Apolipoprotein (Apo) A-I Production and mRNA Abundance Explain Plasma ApoA-I and High Density Lipoprotein Differences between Two Nonhuman Primate Species with High and Low Susceptibilities to Diet-induced Hypercholesterolemia*

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Earlier studies have shown that African green monkeys develop a more modest hypercholesterolemia, higher high density lipoprotein (HDL) concentrations, and less atherosclerosis than cynomolgus monkeys fed diets with the same cholesterol content. In the present study, cynomolgus monkeys were fed less cholesterol than was fed to African green monkeys to induce equivalent hypercholesterolemia in both species. African green monkeys developed 2-fold higher plasma HDL cholesterol concentrations and 2.7-fold higher plasma apolipoprotein (apo) A-I concentrations. Therefore, the higher HDL concentration in African green monkeys appears to result from factors that act independently of dietary cholesterol intake or total plasma cholesterol concentration.

Two aspects of HDL production were examined to determine the metabolic basis of the species difference in HDL concentration. The rate of hepatic apoA-I secretion, as estimated by the accumulation of apoA-I in the medium during recirculating liver perfusion, was 5-fold higher in livers of African green monkeys. In addition, the concentration of apoA-I mRNA was 2-fold higher in the liver and 3.7-fold higher in the intestine of African green monkeys. Taken together, these findings indicate that differences in apoA-I production in the liver and small intestine are large enough to be responsible for the differences in the plasma concentrations of HDL and apoA-I between these species. Factors which regulate apoA-I secretion, including modulation of tissue apoA-I mRNA concentrations, are important determinants of plasma HDL concentrations and may contribute to the relative resistance of African green monkeys to dietary cholesterol-induced hypercholesterolemia and atherosclerosis.

ApoA-I mRNA was also detected at low levels in the kidney and testis of African green and cynomolgus monkeys but not in the adrenal or brain. The tissue distribution and abundance of apoA-I mRNA in peripheral tissues was very different than that seen for apoE mRNA. Kidney and testis apoA-I mRNAs were the same size as liver apoA-I mRNA when examined by Northern blot analysis. Testis apoA-I mRNA appeared to be functionally active as judged by its presence in cytoplasmic polyribosomes. The low levels of apoA-I expression in kidney and testis are unlikely to contribute significantly to the plasma apoA-I pool but might function in some aspect of local lipid metabolism within these tissues.

HDL†, one of the major plasma lipoproteins involved in the transport and disposition of cholesterol and cholesteryl esters, is removed from peripheral tissues and delivered to the liver for metabolism and elimination. This role of HDL may be of critical importance in preventing the deposition of cholesterol in the coronary and other arteries as occurs in atherosclerosis. A number of studies in *vitro* have shown that HDL and HDL apolipoproteins can serve as acceptors of cholesterol released from cells (2-4). Epidemiological studies have also identified HDL cholesterol as a negative risk factor in the development of atherosclerotic heart disease; decreased HDL levels are associated with increased risk (5). Thus, it is important to understand the factors which determine the levels of plasma HDL.

Previous suggestions have been made, based on apoA-I turnover studies in *vivo*, that apoA-I production may be important in the regulation of plasma HDL concentrations (6, 7). Other studies suggest that differences in HDL concentrations are more closely related to differences in the fractional catabolic rate of apoA-I (8, 9). With the exception of these turnover studies, little is known about the importance of production rates (versus catabolism) in explaining the substantial differences in HDL levels seen among mammalian species or within the human population. A genetic basis for low HDL concentrations in humans has been identified in several studies (10-12), and in at least one instance a DNA polymorphism closely linked to the apoA-I gene was found to be associated with HDL deficiency (13). It is not known whether species differences or genetic differences are due to factors that act at the level of plasma HDL production or catabolism or both.

*The abbreviations used are: HDL, high density lipoprotein; apo, apolipoprotein.

