Rapid Serum Lipoprotein Changes in Spider Monkeys on Short-Term Feeding of High Cholesterol–High Saturated Fat Diet[1] (36735)

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The advantage of using subhuman primates in the study of diet-induced hyperlipoproteinemia and atherosclerosis is well recognized. Although several species of Old and New World monkeys have been extensively used for this purpose (1–3), information about the response of serum lipids to various dietary regimens and the nature and extent of atherosclerotic lesions induced in spider monkeys is scanty.

The use of the spider monkey, a New World primate, as an experimental model of human atherosclerosis needs to be explored, since it has certain advantages. For example, spider monkeys, which are comparable to the rhesus in size and weight, are less susceptible to tuberculosis and dysenteric diseases, unlike the commonly used Old World primates, especially rhesus (4).

Serum lipid changes induced by hypercholesteremic diets are often measured in terms of cholesterol and, less often, triglyceride. However, little information is available on the changes of the different serum lipoprotein classes immediately following the high cholesterol-fat diet, perhaps due to the lack of simple methodology to quantitate serum lipoproteins. Recently, an easy procedure for measuring serum β- and pre-β-lipoproteins or the cholesterol carried by these two classes have been developed (5, 6) and extensively applied (7, 8). Further preliminary studies have indicated the usefulness of this method in studying the serum lipoproteins of different primate species. In the present investigation, serum lipoprotein changes in spider monkeys have been studied before, during, and after short-term high cholesterol-fat feeding. Investigations of this nature may lead to methods of observing responses to various dietary changes and of individual responses to specific diet compositions.

Materials and Methods. Animals and Diets. Five adult spider monkeys (*Ateles geoffroyi*) weighing 5.7 to 7.5 kg were used in this study. Each animal served as its own control and received (*ad libitum*) a regular diet of Purina Monkey Chow 25, which contains approximately 55% carbohydrate, 25% protein and 5% fat. The protein content of this monkey chow is similar to the basal semipurified diet described by Mann *et al.* (9). The monkeys were on this diet for about 13 wk prior to starting the experimental diets. During this period serum lipoprotein analysis was performed at weekly intervals. When the serum lipid levels reached a steady state, the monkeys were fed experimental diets. A butter–cholesterol diet used was similar to the one described by Malinow, Maruffo and Perley (10) and contained by weight: Purina Chow, 38.6%; banana, 8.5%; butter, 4.3%; cholesterol, 2% (or 0.5%). Each animal was given 250 g (approx 450 cal) of this diet once a day for 3 wk. This amount was based on studies of diet consumed *ad libitum* and on observations of maintenance of body weights. In between the experimental diets, the animals were maintained on regular chow for 27 days. The diets were well accepted by the monkeys, with very little wastage of food. In addition, each animal received an orange twice a week both during regular and experimental diets.

There were no significant changes in the body weight of the monkeys throughout the

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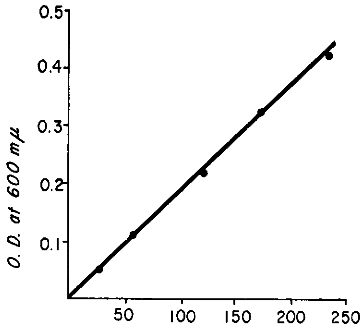

FIG. 1. Relationship between turbidity and serum β- plus pre-β-lipoprotein cholesterol content of spider monkeys. Different aliquots of serum were incubated with solutions of heparin and $CaCl_2$. The turbidity was related to the corresponding cholesterol content of the precipitated complex (5).

experiment. Blood specimens (12 hr fasting) were obtained at specific intervals under Serynylan[2] anesthesia, and serum lipoprotein patterns and lipid levels were determined.

