

Effects of dietary fats and cholesterol on liver lipid content and hepatic apolipoprotein A-I, B, and E and LDL receptor mRNA levels in cebus monkeys

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Abstract The effects of the long-term administration of the dietary fats coconut oil and corn oil at 31% of calories with or without 0.1% (wt/wt) dietary cholesterol on plasma lipoproteins, apolipoproteins (apo), hepatic lipid content, and hepatic apoA-I, apoB, apoE, and low density lipoprotein (LDL) receptor mRNA abundance were examined in 27 cebus monkeys. Relative to the corn oil-fed animals, no significant differences were noted in any of the parameters of the corn oil plus cholesterol-fed group. In animals fed coconut oil without cholesterol, significantly higher ($P < 0.05$) plasma total cholesterol (145%), very low density lipoprotein (VLDL) + LDL (201%) and high density lipoprotein (HDL) (123%) cholesterol, apoA-I (103%), apoB (61%), and liver cholesteryl ester (263%) and triglyceride (325%) levels were noted, with no significant differences in mRNA levels relative to the corn oil only group. In animals fed coconut oil plus cholesterol, all plasma parameters were significantly higher ($P < 0.05$), as were hepatic triglyceride (563%) and liver apoA-I (123%) and apoB (87%) mRNA levels relative to the corn oil only group, while hepatic LDL receptor mRNA (-29%) levels were significantly lower ($P < 0.05$). Correlation coefficient analyses performed on pooled data demonstrated that liver triglyceride content was positively associated ($P < 0.05$) with liver apoA-I and apoB mRNA levels and negatively associated ($P < 0.01$) with hepatic LDL receptor mRNA levels. Liver free and esterified cholesterol levels were positively correlated ($P < 0.05$) with liver apoE mRNA levels and negatively correlated ($P < 0.025$) with liver LDL receptor mRNA levels. Interestingly, while a significant correlation ($P < 0.01$) was noted between hepatic apoA-I mRNA abundance and plasma apoA-I levels, no such relationship was observed between liver apoB mRNA and plasma apoB levels, suggesting that the hepatic mRNA of apoA-I, but not that of apoB, is a major determinant of the circulating levels of the respective apolipoprotein. ■ Our data indicate that a diet high in saturated fat and cholesterol may increase the accumulation of triglyceride and cholesterol in the liver, each resulting in the suppression of hepatic LDL receptor mRNA levels. We hypothesize that such elevations in hepatic lipid content differentially alter hepatic apoprotein mRNA levels, with triglyceride increasing hepatic mRNA concentrations for apoA-I and B and cholesterol elevating hepatic apoE mRNA abundance.—Hennessy, L. K., J. Osada, J. M. Ordovas, R. J. Nicolosi, A. F. Stucchi, M. E. Brousseau, and E. J. Schaefer. Effects of dietary fats and cholesterol on liver lipid content

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Circulating lipoprotein levels play a significant role in the pathogenesis of coronary artery disease (CAD). Data derived from both laboratory animal experiments and from human epidemiologic studies provide evidence of a positive correlation between elevated low density lipoprotein (LDL) concentrations and CAD risk, while the inverse is true for increased levels of high density lipoproteins (HDL) (1–3). Plasma lipoprotein levels are determined by the interaction of a number of genetic and environmental factors, with diet being of great significance in the latter category.

The relevance of dietary fatty acid and cholesterol content with respect to the regulation of plasma lipids is well documented (4–6). Both in the presence and absence of significant dietary cholesterol, saturated fat compared with polyunsaturated fat causes significant increases in LDL cholesterol levels (4, 7), with chain length being a determinant in the hypercholesterolemic effect of saturates (8–10). The influence of these nutritional factors on HDL cholesterol levels, however, is somewhat more controversial. While it appears conclusive that saturated fatty acids increase HDL cholesterol concentrations (5), the evidence concerning

Abbreviations: CAD, coronary artery disease; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; IDL, intermediate density lipoproteins.

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polyunsaturates is equivocal. High polyene diets have been reported to lower HDL cholesterol (4, 11, 12), or show no effects (13, 14).

