Dietary Palmitic Acid (16:0) Enhances High Density Lipoprotein Cholesterol and Low Density Lipoprotein Receptor mRNA Abundance in Hamsters (43145)

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Abstract. **In order to examine the qualitative effect of different fats and specific fatty acids on plasma lipids and lipoprotein metabolism, six low fat, cholesterol-free diets were fed to young male hamsters (10/group) for a 4-week period. Fat blends were formulated with coconut oil, palm oil, soybean oil, high oleic acid safflower oil, butter, corn oil, and canola oil. Diets contained 13% energy as fat and dietary polyunsaturate/saturate ratios ranged from 0.12 to 1.04, one of which incorporated the American Heart Association-recommended concentrations of saturates, monoenes, and polyenes and another reflected the current American Fat Blend. In three diets the polyunsaturate/monounsaturate/saturate ratio was held constant while only the 12:0, 14:0, and 16:0 were varied. Plasma lipoproteins and apoproteins were assessed in conjunction with the abundance of specific hepatic and intestinal mRNA for the low density lipoproteins (LDL) receptor and various apolipoproteins associated with cholesterol metabolism. The plasma cholesterol response was lowest with the American Heart Association blend and equally elevated by the more saturated, low polyene diets (polyunsaturate/saturate, 0.12-0.38). Replacing 12:0 plus 14:0 from coconut oil with 16:0 as palm oil induced a significant increase in high density lipoprotein (HDL) cholesterol with a trend toward decreased LDL. These shifts in lipoprotein cholesterol were corroborated by measures of the LDL/HDL ratio, the plasma apolipoprotein B/apolipoprotein A1 ratio, and differences in the synthesis of apolipoproteins and the LDL receptor based on estimates of the mRNA for these proteins in the liver and gut, using specific cDNA probes for apolipoprotein A1, apolipoprotein B, apolipoprotein E, and the LDL receptor. Although it has been suggested that dietary polyenes lower total plasma cholesterol, including HDL, and that saturated fat increases both these pools of cholesterol, the current data represent the first evidence that a specific saturated fatty acid, i.e., palmitic acid, may enhance HDL production.** [P.S.E.B.M. 1990, Vol 195]

Many studies (1–5) have compared the hypocholesterolemic effect of dietary polyunsaturated fat with the hypercholesterolemic effect of saturated fat, both in humans and in animal models. Furthermore, elevated plasma low density lipoprotein (LDL) cholesterol and apolipoprotein B (apo B) are associated with increased risk for atherosclerosis, whereas elevated plasma high density lipoprotein (HDL) cholesterol and its associated apolipoprotein A1 (apo A1) are thought to be protective (6, 7). The concentrations of these circulating lipoproteins are determined in part by diet and, although most studies indicate that dietary fat composition modulates the plasma cholesterol concentration, considerable variation in this response exists between individuals and species (8–12), particularly in the specific lipoprotein response.

Based on their hepatic cholesterol dynamics, male hamsters have been recommended as an animal model for the study of cholesterol metabolism (13). In addition they demonstrate down-regulation of hepatic LDL receptor (LDLr)-mediated activity when fed diets containing cholesterol in conjunction with high saturated fat, compared with highly polyunsaturated fat (14). Based on current data, it is not clear whether all the dietary saturated fatty acids, i.e., 12:0, 14:0, 16:0, and 18:0, are equally responsible for the adverse effect on

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cholesterol homeostasis or whether one fatty acid is more responsible than another. Nor is it obvious that extremes of fat saturation or polyunsaturation are an appropriate physiologic test of the highly varied mixture of saturates and polyunsaturates consumed in the typical diet. Although it is generally agreed in humans that 18:0 is neutral (1, 2), the contribution of 12:0, 14:0, and 16:0 is less certain. Whereas studies by Keys *et al.* (2) suggest that these fatty acids are equally cholesterolemic, the original report by Hegsted *et al.* (1) places the primary cholesterol-elevating emphasis on 14:0. Our recent monkey study also suggests that 12:0 plus 14:0 were more cholesterolemic than 16:0 in a diet containing 31% fat calories (15).

The present experiment was designed to extend these previous studies by evaluating the qualitative impact of specific saturated fatty acids over a practical range of dietary polyunsaturate/saturate (P/S) ratios. Cholesterolemia was measured in hamsters fed purified diets in which levels of monounsaturates, polyunsaturates, or saturates were varied or were held constant while specific saturated fatty acids were varied. Under these conditions, the plasma lipids were compared with the abundance of specific hepatic and intestinal mRNA for the LDLr and various apolipoproteins associated with cholesterol metabolism.

