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Diet and Lipoprotein Influence on Primate Atherosclerosis (39863)

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One of the puzzling aspects of atherosclerosis has been the occasional discrepancy between hypercholesterolemia and the incidence of atherosclerosis or ischemic heart disease. Whereas hypercholesterolemia is considered a primary risk factor in atherosclerosis, some individuals with elevated levels of circulating cholesterol do not develop significant atherosclerotic vascular disease while other normocholesterolemic persons do.

Recent epidemiologic evidence suggests that an increased concentration of plasma high-density lipoproteins (HDL) may reduce the risk of ischemic heart disease (1–4). Conversely, a high concentration of circulating cholesterol associated with the low-density lipoproteins (LDL) is particularly damaging to the arterial wall and correlates with a high degree of risk for ischemic heart disease (5–9). It is also noteworthy that species of animals transporting their cholesterol primarily as HDL are resistant to atherosclerosis, in contrast to those few species, such as man, the pig, and most monkeys, which circulate much of their cholesterol as LDL and are prone to atheromatous change (10).

During investigation of atherogenesis in monkeys, our objective has been to relate diet to those factors responsible for the atherosclerotic susceptibility of the squirrel monkey (*Saimiri sciureus*) in comparison with the resistance of the cebus (*Cebus albifrons*), both New World monkeys from the same family, *Cebidae*. Although nonhuman primates seldom develop appreciable atherosclerosis in the wild, it is known that squirrel monkeys have more fatty streaks and atheromatous plaques than most other nonhuman primates, whereas the cebus are relatively free of intimal proliferation and lipid deposition (11, 12).

In a previous investigation (13) we reported that these two species develop comparable hypercholesterolemias when fed saturated fat and/or cholesterol. Subsequent analysis revealed that saturated fat reduced the fractional clearance of the circulating lipoprotein cholesterol, but the two species did not differ in this regard. Nor were there differences between species for rates of total-body cholesterol synthesis measured *in vivo* from analysis of kinetic data on cholesterol turnover (14).

Despite these similarities in cholesterol metabolism, recent ultrastructural study (15, 16) has confirmed the difference in character between the arterial response of the two primates when their hypercholesterolemia ranged from 250–325 mg/dl as a result of saturated fat feeding. The squirrel monkey developed intimal smooth muscle cell proliferation, a connective tissue response including collagen, elastin, glycosaminoglycans, and extensive intra- and extracellular lipid. The cebus intima revealed a less cellular, connective tissue proliferation reminiscent of diffuse intimal thickening associated with developmental changes in human arteries (17, 18), but lacked the accumulation of either intra- or extracellular lipid (Fig. 1).

These collective observations suggested that the differences in susceptibility or resistance of these two species to diet-induced atherosclerosis might reside either in the arterial wall itself or in the "circulating insult" delivered to the wall by the lipoproteins. To obtain more quantitative estimates of the atherosclerotic response to diet-induced hypercholesterolemia, aortas from these two species were analyzed histopathologically and biochemically following the characterization of plasma lipoproteins.

Materials and methods. Six squirrel and six cebus monkeys of both sexes born and raised in our primate colony were fed semipurified diets of our own design (13). Three monkeys of each species were fed diets containing saturated fat (coconut oil) or unsaturated fat (corn oil) for a period of 3 to 4 years from birth.