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Nonhuman primates are of interest for studies of HDL because of the close phylogenetic relationship to man and because hypercholesterolemia and atherosclerosis can be induced by the addition of moderate amounts of cholesterol to the diet (14–18). Plasma HDL is also a negative risk factor for atherosclerosis in monkeys (16–18) as it is in human beings (5). Several monkey species have been studied, and marked differences in the magnitude of diet-induced hypercholesterolemia have been observed among species (17–20). For example, when fed the same cholesterol-enriched atherogenic diet, African green monkeys develop less severe hypercholesterolemia and atherosclerosis than do cynomolgus monkeys (15–20). An important feature of this difference is that African green monkeys have substantially higher levels of plasma HDL (17–19) and apoA-I (21), factors which may contribute to their greater ability to resist diet-induced hypercholesterolemia.

The metabolic basis for the different plasma HDL levels in these species is not understood. Species-specific plasma HDL levels could reflect differences in apolipoprotein production or differences in the catabolism of lipoprotein particles in the plasma. One possibility is that the species difference in HDL concentration occurs secondarily to the marked difference in total plasma cholesterol, since cynomolgus monkeys become massively hypercholesterolemic when fed dietary cholesterol at levels that only introduce modest cholesterol elevations in African green monkeys (17). To avoid this potential confounding variable in the present study, dietary cholesterol levels were adjusted so that an equivalent degree of hypercholesterolemia was present in both species. HDL and apoA-I concentrations were still much lower in cynomolgus monkeys compared with African green monkeys, suggesting that the species-specific difference in HDL is independent of total plasma cholesterol concentration or dietary cholesterol intake. The basis for this difference in plasma HDL concentrations was examined by determining hepatic apoA-I production rates and by monitoring the absolute amounts of apoA-I mRNA in the liver and small intestine. Both the rate of perfusate apoA-I accumulation during liver perfusion and the apoA-I mRNA concentrations in the liver and intestine were higher in African green monkeys, and the degree of difference between species was as high or higher than for plasma HDL concentrations. These findings support the idea that production rates of apoA-I are the major factor in determining the species difference in plasma HDL. This species difference itself suggests that factors that modulate apoA-I production, including regulation at the mRNA level, may be important determinants of the susceptibility to dietary cholesterol-induced hypercholesterolemia.

**Experimental Procedures**

**Animals and Diets**—The animals for these studies were part of ongoing studies of effects of diet on atherosclerosis, and tissues were obtained at the time of sacrifice for atherosclerosis evaluations. Four diet groups of each species were used. The cynomolgus monkeys (Macaca fascicularis) were fed for three years diets containing 40% of calories as fat (eight lard or safflower oil) and cholesterol (either 0.03 or 0.4 mg/kcal). While these different diet manipulations induced differences in some plasma lipoprotein end points, hepatic apoA-I production and mRNA levels were found to be unaffected by these diet manipulations. The African green monkeys (Cercopithecus aethiops) were fed for five years diets containing 40% of calories as fat (saturated fat-enriched with polyunsaturated/saturated = 0.3 or polyunsaturated fat-enriched with polyunsaturated/saturated = 2.2) and containing cholesterol at either 0.03 or 0.8 mg/kcal. Dietary effects on hepatic apoA-I production and mRNA levels in this species were small (approximately 25% differences) but statistically significant and will be the topic of a separate publication. Therefore, the comparisons made between species in the present report accurately reflect the differences between the species and are not due to separate diet effects. Necropsies were carried out beginning about 9:30 a.m. After animals were fasted overnight. At the time of necropsy, animals were anesthetized with ketamine-HCL (15 mg/kg body weight) and their bodies were exsanguinated and flushed with 0.9% NaCl. Whole organs were removed, weighed, and quick frozen in liquid nitrogen, with subsequent storage at −70°C.

**Isolation and Characterization of Cellular RNA**—Total cellular RNA was prepared by either the method of Cox (22) with modifications (23), or by the guanidine thiocyanate method (24) combined with CsCl centrifugation (25). RNA was prepared from large sections of liver (1–2 g) to minimize sampling error. To test for regional regulation on the intestine, RNA was prepared from different segments along the small intestine in a subset of animals. Since major regional variations were observed, RNA was prepared from a sample of tissue representative of the entire organ. This was accomplished by freezing the tissue and then shattering the entire small intestine into small fragments under liquid N2. A pool (1–2 g) of randomly selected fragments was used for RNA isolation. RNA was isolated from Hep G2 cells (26) as described (27). The integrity of all RNA samples was assessed by electrophoresis in 1.2% agarose gels containing 2.2 M formaldehyde (28).

