** 2183
40 Rivellese, A.A., Auletta, P., Marotta, G., Saldalamacchia, G., Giacco, A., Mastrilli, V., Vaccaro, O., and Riccardi, G. (1994). Long term metabolic effects of two dietary methods of treating hyperlipidaemia. *Brit. Med. J.* **308,** 227–231
41 Hjermann, I., Enger, S.C., Helgeland, A., Holme, I. Leren, P., and Trygg, K. (1979). The effect of dietary changes on HDL-C. The Oslo Study. *Am. J. Med.* **66,** 105–109
42 Connor, W.E., Cerqueira, M.T., Connor, R.W., Wallace, R.B., Malinow, M.R., and Casdorph, H.R. (1978). The plasma lipids, lipoproteins, and diet of the Tarahumara Indians of Mexico. *Am. J. Clin. Nutr.* **31,** 1131–1142
43 Nestel, P., Noakes, M., Belling, B., McArthur, R., Clifton, P., James, E., and Abbey, M. (1992). Plasma lipoprotein lipid and

Lp(a) changes with substitution of elaidic acid for oleic acid in the diet. *J. Lipid Res.* **33,** 1029–1036

44 Truswell, A.S., Choudhury, N., and Roberts, D.C.K. (1992). Double blind comparison of plasma lipids in healthy subjects eating potato crisps fried in palm olein or canola oil. *Nutr. Res.* **12** (Suppl. 1). S43–S52

45 Gartside, P.S., and Glueck, C.J. (1993). Relationship of dietary intake to hospital admission for coronary heart and vascular disease: The NHANES II National Probability Study. *J. Am. Coll. Nutr.* **12,** 676–684

46 Woollett, L.A., Spady, D.K., and Dietschy, J.M. (1992). Saturated and unsaturated fatty acids independently regulate LDL receptor activity and production rate. *J. Lipid Res.* **33,** 77–78

47 Tall, A.S., and Small, D.M. (1978). Plasma high-density lipoproteins. *N. Eng. J. Med.* **299,** 1232–1236

48 Mensink, R.P., and Katan, M.B. (1992). Effect of dietary fatty acids on serum lipids and lipoproteins: a meta-analysis of 27 trials. *Arterioscler. Thromb.* **12,** 911–919

49 Lin, E.C., Fernandez, M.L., Tosca, M.A., and McNamara, D.J. (1994). Regulation of hepatic LDL metabolism in the guinea pig by dietary fat and cholesterol. *J. Lipid Res.* **35,** 446–457

50 Khosla, P., and Hayes, K.C. (1991). Dietary fat saturation in rhesus monkeys affects LDL concentrations by modulating the independent production of LDL apolipoprotein B. *Biochim. Biophys. Acta* **1083,** 46–56

51 Baudet, M.F., Dacket, C., Lasserre, M., Esteva, O., and Jacotot, B. (1984). Modification in the composition and metabolic properties of human low density lipoproteins by different dietary fats. *J. Lipid Res.* **25,** 456–468

52 Laine, C.W., Snodgrass, C.M., Dawson, E.A., Ener, M.A., Kuba, K., and Frantz, I.D.,Jr. Lightly hydrogenated soya oil versus other vegetable oils as a lipid lowering dietary constituent. *Am. J. Clin. Nutr.* **35,** 683–690

53 Kritchevsky, D. (1988). Effects of triglyceride structure on lipid metabolism. *Nutr. Rev.* **46,** 177–181

54 Hornstra, G., Houwelingen, A.C.V., Kester, A.D.M., and Sundram, K. (1991). A palm oil enriched diet lowers serum lipoprotein(a) in normocholesterolemic volunteers. *Atherosclerosis* **90,** 91–93

55 Mensink, R.P., Zock, P.L., Katan, M.B., and Hornstra, G. (1992). Effect of dietary cis and trans fatty acids on serum lipoprotein(a) levels in humans. *J. Lipid Res.* **33,** 1493–1501