Materials and Methods

Hamsters and Diets. Sixty 6-week-old male Syrian hamsters, weighing 83–90 g, were obtained from the Charles River Breeding Laboratories (Wilmington, MA). Hamsters were assigned to one of six dietary groups, 10 in each group, for a 4-week experimental period. All diets contained 5% fat by weight (13% of total calories) without added cholesterol and were designed to meet several criteria. First, diets were low in fat, because hamsters reportedly prefer that (16) and we wished to determine whether a qualitative fat effect (versus a quantitative effect associated with high fat diets) could be demonstrated when feeding a minimal fat load. Second, the range in the dietary P/S ratio should span the current U.S. dietary value of 0.5–0.6, rather than the extremes in P/S ratio often examined in such studies. One diet represented an American Fat Blend (AFB; Diet 5) and another the American Heart Association's recommended P/S ratio of 1.0 (AHA; Diet 6). An additional objective was to keep the polyunsaturate/monounsaturate/saturate ratio constant while altering only the saturated fatty acids (12:0, 14:0, and 16:0) in Diets 2, 3, and 4. Finally, a graded decrease in total saturates was coupled to a balanced increase in polyunsaturates while monounsaturates were held constant across Diets 4, 5, and 6. The diets are listed in Table I, and the fatty acid profile for each diet is presented in Table II. Hamsters were housed 4/cage on a 12-hr light-dark cycle (6 AM to 6 PM) and were given access to food and water *ad libitum.* They were weighed weekly.

Isolation of Total RNA. At the end of 4 weeks, hamsters were fasted overnight in individual wire-bottomed cages and exsanguinated under sodium pentobarbital anesthesia. Plasma samples, with 0.15 mM EDTA, were collected for analysis of apolipoproteins and lipids. Liver and intestine samples were excised, quickly frozen in liquid nitrogen, and stored at −70°C. Total RNA was isolated from the liver and intestine (100 mg) by homogenization, in 10 ml of 6 *M* urea/3 *M* lithium chloride, at full speed in a Tekmar Tissumizer (Cinncinatti, OH). The homogenate was kept at 4°C overnight and the RNA was pelleted by centrifugation at 16,000 rpm before being dissolved and incubated for 45 min at 37°C in buffer containing 10 m*M* Tris (pH 7.5), 0.5% sodium dodecyl sulfate (SDS), and 50 mg/ml proteinase K. The RNA was extracted three times with 0.5 vol of water-saturated phenol and twice with 0.5 vol of chloroform/isoamyl alcohol (24:1) before ethanol precipitation and was stored at −70°C (17).

Determination of apo B, Apolipoprotein E (apo E), apo A1, and LDLr mRNA. RNA in increasing quantities of 2, 4, and 6 μg was analyzed by slot-blot hybridization (18). Yeast tRNA and the murine actin gene (cytoplasmic class, β-isoform) were used as negative and positive controls, respectively. The nitrocellulose filters were dried overnight, baked for 2 hr at 80°C, and prehybridized and hybridized for 1 and 16 hr, respectively. All RNA blots were hybridized to ^{32}P-labeled recombinant plasmids containing specific cDNA inserts corresponding to an individual mRNA species. Plasmid DNA was labeled by nick translation (>1 × 10^8 cpm/μg) using a labeling kit (Amersham, Arlington Heights, IL), according to the manufacturer's directions. Each filter was probed consecutively with human apo A1 cDNA (courtesy of J. Breslow, Rockefeller University), human apo B cDNA (courtesy of V. Zannis, Boston University), human LDLr cDNA (clone pLDLR3; American Type Culture Collection, Rockville, MD) hybridized under 30% formamide conditions, and rat apo E cDNA (courtesy of J. Taylor, University of San Francisco) hybridized under 40% formamide conditions at 42°C. The specificity of the cDNA probes for each hamster mRNA in question was confirmed by Northern blots (19). The labeled probes hybridized to a single electrophoretic mRNA species (data not shown). The filters were stripped of probe before each subsequent hybridization and re-exposed to film to confirm its removal. After the final analysis, the first probe was again hybridized to the filter to confirm that multiple probe analysis had not altered the RNA binding affinity. Following each hybridization, the filters were washed with 2× standard saline citrate (0.3 *M* sodium citrate, 3 *M* NaCl, pH 7.0), 0.1% SDS, 1% $NaPP_i$ for 1 hr, followed by a second 1-hr

Table I. Purified Diets for Hamsters Fed 5% Fat Blends

Ingredients	Diets[a]					
	1 (Hi-SATS)	2 (12:0 + 14:0)	3 (Mixed)	4 (16:0)	5 (AFB)	6 (AHA)
Casein	20	20	20	20	20	20
Glucose	17.5	17.5	17.5	17.5	17.5	17.5
Rice flour	30.4	30.4	30.4	30.4	30.4	30.4
Fat blends						
Coconut oil	4.5	2.3	1.1	—	—	—
Palm oil	—	—	2.3	4.5	—	2.3
Soybean oil	0.5	0.7	0.6	0.5	—	2.0
High oleic safflower oil	—	2.0	1.0	—	—	0.7
Butter	—	—	—	—	2.6	—
Canola oil	—	—	—	—	1.6	—
Corn oil	—	—	—	—	0.8	—
Cellulose	10	10	10	10	10	10
Wheat bran	5	5	5	5	5	5
Mineral mix[b]	4.6	4.6	4.6	4.6	4.6	4.6
Vitamin mix[c]	1.2	1.2	1.2	1.2	1.2	1.2
Choline Cl_2	0.3	0.3	0.3	0.3	0.3	0.3

