# Effect of Dietary Lipids on Hepatic and Extrahepatic Sterol 27-Hydroxylase Activity in High- and Low-Responding Baboons

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**Our previous studies found that low low-density lipoprotein (LDL)–responding baboons compared with high LDL–responding baboons have higher hepatic sterol 27-hydroxylase activity when consuming a high-cholesterol and high-fat (HCHF) diet. The present studies were conducted to determine whether the extrahepatic activity of sterol 27-hydroxylase is also higher in low-responding baboons and to assess whether the enzyme is regulated at the protein level. We measured the hepatic sterol 27-hydroxylase activity and protein level and plasma 27-hydroxycholesterol concentration in six low- and six high-responding baboons on both the basal and the HCHF diet. We also compared the sterol 27-hydroxylase activity in the adrenal gland and 27-hydroxycholesterol concentration in blood lymphocytes from high- and low-responding baboons consuming the HCHF diet. With the HCHF diet, the plasma 27-hydroxycholesterol concentration and hepatic sterol 27-hydroxylase activity and protein level increased rapidly in low responders, but not in high responders. Blood lymphocytes of low-responding baboons cultured in the presence of lipoprotein-deficient serum (LPDS) had lower cholesterol concentrations than those from high-responding baboons. Addition of exogenous 27-hydroxycholesterol to the culture medium of blood lymphocytes decreased the cellular cholesterol concentration. Plasma 27-hydroxycholesterol and hepatic sterol 27-hydroxylase activity and protein levels were negatively correlated with the plasma VLDL + LDL cholesterol concentration and VLDL + LDL/HDL cholesterol ratio after 6 weeks on the HCHF diet, but not on the chow diet. The results suggest that sterol 27-hydroxylase activity in both hepatic and extrahepatic tissues attenuates the dietary responsiveness in baboons, and the enzyme activity is not regulated by the specific activity of the protein.**



DIETARY CHOLESTEROL and saturated fatty acids increase plasma cholesterol concentrations in most animal species, including humans.[1] However, the degree of response to dietary lipids differs among species and among individual animals within species.[2-5] Mechanisms controlling the responsiveness differ among species,[2-6] but identification of metabolic characteristics that predict dietary responsiveness in a nonhuman primate would be helpful in searching for the mechanisms controlling responsiveness in humans.

Our studies of baboons (*Papio* species) selectively bred for high and low responses to dietary lipids suggested that the plasma cholesterol response to a cholesterol and fat–enriched diet is inversely associated with the ability to induce hepatic sterol 27-hydroxylase.[7,8] Sterol 27-hydroxylase is an important enzyme of hepatic bile acid synthesis and may influence dietary responsiveness by affecting bile acid metabolism.[9] However, sterol 27-hydroxylase is also present in the adrenal gland, macrophages, arterial endothelium, and other extrahepatic tissues that do not synthesize bile acids.[10-14] The presence of sterol 27-hydroxylase enables extrahepatic tissues to synthesize 27-hydroxycholesterol, an inhibitor of hepatic hydroxymethyl glutaryl coenzyme A-(HMG-CoA) reductase.[15] Since most of the body cholesterol is synthesized in extrahepatic tissues,[16] the increased activity of sterol 27-hydroxylase in extrahepatic tissues would be hypolipidemic. However, it is not known whether the dietary cholesterol and fat that induce sterol 27-hydroxylase in hepatic tissues also induce it in extrahepatic tissues.

The purpose of the present study was to determine whether sterol 27-hydroxylase activity differs in hepatic and extrahepatic tissues between high- and low-responding baboons consuming a high-cholesterol and high-fat (HCHF) diet, and whether endogenous and exogenous 27-hydroxycholesterol affects the cholesterol concentration in their lymphocytes. We also investigated the relationship of sterol 27-hydroxylase activity to sterol 27-hydroxylase protein levels to determine whether sterol 27-hydroxylase activity is regulated by the specific activity of enzyme protein.

## MATERIALS AND METHODS

### *Experimental Design and Subject Selection*

We used three groups of selectively bred pedigreed baboons for three experiments.

The first experiment was conducted to determine the effect of dietary cholesterol and fat on the plasma 27-hydroxycholesterol concentration, hepatic sterol 27-hydroxylase protein concentration, and hepatic sterol 27-hydroxylase activity. We selected six low-responding baboons (progeny of low-responding sires and dams with plasma very–low-density and low-density lipoprotein [VLDL + LDL] cholesterol <75 mg/dL while consuming the HCHF diet) and six high-responding baboons (progeny of high-responding sires and dams with plasma VLDL + LDL cholesterol > 150 mg/dL while consuming the HCHF diet). All baboons were progeny of different dams, and were adults aged 4 to 9 years and weighing 12 to 28 kg. In each group, half were males and half were females. Initially, the baboons were maintained on a basal diet (Wayne Teklad, Madison, WI) low in both cholesterol (0.03 mg/kcal) and fat (10% of total calories) for at least 12 weeks. After baseline observations, they were fed a high-cholesterol (0.45 mg/kcal) and high-fat (40% of total calories from coconut oil) diet for 18 weeks. The animals were fed once per day and had access to water at all times. They were housed in indoor-outdoor gang cages except during liver biopsy.