As a result of their phylogenetic similarity to humans, their diet responsiveness, and their ability to develop atherosclerosis, nonhuman primates provide an excellent model for studies of diet-induced atherosclerosis. In addition to providing evidence of the involvement of dietary fat and cholesterol in the regulation of the receptor-mediated catabolism of LDL (15, 16), recent investigations in nonhuman primates have demonstrated that these nutrients may mediate the expression of genes encoding apolipoproteins and the LDL receptor. In studies in African green monkeys, Sorci-Thomas et al. (17) have reported that dietary fat type can differentially alter apoA-I gene expression in a tissue-specific manner, with saturates enhancing and polyunsaturates reducing expression, respectively. This group has further shown that, when fed similar atherogenic diets, cynomolgus monkeys synthesize comparatively less apoA-I than do African green monkeys, the latter being much less susceptible to diet-induced hypercholesterolemia (18). While other studies by this group (19) have failed to demonstrate an influence of such nutrients on hepatic apoB mRNA abundance, alterations in this parameter have been induced by cholesterol feeding in both rabbits (20) and rats (21), suggesting species-specific regulation at the molecular level. Moreover, hepatic apoE gene expression appears to be regulated by dietary saturated fat and cholesterol, as rhesus monkeys fed diets enriched in these nutrients have demonstrated increased synthesis of apoE during liver perfusion studies (22). In baboons, Fox et al. (23) have also reported significant reductions in mRNA for hepatic LDL receptors, consequent to the consumption of diets enriched in saturated fat and cholesterol.

In order to further define the mechanisms by which dietary fatty acids and cholesterol affect circulating lipid and apolipoprotein levels, we assessed the relationships between these parameters and hepatic lipid and mRNA content in cebus monkeys fed diets of differing fatty acid composition in the presence or absence of cholesterol. We hypothesized that, relative to a corn oil-enriched diet without cholesterol, a diet high in saturated fat and cholesterol may induce changes at the molecular level by increasing hepatic lipid content, resulting in the differential regulation of apoA-I, B, and E and LDL receptor mRNA levels.

MATERIALS AND METHODS

Animals and diets

Twenty-seven adult cebus monkeys (*Cebus albifrons*) between the ages of 4 and 10 years were randomized

into four groups and fed semipurified diets that supplied 31% of calories as fat, either as corn oil (Best Foods, Englewood, NJ) or as coconut oil (Capital City Products, Columbus, OH) with or without 0.1% (wt/wt) dietary cholesterol for 3 to 10 years. Fatty acid analyses of the two dietary fats have been reported elsewhere (24). As we have previously stated (16), non-hydrogenated coconut oil, rather than butter or animal fat, was used as the saturated fat because it does not contain any cholesterol and is the only fat capable of elevating plasma cholesterol levels in the absence of dietary cholesterol, at least in this animal model. The experimental protocol was in accordance with the guidelines of the Committee on Animals of the University of Lowell Research Foundation and those of the Committee on Care in Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW publication #85-23, revised 1985).

Biochemical analyses

Subsequent to a 16-h fast, blood samples from the femoral vein were collected in 0.1% EDTA, using aseptic technique. Plasma was isolated by centrifugation at 2500 rpm, 4°C, for 20 min, and phenylmethyl sulfonyl fluoride (PMSF) (2×10^{-4} M) and N-ethylmaleimide (1.25 mg/ml) were added as proteolytic and lecithin:cholesterol acyltransferase inhibitors, respectively. VLDL + IDL ($d < 1.019$ g/ml), LDL (1.019 g/ml $< d < 1.063$ g/ml), and HDL (1.063 g/ml $< d < 1.21$ g/ml) were isolated by sequential ultracentrifugation (25) at 4°C using a Beckman 50.2 Ti rotor. Protein was analyzed by the method of Lowry et al. (26), using bovine serum albumin as a standard. Total cholesterol, free cholesterol, phospholipids, and triglycerides were determined on an Abbott Diagnostics ABA 200 Bichromatic analyzer using enzymatic reagents (27). HDL-cholesterol was determined in a similar manner after dextran-magnesium sulfate precipitation of VLDL and LDL (28). VLDL + LDL cholesterol was estimated by subtracting HDL-cholesterol from total cholesterol. Apolipoproteins A-I and B were measured using radial immunodiffusion (RID), as described by Chong et al. (24), using goat polyclonal antiserum directed against nonhuman primate apolipoproteins. The apoE content of lipoprotein fractions was assessed semiquantitatively by laser densitometric scanning of 4–22.5% gradient gels subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Liver biopsy

Liver wedge biopsies were obtained from each animal by laparotomy after anesthesia with ketamine-HCl (5 mg/kg). A section of liver was placed in 4% formal-

dehyde, phosphate-buffered saline, pH 7.4, for the preparation of histological slides. The remaining biopsy specimen was flash-frozen in liquid nitrogen and stored at -70°C until use. An additional tissue sample was obtained from each animal 16 months subsequent to the initial biopsy and was treated in the same manner as described above.