[a] See Table II for diet designations.
[b] Ausman-Hayes salt mix. Salt mix contained (g/kg): magnesium oxide, 320; calcium carbonate, 290.5; potassium phosphate dibasic, 312.2; calcium phosphate dibasic, 72.6; magnesium sulfate, 98.7; sodium chloride, 162.4; ferric citrate, 26.6; potassium iodide, 0.77; maganese sulfate, 3.66; zinc chloride, 0.24; cupric sulfate, 0.29; chromium acetate, 0.044; sodium selenite, 0.004.
[c] Hayes-Cathcart vitamin mix. Vitamin mix contained (g/kg): DL-δ-tocopheryl acetate (500 IU/g), 15; inositol, 5; niacin, 3; calcium pantothenate, 1.6; vitamin A palmitate (500,000 IU/g), 1.5; vitamin D_3 (400,000 IU/g), 0.100; menadione, 0.200; biotin, 0.020; folic acid, 0.200; riboflavin, 0.700; thiamin, 0.600; pyridoxine HCl, 0.700; cyanocobalamin, 0.001; dextrin, 972.

Table II. Fatty Acid Composition of Experimental Fat Blends as Determined by GLC

Fatty acid (% of total fatty acids)	Fat blend[a]					
	Diet 1 (Hi-SATS)	Diet 2 (12:0 + 14:0)	Diet 3 (Mixed)	Diet 4 (16:0)	Diet 5 (AFB)	Diet 6 (AHA)
<12:0	47.8	23.8	13.4	0.2	2.3	0.4
14:0	18.8	9.6	5.8	1.0	7.5	0.7
16:0	10.7	8.6	25.1	40.3	21.9	23.4
18:0	3.3	3.0	3.6	4.1	6.5	3.9
20:0	0.0	0.2	0.2	0.3	0.4	0.3
Total saturates	80.6	45.2	48.1	45.9	38.6	28.7
16:1	0.0	0.2	0.0	0.0	0.9	0.0
18:1	9.4	37.0	37.2	37.0	35.8	41.1
Total monounsaturates	9.4	37.2	37.2	37.0	36.7	41.1
18:2	8.5	16.0	13.3	15.4	20.4	27.2
18:3	0.9	1.2	0.8	1.0	4.3	2.7
Total polyunsaturates	9.4	17.2	14.1	16.4	24.7	29.9
P/S	0.12	0.38	0.29	0.38	0.64	1.04

[a] Blend 1, 90% coconut oil + 10% soy oil; referred to as hi-SATS; blend 2, 45% coconut oil + 15% soy oil + 40% high oleic acid safflower oil; referred to as 12:0 + 14:0-enriched; blend 3, 22.5% coconut oil + 12.5% soy oil + 45% palm oil + 20% high oleic acid safflower oil; referred to as mixed in 12:0, 14:0, 16:0; blend 4, 90% palm oil + 10% soy oil; referred to as 16:0-enriched; blend 5, AFB, 52% butter + 32% canola oil + 16% corn oil; blend 6, AHA, Step I, 45% palm oil + 40% soy oil + 15% high oleic acid safflower oil.

wash with 0.2× standard saline citrate; 0.1% SDS, 1% $NaPP_i$. The filters were placed on film, the resulting autoradiographs were scanned densitometrically (E-C Apparatus Corporation, St. Petersberg, FL), and the relative mRNA levels were determined.

Plasma Cholesterol, Triglyceride, Apolipoproteins, and Dietary Fatty Acids. Total cholesterol, HDL-cholesterol (HDL-C), and total triglyceride were determined by enzymatic assays (Sigma kit; procedures 351 and 336). HDL was obtained following sodium phosphotungstate-Mg^{2+} precipitation of the apo B- and apo E-containing lipoproteins (20). LDL-cholesterol (LDL-C) was estimated after adjustment for very low density lipoprotein-cholesterol (VLDL-C), based on plasma triglycerides, according to the procedure of Friedwald *et al.* (20).

Plasma apolipoprotein content was quantitated by an immunoturbidometric method (21). Hamster apo A1, E, and B were isolated and purified from HDL, VLDL, and LDL, respectively, and purity was checked

by SDS-polyacrylamide gel electrophoresis as described by Davis (22). Specific antibodies were raised in rabbits and apolipoproteins were assessed in hamster plasma by an immunoturbidometric procedure, measured at 340 nm in a Perkin-Elmer spectrophotometer (Perkin-Elmer Co., Oak Brook Instrument Division, Oak Brook, IL). The concentration was established from a standard curve generated with purified apoprotein.

