Effect of Corn and Coconut Oil–Containing Diets With and Without Cholesterol on High Density Lipoprotein Apoprotein A-I Metabolism and Hepatic Apoprotein A-I mRNA Levels in Cebus Monkeys


The mechanism(s) by which diets containing corn or coconut oil (31% of energy as fat) totally free of cholesterol or with 0.1% added cholesterol by weight (0.3 mg/kcal) affect plasma high density lipoprotein cholesterol (HDL-C), apoprotein (apo) A-I levels, apo A-I kinetics, and hepatic apo A-I mRNA concentrations were investigated in 26 cebus monkeys. Coconut oil-fed monkeys had elevated levels of plasma total cholesterol (217%), very low density lipoprotein plus low density lipoprotein cholesterol (331%), HDL-C (159%), and apo A-I (117%) compared with corn oil–fed animals. Although the addition of cholesterol to the corn oil diet significantly increased these parameters, no such effects were seen when cholesterol was added to the coconut-oil diet. Both the type of fat and cholesterol in the diet significantly affected HDL apo A-I metabolism by decreasing apo A-I fractional catabolic rate and increasing apo A-I production rate in the coconut oil–fed groups. The decrease in apo A-I fractional catabolic rate in the coconut oil–fed animals was also associated with an increase in the HDL core lipid to surface ratio. Liver apo A-I mRNA abundance was elevated in the coconut oil–fed groups; however, dietary cholesterol had no affect on these levels. The lack of parallel effects of dietary fat and cholesterol on apo A-I production rate and liver apo A-I mRNA levels suggests that the increase in the apo A-I production rate observed in the coconut oil–fed groups resulted from the fat-induced rise in liver apo A-I mRNA abundance, whereas the cholesterol-induced rise in the apo A-I production rate resulted from a mechanism other than changes in liver apo A-I mRNA levels. (Arteriosclerosis and Thrombosis 1991;11:1719–1729)

High density lipoproteins (HDLs) play a key role in lipoprotein metabolism by regulating both the flux of lipoprotein components as well as the extracellular transport of cholesterol. Numerous studies have demonstrated an inverse relation between plasma HDL cholesterol (HDL-C) and/or apoprotein (apo) A-I levels and cardiovascular disease in both human and nonhuman primates. Recent evidence has shown that saturated-fat feeding and/or moderate intakes of dietary cholesterol typically elevate plasma HDL-C and apo A-I concentrations, whereas polyunsaturated-fat feeding and/or reduced levels of dietary cholesterol lower plasma HDL-C and apo A-I levels. These studies suggest that the quality of dietary fat can modify both the degree of fatty acid enrichment and the lipid class composition of specific lipoproteins that may subsequently alter their metabolism.

Nonhuman primate studies by Chong et al and Parks and Rudel have demonstrated that diet-induced alterations in the apo A-I fractional catabolic rate (FCR) can mediate plasma HDL levels. In contrast, studies of humans by Shepherd et al and Brinton et al have shown that diet-induced changes in the apo A-I production rate (PR) may play a more
central role in determining plasma HDL levels. In either case, the molecular mechanism(s) underlying the role of diet in regulating apo A-I kinetics, especially PR, are poorly understood. Recently, Sorci-Thomas et al.\(^{16}\) have demonstrated that saturated fat–induced elevations in liver apo A-I mRNA levels in African green monkeys were associated with increases in apo A-I PR, suggesting that dietary fat may play an important role in regulating plasma apo A-I concentrations by modifying hepatic apo A-I mRNA transcriptional or posttranscriptional events.

To further investigate the underlying molecular mechanism(s) whereby the type of dietary fat and cholesterol either independently or in concert regulate plasma HDL and apo A-I levels, apo A-I kinetics were investigated in cebus monkeys fed either corn or coconut oil containing diets that were either totally free of cholesterol or that contained 0.1% added cholesterol by weight (0.3 mg/kcal). Liver apo A-I mRNA levels were also measured in the same animals to ascertain if dietary fat and cholesterol-induced alterations in liver apo A-I mRNA levels were related to apo A-I kinetic parameters, especially PR, which might explain the observed changes in plasma apo A-I levels. The concurrent measurement of apo A-I kinetics and hepatic apo A-I mRNA levels in the same animals represented a unique opportunity to evaluate these potentially dependent parameters together in vivo. Our results indicate that both the type of dietary fat and cholesterol play a significant role in regulating plasma lipids and apo A-I levels as well as HDL apo A-I FCR and PR. In contrast, it appears that at the levels of cholesterol fed in this study, only the type of dietary fat affects hepatic apo A-I mRNA levels, suggesting that dietary cholesterol may influence apo A-I PR via mechanism(s) not related to changes in hepatic apo A-I mRNA levels.