Tissue lipid analyses

Tissue lipid was analyzed in homogenates of small portions of liver biopsy specimens in four animals from each diet group. Samples of 40–60 mg of liver were homogenized on ice in 10 mM Tris-HCl, pH 8.0, 1 mM PMSF, 150 mM sodium chloride, 0.1 mM leupeptin with a Polytron. Aliquots were taken for protein and lipid measurements. Lipid was obtained from liver homogenates by chloroform–methanol extraction, as described by Bligh and Dyer (29). Total and free cholesterol were measured by gas–liquid chromatography, according to the method of Bates et al. (30). Cholesteryl ester was calculated after subtracting free cholesterol from total cholesterol and multiplying by 1.68 to correct for fatty acid content. Triglycerides were quantitated by the thin-layer chromatography method of Kritchevsky et al. (31).

Preparation of RNA

Hepatic biopsy specimens were homogenized by guanidine thiocyanate, extracted with phenol–chloroform–isoamyl alcohol 50:48:2, and applied to a cushion of cesium chloride, as described by Chirgwin et al. (32). HepG2 and HeLa cells were used in the procedure as positive and negative controls, respectively. The $A_{260}:A_{280}$ ratios of the resultant samples were greater than 2.0. To evaluate the integrity of the RNA, 2- μl aliquotes were electrophoresed on 1.0% agarose gels containing Tris–acetate–EDTA buffer and 0.01% ethidium bromide. The 28S and 18S RNA components were visualized by ultraviolet light, and a ratio equivalent to, or exceeding, 2:1 was considered indicative of acceptable RNA quality.

Preparation of DNA probes

Inserts containing cDNA probes for human apoA-I (33), apoB (34), apoE (35), and the LDL receptor (ATCC #57004) were purified by gel electrophoresis and electroelution. A 1.6-kb insert of rat beta tubulin, known to cross-react specifically with cebus and human DNA and RNA, was subcloned into pGM4 and used as a standard. The purified DNA inserts were radiolabeled with [^{32}P]dCTP by random priming (BRL, Bethesda, MD) to a specific activity of 10^8 – 10^9 cpm/ μg .

Analysis of apolipoprotein and LDL receptor mRNA

RNA was denatured in 40 mM 3-(N-morpholino)propane sulfonic acid (MOPS), pH 7.0, 1 mM sodium acetate, 1 mM EDTA, 6% formaldehyde, and 50% formamide by heating to 65°C for 15 min. Thirty μg of sample was electrophoresed on 1.0% agarose gels containing 2.2 M formaldehyde in 20 mM MOPS buffer, pH 7.0, 5 mM sodium acetate, 0.1 mM EDTA for a period of 20 h at 15 volts in a running buffer consisting of 40 mM MOPS, pH 7.0, 1 mM sodium acetate, 1 mM EDTA. The RNA was blotted onto nylon filters (Amersham Corporation) by capillary transfer and fixed by exposure to ultraviolet light for 2 min. Prehybridization and hybridization were performed for 2 and 18 h, respectively, at 42°C . Filters were washed at 50°C with $1 \times \text{SSC}$ (15 mM sodium chloride, 1.5 mM sodium citrate $\bullet 2\text{H}_2\text{O}$), 0.1% sodium dodecyl sulfate, and dried, mounted, and exposed to Kodak film at -70°C using intensifying screens. For slot-blot analysis, RNA was resuspended in 6.15 M formaldehyde, $10 \times \text{SSC}$ (1.5 M sodium chloride, 0.15 M sodium citrate $\bullet 2\text{H}_2\text{O}$), and denatured at 65°C for 15 min. One-, 5- and 10- μg quantities of RNA were applied to nylon filters using a slot-blot apparatus (Schleicher and Schuell). The RNA was fixed to the filters as described above and processed similarly to the Northern blots. Blots were stripped and rehybridized with various probes. Films were scanned by laser densitometry in the two-dimensional mode utilizing an LKB 2202 densitometer (LKB Instruments, Inc., Paramus, NJ) with LKB 2400 GSXL software. The relative abundance of each message was measured in absorbance units and normalized using beta tubulin as an internal standard.

Statistical analyses

The data collected in this study were entered and stored in a VAX II/780 computer (Digital Equipment Co., Maynard, MA) using the RS/1 scientific package (BBN Research Systems). Analysis of variance was performed with Statistical Analysis Systems (SAS) software (SAS Institute, Cary, NC), while SPSS-X software (SPSS Inc., Chicago, IL) was used to test for correlations between parameters. Correlations between hepatic message levels and other biological parameters were performed on pooled data due to the inability to study each animal on each dietary treatment. Because a major goal of our study was to evaluate the independent, as well as concerted, effects of dietary saturated fat and cholesterol, our data in the text are expressed as percentage change relative to the mean values of corn oil-fed animals which were considered as baseline.