Lipoprotein analyses. Plasma lipoproteins

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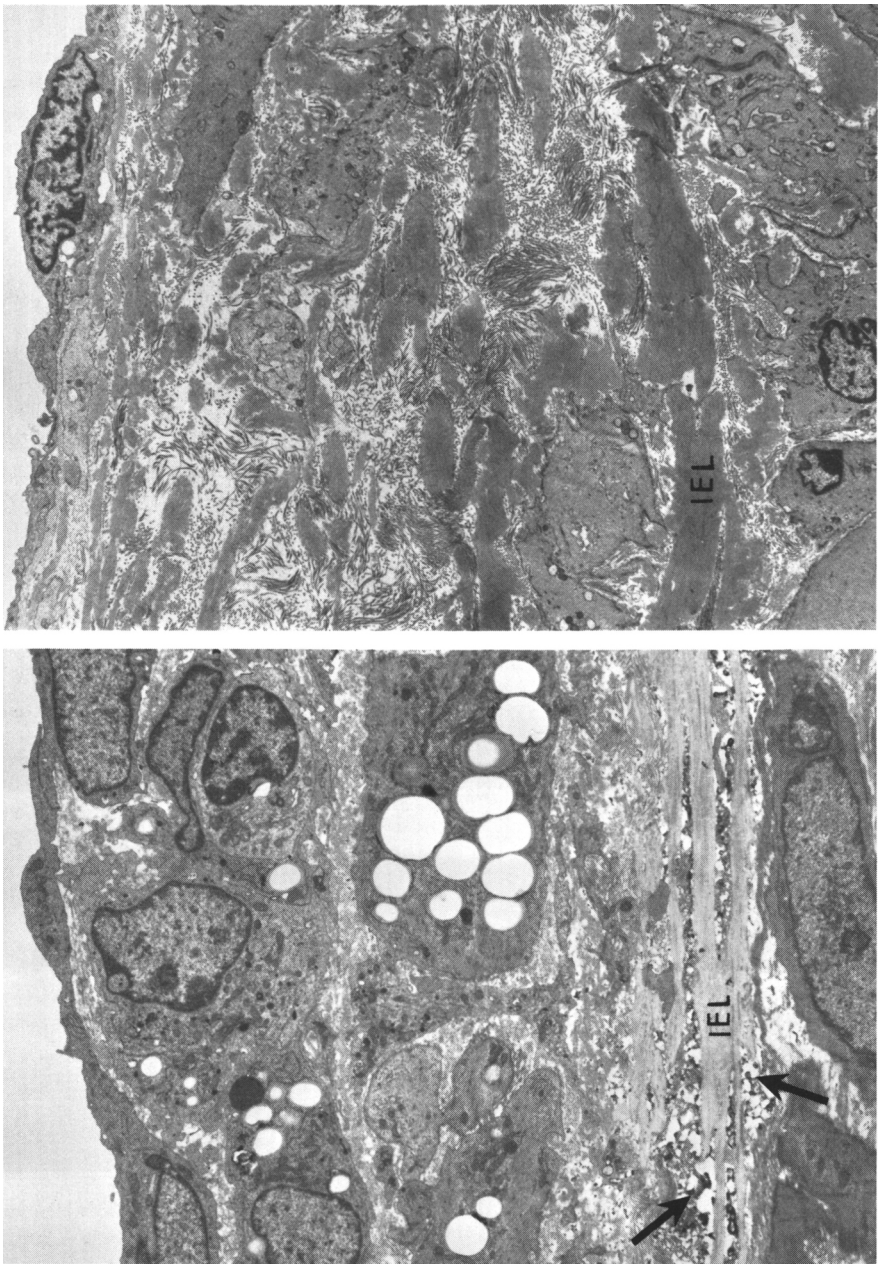

FIG. 1. Electron micrographs of the intima from a squirrel monkey (left) and cebus (right) fed the coconut oil diet depict differences in the responses of the two species when hypercholesterolemias were comparable (288 versus 294 mg/dl, respectively). The squirrel monkey reveals numerous lipid-laden smooth muscle cells and extracellular pleomorphic lipid deposits (arrows) along the internal elastic lamina (IEL). In the equally thickened, less-cellular intima of the cebus, elastin and collagen predominate within the internal elastic lamina and lipid deposition is absent.

and cholesterol were analyzed after an overnight fast within a week of sacrifice. Eight milliliters of blood were collected in EDTA with merthiolate and centrifuged at 4°C. Plasma lipoproteins were separated by the method of differential ultracentrifugation (19) utilizing a fixed-angle 40.3 rotor into very-low-density lipoproteins (VLDL, density < 1.006), intermediate-low-density lipoproteins (LDL_1, density 1.006–1.019), low-density lipoproteins (LDL_2, density 1.019–1.063), and high-density lipoproteins (HDL, density 1.063–1.210). Lipoprotein fractions were exhaustively dialyzed against 5 m*M* Tris-EDTA (pH 7.4) and aliquots were taken for protein (20) and purity verification by agarose electrophoresis (21). The remaining samples were delipidated (22) and the extracted lipid was separated into neutral lipid classes by thin-layer chromatography and quantitated by direct *in situ* charring densitometry (23). Phospholipid phosphorus was assayed by the procedure of Bartlett (24) utilizing a multiplication factor of 25 to convert phosphorus to phospholipid. Total plasma cholesterol was assayed colorimetrically (25).