### Methods

#### Animals and Diets

Twenty-six adult male cebus monkeys (Cebus albifrons) ranging in age from 4 to 8 years were divided into four groups and fed semipurified isocaloric diets containing 31% of calories as fat (see Table 1). Each diet contained 12.6% by weight of either corn oil (polyunsaturated to saturated fat ratio \([P/S]\) = 4.79) with \((\text{corn} + \text{+})\) or totally free \((\text{corn})\) of 0.1% added cholesterol (0.3 mg cholesterol/kcal), or nonhydrogenated coconut oil \((P/S=0.02)\) with \((\text{coco}+\text{+})\) or totally free \((\text{coco})\) of 0.1% cholesterol. Animals had been maintained on their respective diets for 3–8 years. Fatty acid analyses of the two dietary fats have been reported elsewhere.\(^{11}\) As we have previously stated,\(^{17}\) nonhydrogenated coconut oil rather than butter or animal fat was used as the saturated fat because it does not contain any cholesterol, and its consumption is associated, in nonhuman primates, with plasma lipoproteins that are similar in lipid composition to those produced by an average American diet.\(^{18}\) In addition, at least in this species, only coconut oil significantly elevates plasma cholesterol levels without added dietary cholesterol \((\text{Nicolosi et al., unpublished observations})\), an important consideration, as one of our objectives was to distinguish between the effects of the type of dietary fat and cholesterol on the regulation of HDL apo A-I levels and metabolism.

The animals used in these studies were maintained in accordance with the guidelines of the Committee on Animal Care and Use of the University of Lowell Research Foundation and the guidelines prepared by the Committee on Care in Use of Lab Animals of the Institute of Lab Animal Resources, National Research Council \((\text{DHEW publication No. 85-23, revised 1985})\).

#### High Density Lipoprotein Isolation

After a 16-hour fast, blood samples were collected from the femoral vein into EDTA-containing \((1.5 \text{ mg/ml})\) tubes. Plasma was separated at 2,500 rpm for 30 minutes at 4°C, followed by the addition of \(2 \times 10^{-4} \text{ M} \) phenylmethylsulfonyl fluoride and \(0.125\% \text{ N-ethylmale-}

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/100 g anhydrous mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>17.0</td>
</tr>
<tr>
<td>Dextrin</td>
<td>22.2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>22.6</td>
</tr>
<tr>
<td>Oil (corn or nonhydrogenated coconut)</td>
<td>12.6</td>
</tr>
<tr>
<td>Fiber (cellulose)</td>
<td>20.0</td>
</tr>
<tr>
<td>Cholesterol (with or without)</td>
<td>0.10</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.30</td>
</tr>
<tr>
<td>Vitamin mix*</td>
<td>0.43</td>
</tr>
<tr>
<td>Salt mix†</td>
<td>4.30</td>
</tr>
<tr>
<td>Banana flavoring</td>
<td>0.50</td>
</tr>
</tbody>
</table>

*The composition of the vitamin mix (grams per kilogram) consisted of thiamine hydrochloride 0.80, riboflavin 1.60, pyridoxine hydrochloride 0.08, calcium pantothenate 5.00, nicotinamide 8.00, folic acid 0.80, biotin 0.04, cyanocobalamin 0.03, menadione 0.1, d,l-a-tocopherol acetate (500 IU/g) 20.00, vitamin A acetate (500,000 IU/g) 5.00, vitamin D₃ (4,000,000 IU/g) 0.62, ascorbic acid 120, inositol 10, and tauroine 50, made up to 1 kg with 776 dextrin (Bioserv, Inc., Frenchtown, N.J., catalog No. F8280).

†Salt mix (grams per kilogram) contained magnesium oxide 32.0, calcium carbonate 290.5, potassium phosphate dibasic 347.9, calcium phosphate dibasic 72.6, magnesium sulfate 1.22, zinc chloride 0.91, cupric sulfate 0.29, chromium acetate 0.044, sodium selenite 0.004, and sodium fluoride 0.02 (Bioserv, Inc., Frenchtown, N.J., catalog No. F8530).
**Analytical Methods**

Total protein determinations were performed by the method of Markwell et al., with bovine serum albumin as a standard. Plasma total and HDL cholesterol and triglyceride levels were quantified by enzymatic methods. HDL-C was obtained after heparin–manganese precipitation of very low density lipoprotein (VLDL) and low density lipoprotein (LDL). Completeness of precipitation was assessed via agarose gel electrophoresis of the supernatant. VLDL-C and LDL-C were determined by subtracting LDL-C from total cholesterol. Because cebus monkeys in the fasting state have negligible amounts of VLDL-C, the VLDL-C and LDL-C fraction will be referred to as LDL-C for purposes of brevity. Plasma lipid assays were standardized by participation in the Centers for Disease Control/National Heart, Lung, and Blood Institute Standardization Program.