Serum lipid measurements. Serum cholesterol was measured by the method of Pearson, Stern and McGavack (11). The determination of cholesterol in lipoproteins was obtained by a specific and quantitative precipitation procedure using heparin in the presence of Ca^{2+} (5, 6). Briefly, the method consists of mixing serum (0.2 ml), distilled water (3.2 ml), heparin (0.25%, ≅ 140 units/mg, 0.1 ml) and $CaCl_2$ (0.5 *M*, 0.5 ml), in the order given, and measuring the turbidity obtained after 15 min at 600 mμ against a blank containing a similar mixture but omitting heparin. The cholesterol content of β- plus pre-β-lipoprotein was related to turbidimetric measurements by constructing a standard curve (Fig. 1). This curve was obtained by measuring turbidity with increasing amounts of serum, the addition of distilled water to make the total volume of 4 ml, and analyzing the corresponding cholesterol content of the precipitate after centrifugation and washing. From this standard curve the β- plus pre-β-lipoprotein cholesterol value of any given serum could be calculated by measuring the turbidity alone. Earlier observations on human serum indicated that the difference between the values of the β-plus pre-β-lipoprotein cholesterol estimated by the turbidimetric procedure and the actual values obtained by chemically analyzing the precipitated complex would be less than 2%, provided there was no significant variation in the proportion of pre-β-lipoprotein. (On the standard chow and these experimental diets, pre-β-lipoproteins in these monkeys were very low in concentration.) The cholesterol value obtained from the standard curve (Fig. 1) for a given turbidity (OD) was almost identical to the values obtained in human serum, indicating the marked similarity in composition of lipoproteins in spider monkey and human serum. This similarity in lipoprotein compositions between human and subhuman primates has also been reported (12, 13). The difference between total cholesterol and β- plus pre-β-lipoprotein cholesterol was considered as α-lipoprotein cholesterol (14).

Agarose-agar gel electrophoresis. Electrophoresis of serum lipoproteins was performed according to the method of Noble (15) with some modifications (6). The lipoprotein bands obtained were scanned with densitometer and the curves integrated to estimate the proportion of β- to pre-β-lipoproteins.

Estimation of β- and pre-β-lipoproteins. Serum β- and pre-β-lipoprotein concentrations have been calculated as described previously (6). Assuming that cholesterol content per unit β- or pre-β-lipoprotein molecule is similar to human, the approximate serum content of these two classes of lipoproteins were calculated based on the densitometric ratios of β- to pre-β-lipoprotein, β- plus pre-β-lipoprotein cholesterol content.

Ultracentrifugal fractionation. β- and pre-β-lipoproteins were fractionated from other serum proteins in a preparative ultracentrifuge, Spinco Model L2-65, using a Type 65 rotor at density 1.063 by the method described by Hatch and Lees (14).

Results. Figure 2 illustrates the changes in serum total cholesterol, β- plus pre-β-lipoprotein cholesterol and α-lipoprotein cholesterol in spider monkeys during regular and hypercholesteremic diets. It can be seen that there were slight variations in serum

[2] Bio-Ceutic Laboratories, Inc., St. Joseph, MO.