RESULTS

Plasma analyses

Table 1 shows the total plasma lipid, HDL-cholesterol, VLDL + LDL cholesterol, and apoA-I and apoB concentrations for each of the four diet groups during the experimental period. The addition of cholesterol to the corn oil diet did not produce statistically significant differences in any of the measured parameters. As expected, saturated fat feeding was associated with significantly higher plasma total cholesterol (145% higher), VLDL + LDL cholesterol (201% higher), and HDL cholesterol (123% higher) concentrations as compared with those of the corn oil group. ApoA-I (103% higher), apoB (61% higher), VLDL apoE (330% higher), and LDL apoE (120% higher) levels were also higher in these animals relative to those of the corn oil group (**Fig. 1**). Similar effects were demonstrated in the animals consuming coconut oil in the presence of dietary cholesterol, with plasma total cholesterol (136% higher), VLDL + LDL cholesterol (239% higher), and HDL-cholesterol (95% higher) values being significantly greater relative to those of the corn oil group. Increments in apoA-I (79% higher) and apoB (82% higher) levels paralleled those of their constituent lipoproteins, although to a lesser extent, while dramatic increases in both VLDL apoE (755% higher) and LDL apoE (680% higher) were observed in these animals (**Fig. 1**). Additionally, plasma triglyceride (60% higher) levels were significantly different in this group compared with those of corn oil-fed animals. Of interest is the demonstration that increased dietary fat saturation was associated with higher levels of both HDL cholesterol and apoA-I concentrations in the absence of dietary cholesterol.

Hepatic lipid content

Histological examination of the biopsy specimens revealed normal morphology in all diet groups; however, increased fat deposition was noted in the livers of

those animals fed coconut oil both in the presence and absence of dietary cholesterol (data not shown). Relative to animals fed corn oil, livers from coconut oil-fed monkeys had significantly elevated cholesteryl ester (263% higher) and triglyceride (325% higher) content (**Table 2**). While the addition of dietary cholesterol to coconut oil exacerbated the hepatic triglyceride response (563% higher), no such effect on hepatic cholesteryl ester concentrations was observed in this group. Diet did not significantly influence either hepatic total or free cholesterol content.

Apolipoprotein A-I, B, E, and LDL receptor mRNA determination

Labeled cDNA probes were hybridized to a single electrophoretic mRNA species of the appropriate molecular weight (apoA-I, 1.1 kb; apoB, 14 kb; apoE, 1.2 kb; LDL receptor, 5.3 kb; data not shown), and slot-blot hybridizations were performed to evaluate the relative abundance of specific mRNAs, utilizing beta tubulin as a standard to correct for differences in total RNA loading.

Table 3 illustrates the effect of the degree of dietary fat saturation and cholesterol on hepatic mRNA levels. Relative to corn oil feeding alone, the only statistically significant changes in hepatic mRNA abundance were demonstrated by the animals fed the coconut oil diet supplemented with dietary cholesterol, although a 78% increase in liver apoA-I mRNA was observed in the coconut oil only group. In the coconut oil plus cholesterol group, hepatic apoA-I mRNA (123% higher) and apoB mRNA (87% higher) were significantly greater, while hepatic LDL receptor message abundance (-29% lower) was significantly lower relative to the corn oil without cholesterol-fed animals. Although a 54% increase in hepatic apoE mRNA abundance was demonstrated in these animals, it was not statistically significant, perhaps consequent to the intragroup variability in response to diet and to the small number of animals studied. As expected, the

TABLE 1. Effect of dietary fat saturation and cholesterol on plasma lipids and apolipoproteins

Diet Group	Corn (n = 5)	Corn + Chol (n = 7)	Coco (n = 8)	Coco + Chol (n = 7)
Parameter				
			<i>mg/dl</i>	
Total cholesterol	152 ± 32	187 ± 23	372 ± 51 ^{ab}	358 ± 65 ^{ab}
HDL cholesterol	101 ± 10	112 ± 18	225 ± 32 ^{ab}	197 ± 40 ^{ab}
VLDL-LDL cholesterol	44 ± 25	67 ± 27	137 ± 29 ^{ab}	149 ± 45 ^{ab}
Triglyceride	37 ± 21	42 ± 7	46 ± 12	59 ± 12 ^b
ApoA-I	202 ± 23	231 ± 24	411 ± 74 ^{ab}	362 ± 80 ^{ab}
ApoB	67 ± 3	61 ± 15	108 ± 18 ^{ab}	122 ± 23 ^{ab}

Values are given as means ± SD. Corn, corn oil; coco, coconut oil; chol, cholesterol.