Aorta analyses. Only the thoracic aorta was available for analysis. This portion of aorta was split longitudinally and one-half was lyophilized for dry weight determinations. Lipids were extracted (22) and quantitated by *in situ* charring densitometry following separation into various lipid classes by thin-layer chromatography (23). From the remaining one-half of the aorta, histopathologic data were obtained from 16 sections of the arch and descending aorta as previously described (26). An atherosclerosis index was obtained by totaling the observed intimal thickness (based on diameters or layers of smooth muscle cells), the amount of intracellular (0–5) and extracellular (0–5) lipid, the degree of collagen (0–5), and the number of replicating internal elastic laminae observed within the intima from the 16 sections.

Results. Lipoprotein analyses. The data obtained revealed several important points (Table I). First, both species transported

TABLE I. LIPOPROTEIN LIPIDS AND PROTEIN FROM CEBUS AND SQUIRREL MONKEYS FED DIETARY CORN OIL AND COCONUT OIL.[a]

	Lipoprotein fraction by diet			
	Corn oil (mg/dl)		Coconut oil (mg/dl)	
Lipid class and species	LDL_2	HDL	LDL_2	HDL
Cholesteryl ester				
Cebus	39.0 ± 7.2	99.1 ± 5.4*, **	51.4 ± 5.2**	186.1 ± 21.9**
Squirrel	26.6 ± 7.9*	63.8 ± 2.6*	117.1 ± 22.0	93.9 ± 10.3
Cholesterol				
Cebus	7.3 ± 1.5	8.7 ± 2.5	10.5 ± 1.3**	12.3 ± 1.9
Squirrel	7.7 ± 1.3*	10.9 ± 2.7	30.5 ± 3.8	16.7 ± 3.3
Phospholipid				
Cebus	13.8 ± 1.9*	38.6 ± 2.5*	32.3 ± 1.7**	146.2 ± 10.9**
Squirrel	14.4 ± 2.6	53.4 ± 9.9	20.2 ± 0.5	53.5 ± 16.3
Triglyceride				
Cebus	1.9 ± 0.5	6.5 ± 2.1	3.5 ± 1.3	11.2 ± 0.2**
Squirrel	1.9 ± 1.1	7.3 ± 3.3	1.7 ± 0.5	2.3 ± 0.5
Total lipid				
Cebus	62.0 ± 9.9*	147.9 ± 11.7*	97.6 ± 5.3**	356.0 ± 32.9**
Squirrel	50.6 ± 6.5*	135.5 ± 17.5	169.5 ± 22.9	166.0 ± 28.8
Total protein				
Cebus	18.4 ± 3.6*	196.0 ± 11.7*, **	36.5 ± 0.6**	272.5 ± 18.0**
Squirrel	16.7 ± 0.8*	130.1 ± 2.9	61.7 ± 6.2	113.2 ± 12.3
Total lipoprotein[b]				
Cebus	80.4 ± 13.1*	343.9 ± 9.4*, **	134.1 ± 5.1**	628.5 ± 48.5**
Squirrel	67.4 ± 5.8*	265.6 ± 17.2	231.0 ± 17.6	279.3 ± 40.6

[a] Values are means ± SE for three monkeys per group.
[b] Sum of total lipid and protein.
* Difference between dietary fats significant at the 0.05 level.
** Difference between species significant at the 0.05 level.

approximately 70% of their lipids as HDL when fed *corn oil* (plasma cholesterol levels were 155 ± 5 and 119 ± 13 mg/dl for cebus and squirrel monkeys, respectively), and, although their lipid, protein, and total lipoprotein concentration for LDL_2 did not differ, the corn oil-fed cebus had significantly more HDL cholesteryl ester and protein than similarly fed squirrel monkeys.