Dietary fatty acids were analyzed using the one-step transesterification method of Lapage and Roy (23). Gas-liquid chromatography (GLC) of fatty acid methyl esters was performed on a Hewlett-Packard 5790A GLC equipped with a flame ionization detector (Hewlett-Packard Co., Avondale, PA) and a 30-m fused silica column (SP-2330; Supelco Inc., Bellefonte, PA).

Statistical analysis was performed on the Macintosh Plus computer using appropriate statistical packages, StatView 512$^+$ (Brain Power, Inc. Calabasas, CA), and Cricket Graph 1.2 (Cricket Software, Inc. Philadelphia, PA). Statistical assessment of dietary treatments utilized one-way analysis of variance and Fisher's protected least significant difference between groups when indicated by a significant one-way analysis of variance.

Results

Body Weight Data. Body weight gain per day as well as the final body weights were comparable between groups and averaged approximately 10 g/week. No significant differences existed between groups (Table III).

Plasma Lipids and Apolipoproteins. The circulating cholesterol values in these hamsters fed low fat purified diets ranged from 165 mg/dl (AHA, Diet 6; highest polyenes, lowest saturates) to almost 200 mg/dl (Diets 1 and 4; high to moderate saturates) (Table IV). The plasma cholesterol concentration declined significantly between Diets 1 and 2, when saturates were reduced by half, polyenes were doubled, and monoenes were quadrupled. For Diets 2 to 3 to 4, a progressive significant increase occurred in total plasma cholesterol, even though the only dietary fat change was a progressive increase in 16:0 supplied by palm oil at the expense of 12:0 plus 14:0 in coconut oil. However, the cholesterol increase was attributable to a significant rise in HDL-C and a lesser rise in VLDL-C, with a modest decline in LDL-C (Diet 4). Finally, Diets 5 and 6, which incorporated incremental increases in polyenes and a decrease in total saturates (primarily 16:0) compared with Diet 4, led to a progressive decline in total cholesterol attributable to HDL-C and VLDL-C. The AFB (Diet 5) produced the highest LDL-C, whereas the highest 16:0 intake (Diet 4) produced the lowest LDL-C.

The shifting distribution of cholesterol among lipoprotein fractions was reflected in the LDL/HDL ratio, such that the highest ratio was induced by the AFB (Diet 5) and the lowest by Diet 4 (highest 16:0). Similarly, the plasma apo A1 was elevated in association with increasing dietary 16:0 (Diets 2→3→4) and was substantially lower when dietary 16:0 was low (Diet 1) or when polyenes were increased at the expense of 16:0 (Diets 5 and 6). Plasma apo B, on the other hand, was lowest when triglycerides (VLDL) were low (Diets 2 and 6) and highest with the AFB (Diet 5). These relationships caused the plasma apo B/apo A1 ratio to be lowest with Diets 2, 3, and 4, with Diets 3 and 4 being significantly lower than Diets 1 and 5 (AFB). The AFB caused this ratio to be strikingly elevated.

When human-based regression equations derived by Keys *et al.* (2) and Hegsted *et al.* (1) were used to predict changes in plasma cholesterol based on the dietary fatty acid profile of these hamster diets, the predictions were generally in the right direction but the fit was poor, i.e., $r = 0.4$, explaining only 16% of the variance.

mRNA Abundance. For purposes of comparison, the mRNA abundance for hamster liver and intestine (Table V) is expressed as a percentage of difference from the control fat (AFB, Diet 5). As expected, actin mRNA was detected in both liver and gut preparations and was not affected by diet. The relative mRNA abundance for the various apolipoproteins generally varied less than 30% between dietary groups. For example, the variation in apo A1 mRNA abundance in the liver ranged from −9% to +18% of the control group.

The lowest hepatic and gut apo A1 mRNA abundance was associated with the highest concentration of dietary saturates, especially 12:0 plus 14:0, and low monoenes in Diet 1, followed by Diet 5 (AFB), which also produced the lowest concentration of plasma apo A1. The AHA blend rich in 18:1 and 16:0 (Diet 6) and

Table III. Weights and Weight Gain of Hamsters Fed 5% Fat Blends[a]

	Diets					
	1 (Hi-SATS)	2 (12:0 + 14:0)	3 (Mixed)	4 (16:0)	5 (AFB)	6 (AHA)
Initial weights (g)	87 ± 4	89 ± 4	90 ± 3	90 ± 3	83 ± 8	90 ± 5
Final weights (g)	134 ± 15	126 ± 13	135 ± 10	134 ± 10	125 ± 13	127 ± 12
Weight gain/day (g)	1.66 ± 0.60	1.33 ± 0.50	1.59 ± 0.40	1.61 ± 0.30	1.53 ± 0.40	1.32 ± 0.40

[a] Values are means ± SD.