**Probes and Solution Hybridization**—ApoA-I and apoE RNAs were measured by DNA-excess solution hybridization assays using single stranded CDNA probes prepared from appropriate subclones in bacteriophage M13 exactly as described (27, 29). ApoA-I and apoE mRNA values were determined by reference to standard curves constructed with the respective M13- apoCDNA templates as the hybridization standards. Reported mRNA values are the mean ± S.E. for the number of animals examined. Four to 12 mRNA determinations were made for each animal.

**S1 Nuclease Protection and Northern Blot Analysis**—Uniformly labeled apoA-I [32P]cDNA probe (106 pg), synthesized as described above, was hybridized to 50 pg of RNA isolated from African green monkey liver, cynomolgus monkey liver, and Hep G2 cells as described (27, 30). Samples were digested with 100 units of S1 nuclease, alkali-treated to hydrolyze RNA (30), and analyzed by electrophoresis on a 7 M urea, 6% polyacrylamide gel (31). The gel was dried and exposed to x-ray film at −70°C. For Northern blot analysis total cellular RNA was electrophoresed on 1.2% agarose gels containing 2.2 M formaldehyde (28), transferred to nitrocellulose, and hybridized (32) with the uniformly labeled apoA-I cDNA probe described above.

**Polyribosome Isolation**—Polyribosomes were prepared from African green monkey testis according to the magnesium precipitation method of Palmiter (33). Polyribosomes were isolated by density gradients (33), and fractions were pooled on the basis of the absorbance profile at 254 nm into four regions: free ribonucleoprotein particles (I), ribosomal subunits and monosomes (II), and polysomes of increasing sedimentation (III, IV). RNA was prepared from each pooled fraction as described above, and assayed for apoA-I mRNA by DNA-excess solution hybridization.

**Liver Perfusion**—Livers were isolated from African green and cynomolgus monkeys, and recirculating perfusion was performed as described (34). Perfusion medium consisted of Krebs-Henseleit bicarbonate buffer, pH 7.4, containing glucose, insulin, amino acids, hydrocortisone, streptomycin, penicillin, and washed human red blood cells at a 19–22% hematocrit (34). For labeling studies, 1 μCi of [3H]leucine (Du Pont-New England Nuclear; >300 mCi/mmol) or 3 μCi of [14C]leucine (ICN; >90 Ci/mmol) was added to the perfusate. An initial 30 min of recirculating perfusion was performed to wash the liver of trapped plasma lipoproteins. The medium was then changed, radiolabeled leucine was added, and perfusion was continued for 4 h with samples taken at 30-min intervals. For estimates of total protein synthesis rates 0.6 ml aliquots of perfusate were precipitated with 10% trichloroacetic acid. The precipitate was pelleted by centrifugation, washed by resuspension in trichloroacetic acid, then dissolved in 1 N NaOH and radioactivity determined by scintillation spectrometry. The rate of incorporation of radiolabeled leucine into secreted protein was linear for at least 120 min of perfusion. Leucine incorporation into protein was expressed as the percentage of total incorporation per hour per gram of liver protein. The apoA-I accumulation rate and the

rate of leucine incorporation into secreted protein were calculated as the best fit regression line obtained from the time course data. In all cases the regression coefficients were $r \geq 0.99$.

**HDL Cholesterol and Apolipoprotein A-I Determinations**—Total plasma cholesterol and HDL cholesterol were determined by the standardized automated procedures described in the Lipid Research Clinics manual (35). ApoA-I in plasma and in liver perfusates was measured by an enzyme-linked immunosorbent assay as described previously (36). The linear range of the assay was 0.15–2.5 ng, and purified apoA-I from each monkey species was used as the standard for samples from that species.

**Statistical Analysis**—Statistical analysis was performed by a Student's $t$ test or by a one-way analysis of variance.