^aSignificantly different from corn, $P < 0.05$.

^bSignificantly different from corn + chol, $P < 0.05$.

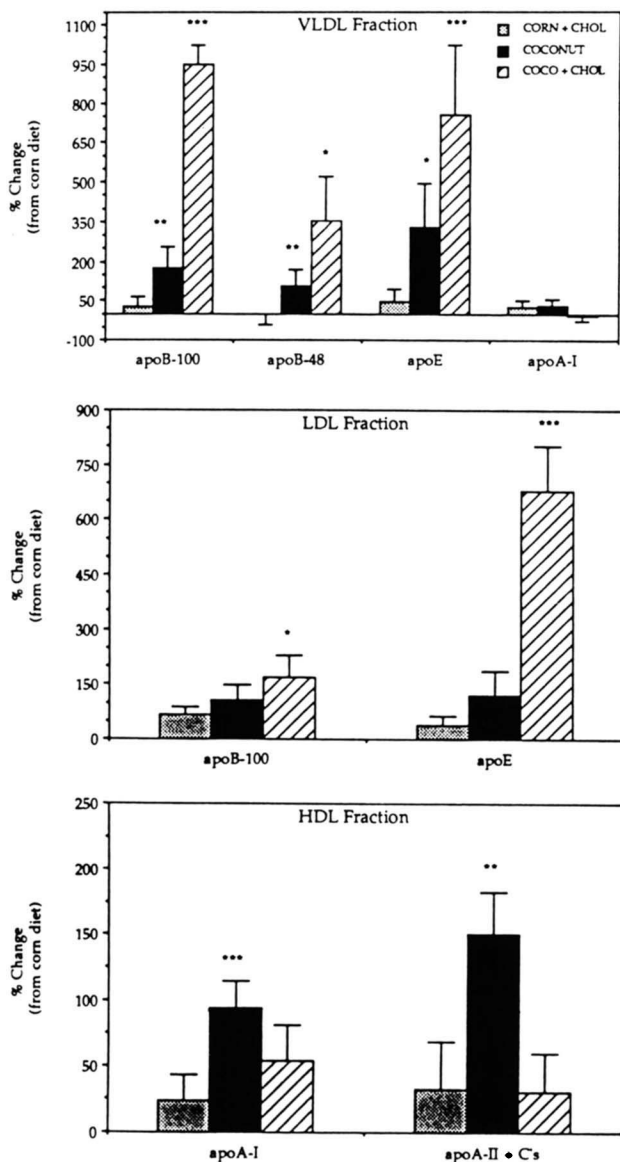


Fig. 1. Effects of dietary fat and cholesterol on lipoprotein apolipoprotein levels relative to animals in the corn oil group. Lipoprotein fraction apolipoprotein content was assessed semiquantitatively by laser densitometric scanning of 4–22.5% gradient gels, as described in Methods. * $P < 0.05$; ** $P < 0.025$; *** $P < 0.01$; Student's t -test.

relative abundance of LDL receptor mRNA was greatest in those animals fed the polyunsaturated fat diet from which cholesterol was excluded.

Hepatic apoA-I, apoB, apoE, and LDL receptor mRNA levels were used for standard correlation coefficient analysis with other study parameters as shown in **Table 4**. Liver apoA-I mRNA abundance was positively correlated with plasma total cholesterol, VLDL + LDL cholesterol, HDL cholesterol, plasma triglyceride, plasma apoA-I, and plasma apoB concentrations. Both hepatic apoB and LDL receptor mRNA levels, on the other hand, were not significantly associated with any

of the plasma parameters, while liver apoE mRNA abundance was significantly correlated with HDL cholesterol, but not with either VLDL (0.404 , $P = 0.085$) or LDL (0.402 , $P = 0.087$) apoE levels. With respect to hepatic lipid content, both liver apoA-I and apoB mRNA levels were positively correlated with liver triglyceride, while hepatic apoE mRNA abundance was positively associated with liver total cholesterol, free cholesterol and cholesteryl ester. Significant inverse correlations were demonstrated between hepatic LDL receptor mRNA and liver total cholesterol, free cholesterol, cholesteryl ester, and triglyceride levels. Interestingly, hepatic apoA-I and apoB mRNA levels were positively correlated, and a negative association was demonstrated between liver apoE and LDL receptor mRNA levels.