Second, although *coconut oil* feeding resulted in comparable plasma cholesterol levels (280 ± 9 and 278 ± 23 mg/dl for cebus and squirrel monkeys, respectively), the major expansion in the cholesterol pool differed between the two species, with the squirrel monkeys primarily expanding the esterified (CE) and unesterified (FC) cholesterol of its LDL_2 fraction, whereas the cebus monkey primarily increased its HDL. In addition, the cebus, unlike the squirrel, significantly increased the phospholipids in its LDL_2 and both phospholipids and protein in its HDL. The result was a total concentration of HDL in cebus fed coconut oil which was twice that observed in the squirrel monkey. Thus, whereas the major response to coconut oil feeding by squirrel monkeys was to expand its LDL_2 fraction, the cebus monkey primarily increased its HDL. A third point was the CE:FC ratio in HDL, where the cebus maintained a ratio greater than 10:1 on both diets and the squirrel managed only a 5:1 ratio.

The contribution of VLDL and LDL_1 to the total circulating lipids was minimal, and they are not included for the sake of brevity.

Aorta analyses. From the limited histologic data (Table II), three observations are appropriate. First, the arterial response determined histologically appeared more variable than that of the circulating lipids. Second, squirrel monkeys fed either diet developed an intimal proliferation which was slightly exacerbated by hypercholesterolemia, particularly in terms of lipid accumulation. Third, cebus monkeys had negligible intimal hyperplasia when normocholesterolemic, but appeared comparable to the squirrel monkey when hypercholesterolemic, with the exception of the absence of observable lipid and the tendency to have increased amounts of connective tissue.

Lipid class analysis of the remaining half of the thoracic aorta (Table III) supported the histologic data, i.e., the squirrel monkeys fed either diet accumulated significantly more total lipid than comparable cebus monkeys. The most remarkable difference between the two species was the 10-fold greater accumulation of cholesteryl ester in the squirrel monkey fed coconut oil. A similar species difference was noted for the corn oil diet, although the amount of lipid that accumulated was less than that for the coconut oil diet. Significantly more triglyceride and free fatty acid also accumulated in coconut oil-fed squirrel monkeys, while aortas from cebus fed corn oil contained more free cholesterol.

Discussion. Analyses of the aortas of these two species biochemically and histologically by light and electron microscopy concur with previous observations on the susceptibility of the squirrel monkey and resistance of the cebus monkey to atherosclerosis (27). The finding that aortas of the susceptible squirrel monkey contained 10 times more cholesteryl ester than those of the resistant cebus lends credence to the species difference, since the accumulation of

TABLE II. HISTOPATHOLOGIC DATA OF THE AORTA OF SQUIRREL AND CEBUS MONKEYS FED DIETARY CORN OIL OR COCONUT OIL.[a]

Dietary treatment and species	Intimal thickness	Lipid	Connective tissue	Total
Corn oil				
Squirrel	16.7 ± 2.0	4.7 ± 2.4	10.3 ± 3.4	31.7 ± 6.7
Cebus	1.3 ± 0.3	0	2.3 ± 0.9	3.7 ± 1.2
Coconut oil				
Squirrel	19.7 ± 5.4	15.7 ± 5.4	13.7 ± 8.2	49.0 ± 16.7
Cebus	17.0 ± 7.9	0	27.3 ± 13.9	44.3 ± 21.8

[a] Values represent means ± SE of the arch and descending thoracic aorta from three monkeys of each species per dietary group. See methods for description of units.

TABLE III. AORTIC LIPIDS FROM SQUIRREL AND CEBUS MONKEYS FED DIETARY CORN OIL OR COCONUT OIL.

Dietary treatment and species	Aortic lipid classes[a]					
	Cholesteryl ester	Triglyceride	Free fatty acid	Cholesterol	Phospholipid	Total
	(mg/g dry wt)					
Corn oil						
Squirrel	1.7 ± 0.1*	1.5 ± 0.3*	2.2 ± 0.1*	3.6 ± 0.0*	8.5 ± 0.1	17.6 ± 0.2**
Cebus	0.2 ± 0.1	0.1 ± 0.0	1.2 ± 0.2	4.8 ± 0.1	8.4 ± 0.8	14.6 ± 0.9
Coconut oil						
Squirrel	6.6 ± 1.2*	1.7 ± 0.1*	4.3 ± 1.0***	4.7 ± 0.7	8.2 ± 1.4	25.4 ± 1.3***
Cebus	0.8 ± 0.1	0.5 ± 0.1	1.3 ± 0.2	5.2 ± 0.7	7.1 ± 0.7	15.8 ± 2.3

[a] Values represent means ± SE of the arch and descending thoracic aorta from three monkeys of each species per dietary group.