**RESULTS**

**Plasma ApoA-I Concentrations and Liver Perfuse ApoA-I Accumulation Rates**—The monkeys used for these studies were adult males of each species that were fed control or atherogenic diets for 3–5 years for the measurement of atherosclerosis progression. The plasma concentrations of total cholesterol, HDL cholesterol, and apoA-I are shown in Table I for all animals in both experimental groups. The cynomolgus monkeys were fed less cholesterol in their diet than the African green monkeys to adjust the hypercholesterolemia to a similar level (see "Experimental Procedures"). These diets the total plasma cholesterol values of the two species were not significantly different. Plasma HDL cholesterol and apoA-I concentrations were 2–3-fold greater in the African green as compared to the cynomolgus monkey. The African green monkey was previously noted to have higher HDL levels, whereas both species were fed the same amount of cholesterol, a situation which produces much greater hypercholesterolemia in the cynomolgus monkey (15, 17, 18). The present data show that the higher plasma HDL and apoA-I levels of the African green monkey were still seen when the total plasma cholesterol values were equivalent to those in cynomolgus monkeys suggesting that the species difference in HDL levels is independent of total plasma cholesterol concentration or dietary cholesterol intake.

The metabolic basis for the difference in plasma HDL concentration was examined by determining hepatic apoA-I accumulation rates in liver perfusates carried out in a subset of animals. In both species the rate of apoA-I accumulation in the perfuse was linear throughout the 4-h recirculating perfusion. As shown in Table II, the rate of apoA-I accumulation was 5–3-fold greater with livers of African green monkeys as compared to livers of cynomolgus monkeys. These data suggest that the difference in plasma HDL concentrations between these species is largely the result of a much greater hepatic apoA-I production rate in the African green monkey. To ensure that the difference in apoA-I production rates was not due to differences in total protein production during the perfusions, the rate of incorporation of perfusate leucine into secreted protein was examined. The data in Table II show that leucine incorporation into perfusate protein was not different for the two species.

**Table I**

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Total plasma cholesterol*</th>
<th>HDL cholesterol*</th>
<th>apoA-I mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>African green monkey</td>
<td>15</td>
<td>275 ± 23</td>
<td>88 ± 4</td>
<td>255 ± 9</td>
</tr>
<tr>
<td>Cynomolgus monkey</td>
<td>20</td>
<td>307 ± 27</td>
<td>40 ± 3</td>
<td>92 ± 5</td>
</tr>
</tbody>
</table>

*Mean ± S.E. The mean for each animal represents multiple determinations ($n > 4$) per animal over at least a 1-year period of study.

**Table II**

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Perfusate apoA-I*</th>
<th>Radiolabeled leucine into perfusate protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µg/liver</td>
<td>%/h/g liver protein</td>
</tr>
<tr>
<td>African green monkey</td>
<td>15</td>
<td>14.93 ± 1.71</td>
<td>1.00 ± 0.23</td>
</tr>
<tr>
<td>Cynomolgus monkey</td>
<td>12</td>
<td>2.99 ± 0.48</td>
<td>0.86 ± 0.05</td>
</tr>
</tbody>
</table>

*Statistically significant difference compared to cynomolgus monkey, $p < 0.001$.