Table IV. Plasma Lipid and Apolipoprotein Concentrations in Hamsters Fed 5% Fat Blends[a]

Criterion	Diets					
	1 (Hi-SATS)	2 (12:0 + 14:0)	3 (Mixed)	4 (16:0)	5 (AFB)	6 (AHA)
Cholesterol (mg/dl)	191 ± 13e	176 ± 20c	183 ± 17b	198 ± 22a,c,d	176 ± 18d	165 ± 25a,b,e
Triglyceride (mg/dl)	122 ± 47	98 ± 40	112 ± 47	148 ± 73b	139 ± 86a	85 ± 18ab
HDL-C[b] (mg/dl)	115 ± 20a	111 ± 35d	113 ± 30c	131 ± 22b,e	85 ± 24a–d	94 ± 15e
LDL-C[b] (mg/dl)	52 ± 18	46 ± 24	47 ± 24	37 ± 18a	63 ± 23a	53 ± 30
VLDL-C (mg/dl)	24 ± 9	20 ± 8	22 ± 9	30 ± 15b	28 ± 17a	17 ± 4ab
LDL/HDL (mg/dl)	0.49 ± 0.2a	0.51 ± 0.4d	0.48 ± 0.3c	0.30 ± 0.2b	0.85 ± 0.5a–d	0.61 ± 0.4
Plasma apolipoproteins						
Apo B (mg/dl)	75 ± 12d,e	59 ± 12a,d,f	68 ± 7b	71 ± 12f,g	81 ± 13a–c	59 ± 7e,g
Apo A1 (mg/dl)	481 ± 152	459 ± 76d,e	575 ± 114b,e,f	560 ± 68a,c,d	390 ± 114a,b	428 ± 112c,f
Apo E (mg/dl)	25 ± 6	25 ± 5	27 ± 3	26 ± 4	25 ± 4	23 ± 3
Apo B/Apo A1	0.17 ± 0.06a,e	0.13 ± 0.04b	0.12 ± 0.03c,e	0.13 ± 0.03a	0.23 ± 0.08a–d	0.15 ± 0.04d

[a] Values are mean ± SD (n = 10/diet group). Means with common letters differ significantly from each other by one-factor ANOVA ($P < 0.05$).
[b] Calculated by correcting for VLDL-C: VLDL-C = triglyceride × 0.2 (18).

Table V. Relative Abundance of mRNA in Hamsters Fed 5% Fat Blends[a]

Criterion	Diets					
	1 (Hi-SATS)	2 (12:0 + 14:0)	3 (Mixed)	4 (16:0)	5 (AFB)	6 (AHA)
Apo A1 mRNA (% of control)						
Liver	91 ± 7c–f	112 ± 5b,e	115 ± 6a,c	118 ± 4d	100 ± 4a,b	115 ± 6f
Gut	90 ± 10a–c	119 ± 12a	119 ± 11b	113 ± 12	100 ± 11	131 ± 10c
Apo E mRNA (% of control)						
Liver	104 ± 5	111 ± 5	121 ± 7a	123 ± 7b,c	100 ± 8a,b	110 ± 7c
Gut	108 ± 3	108 ± 3	102 ± 5	105 ± 6	100 ± 12	113 ± 7
Apo B mRNA (% of control)						
Liver	95 ± 8b	125 ± 10a,b	109 ± 6	112 ± 6	100 ± 9a	112 ± 6
Gut	118 ± 5	125 ± 14	130 ± 8	125 ± 8	100 ± 12	120 ± 12
LDLr mRNA (% of control)						
Liver	137 ± 16a,f,g	142 ± 23b,h	158 ± 20c,f,h,i	154 ± 19d,g	100 ± 11a–e	142 ± 14e,i
Gut	103 ± 15	127 ± 14	119 ± 7	117 ± 11	100 ± 16	128 ± 17

[a] Values are means ± SD (n = 10/diet group). Common letters differ significantly from each other by one-factor ANOVA ($P < 0.05$).

the increase in 18:1 and drop in 14:0 associated with Diets 2, 3, and 4 were reflected in higher apo A1 mRNA abundance in liver.

Apo B mRNA in the liver was significantly elevated only by Diet 2, being 25% higher than the control (AFB, Diet 5) and 35% higher than Diet 1. Although the intestinal apo B mRNA was higher in Diets 3 and 4 than in the AFB (Diet 5) by 30% and 25%, respectively, the effect was not significant.

Liver apo E mRNA abundance was significantly elevated above control (Diet 5, AFB) by Diets 3 and 4, again when 16:0 consumption was greatest. Apo E mRNA abundance in the intestine was less than 40% of that found in the liver, and all diets revealed similar concentrations of this message.