Total and free cholesterol, phospholipids, and triglycerides of HDL were measured with an Abbott Diagnostics ABA 200 Biochromatic analyzer and enzymatic reagents (Abbott A-Gent) as described by McNamara and Schaefer. Cholesterol ester mass represents the difference between total and free cholesterol after correction for fatty acid content. Plasma apo A-I levels were measured by radial immunodiffusion as previously described by Chong et al utilizing squirrel monkey anti-apo A-I. The intra-assay and interassay coefficients of variation for apo A-I were 4.1% and 2.0%, respectively. Fatty acids of HDL lipid fractions were analyzed by gas–liquid chromatography after lipid extraction and thin-layer chromatographic separation of individual lipid classes as we have previously described.

**RNA Isolation**

Liver wedge biopsies were obtained by laparotomy after the monkeys were anesthetized with ketamine hydrochloride (5 mg/kg). A section of each biopsy sample was retained for histological examination to assure typical hepatic morphology. Freshly isolated liver biopsies were homogenized in guanidine thiocyanate and extracted essentially as described by Chirgwin et al. RNA was also extracted (minus the phenol/sevac step) from Hep G2 and HeLa cells for use as positive and negative controls, respectively. The absorbance ratios at 260 and 280 nm were greater than 2.0.

**Preparation of DNA Probe and Quantification of Liver Apoprotein A-I mRNA**

A cDNA probe specific for human apo A-I was purified by gel electrophoresis and electrolution. Rat \(\beta\)-tubulin 1.6-kb insert was subcloned into pGEM4. The purified DNA inserts were radiolabeled with phosphorus-32 deoxyctydine triphosphate by random priming (BRL, Bethesda, Md.) to a specific activity of \(10^9-10^9\) cpmp/\(\mu\)g.

RNA quantification was performed by Northern and slot-blot hybridization as described by Sambrook et al. Autoradiographic signals were scanned by laser densitometry with an LKB 2202 densitometer (LKB Instruments, Inc., Paramus, N.J.) interfaced to an AT&T 6300 personal computer. Specific signals were scanned in the two-dimensional mode and then quantified with the use of an LKB 2400 Gelscan Xl software package. mRNA abundance was measured in absorbance units. Blots were stripped and re-probed with \(\beta\)-tubulin, exposed to film, and scanned. Relative abundance of apo A-I mRNA was normalized to that of \(\beta\)-tubulin.

**Apoprotein A-I Metabolic Studies**

HDLs were radiolabeled with carrier-free iodine-125 sodium iodide (Amersham, Arlington Heights, Ill.) by the iodine monochloride method of MacFarlane as modified by Bilheimer et al. Unbound iodine was removed by eluting the sample through a Sephadex G-25M column (PD-10, Pharmacia, Piscataway, N.J.) with phosphate-buffered saline. Protein-bound radioactivity was assessed by precipitation of an aliquot of the labeled HDL mixture with 10% (wt/vol) trichloroacetic acid (TCA). Labeled HDL infusion mixtures were found to be greater than 98% TCA precipitable, with less than 5% lipid labeling. Specific activities of the labeled HDL preparations ranged from 600 to 800 cpmp/\(\mu\)g protein.

Monkeys were prepared for the metabolic studies as we have previously described, with the modification that metabolic restraining chairs were not used. Instead, after a 16-hour fast, animals were anesthetized with ketamine, and after infusion with 25 \(\mu\)Ci \(^{125}\)I-HDL into the saphenous vein, 2-ml blood samples were collected from the femoral vein at 10 minutes and at 2, 4, 8, 24, 30, 48, 54, 72, 78, 96, 120, 144, and 168 hours from each ketamine-anesthetized monkey. Although ketamine-induced anorexia is initially apparent, feeding was usually resumed within 24–36 hours. Animals that did not resume eating within this period were given an enteral bolus of their regular diet. Earlier studies in this lab (Nicolosi et al., unpublished observations) have also shown that animals that were tethered and infused with radiolabeled lipoproteins (without the use of ketamine) had HDL apo A-I kinetics similar to those that had received repeated ketamine doses, indicating that during long-term studies early ketamine-induced anorexia does not influence steady-state kinetics, so long as feeding is resumed within 24–36 hours.

Thyroidal uptake of radiolabeled iodine was inhibited by the oral administration of approximately 1 ml of a 1% \(\text{KI}\) solution for 7 days before the injection of label and for the duration of the study. After measurement of the total radioactivity in an aliquot of plasma at each time point, apo A-I was immunoprecipitated from whole plasma with the use of squirrel monkey apo A-I antisera as previously described. The FCR of plasma apo A-I was deter-
Kinetic Analyses

The radioactivity in each immunoprecipitated apo A-I sample was expressed as a fraction of the radioactivity in the sample obtained 10 minutes after tracer administration. Plasma volume was determined by isotopic dilution from the counts at the 10-minute point. The FCR was calculated as we have previously described.11,17 using a curve-peeling computer program in accordance with the general principles described by Matthews.32 PRs (synthetic or transport) and apo A-I pool sizes were calculated with the formulas described in Table 5.