**ApoA-I mRNA in Liver and Small Intestine**—To determine whether the difference in apoA-I accumulation was reflected at the level of mRNA, liver apoA-I mRNA was measured by DNA-excess solution hybridization with a single stranded probe derived from human apoA-I cDNA (29). Measurements of intestinal apoA-I mRNA also were made since the intestine is a major site of apoA-I synthesis. To ensure that the human cDNA was sufficiently homologous to monkey apoA-I mRNA for the solution hybridization assay (27, 29), the human cDNA was tested with liver RNA in an S1 nuclease protection assay. Fig. 1A shows that the 246-nucleotide probe (including M13 sequence) (lane $P$) was completely eliminated by digestion with S1 nuclease after hybridization with yeast tRNA (lane $D$). RNA from human Hep G2 cells completely protected the 195 nucleotides of the probe complementary to apoA-I mRNA (lane $C$). RNA from cynomolgus (lane $A$) or African green (lane $B$) monkey liver also afforded nearly complete protection of the probe. These results indicate that the human cDNA is a suitable reagent for solution hybridization measurement of monkey apoA-I mRNA. The recent publication of the cynomolgus apoA-I mRNA sequence (37) indicates 97% homology with the human sequence represented in the hybridization probe. Of the five mismatches in the 195-nucleotide probe, three are scattered throughout the probe while two are within the last six nucleotides at the 3' end of the probe. The two mismatches near the end may lead to S1 nuclease trimming and account for the slightly faster mobility of the cDNA fragments protected by monkey RNA (lanes $A$ and $B$) as compared to human RNA (lane $C$). The absence of smaller protected probe fragments indicates that the isolated internal mismatches are not detected by S1 nuclease under these conditions. Northern blot analysis (Fig. 1B) showed that apoA-I cDNA detected a single hybridizing mRNA of approximately 1100 nucleotides with RNA from monkey liver and small intestine.

Fig. 2 shows results of a typical solution hybridization assay in which apoA-I cDNA was hybridized to template M13-apoA-I DNA (panel $A$) or to monkey liver RNA (panel $B$). In each case S1 nuclease-resistant hybrids increased linearly as a function of input DNA or RNA. Prior to the measurement of apoA-I mRNA in the two species, several livers and small intestines were examined for regional variations in mRNA distribution within these organs. When RNA was prepared from 1–2-g segments removed from each of the major lobes of the liver, no differences were seen in the apoA-I mRNA content (data not shown). In addition, no difference was seen in the apoA-I mRNA content of tissue removed from the smaller caudate lobe as compared to samples from the larger right, left, and quadrate lobes. This result permitted the caudate lobe to be removed for mRNA measurements prior to liver perfusion in some animals. Fig. 3 shows that there were substantial variations in the apoA-I mRNA concentration along the small intestine. ApoA-I mRNA concentration was low in the duodenum, highest in the upper segment of jejunum, and progressively lower in the distal jejunum.
the two segments of ileum. No major differences between the two species of monkey were seen in the distribution of apoA-I mRNA along the intestine. To avoid the regional variation, apoA-I mRNA was measured with RNA prepared from pooled tissue samples representative of the entire small intestine.

Table III shows the tissue apoA-I mRNA concentrations expressed as picograms of mRNA per microgram of RNA and as nanograms of mRNA per gram of tissue for both African green and cynomolgus monkeys. The apoA-I mRNA concentration of African green monkey liver was approximately 2-fold greater than that found for cynomolgus monkey liver. Hybridization measurements with RNA from the small intestine also indicated that the concentration of apoA-I mRNA was much greater in the African green monkey. In this case, the apoA-I mRNA concentration in the small intestine was 3.7-fold greater in the African green monkey as compared to the cynomolgus monkey.

From the nanograms of apoA-I mRNA per gram of tissue and the organ weights, the distribution of total apoA-I mRNA between the liver and small intestine was calculated for each species (Table III; percent total mRNA per organ). The African green monkey displayed an approximately equal distribution of apoA-I mRNA between the liver and small intestine. In the cynomolgus monkey, the liver contained about 12% more and the small intestine 12% less of the total apoA-I mRNA as compared to the African green monkey. The species difference in the organ distribution of apoA-I mRNA is small but statistically significant ($P < 0.01$). These measurements were made with animals that were fasted for at least 12 h prior to sacrifice and would not reflect any acute changes in mRNA content that might occur in response to feeding.