The abundance of hepatic LDLr mRNA was significantly elevated in all hamsters when compared with that of the controls (AFB, Diet 5). As with apo E and apo A1 mRNAs, the highest values were associated with Diets 3 and 4, i.e., fat blends containing the most 16:0 from palm oil. Both Diet 3 and Diet 4 values were significantly higher than those in livers from hamsters fed Diet 1, which was rich in 12:0 plus 14:0 and low in monoenes, or Diet 5, which contained the most polyenes. The LDLr mRNA in the intestine was not affected by diet.

Regressions. When the hepatic LDLr mRNA abundance was regressed against the plasma lipid response variables, utilizing the data from all hamsters combined (Fig. 1), the plasma apo A1 revealed a positive association (r = +0.53) with LDLr mRNA (Fig. 1A), whereas an equally strong inverse association (r = −0.53) was found between the apo B/apo A1 ratio and LDLr mRNA abundance (Fig. 1B). Other significant predictors of LDLr mRNA were apo E mRNA (r = +0.41, $P < 0.01$), a correlation also found in monkeys (24), and apo A1 mRNA (r = +0.39, $P < 0.01$), a correlation noted previously in hamsters (25).

Discussion

Plasma Cholesterol Response to Dietary Fat. It is noteworthy that the hamster plasma cholesterol con-

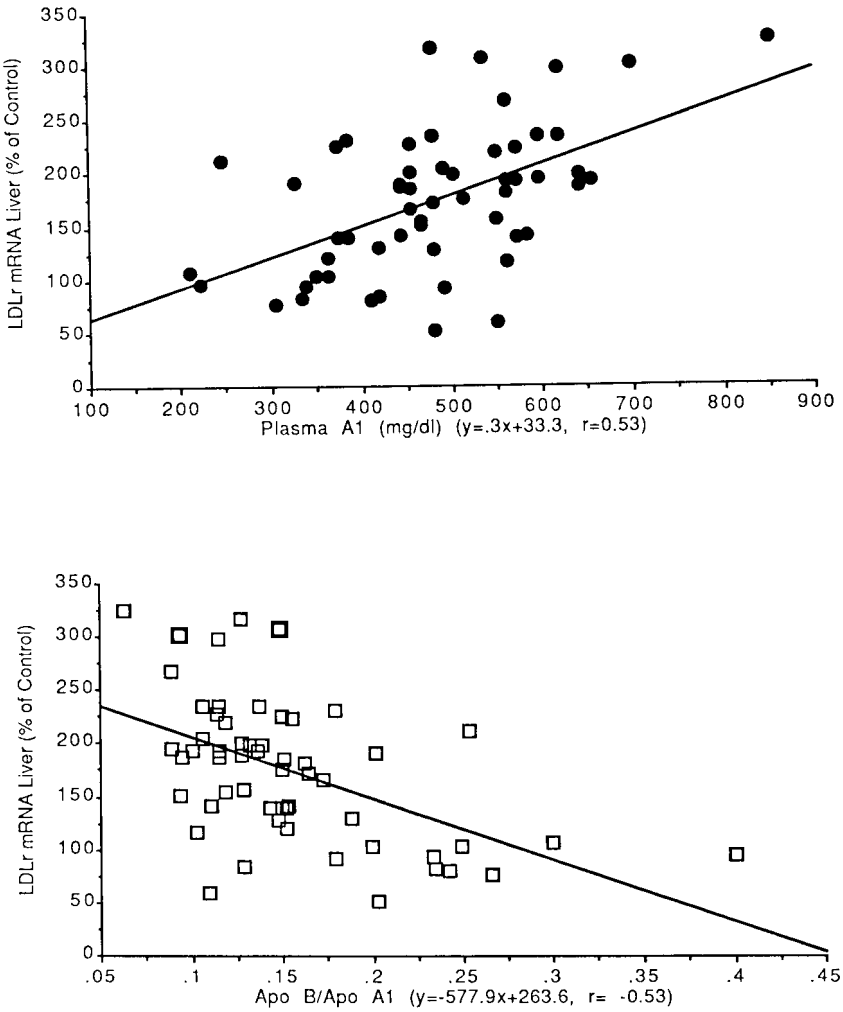

Figure 1. Regression analysis of the various response variables against the hepatic LDLr mRNA abundance revealed significant correlations between the plasma apo A1 concentration (*top*) and the plasma apo B/apo A1 ratio (*bottom*).