Statistical Analyses

The data were analyzed by two-way analysis of variance (ANOVA) to assess the effects of the type of dietary fat and cholesterol. When significant main effects were detected (p<0.05), multiple unpaired Student’s t tests, with the probability value modified by the Bonferroni method,33 were used to isolate differences between the following comparisons of interest: corn versus coco, corn versus corn(+), coco versus coco(+), and corn(+) versus coco(+) diet groups. Pearson’s product-moment correlation coefficients were used to evaluate linear relations between variables. Square-root transformation was performed on all data expressed as percentages before statistical analyses.34 All data are presented in the text, tables, and figures as mean±SEM.

Results

Plasma Lipids, Lipoprotein Cholesterol, and Apoprotein A-I Levels

Cebus monkeys fed coconut oil–containing diets free of added cholesterol had significantly higher levels of plasma total cholesterol (217%), LDL-C (331%), HDL-C (159%), and apo A-I (117%) than did the animals fed corn oil (Table 2). Plasma triglycerides were not significantly altered by the type of dietary fat. Although the addition of cholesterol to the corn oil diet resulted in significant increases in total cholesterol (74%), LDL-C (111%), HDL-C (53%), and apo A-I (41%), no such effects were observed with the addition of cholesterol to the coconut oil diet. Plasma triglycerides were not influenced by dietary cholesterol. No significant interactions between the type of fat and dietary cholesterol were detected for any of the plasma lipid parameters. The distribution of circulating cholesterol, although predominantly in the HDL fraction (75%) in monkeys fed corn oil, was partitioned approximately 60:40 between HDL and LDL of the monkeys fed coconut oil.

High Density Lipoprotein Composition

The HDL of monkeys fed coconut oil, compared with the corn oil–fed group, contained 25% less triglyceride (p<0.008), 23% more phospholipid (p<0.03), 9% less protein (p<0.04), and 11% more cholesteryl ester, which explained the 23% greater (p<0.03) cholesteryl ester to protein ratio in the HDL of the coconut oil–fed animals (Table 3). Although the addition of dietary cholesterol did not alter the cholesteryl ester to protein ratio of HDL from corn oil–fed animals, it resulted in a 19%
Density Lipoprotein Fatty Acid Composition of Dietary Oils and High Density Lipoprotein Metabolism

Two-way ANOVA indicated that the addition of dietary cholesterol appeared to have impacted primarily on HDL triglyceride levels, although significant interactions were noted for all chemical constituents of HDL (Table 3).

Fatty Acid Composition of Dietary Oils and High Density Lipoprotein

Fatty acid analyses of the dietary oils (data not shown) demonstrated that corn oil was richest in linoleate (18:2) and olate (18:1), while the medium-chain fatty acids laurate (12:0) and myristate (14:0) predominated in the highly saturated fatty acid-containing coconut oil.

In view of the greater dietary effects on the HDL triglyceride content (Table 3), it is not surprising that the fatty acid profile of the HDL triglyceride fraction was the most influenced by the differing dietary fats (Table 4). As expected from the dietary fatty acid profile, the HDL triglyceride fraction of corn oil-fed animals was enriched 55% in linoleate-containing coconut oil, whereas those of the coconut oil-fed animals contained threefold more saturated fatty acids (C12-18).

The incorporation of the medium-chain fatty acids (12:0 and 14:0) into the HDL phospholipid fraction of the coconut oil-fed animals was negligible. Interestingly, the concentrations of 16:0 were greater than 25% of the total fatty acids in phospholipids of both groups, despite the fact that neither corn nor coconut oil contains significant amounts of palmitate. Similarly, neither dietary oil contains significant amounts of oleate, while arachidonate levels were reduced by 47% when compared with coconut oil-fed animals.

Cholesteryl esters from the coconut oil-fed animals contained 20% more saturated fatty acids, while cholesteryl esters from animals fed corn oil contained 86% more polyunsaturates (C18:2-C20:4) and approximately 40% less monounsaturated fatty acids.

High Density Lipoprotein Apoprotein A-I Metabolism

Plasma pools of apo A-I in coconut oil-fed animals were significantly larger (10%) than those of corn