ApoE mRNA also was measured in the livers of these animals to determine whether other apolipoprotein mRNAs were higher in the African green monkey. As shown in Table IV, the liver apoE mRNA concentration was approximately 29% lower in African green monkeys. As previously reported...
plasmic polyribosomes were prepared from testis, and apoA-I whether the apoA-I mRNA was functionally active, cyto-
tected  in kidney and  testis RNA a single RNA of the same 
size as was seen in liver and small intestine. To determine 
tissues include kidney, testis,  brain, and adrenal. To deter-
mRNA was measured after polyribosomes were sedimented 
in  a 
peripheral  tissues 
of the assay set at approximately 1 mRNA  molecule/cell. No 
hybridization was due to  authentic apoA-I mRNA.  Fig. 
hybridization occurred with kidney and 
tissues in comparison to  the contents of apoE mRNA. The distribution of liver and small intestine 
apo-I mRNA was determined from the amount of mRNA in each organ as the percentage of the mRNA in both 
statistically significant differences compared to cynomolgus monkey, p < 0.01.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Afro-American monkey</th>
<th></th>
<th></th>
<th></th>
<th>Cynomolgus monkey</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoA-I mRNA</td>
<td>pg mRNA/µg RNA</td>
<td>ng mRNA/µg tissue</td>
<td>% Total mRNA/organ</td>
<td>pg mRNA/µg RNA</td>
<td>ng mRNA/µg tissue</td>
<td>% Total mRNA/organ</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>69.5 ± 3.46</td>
<td>420 ± 21</td>
<td>51%</td>
<td>35.2 ± 1.2</td>
<td>220 ± 14</td>
<td>63%</td>
<td></td>
</tr>
<tr>
<td>Intestine</td>
<td>119.9 ± 7.12</td>
<td>767 ± 45</td>
<td>49%</td>
<td>32.7 ± 2.6</td>
<td>205 ± 13</td>
<td>37%</td>
<td></td>
</tr>
</tbody>
</table>

* Calculation of apoA-I mRNA per gram of tissue is based on the tissue RNA content as determined by colorimetric assay (29).
* Total organ apoA-I mRNA content was determined from the value of apoA-I mRNA per gram of tissue and the organ size from each animal obtained at the time of necropsy. The distribution of liver and small intestine apo-I mRNA was determined from the amount of mRNA in each organ as the percentage of the mRNA in both organs.
* Statistically significant differences compared to cynomolgus monkey, p < 0.01.

**TABLE IV** Comparison of liver apoE mRNA

<table>
<thead>
<tr>
<th>ApoE mRNA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pg mRNA/µg RNA</td>
</tr>
<tr>
<td>African green monkey</td>
</tr>
<tr>
<td>Cynomolgus monkey</td>
</tr>
</tbody>
</table>

* Mean ± S.E. African green n = 24; cynomolgus n = 18.

**TABLE V** Peripheral tissue abundance of apoE and apoA-I mRNA

<table>
<thead>
<tr>
<th>Tissue</th>
<th>ApoE mRNA*</th>
<th>ApoA-I mRNA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pg mRNA/µg RNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>13.9 ± 1.2</td>
<td>0.63 ± 0.11</td>
</tr>
<tr>
<td>Testis</td>
<td>7.45 ± 0.7</td>
<td>1.53 ± 0.21</td>
</tr>
<tr>
<td>Brain</td>
<td>4.36 ± 0.4</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Adrenal</td>
<td>3.06 ± 0.5</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

* The number of African green monkeys used for each peripheral tissue analysis were kidney n = 15; testis n = 8; brain n = 10; adrenal n = 21. All values represent the mean ± S.E.

(27) only low levels of apoE mRNA were present in the small intestine (data not shown).

ApoA-I mRNA in Peripheral Tissues—Apo-A-I synthesis occurs primarily in the liver and small intestine in mammals (38-42) and apoA-I mRNA appears to be restricted to these tissues (43-49). In contrast, in the chicken apo-A-I is synthe-
sized at substantial rates in a wide variety of peripheral tissues in addition to the liver and small intestine (50-52). Such tissues include kidney, testis, brain, and adrenal. To deter-
mapper whether apoA-I synthesis might occur at low levels in peripheral tissues of African green monkeys, RNA samples from brain, kidney, testis, and adrenal were tested with the DNA-excess solution hybridization assay with the sensitivity of the assay set at approximately 1 mRNA molecule/cell. No 
hybridization was detected with brain or adrenal RNA, but readily detectable hybridization occurred with kidney and testis RNA. Table V shows the apoA-I mRNA contents of these tissues in comparison to the contents of apoE mRNA. ApoA-I mRNA was also detected at similar levels in kidney and testis of cynomolgus monkeys (data not shown).