* Difference between species significant at the 0.001 level.

** Difference between species significant at the 0.05 level.

*** Difference between species significant at the 0.02 level.

cholesteryl ester is often correlated with the degree of atherosclerotic involvement (27).

The marked increase in total HDL in the cebus monkey fed coconut oil, coupled with the lack of response in this lipoprotein by the squirrel monkey, which primarily elevates its LDL, tend to support current hypotheses on the atherogenicity of LDL and the seemingly protective effect of HDL. It has been demonstrated that LDL from hypercholesterolemic serum stimulates growth of primate smooth muscle cells in tissue culture and leads to intracellular lipid accumulation, while HDL has little effect (28, 29). In this context, HDL has been shown to compete with LDL for cell surface receptors in porcine smooth muscle cells and may not be internalized as extensively as the latter, in effect reducing the cholesterol uptake and load on the smooth muscle cell (30). In addition, HDL in concert with phospholipids may remove cholesterol from cholesterol-laden tissues, such as the arterial intima, as demonstrated *in vitro* (31–33). Although the mechanism of cholesterol removal is unknown, it has been hypothesized that the lecithin cholesterol acyl transferase (LCAT) enzyme may play a role in transport of cholesterol from peripheral tissues to the liver (34, 35).

It has been further suggested that membrane and lipoprotein phospholipids may be critical to normal membrane fluidity, enzymatic interactions, and mobilization of lipids including cholesterol (36, 37). Thus, the numerous phospholipid-rich HDL particles of the cebus monkey may enhance LCAT activity, as evidenced by the greater CE:FC ratio in cebus, and thereby modulate the expansion of the intravascular cholesterol pool during saturated fat feeding, precluding the lipid deposition and atheromatous change associated with the LDL-rich, phospholipid-poor lipoproteins of the squirrel monkey under similar dietary circumstances. Study of LCAT activity in these two species is in progress.

From our collective observations on these monkeys we hypothesize that the diffuse intimal thickening of the cebus represents a proliferative response of the intima under conditions of physiologically controlled lipemia. That is, if hypercholesterolemia (character undefined) damages endothelium and enhances platelet aggregation as currently proposed (38, 39), the intima would be exposed to circulating growth factors and lipoproteins which can either induce atheromatous change when cholesterol-rich LDL particles penetrate the exposed intima (squirrel monkey) or simply induce a relatively benign intimal hyperplasia without significant lipid accumulation when phospholipid-rich HDL particles predominate (cebus monkey).

The possible implications of these diet-induced variations in primate atherogenesis are intriguing, particularly when one considers that these monkeys are primates of the same family (*Cebidae*) and that they may represent models of the variable response observed among humans subjected to the

"dietary stresses" associated with affluence. Agrarian nonstressed populations (underdeveloped countries), not unlike our two species of monkeys fed corn oil, might be expected to have few differences in their circulating lipoproteins and minimal vascular disease (atherosclerosis). The saturated fat-cholesterol-sugar diet of the affluent society may unmask genetic variations within a population, revealing individual variability in packaging, transport, and metabolism of lipoproteins which has specific ramifications in terms of arterial degeneration.

Summary. Squirrel and cebus monkeys fed a coconut oil diet develop comparable hypercholesterolemias, but the squirrel monkey primarily expands its low-density lipoprotein cholesterol pool, whereas the cebus primarily increases its HDL pool of cholesterol. These results, coupled with the greater accumulation of aortic lipid, particularly cholesteryl ester, in the atherosclerotic-susceptible squirrel monkey, support the concept of the protective nature of high-density lipoproteins and the atherogenic potential of LDL. They also suggest that a species' genetic control of the lipoprotein response to diet is variable and has important biological implications.

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