| TABLE 3. Effects of Dietary Fat Saturation and Cholesterol on High Density Lipoprotein Composition |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Diet group/ANOVA                              | Component (percent by weight)                    | Constituent ratio                               |
|                                                 | FC                                              | CE                                              | TG                                              | PL                                              |
|                                                 | (%)                                             | (%)                                             | (%)                                             | (%)                                             |
|                                                 |                                                 |                                                 |                                                 |                                                 |
| Corn                                            | 2.2±0.22                                        | 13.5±0.56                                       | 4.4±0.25*                                       | 19.1±0.82†                                      | 60.8±1.40†                                      | 0.22±0.01†                                      |
| Coco                                            | 3.0±0.18                                        | 15.0±0.68                                       | 3.3±0.26†                                       | 23.5±0.22                                       | 55.2±0.77†                                      | 0.27±0.01†                                      |
| Corn(+)                                         | 2.4±0.12                                        | 13.7±0.30                                       | 2.7±0.25†                                       | 21.6±0.91                                       | 59.6±1.10                                      | 0.23±0.07                                      |
| Coco(+)                                         | 2.1±0.09                                        | 12.8±0.48                                       | 4.9±0.36                                        | 21.1±0.63                                       | 59.1±0.60                                      | 0.22±0.07                                      |
| Two-way ANOVA (p<)                              |                                                 |                                                 |                                                 |                                                 |                                                 |                                                 |
| Type of fat                                     | NS                                              | NS                                              | 0.04                                            | 0.01                                            | 0.008                                          | 0.10                                            |
| Dietary cholesterol                            | 0.04                                            | 0.04                                            | 0.0001                                          | 0.003                                           | 0.02                                           | 0.02                                            |
| Interaction                                    | 0.004                                          | 0.04                                            |                                                 |                                                 |                                                 |                                                 |
| Values are mean±SEM for four animals per diet group. |
*Significantly different (p<0.05) from coco and corn(+) by unpaired t test with the Bonferroni modification. |
†Significantly different (p<0.05) from coco by unpaired t test with the Bonferroni modification.
‡Significantly different (p<0.05) from coco and corn(+) by unpaired t test with the Bonferroni modification.

reduction (p<0.02) in the cholesteryl ester to protein ratio in the coconut oil-fed animals.

| TABLE 4. Fatty Acid Composition of High Density Lipoprotein From Cebus Monkeys Fed Corn Oil or Coconut Oil |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Fatty acid                                      | Cholesteryl esters                              | Triglycerides                                  | Phospholipids                                   |
|                                                 | Corn oil                                        | Coconut oil                                    | Corn oil                                        | Coconut oil                                    |
| 12:0                                            | 0.1                                             | 0.6                                            | ND                                              | 5.0                                            | <0.1                                           | 0.5                                             |
| 14:0                                            | 0.3                                             | 5.8                                            | 0.4                                            | 9.5                                            | 0.2                                            | 2.4                                             |
| 16:0                                            | 8.7                                             | 22.9                                           | 14.6                                           | 36.1                                           | 26.5                                           | 27.4                                            |
| 18:0                                            | 1.0                                             | 2.0                                            | 8.0                                            | 12.3                                           | 15.5                                           | 17.8                                            |
| 18:1                                            | 18.0                                            | 30.1                                           | 29.5                                           | 28.5                                           | 10.2                                           | 9.6                                             |
| 18:2                                            | 68.0                                            | 29.2                                           | 47.6                                           | 7.3                                            | 37.1                                           | 22.8                                            |
| 20:4                                            | 3.9                                             | 9.5                                            | Trace                                          | 1.3                                            | 10.4                                           | 19.5                                            |
| Values were derived from pooled high density lipoprotein within each diet group and are expressed as mass percents. |
| Experiments were performed as described in the "Methods" section. Each value represents the average of at least two measurements. ND, not detectable.
oil-fed monkeys (Table 5). Although the addition of dietary cholesterol to the coconut oil-fed animals did not alter plasma apo A-I pool size, cholesterol supplementation to the corn oil-fed monkeys further increased pool sizes by 40%.

The decay curves, which were biexponential, demonstrated that the FCR of apo A-I in monkeys fed coconut oil alone was 30% slower ($p<0.01$) than that in those fed the corn oil diet (Table 5 and Figure 1); however, the fat effect on FCR was diminished to a 13% reduction ($p<0.10$) by the addition of dietary cholesterol. Although the addition of dietary cholesterol had no significant effect on apo A-I FCR in corn oil-fed monkeys, it resulted in a 27% increase in apo A-I FCR in the coconut oil-fed monkeys, which was not statistically significant. The prolonged residence times in the coconut oil-fed groups mirrored the reduced clearance rates.

Apo A-I PR was 48% higher ($p<0.01$) in coconut oil-fed monkeys than in monkeys fed corn oil. The addition of dietary cholesterol to either the corn or the coconut oil diets further increased apo A-I production rates by 49% ($p<0.02$) and 31%, respectively, although the latter was not statistically significant.

Hepatic apo A-I mRNA levels were elevated in the coconut oil-fed monkeys both with (136%) and without (78%) dietary cholesterol. Surprisingly, while there were significant fat and cholesterol effects on apo A-I PR, parallel dietary effects on hepatic apo A-I mRNA relative abundance were not observed. Two-way ANOVA (Table 5) indicated that at the level of dietary cholesterol fed in this study, only the type of dietary fat affected hepatic apo A-I mRNA levels, with the higher abundance being found in the coconut oil-fed groups. It should also be noted that one liver apo A-I mRNA value from the corn oil-fed group, denoted by an asterisk in Figure 4, was statistically omitted from the data base as an outlier.35

### Figures

**Figure 1.** Plasma decay curves of high density lipoprotein apoprotein (apo) A-I from corn and coconut oil-fed cebus monkeys, demonstrating the reduced rate of apo A-I clearance in saturated fat-fed animals. Cholesterol-fed animals had similar biexponential patterns of apo A-I decay.