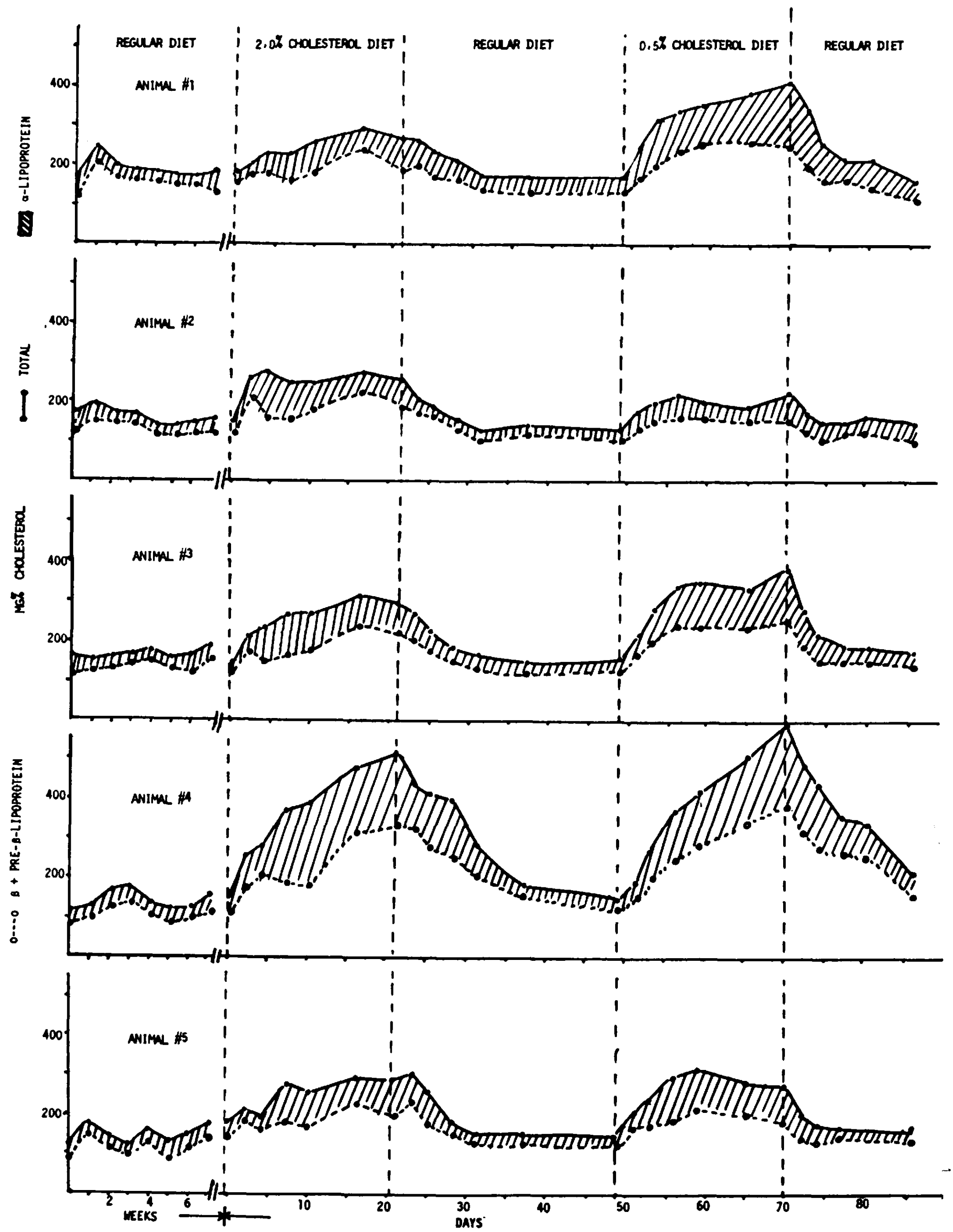

FIG. 2. The effect of short-term high cholesterol-fat feeding upon the serum lipoprotein cholesterol level of spider monkeys. The difference between total cholesterol (—) and β- plus pre-β-lipoprotein cholesterol (- - - -) was considered α-lipoprotein cholesterol (hatched area). A significant elevation in α-lipoprotein cholesterol and marked variation in individual responses to 2.0 and 0.5% cholesterol feeding can be seen. Significant changes can be detected within 48 hrs.

cholesterol content during the 13 wk of conditioning, the average level being 165 mg/100 ml. About 80% of the lipoprotein cholesterol seems to be carried by β- and pre-β-lipoproteins. Upon 2% cholesterol feeding, increases of serum total cholesterol were observed in the first blood sample taken, as early as two days, and there was a marked variation in the degree of response among the monkeys; for example, monkey No. 4 with a serum cholesterol level reaching 520 mg/100 ml compared to 260 mg/100 ml attained by monkey No. 2 (av, 324 mg/100 ml for the 5 monkeys).

Interestingly, the increase in serum cholesterol during the 3-wk period was reflected not only in β- plus pre-β-lipoprotein cholesterol but also in the α-lipoprotein cholesterol. This is in contrast to the general observation that on such diets most of the total cholesterol elevation was due to β-lipoprotein. A similar trend was observed when the animals were refed a 0.5% cholesterol diet after being fed a regular diet for a period of 27 days. However, the animals seem to respond as much or possibly more as on the 2% intake when they were maintained on 0.5% cholesterol diet. This can be seen from the overall increase in serum lipoprotein cholesterol, in all monkeys except No. 2. Again, on the lower cholesterol intake, the monkeys varied significantly in their degree of response. No. 4 consistently remained the highest responder, and No. 2 the least, as reflected by serum lipid studies. It is also interesting to note that both of the high and low responding monkeys were females. When the animals were returned to a regular diet, the serum lipoprotein cholesterol levels fell rapidly and reached their original levels within 2 to 3 wk. The serum levels on standard chow could not be used to predict which of the monkeys would respond to the diets by developing high serum lipid levels.