centration responded to changes in the dietary P/S ratio even though fat represented only 13% of the dietary calories. Thus, a decrease in plasma cholesterol was observed between Diets 1 and 2 and between Diets 4 and 6, when major portions of the saturates were replaced by polyenes. The AHA-recommended polyunsaturate/monounsaturate/saturate ratio (Diet 6) produced the most favorable plasma lipid results, whereas the control AFB (Diet 5) resulted in the least favorable LDL/HDL and apo B/apo A1 ratios. In addition, an impact attributable to the *type* of saturated fat ingested was associated with the shift from coconut oil to palm oil across Diets 2, 3, and 4. As evidenced by specific lipoprotein cholesterol concentrations, apoprotein distribution, and changes in triglyceride concentration (i.e., VLDL), progressively replacing 12:0 plus 14:0 (Diet 2) with 16:0 (Diets 3 and 4) led to a direct increase in plasma HDL (apo A1 and HDL-C) along with a tendency for increased triglyceride (and VLDL-C) and decreased LDL-C. This lipoprotein shift in cholesterol occurred while total saturates, monoenes, and polyenes were held constant and only 12:0, 14:0, and 16:0 were varied, clearly linking the consumption of 16:0 as palm oil (compared with 12:0 plus 14:0 as coconut oil) with this shift. Although it is well acknowledged that saturated fat is associated with an increase in total cholesterol, including HDL (and that polyenes generally lower the HDL along with LDL) in humans (26), evidence exists in monkeys (27) that the depression in HDL by polyenes may depend on a high percentage (40%) of the dietary calories being derived from fat. These hamster data provide the first indication that the primary increase in HDL-C associated with associated with saturated fat consumption may be specifically attributable to the dietary 16:0 content.

When saturated fatty acids from Diet 4 were replaced by polyunsaturates (Diets 5 and 6) while monounsaturates were held more or less constant, the effect on plasma apo A1, apo B, and apo B/apo A1 ratio was demonstrably influenced by the type and amount of saturated fat remaining. On the one hand, Diet 6 (AHA) induced the lowest total plasma cholesterol while main-

taining an apo B/apo A1 ratio similar to that observed with most other diets. This occurred when 50% of the 16:0 in Diet 4 was removed and monoenes plus polyenes were increased by 15%. By contrast, even though the AFB (Diet 5) reduced total saturates and increased polyunsaturates, doubling the P/S ratio from 0.38 to 0.64 between Diets 4 and 5, these substitutions doubled the apo B/apo A1 ratio and caused the greatest expansion in LDL-C. This occurred as 16:0 was reduced 50% and 14:0 increased 8-fold, re-emphasizing the negative impact of 14:0 on the LDL/HDL ratio and the strong positive effect of 16:0 on the HDL level.

Caveats in the Plasma Cholesterol Data. The plasma cholesterol data obtained with these hamsters did not fit the Hegsted or Keys regression equations very well. This indicates that hamsters fed low fat diets (13% total energy) fail to model the typical human cholesterolemic response to dietary saturated fat fed at 40% dietary energy. Part of the discrepancy may reflect the fact that the butter as fed included 16% water, which reduced the saturated fat content of the AFB slightly (1.3% of the total energy), but ongoing experiments with hamsters fed fat at 40% total energy reveal good correlations between the observed plasma cholesterol concentrations and those predicted by the Keys and Hegsted formulas (unpublished observations). Thus, the quantity of fat consumed appears to contribute significantly to the magnitude of the shift and the goodness of fit.

An additional caveat associated with these data is our recent observation (unpublished) that the typical dextran sulfate-Mg^{2+} or phosphotungstic acid precipitation of apo B and E tends to overestimate the LDL and underestimate the VLDL contribution to the lipoprotein cholesterol pool in the hamster, even though the HDL cholesterol estimate is relatively accurate. Thus, although the relative estimates of the dietary fat effect on the LDL/HDL ratio between diets is considered valid, the absolute estimate of cholesterol distribution among lipoproteins probably is not. Nonetheless, our basic argument is supported by the fact that the LDL/HDL ratios were generally substantiated by the plasma apo B/apo A1 ratios.

In this context, it should be emphasized that the LDL/HDL profile of Syrian hamsters is essentially the reverse of that in humans, i.e., generally two to four times as much cholesterol is transported as HDL compared with LDL, even when the plasma cholesterol pool is expanded by dietary saturated fat (e.g., compare Diet 4 with Diet 6). In addition, with the expansion of the lipoprotein pool during cholesterol supplementation, the primary increase in cholesterol mass was distributed in VLDL, HDL, intermediate density lipoprotein, and LDL in that order.[1] These observations serve to emphasize that the saturated fat elevation in plasma cholesterol in hamsters is not restricted to (or even dependent upon) increased plasma LDL and decreased LDLr activity (14) but reflects the expansion of other lipoprotein cholesterol pools as well, depending on the chain length of the dietary saturated fatty acids and amount of cholesterol fed.