**Table 5.** Effect of Dietary Fat Saturation and Cholesterol on Kinetic Parameters of High Density Lipoprotein Apoprotein A-I and Liver Apoprotein A-I mRNA

<table>
<thead>
<tr>
<th>Diet group/ANOVA</th>
<th>n</th>
<th>Apo A-I pool size (mg/kg)</th>
<th>Fractional catabolic rate (pools/day)</th>
<th>Production rate (mg/kg/day)</th>
<th>Residence time (days)</th>
<th>Liver Apo A-I mRNA (relative abundance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>5</td>
<td>82.9±8.8†</td>
<td>0.37±0.03♀</td>
<td>29.8±2.5†</td>
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<td>1.67±0.09♀</td>
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<tr>
<td>Coco</td>
<td>7</td>
<td>172.5±8.9</td>
<td>0.26±0.01</td>
<td>44.0±2.8†</td>
<td>4.1±0.3</td>
<td>2.98±0.22</td>
</tr>
<tr>
<td>Corn(+)</td>
<td>7</td>
<td>116.1±3.7♀</td>
<td>0.38±0.02</td>
<td>44.5±3.0</td>
<td>2.7±0.1</td>
<td>1.58±0.38♀</td>
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<tr>
<td>Coco(+)</td>
<td>7</td>
<td>176.6±18.2</td>
<td>0.33±0.03</td>
<td>57.7±6.6</td>
<td>3.2±0.4</td>
<td>3.73±0.32</td>
</tr>
</tbody>
</table>

Two-way ANOVA ($p<\text{•}$)

- Type of fat: 0.0001, 0.002, 0.004, 0.01, 0.0001
- Dietary cholesterol: NS, 0.052, 0.003, NS, NS
- Interaction: NS, NS, NS, NS, NS

Values represent mean±SEM.

(+) Signifies 0.1% added cholesterol. Coco, coconut; ANOVA, analysis of variance; NS, not significant.

*Kinetic parameters were calculated, assuming a biphasic exponential decay of high density lipoprotein (HDL) apoprotein (apo) A-I and plasma apo A-I concentration. Plasma volume was estimated by isotopic dilution of iodine-125-labeled HDL at 10 minutes. Production rate is equal to fractional catabolic rate×pool size.

†Significantly different ($p<0.05$) from coco and corn(+) by unpaired t test with the Bonferroni modification.

§Significantly different ($p<0.05$) from both coco and corn(+) by unpaired (test with the Bonferroni modification.

*Significantly different ($p<0.05$) from coco(+) by unpaired t test with the Bonferroni modification.

$+$Significantly different ($p<0.05$) from coco by unpaired t test with the Bonferroni modification.

Relations Between High Density Lipoprotein Cholesterol Apoprotein A-I Levels, Apoprotein A-I Metabolism, and Hepatic Apoprotein A-I mRNA Levels

Among all animals, plasma HDL-C was significantly correlated with plasma apo A-I levels ($r=0.960$, $p=0.0001$). Plasma apo A-I levels were inversely related to apo A-I FCR ($r=-0.562$, $p<0.003$) and positively correlated with apo A-I PR ($r=0.712$, $p=0.0001$) (Figure 2). Because HDL-C and apo A-I correlated so highly, it was not surprising that HDL-C was also inversely related to apo A-I FCR ($r=-0.540$, $p=0.004$) and positively related to apo A-I PR ($r=0.715$, $p=0.0001$). FCR did not correlate with PR ($r=0.111$). The calculated apo A-I pool size correlated with plasma apo A-I levels ($r=0.977$, $p<0.0001$) as determined by radial immunodiffusion.

Figure 3 shows the significant inverse relation between HDL composition, as measured by the ratio...
of HDL core to surface components (HDL-C/HDL-protein), and apo A-I FCR ($r = -0.523$, $p = 0.006$).

The ratio of HDL core to surface components was calculated as described by Brinton et al. 13

A significant positive relation was found between liver apo A-I mRNA relative abundance and plasma apo A-I levels ($r = 0.759$, $p = 0.0001$) and apo A-I PR ($r = 0.559$, $p = 0.003$) (Figure 4). Hepatic apo A-I mRNA relative abundance was also related to plasma HDL-C ($r = 0.749$, $p < 0.0001$). Liver apo A-I mRNA did not correlate with apo A-I FCR ($r = -0.325$).

Because the relations shown in Figures 2–4 were derived by pooling data from all diet groups, the significance of the correlations between variables only suggests the existence of a supporting relation. Although the trends remained the same, significant correlations within individual diet groups were not detected.

**Discussion**

The main goals of this study were to determine whether the type of dietary fat and cholesterol play a role in the regulation of plasma levels of HDL-C and
apo A-I and to investigate the mechanism(s) underlying these diet-induced effects by measuring apo A-I kinetics and hepatic apo A-I mRNA levels in these same animals.