Since the apoA-I mRNA levels in kidney and testis were very low, independent means were used to confirm that the hybridization was due to authentic apoA-I mRNA. Fig. 1B shows a Northern blot analysis in which apoA-I CDNA de-
tected in kidney and testis RNA a single RNA of the same size as was seen in liver and small intestine. To determine whether the apoA-I mRNA was functionally active, cyto-
plasmic polyribosomes were prepared from testis, and apoA-I mRNA was measured after polyribosomes were sedimented in a 0.5-1.5 M sucrose gradient. Fig. 4 shows that apoA-I mRNA was present primarily in higher order polyribosomes with only small amounts in gradient fractions containing monosomes or material of lower sedimentation rates. In an additional experiment kidney tissue was metabolically labeled with radiolabeled amino acid in short term organ culture (53, 54) to determine whether newly synthesized apoA-I could be detected. Tissue extracts were reacted with anti-apoA-I anti-
serum, and the immunoprecipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluo-
rography (53, 54). A newly synthesized band with the mobility of plasma apoA-I was seen with anti-apoA-I serum but not with preimmune serum or anti-apoE serum (data not shown). These data indicate that the low levels of kidney and testis apoA-I mRNA are functionally active in the synthesis of apoA-I.
The African green monkey is less responsive to dietary cholesterol induction of hypercholesterolemia and the development of atherosclerosis than the cynomolgus monkey (15-20). When fed similar amounts of dietary fat and cholesterol, the African green monkey consistently has higher plasma HDL levels than the cynomolgus monkey (17-19). The capacity to produce or maintain higher plasma HDL levels may be a significant factor in the relative resistance of the African green monkey to dietary induced hypercholesterolemia and atherosclerosis. In the present study, we have shown that higher plasma HDL and apoA-I concentrations still occur in the African green monkey even when diets are adjusted to produce the same degree of hypercholesterolemia in both monkey species. This result argues that the African green monkey has higher HDL and apoA-I concentrations due to factors that act independently of dietary cholesterol intake or total plasma cholesterol concentration.

The basis for the species difference in plasma HDL and apoA-I concentrations appears to reside primarily in a much greater rate of apoA-I production in the African green monkey. This conclusion is supported by the greater rate of perfusate apoA-I accumulation in liver perfusions in African green monkeys. Furthermore, the concentration of apoA-I mRNA was 2-fold greater in the liver and 3.7-fold greater in the small intestine of the African green monkey as compared to the cynomolgus monkey. As an index of the potential for apoA-I production, the measurements of apoA-I mRNA support the conclusion from the liver perfusion data and suggest further that intestinal apoA-I production is also much greater in the African green monkey. The degree of difference between these species in hepatic apoA-I production rate and tissue apoA-I mRNA concentrations was as great or greater than the difference in plasma HDL concentration. In addition, in studies to be reported elsewhere (55), we have seen little or no difference between these species in the fractional catabolic rate of apoA-I measured in turnover studies in vivo. These results indicate that the difference in apoA-I production is primarily responsible for the species difference in plasma HDL and apoA-I concentrations. This species difference itself suggests that factors that regulate apoA-I production, including regulation at the mRNA level, may be important determinants of the susceptibility to dietary cholesterol-induced hypercholesterolemia.

The suggestion that the different susceptibilities of the cynomolgus and African green monkeys to hypercholesterolemia is due in some measure to apoA-I mRNA levels and to the capacity for apoA-I production has support in studies with other species. Rabbits are probably the species most sensitive to dietary cholesterol-induced hypercholesterolemia. They express little or no apoA-I mRNA in the liver and produce little apoA-I during liver perfusion (43, 56, 57). Guinea pigs are also very sensitive to dietary cholesterol, have a low plasma HDL concentration, and have a very low hepatic apoA-I production rate (58, 59). In contrast, rates are relatively insensitive to dietary cholesterol-induced hypercholesterolemia and have a much higher rate of hepatic apoA-I production (60). As summarized in Table VI, cynomolgus monkeys fit between the rat and guinea pig in apoA-I production rates while African green monkeys have the highest hepatic apoA-I production rate among the species for which it has been measured. Therefore, the available data lead us to speculate that the capacity for hepatic apoA-I production is a major determinant of the susceptibility to dietary cholesterol-induced hypercholesterolemia.