DISCUSSION

The present experiment was designed to assess the lipemic response of cebus monkeys to dietary fat saturation and cholesterol. We selected this model because of its documented sensitivity to saturated fat consumption, particularly to the 12:0 and 14:0 fatty acids which predominate in coconut oil, in the presence of physiologic cholesterol levels (36). This allowed for a distinction to be made between dietary fat type and cholesterol effects. Although the cebus is primarily an HDL animal with respect to cholesterol transport, data reported in other nonhuman primates (37) indicate that the elevation in plasma lipid levels caused by saturated fat feeding is largely the result of an increased LDL pool, irrespective of the major circulating lipoprotein class.

Effects of dietary fat saturation and cholesterol on plasma and hepatic lipid levels

Our data demonstrate that the ingestion of saturated fat (coconut oil) in the presence of 0.1%

TABLE 2. Effect of diet on hepatic lipid content

Diet Group	Corn (n = 4)	Corn + Chol (n = 4)	Coco (n = 4)	Coco + Chol (n = 4)
Parameter				
	$\mu\text{g}/\text{mg}$ tissue protein			
Total cholesterol	18 ± 2	24 ± 3 (33%) ^a	34 ± 9 (89%)	26 ± 3 (44%)
Free cholesterol	14 ± 1	15 ± 2 (7%)	17 ± 4 (21%)	17 ± 2 (21%)
Cholesteryl ester	8 ± 1	15 ± 4 (88%)	29 ± 9 ^b (263%)	15 ± 3 (88%)
Triglyceride	40 ± 4	40 ± 7 (0%)	170 ± 58 ^c (325%)	265 ± 57 ^c (563%)

Values given as mean ± SEM.

^a Percent change from corn oil diet; data previously published in ref. 24.

^b Significantly different from corn, $P < 0.05$.

^c Significantly different from corn + chol, $P < 0.05$.

TABLE 3. Effect of dietary fat and cholesterol on relative hepatic mRNA abundance

Diet Group	Corn + Chol (n = 7)	Coco (n = 8)	Coco + Chol (n = 7)
ApoA-I mRNA	0.95 ± 0.45	1.78 ± 0.20	2.23 ± 0.39 ^a
ApoB mRNA	0.86 ± 0.17	1.01 ± 0.34	1.87 ± 0.51 ^a
ApoE mRNA ^b	1.40 ± 0.44	1.45 ± 0.43	1.54 ± 0.33
LDL receptor mRNA	0.86 ± 0.26	0.79 ± 0.26	0.71 ± 0.24 ^c

Results are expressed as the means ± SD for the monkeys in each diet group relative to corn oil-fed animals (n = 5) (assigned a value of 1).

^aSignificantly different from corn, *P* < 0.05.

^bOnly four animals per diet group.

cholesterol, as compared with that of polyunsaturated fat (corn oil) alone, results in elevations of the following parameters: 1) plasma lipoprotein cholesterol, triglyceride, and apolipoprotein levels, 2) hepatic triglyceride content, and 3) hepatic apoA-I, apoB, and apoE mRNA abundance, while suppressing LDL receptor gene expression. Surprisingly, hepatic cholesteryl ester content was only significantly increased (29%) in those animals fed coconut oil in the absence of dietary cholesterol, with both of the cholesterol-containing diets causing identical elevations (15%) in this parameter relative to corn oil only-fed animals. While our study indicates that dietary cholesterol does raise liver cholesteryl ester content relative to a polyunsaturated fat diet without cholesterol, these data also suggest that saturated fat (coconut oil) may have a substantial independent effect on hepatic cholesteryl ester levels. Since each animal was not studied on each dietary treatment, it should be noted that biological variability between animals could be partially responsible for such results, as could the low cholesterol content of our diets relative to those used in investigations where a significant increase in the hepatic cholesteryl ester content of animals fed safflower oil plus 0.78 mg cholesterol/kcal was observed (38).

Effects of dietary fat saturation and cholesterol on apoA-I mRNA levels

While the addition of dietary cholesterol did not significantly magnify the increases in VLDL + LDL cholesterol and apoB levels caused by coconut oil feeding alone, this amount of dietary cholesterol was sufficient to achieve relative decrements in HDL cholesterol and apoA-I concentrations. This latter observation confirms that of previous investigations (39, 40), where levels of dietary cholesterol substantially exceeded those of our study. In view of the decline in circulating levels of HDL cholesterol and apoA-I witnessed in the coconut oil plus cholesterol group, it is interesting that hepatic apoA-I mRNA abundance was greatest in the former group (coconut oil alone). Seemingly, this fur-