Triglyceridemia. One striking aspect of the hamster plasma lipid profile is the relative triglyceridemia that is typically encountered in this species and that is exacerbated by purified diets containing saturated fat or cholesterol (28) and even more dramatically by a high level of fish oil consumption in the absence of dietary cholesterol (29). The abundant plasma triglyceride pool is unlike most other animal models but similar to humans, whereas the hypertriglyceridemia due to fish oil is opposite to the response in humans (5). The AHA diet, with the highest P/S ratio, resulted in the lowest triglycerides. In contrast, plasma triglycerides were elevated by saturated fat (witness Diet 1, with the lowest P/S ratio) but especially by 16:0 (witness the steady increase in triglycerides between Diets 2, 3, and 4 when total saturates were held constant and 16:0 replaced 12:0 plus 14:0).

mRNA Abundance and Dietary Fat. Previous studies in monkeys (15) revealed decreased total plasma cholesterol and HDL-C with no change in plasma apo A1 when either polyenes or 16:0 were increased at the expense of saturates, in a similar dietary comparison where fat represented 31% of calories. In the present study, dietary 16:0 (Diets 3 and 4) increased HDL-C and plasma apo A1 levels and was coupled with the highest apo A1 mRNA abundance in the liver and gut. Differences in apo A1 mRNA abundance were significant between Diets 4 and 1 (liver) and Diets 1, 2, and 3 (gut). Thus, the greatest intake of dietary saturates per se (represented by 12:0 plus 14:0 from coconut oil, but almost *no* 16:0 (Diet 1) failed to enhance apo A1 mRNA, plasma apo A1, or HDL-C, whereas addition of 16:0 as palm oil elicited elevations in all these parameters. The difference between monkeys (15) and these hamsters in their cholesterolemic response to 16:0 can be attributed to the low percentage of fat energy in the present hamster diets (unpublished data). However, both sets of data reveal positive attributes of the 16:0 associated with palm oil on lipoprotein metabolism, i.e., in a high fat diet 16:0 reduces total cholesterol relative to 12:0 plus 14:0 (15) (unpublished data), whereas in a low fat high carbohydrate diet 16:0 greatly expands the HDL-C at the expense of LDL-C. These data, now obtained from two species, emphasize the need for rigorous differentiation between the metabolic aspects of the various dietary saturated fatty acids. Also, the relative effect of high fat (31% kcal in the monkey study) versus low fat (13% kcal herein) diets in this scenario requires further elaboration.

It is noteworthy that rather minor increments in apo A1 mRNA abundance (<25% difference) generally

reflected the magnitude of change in plasma HDL-C (generally <25%). This is consistent with similar data in African green monkeys, where the plasma apo A1 correlated with small increments in hepatic mRNA abundance (30). The liver mRNA data might be expected to play a more important role than gut mRNA in this study, because these were high carbohydrate diets and the liver would presumably have a greater triglyceride and VLDL synthesis and secretory role than the gut, where a high fat diet would favor chylomicron formation and secretion.

Another noteworthy aspect of the mRNA data was the greater abundance of hepatic LDLr mRNA associated with the high dietary 16:0 intake from palm oil (Diets 3 and 4), an observation similar to our previous results involving the feeding of a 5% palm oil diet in this model (25). Both Diets 3 and 4 induced more than 50% higher LDLr mRNA abundance than the control diet (AFB, Diet 5), which also revealed the least favorable LDL/HDL ratio. The LDLr mRNA abundance associated with Diets 3 and 4 was also greater than Diet 1 (with higher plasma apo B/apo A1 and LDL/HDL ratios). This corroborates the overall pattern of lipoprotein metabolism associated with these diets, i.e., that the AFB induced the lowest plasma HDL-C, highest LDL-C, and the least favorable LDL/HDL and apo B/apo A1 ratios, whereas Diet 4 (16:0) provided the most favorable ratios. And even though 16:0 was associated with the highest total cholesterol, the increase was transported as HDL-C, which is considered favorable (7). Furthermore, failure to synthesize the LDLr and failure to clear plasma LDL would predictably lead to the unfavorable ratios observed, whereas enhanced LDLr activity seemed to be associated with enhanced HDL-C levels and favorable LDL/HDL and apo B/apo A1 ratios. Because HDL is largely derived from VLDL (7), it would appear that the 16:0 in palm oil increased VLDL production (witness the elevated triglycerides with 16:0 feeding) and turnover with production of HDL.

The fatty acid profile of the AFB provides no clue why it should depress the LDLr mRNA abundance and increase the LDL/HDL ratio so strikingly in comparison with all other diets. A trace amount of cholesterol (0.01% of diet) would be contributed by the butter present in that diet, which seems nominal. Nor was the total plasma cholesterol elevated to the level expected from a dietary cholesterol load in hamsters (28). The AFB diet also had the highest level of 18:3n3, which may have been a factor, but no trend for 18:3n3 was observed among the other parameters. Another possibility under investigation is that the 16:0 in palm oil was associated with a nonsaponifiable factor(s) capable of modulating cholesterol metabolism and that this agent was absent in the AFB and coconut oil.

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