The results of this study demonstrate significant effects of the type of dietary fat and cholesterol on 1) plasma lipids (except triglycerides) and apo A-I levels, 2) HDL lipid composition, and 3) HDL apo A-I FCR and PR. Interestingly, hepatic apo A-I mRNA abundance was significantly influenced only by the type of fat in the diet and not by dietary cholesterol, with the higher levels being found in the coconut oil-fed groups. In general, dietary cholesterol was not as effective in modulating these parameters in diets containing coconut oil. In general, dietary cholesterol was not as effective in modulating these parameters in diets containing coconut oil. It is possible that this phenomenon was due to the highly saturated nature of this oil, and a more typical saturated fat might not have attenuated the cholesterol effect to this extent. However, recent studies in our lab (Stucchi et al, unpublished data) of cynomolgus monkeys consuming a blend of oils that approximates a more typical American type diet (P/S=0.45, 0.3 mg cholesterol/kcal) have shown LDL apo B and HDL apo A-I kinetics similar to those observed with coconut oil. Thus, these results suggest that although coconut oil contains a more atypical fatty acid profile than do other more common saturated fats, the metabolic response to this oil appears to be comparable to that observed in more responsive species consuming lesser saturated fatty acid-containing diets.

In this study, we report that the consumption of coconut oil compared with corn oil resulted in a significant reduction in apo A-I FCR, which partially explains the rise in plasma apo A-I levels in these groups. Among all animals, the significant inverse correlation (Figure 2, upper panel) between apo A-I FCR and plasma apo A-I levels lends support to this relation. While this finding confirms other studies in human and nonhuman primates, which have shown that diet-induced changes in HDL apo A-I FCR contribute to the regulation of plasma apo A-I and HDL levels, the mechanisms have been largely unexplained. Our observation that HDL apo A-I FCR appears to be related to HDL composition may further substantiate our understanding of the mechanism(s) by which diet regulates apo A-I FCR and subsequently HDL levels. Brinton et al have demonstrated that HDLs with greater core lipid to surface ratios were cleared more slowly. Our results support these studies, as HDL from the coconut oil–fed group compared with the corn oil–fed group had significantly greater HDL cholesteryl ester to protein (core lipid to surface) ratios (Table 3), which were accompanied by a significant reduction in HDL
apo A-I FCR (Table 5). The addition of cholesterol to the corn oil–fed group altered neither the HDL cholesteryl ester to protein ratio nor the HDL apo A-I FCR. However, the addition of cholesterol to the coconut oil diet resulted in a 19% reduction in the HDL cholesteryl ester to protein ratio, concomitant with a 27% increase ($p < 0.05$) in HDL apo A-I FCR. Among all animals, the significant inverse relation ($r = -0.523, p = 0.006$) between the HDL-C to HDL-protein ratio and apo A-I FCR (Figure 3) suggests that the degree of HDL lipid enrichment may play a significant role in determining the rate of HDL apo A-I clearance. The importance of HDL lipid enrichment has been further emphasized by the recent observations that apo A-I epitope expression$^{36}$ and apo A-I conformation$^{37}$ can be modulated by the degree of HDL free cholesterol and phospholipid enrichment. In our studies, HDL from coconut oil–fed monkeys was enriched in free cholesterol (36%) and phospholipid (23%) compared with that from corn oil–fed monkeys, suggesting that the potential exists for lipid-induced changes in apo A-I epitope expression that may subsequently alter HDL apo A-I catabolism.

The degree of fatty acid enrichment of HDL lipids noted in these studies may also play a role in regulating HDL catabolism by modulating the fluidity of these particles. Recently, Dachet et al$^{38}$ have shown that HDLs from hypercholesterolemic humans are significantly less fluid than those of normocholesterolemic subjects. The saturated fatty acid enrichment of HDL lipids from the coconut oil–fed group reported in the present study (Table 4), as well as other studies in monkeys$^{7,11}$ and in humans$^{8,12}$ fed saturated-fat diets, has been shown to decrease the fluidity of these particles, which as Morrisett et al$^{12}$ have suggested, could reduce catabolism. In this study, the significant enrichment of HDL lipids by saturated fatty acids coupled with the increased HDL core to surface ratio in coconut oil–fed monkeys (Table 3) would imply a less-fluid HDL particle, which did, in fact, have a slower FCR. Hence, these data suggest that HDL catabolism may be influenced by particle fluidity as well as by lipid composition, factors that may alter the functional integrity of apo A-I.$^{38}$

As mentioned earlier, the results of this study also demonstrate that both the type of dietary fat and cholesterol affect the metabolism of HDL apo A-I by increasing apo A-I PR (Table 5), suggesting that apo A-I PR may also be a significant factor in regulating plasma apo A-I levels. The rise in apo A-I PR in the coconut oil–fed groups also appears to underlie the observed rise in plasma apo A-I levels in these animals, a finding in agreement with studies in human$^{8,15}$ and nonhuman$^{16}$ primates. This relation is supported by the significant positive correlation among all animals (Figure 2, lower panel).