In order to ascertain that β- plus pre-β-lipoprotein cholesterol calculated from turbidimetric method actually represented the cholesterol content of these lipoproteins, some selected samples of serum (drawn during high cholesterol diet) were studied further. The β- and pre-β-lipoproteins were precipitated by heparin and $CaCl_2$ and analyzed for cholesterol as before but, in addition, the β- and pre-β-lipoproteins were separated by preparative ultracentrifugation and their cholesterol content was determined. The difference between turbidimetric method and chemical analysis of the precipitated lipoproteins was found to be less than 3%, indicating

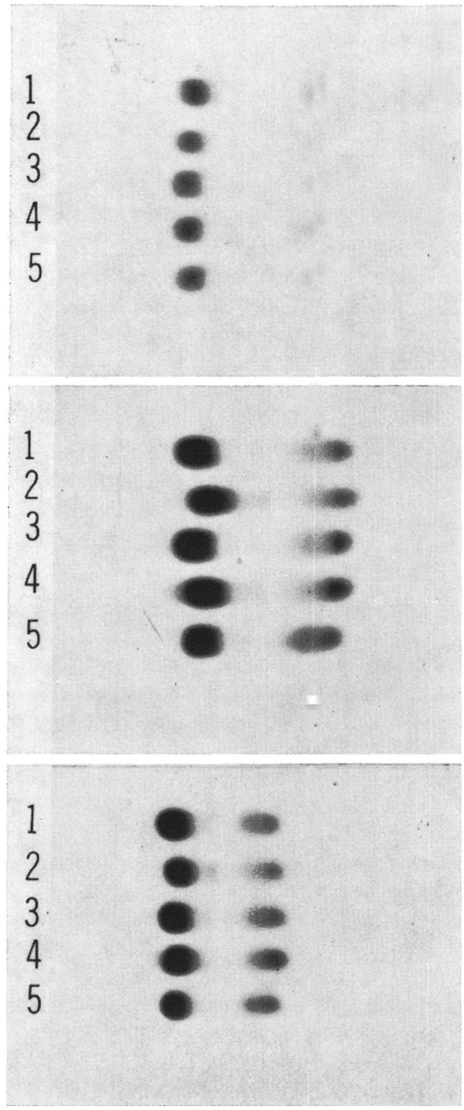

FIG. 3. Agarose-agar gel electrophoresis pattern of serum lipoproteins of spider monkeys before and after hypercholesteremic diets. (top) regular diet; (middle) 2.0% cholesterol diet; (bottom) 0.5% cholesterol diet. An elevation in β- as well as α-lipoproteins with very little variation in pre-β-lipoprotein upon cholesterol feeding was observed.

TABLE I. Serum Lipoprotein (LP) Levels of Spider Monkeys on High Cholesterol–High Saturated Fat Diet.[a]

Spider monkey (no.)	Regular diet (mg/100 ml) β-LP	Regular diet (mg/100 ml) Pre-β-LP	Regular diet α-LP[b] / β + pre-β-LP	2.0% Cholesterol diet (mg/100 ml) β-LP	2.0% Cholesterol diet (mg/100 ml) Pre-β-LP	2.0% Cholesterol diet α-LP / β + pre-β-LP	0.5% Cholesterol diet (mg/100 ml) β-LP	0.5% Cholesterol diet (mg/100 ml) Pre-β-LP	0.5% Cholesterol diet α-LP / β + pre-β-LP
1	363	15	0.18	466	19	0.39	649	19	0.69
2	283	28	0.17	460	25	0.35	350	23	0.51
3	298	13	0.17	553	13	0.38	640	20	0.52
4	238	32	0.42	830	35	0.58	979	30	0.51
5	349	15	0.23	470	15	0.54	409	13	0.61

[a] After 21 days of hypercholesteremic diet.