The basis for the difference in hepatic apoA-I production between the African green and cynomolgus monkeys appears to reside, at least in part, in the steady state level of hepatic apoA-I mRNA. ApoA-I mRNA was present at 2-fold greater concentration in livers of African green monkeys as compared to cynomolgus monkeys. The fact that this difference in mRNA content is less than the difference in apoA-I accumulation rates in liver perfusions may be the result of several factors. First, some physiologic factors affecting apoA-I accumulation during liver perfusion may not be reflected in the pre-perfusion mRNA measurements. Second, species differences in translational efficiency or posttranslational processing of apoA-I may perturb the proportionality between mRNA concentration and actual secretion rates of the protein. Third, for the African green monkeys most of the liver mRNA measurements and the liver perfusions were not performed on the same individual animals but rather on two subgroups drawn from the same pool of animals. This may have introduced some degree of variation that was not present with the cynomolgus monkeys for which many of the same animals were used for liver perfusion and mRNA measurements. Interestingly, the intestinal apoA-I mRNA values showed a 3.7-fold difference between the African green and cynomolgus monkeys. Since 40-50% of the total body apoA-I mRNA is present in the small intestine in both species, it is likely that intestinal production of apoA-I also plays a major role in determining the plasma HDL and apoA-I levels.

The difference in apoA-I mRNA levels between cynomolgus and African green monkeys may be due to some feature of the apoA-I gene that influences the steady state mRNA level. Potential candidates are promoter elements which regulate the rate of transcription or sequences within the mRNA transcript that influence mRNA processing or the stability of the mature mRNA in the cytoplasm. Alternatively, the differences could be due to trans-acting factors that influence any of these steps in mRNA metabolism. However, the measurements of apoE mRNA levels (Table IV) and the similar utilization of leucine in the liver perfusions (Table II) in both species argue that the difference in apoA-I expression is specific to apoA-I and not a general effect that influences other mRNAs or total protein production.

The significance of apoA-I expression in peripheral tissues of the monkey is not understood. ApoA-I mRNA is present in the kidney and testis at 1-3% of the concentration in the liver. When the small sizes of these organs are considered, the absolute amounts of apoA-I mRNA in the kidney and testis represent only fractions of a percent of the apoA-I mRNA in the liver and small intestine. Thus, it is unlikely that apoA-I produced by kidney and testis contribute significantly to the pool of plasma apoA-I. Nevertheless, testis apoA-I mRNA appears to be functionally active as judged by its presence in actively translating polyribosomes, and the kidney appears to synthesize low amounts of apoA-I in short term organ culture. Interestingly, this low level of apoA-I mRNA is tissue specific since it was not seen in other peripheral tissues such as adrenal and brain. The distribution of apoA-I...
mRNA in peripheral tissues is quite distinct from the distribution of apoE mRNA which is present at high levels in many peripheral tissues including brain and adrenal (27, 61, 63). The distribution of apoA-I mRNA in peripheral monkey tissues is also very different than in the chicken in which apoA-I mRNA is present at high levels in many tissues (50–52). Testis and kidney apoA-I synthesis might be restricted to particular cell types and play some role in local lipid metabolism. Alternatively, the low level expression in kidney and testis may reflect a down-regulated state that persists from an earlier developmental stage in which expression was much higher in these tissues. A situation such as this occurs in chicken adult skeletal muscle in which apoA-I mRNA is present at only a few percent of the level seen at the time of hatching (52). In this regard it is of interest that apoA-I mRNA has been detected in a number of embryonic human tissues including gonads, adrenal, and kidney (44). Additional studies are required to determine whether apoA-I made in peripheral tissues in mammals plays a role in development or in other processes not yet understood.

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