ther substantiates the role of enhanced catabolism (40), rather than decreased production, in explaining the reduction of HDL apoA-I levels when considering the action of dietary cholesterol. In fact, recent kinetic experiments performed on a subset of these animals demonstrated that while decreased production rate was the primary determinant of the lower apoA-I levels in those animals consuming polyunsaturated fat versus saturated fat, increased particle clearance was responsible for the reduced apoA-I values observed in saturated fat plus cholesterol fed animals relative to those fed saturated fat alone (41). Additionally, similar to the finding of Sorci-Thomas et al. (17) in African green monkeys, a significant, positive correlation between hepatic apoA-I mRNA abundance and plasma apoA-I concentrations was observed in our study, suggesting that hepatic production rate directly contributes to the regulation of circulating apoA-I levels. It is possible that diet could have influenced intestinal apoA-I mRNA abundance, which was not measured in our experiments, although other investigators have not demonstrated such an effect (17).

Effects of dietary fat saturation and cholesterol on apoB mRNA levels

A novel finding in this study concerns a differential effect of dietary fat saturation and cholesterol with respect to hepatic apoB mRNA abundance. Significantly higher apoB mRNA levels were noted in the livers of those animals fed coconut oil with cholesterol, relative to the corn oil group. Although other reports have not shown that diets enriched in saturated fat

TABLE 4. Correlation coefficient analysis (*r* values)

Parameter	Liver			
	ApoA-I mRNA	ApoB mRNA	ApoE mRNA	LDL Receptor mRNA
Plasma (n = 27)				
Total cholesterol	0.592 ^a	0.275	0.368	-0.298
VLDL + LDL cholesterol	0.564 ^a	0.255	0.262	-0.322
HDL cholesterol	0.540 ^a	0.269	0.412 ^b	-0.244
Triglyceride	0.494 ^a	0.318	0.270	-0.092
ApoA-I	0.535 ^a	0.264	0.405	-0.333
ApoB	0.622 ^a	0.362	0.188	-0.256
Liver (n = 16)				
Cholesterol	0.351	0.188	0.519 ^c	-0.598 ^a
Free cholesterol	0.249	0.199	0.508 ^b	-0.634 ^a
Cholesteryl ester	0.370	0.162	0.468 ^b	-0.512 ^c
Triglyceride	0.496 ^b	0.600 ^a	0.371	-0.663 ^a
Liver mRNA (n = 27)				
ApoA-I		0.480 ^b	0.230	-0.242
ApoB			0.259	-0.364
ApoE				-0.515 ^a

^aSignificant correlation, *P* < 0.01.

^bSignificant correlation, *P* < 0.05.

^cSignificant correlation, *P* < 0.025.

and cholesterol increase hepatic abundance of mRNA for apoB (19, 20), our findings are in agreement with those of Matsumoto et al. (21) in cholesterol-fed rats. However, it should be noted that a significant correlation was not observed between hepatic apoB mRNA abundance and plasma apoB levels in our study, indicating that liver apoB production may not be a major determinant of plasma apoB concentration in this species. Consequently, the fact that hepatic apoB mRNA was not significantly increased in those animals fed saturated fat without cholesterol is not surprising. In fact, in previously reported kinetic studies in these animals (16), the higher plasma LDL apoB levels associated with the coconut oil only and coconut oil plus cholesterol diet groups, as compared with those of the corn oil only diet group, were attributable to reductions in LDL apoB fractional catabolic rate, with no difference in synthesis. In light of recent evidence suggesting that a significant amount of intracellular apoB is degraded prior to secretion (42), it is plausible that elevated apoB mRNA abundance may not always lead to increased apoB secretion. Alternatively, dietary saturated fat and cholesterol may act synergistically to enhance apoB secretion and/or apoB gene expression. It is also possible that coconut oil plus cholesterol-fed animals secrete more VLDL apoB which is catabolized directly prior to conversion to LDL apoB. Yet another, although less likely, explanation for our results is individual animal variability in response to dietary manipulation, since different animals were examined on these long-term diet studies.

Effects of dietary fat saturation and cholesterol on apoE mRNA levels

Although values were not quite statistically significant, hepatic apoE mRNA abundance was clearly increased in those animals consuming coconut oil both with and without dietary cholesterol relative to those animals fed corn oil alone. Moreover, the relative concentrations of apoE in VLDL and LDL particles were significantly greater in the saturated fat-fed animals as compared with those of the polyunsaturated fat-fed animals, with dietary cholesterol greatly exacerbating this effect. Although correlations between lipoprotein apoE content and hepatic apoE mRNA levels did not quite reach statistical significance, these data suggest that the increase in hepatic apoE mRNA is associated with the increase in plasma apoE concentrations. It has been reported that cebus monkeys fed a similar saturated fat-enriched diet demonstrated significant increases in plasma apoE levels (43). Although diets high in saturated fat have been shown to cause an increase in apoE-containing

HDL, analysis of our cebus HDL by SDS-PAGE revealed no detectable apoE even when gels were overloaded with protein (data not shown). This finding was similarly demonstrated by Babiak, Lindgren, and Rudel (44) in African green monkeys fed an atherogenic diet consisting of saturated fat plus 0.4% cholesterol, four times the level of cholesterol used in the present study.