While there were significant effects of both fat and cholesterol on apo A-I only the type of dietary fat affected hepatic apo A-I mRNA relative abundance. Thus, the significant rise in liver apo A-I mRNA levels in the coconut oil–fed groups compared with the corn oil–fed animals (Table 5) was associated with similar elevations in both apo A-I PR and plasma apo A-I levels, respectively. When the data from all animals were pooled, the significant correlation between liver apo A-I mRNA levels and both apo A-I PR and plasma apo A-I levels (Figure 4) suggested that a relation existed between these parameters. This significant relation was also observed in the coco versus coco(+) and corn(+) versus coco(+) comparisons. Hence, these data suggest that the increase in apo A-I PR with coconut oil feeding is related to increased concentrations of hepatic apo A-I mRNA, a finding that concurs with those of Sorci-Thomas et al$^{16}$ who used African green monkeys fed a more typical saturated fat–containing diet with a P/S ratio of 0.3. Thus, the reduced apo A-I FCR$^{14}$ as well as the increased hepatic apo A-I mRNA levels and apo A-I PR with saturated-fat feeding, has been demonstrated in both the cebus and African green monkey, an HDL-C– and an LDL-C–transporting species, respectively. Therefore, these studies suggest that similar mechanisms underlie the regulation of HDL apo A-I metabolism by dietary fat, despite the obvious species differences in plasma lipid profiles.

The lack of a parallel dietary cholesterol effect on liver apo A-I mRNA abundance and apo A-I PR (Table 5) indicates that the mechanism by which dietary cholesterol raises apo A-I PR differs from that of dietary fat. As our data suggest, dietary fat (coconut oil) appears to raise apo A-I PR and plasma apo A-I levels by elevating hepatic apo A-I mRNA abundance, most likely via the modification of hepatic apo A-I mRNA transcription or posttranscriptional events. However, because hepatic apo A-I mRNA levels were not affected by dietary cholesterol, then it would appear that the cholesterol–induced rise in apo A-I PR could result from changes in the translational activity of apo A-I mRNA or from some other posttranslational processing or modification$^{39}$ that would result in an increased apo A-I PR. The lack of a similar cholesterol effect on apo A-I PR and hepatic apo A-I mRNA levels (Table 5) may confound the significance of the relation among all animals shown in Figure 4. However, significant relations do exist among groups that had higher apo A-I PR and hepatic apo A-I mRNA levels. These parameters were found to be significantly related between the coco versus coco(+) groups ($r = 0.54, p < 0.04, n = 14$) as well as between the corn(+) versus coco(+) groups ($r = 0.56, p < 0.04, n = 14$) and the corn versus coco groups ($r = 0.69, p < 0.01, n = 12$). Similar significant relations were observed among these groups for plasma apo A-I levels and hepatic apo A-I mRNA abundance. It is also possible that a significant effect of 0.1% added dietary cholesterol on hepatic apo A-I mRNA levels may have been demonstrable had either the P/S ratio of the saturated-fat...
diet or the levels of dietary cholesterol fed been higher.

Although both dietary saturated fat (coconut oil) and cholesterol raise plasma apo A-I levels, the fact that these dietary components appear to affect apo A-I PR via different metabolic pathways argues against a common regulatory mechanism. Monge et al demonstrated in Hep G2 cells that increased levels of intracellular cholesterol were associated with increased apo A-I mRNA concentrations, and previous studies from this lab in the same cohort of cebus monkeys did, in fact, show increased hepatic levels of free (21%) and esterified (263%) cholesterol in the coconut oil-fed animals. Although these findings differ from previous reports demonstrating that polyunsaturated fat-fed monkeys accumulate more hepatic cholesteryl ester, it may provide an explanation for the results obtained in this study. Coconut oil feeding did significantly raise hepatic cholesterol levels in these cebus monkeys, which, in accordance with the findings of Monge et al, may partially explain the observed increase in hepatic apo A-I mRNA abundance. Interestingly, dietary cholesterol did not significantly affect hepatic cholesterol levels in these animals, which may be the underlying reason for the lack of a cholesterol effect on hepatic apo A-I mRNA levels. Whether this phenomenon is species related or is an effect of long-term coconut oil feeding is unclear, but it does appear to be a plausible explanation for the discordant effects of dietary fat and cholesterol on hepatic apo A-I mRNA abundance.

In conclusion, these studies suggest that the type of dietary fat and cholesterol modulate apo A-I FCR and PR, both of which play significant roles in regulating plasma HDL and apo A-I levels. These data also suggest that diet-induced changes in the fatty acid and lipid composition of HDL can influence its catabolism. Because only the type of dietary fat and not cholesterol affects hepatic apo A-I mRNA abundance, a common regulatory mechanism linking these dietary factors to the observed elevations in apo A-I PR and plasma apo A-I levels could not be established.

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