[b] Ratio of α- to β- + pre-β-lipoprotein cholesterol.

that cholesterol content per unit lipoprotein molecule remained relatively constant under the present conditions. There was a 3–10% variation between the polyanionic precipitation method and values analyzed by the ultracentrifuge method. These results are in agreement with Morris and Fitch (16), who obtained similar agreement in cholesterol distribution by ultracentrifuge and polyanionic precipitation methods in hypercholesteremic rhesus monkeys.

Agarose-agar gel electrophoresis was performed on all blood samples drawn when the animals were on regular and hypercholesteremic diet. β- and pre-β-lipoprotein concentrations as well as the ratio of α- to β- plus pre-β-lipoprotein cholesterol were calculated from the electrophoretic and lipoprotein cholesterol data. Figure 3 and Table I demonstrate qualitative and quantitative changes in serum lipoprotein patterns after cholesterol feeding. As shown, spider monkeys have predominantly β-lipoproteins and very little pre-β-lipoproteins, α-lipoproteins being intermediate in concentration. After 3 wk on hypercholesteremic diet, there was an increase in β-lipoprotein and a definite increase in α-lipoprotein, with no significant change in pre-β-lipoproteins. The methodology used in these experiments was able to detect rapid and selective changes of the serum lipoproteins and reflected not only distinct lipoprotein patterns but differences in individual responses in short time periods.

Discussion. The serum lipoprotein pattern in spider monkeys and their response to short-term cholesterol feeding reveal some interesting observations. The low level of serum α-lipoprotein cholesterol (less than 20%) observed in this primate species seems to be in contrast to observations made in other primate species, like baboon, rhesus and Cebus, in which more than 50% of the total cholesterol was found in α-cholesterol (16, 17). A preliminary survey of serum lipoproteins of 12 different primate species made in this laboratory showed that chimpanzee and squirrel monkeys also have comparatively less α-lipoprotein cholesterol. Lofland *et al.* (18) made similar observations in New World primates, including spider monkey. This low level of α-lipoprotein has been related to susceptibility to atherosclerosis (19).

Marked variation in individual response to cholesterol diet is in agreement with studies conducted on other primate species (20, 21). It is of further interest that decreasing dietary cholesterol levels from 2.0 to 0.5% were equally effective in achieving lipoprotein changes, suggesting some saturation of absorption occurs similar to humans (22, 23). Earlier studies on cholesterol feeding have indicated a decrease in the α-lipoprotein in primates and in rats (17, 24). However, these observations were based on prolonged (3 to 18 mo) feeding of high cholesterol diets. Studies on cholesterol metabolism in baboon have also indicated that both exogenous and endogenous cholesterol are transported generally in a nonpreferential fashion by serum α- and β-lipoproteins (25). Interestingly, at the time of this writing, Morris and Greer reported (26) a transitory hyper-α-lipoproteinemia in cholesterol-fed rhesus monkeys which is in agreement with our observations on spider monkeys.

α-Lipoprotein is known to play a key role in serum lipid clearing and cholesterylester transport and metabolism through the enzymes lipoprotein lipase and lecithin-cholesterol acyltransferase, respectively (12). Recent studies have indicated that low and high density lipoproteins probably are the degradation products of chylomicrons and very low density lipoproteins. This evidence suggests a precursor–product relationship between these lipoprotein classes (12). Therefore, any increase in α-lipoprotein seems to suggest an active lipid transport, necessary to cope with ingested cholesterol. These ideas are speculative and since very little is known about the changes in α-lipoprotein and its physiological significance upon cholesterol feeding, more work is needed in this direction, but obviously with methods of quantitating each of the serum lipoproteins at brief intervals.

Summary. Serum lipoprotein changes resulting from high cholesterol (0.5% and 2.0%)–high saturated fat diets have been studied in spider monkeys (*Ateles geoffroyi*). Rapid increase in β-lipoprotein, significant elevation in α-lipoprotein, and marked variations in individual responses to cholesterol intake were observed during these dietary periods.

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