Effects of dietary fat saturation and cholesterol on LDL receptor mRNA levels

As demonstrated in Table 3, a steady decline in hepatic LDL receptor mRNA levels was also observed as we progressed from the corn oil only diet to the regimen containing coconut oil plus cholesterol, with only the addition of dietary cholesterol to saturated fat resulting in a statistically significant suppression of such levels. Although it was no surprise that animals consuming the corn oil diet had the greatest abundance of mRNA for the hepatic LDL receptor, it was somewhat unexpected that coconut oil feeding alone was not sufficient to induce significant reductions in this mRNA relative to the former group. These findings are, however, in accord with those of two other investigations (19, 23), both of which support the idea that dietary saturated fat and cholesterol act in concert to reduce hepatic LDL receptor mRNA abundance. The fact that the VLDL + LDL cholesterol levels of those animals fed coconut oil without cholesterol, relative to those fed corn oil alone, were dramatically increased in the absence of decreased liver LDL mRNA levels suggests that the influence of dietary fatty acid composition may be considerable with respect to LDL receptor activity, independent of production. In fact, as previously noted, studies in a subset of these same animals reported elsewhere (16) indicate a significant reduction in the receptor-mediated catabolism of LDL associated with coconut oil feeding in both the presence and absence of cholesterol. In vitro studies performed with peripheral mononuclear cells from these animals further substantiate the involvement of membrane fatty acyl content in this scenario by demonstrating that reductions in LDL clearance are highly correlated with membrane fluidity (45). Thus, saturated fat consumption may not only induce reductions in LDL receptor mRNA levels, but may enhance the incorporation of saturated fatty acids into the LDL receptor membrane and, ultimately, inhibit function, as well (46). However, the possibility also exists that such increases in LDL uptake, as cited from our earlier study (45), were the consequence of the relatively lower concentration of LDL in the plasma from which the mononuclear cells were isolated.

Correlations of hepatic message levels and hepatic lipid content

The negative correlation observed between hepatic cholesterol content and hepatic LDL receptor mRNA abundance in our study corroborates the results of *in vitro* experiments that have demonstrated that LDL receptor gene expression may be suppressed by increased cellular cholesterol levels (47, 48). While increments in liver cholesteryl ester concentration were not statistically significant in coconut oil plus cholesterol-fed animals, this diet attenuated the accumulation of hepatic triglycerides relative to animals fed coconut oil alone. This observation is not unlike that of Fox et al. (23), where excessive triglyceride accumulation in the livers of baboons fed coconut oil plus 1% cholesterol resulted in suppression of hepatic LDL receptor synthesis. Although the relationship between liver triglyceride content and hepatic LDL receptor mRNA levels is not known, it is possible that the aforementioned changes in cellular membrane fluidity, consequent to the diet-dependent incorporation of fatty acids into hepatic tissues (16), affect the recycling and/or processing of receptors. Of interest, strong positive associations were noted between hepatic triglyceride content and liver apoA-I and apoB mRNA abundance, implicating enhanced liver triglyceride storage in the regulation of their mRNA levels. Moreover, hepatic apoE mRNA abundance was positively correlated with all hepatic cholesterol parameters, thus suggesting that the differential regulation of intracellular lipid pools may be a determinant of lipoprotein apoE levels.

Although responsiveness to dietary fat and cholesterol does vary among individuals and species, our data are consistent with the concept that dietary saturated fat and cholesterol increase hepatic lipid stores which results, in turn, in the up-regulation of hepatic apolipoprotein A-I, B, and E mRNA levels, while suppressing hepatic LDL receptor mRNA levels. As noted previously, a limitation of our study focusses on the fact that each animal did not serve as its own control, thus increasing the variability in response observed on these diets and decreasing the differences between groups with respect to hepatic lipid and mRNA content. It must further be considered that additional factors that ultimately affect functional protein levels such as mRNA stability, post-transcriptional and translational modifications were not within the scope of this study and may account for some of our observations. Regardless, we believe that while the enhanced incorporation of saturated fatty acids and cholesterol into hepatic cell membranes primarily affects LDL receptor activity, increases in intracellular hepatic lipid are likely responsible for the alterations in liver apoA-I, B, and E mRNA levels. ■

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