The second experiment was conducted to compare the concentration

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of 27-hydroxycholesterol in blood lymphocytes and the effect of endogenous and exogenous 27-hydroxycholesterol on the cellular cholesterol concentration. For this experiment, we selected three high-responding (VLDL + LDL cholesterol >150 mg/dL), three low-responding (VLDL + LDL cholesterol <75 mg/dL), and six average-responding (VLDL + LDL cholesterol 100 to 150 mg/dL) adult (aged 9 to 16 years) female baboons. High- and low-responding baboons were the progeny of high- and low-responding sires and dams, respectively. Baboons were maintained on the HCHF diet for 6 weeks. Blood (50 mL) from each baboon was collected aseptically with heparin (10 U/mL). Blood lymphocytes were isolated by the method described by Ho et al.[17] Half of the cells from each high- and low-responding baboon were used to measure 27-hydroxycholesterol and cholesterol concentrations by the high-performance liquid chromatography (HPLC) method,[7] and the other half were incubated for 72 hours in the presence of 20% lipoprotein-deficient serum (LPDS). Lymphocytes from the six average-responding baboons were pooled and $40 \times 10^6$ cells were incubated in duplicate with 20% LPDS and 27-hydroxycholesterol at 0 (control), 0.25, 0.5, 1, and 2 μg/mL for 72 hours. After incubation, the cells were washed and sonicated with lysis buffer. Cholesterol and 27-hydroxycholesterol levels were measured in the lysate.

In the third experiment, we selected frozen adrenal glands from six high- and six low-responding baboons that underwent necropsy in a previous experiment. These baboons were maintained on the HCHF diet for 18 months before necropsy. Adrenal glands removed at necropsy were quick-frozen in liquid nitrogen and stored at −80°C until used.

### *Blood Sampling and Liver Punch-Biopsies*

For the first experiment and for lymphocyte studies, fasting (approximately 16 hours) baboons were immobilized by ketamine hydrochloride (10 mg/kg) and blood was obtained by venipuncture. At the time of blood sampling, three 25-mg liver cores were obtained from each animal by punch-biopsy for the first experiment. In the first experiment, blood and liver samples were obtained on the basal diet and after 3, 6, 10, and 18 weeks of the HCHF diet.

The protocol of this experiment was approved by the Animal Research Committee of the Southwest Foundation for Biomedical Research (SFBR). The SFBR is accredited by the American Association for Accreditation of Laboratory Animal Care and is registered with the US Department of Agriculture.

### *Measurement of Plasma 27-Hydroxycholesterol*

27-Hydroxycholesterol levels were measured by HPLC as described by us,[7] with a modification in which esterified oxysterols were hydrolyzed with cholesterol esterase.

### *Measurement of Hepatic and Adrenal 27-Hydroxylase Activity*

Mitochondria from the liver and adrenal gland were isolated as described by Griffith.[18] Briefly, liver biopsy cores and minced adrenal glands were homogenized in 10 mmol/L HEPES buffer (pH 7.4) containing 0.2 mol/L sucrose using a Teflon-glass homogenizer. The homogenate was centrifuged for 10 minutes at $2,000 \times g$, the resulting supernatant was centrifuged for 20 minutes at $9,000 \times g$, and the pellet was washed twice and suspended in the same buffer. The protein concentration was determined by the Bradford method[19] using bovine serum albumin as a standard. The aliquots were stored at −80°C until activity assays were performed within 1 week.

Sterol 27-hydroxylase activity in hepatic and adrenal mitochondria was measured by the HPLC method of Petrack and Latario.[20] In short, 400 μg protein of liver mitochondria or 1 mg adrenal gland mitochondria was incubated at 37°C for 15 minutes in 1 mL 100-mmol/L potassium phosphate buffer, pH 7.5, containing 1 mmol/L DTT, 0.2 mmol/L EDTA, 1.2 mmol/L NADPH, 5 mmol/L D,L-trisodium isocitrate, 0.2 U isocitrate dehydrogenase, and 200 nmol cholesterol in 45% 2-hydroxypropyl-β-cyclodextrin. The reaction was initiated by adding isocitrate/NADPH. At the same time, a control experiment was conducted in which reaction mixtures were incubated at 4°C for 15 minutes. The reaction was terminated by adding 50 μL 40% sodium cholate. Afterward, 2 U cholesterol oxidase along with 200 ng 7β-hydroxycholesterol (internal standard) were added to the reaction media and incubated again at 37°C for 20 minutes to generate the α,β-unsaturated ketones. Oxysterol (ketone derivative) peaks were identified by the retention time, and the area ratio method was used to measure 27-hydroxycholesterol concentrations as described previously.[7]

### *Measurement of Sterol 27-Hydroxylase Protein*

Sterol 27-hydroxylase protein levels were measured by Western blotting. Liver or arenal mitochondrial protein (20 μg) was subjected to electrophoresis on 10% sodium dodecyl sulfate–polyacrylamide gels and transferred to nitrocellulose membrane. The immunoreactive protein was detected using the ECL kit (Boehringer Mannheim, Indianapolis, IN). The primary antibody was prepared in rabbits against a synthetic peptide identical to residues 15 to 28 of the sterol 27-hydroxylase protein in our laboratory. Only a single band was detected by this antibody. The sterol 27-hydroxylase protein band was scanned using the densitometer and compared with a reference standard. The amount of sterol 27-hydroxylase protein was expressed as relative units (Fig 1).

### *Measurement of Plasma and Lipoprotein Cholesterol*

Total plasma cholesterol levels were measured by an enzymatic method using a Wako cholesterol assay kit (Wako Chemical, Richmond, VA). The plasma high-density lipoprotein (HDL) cholesterol level was

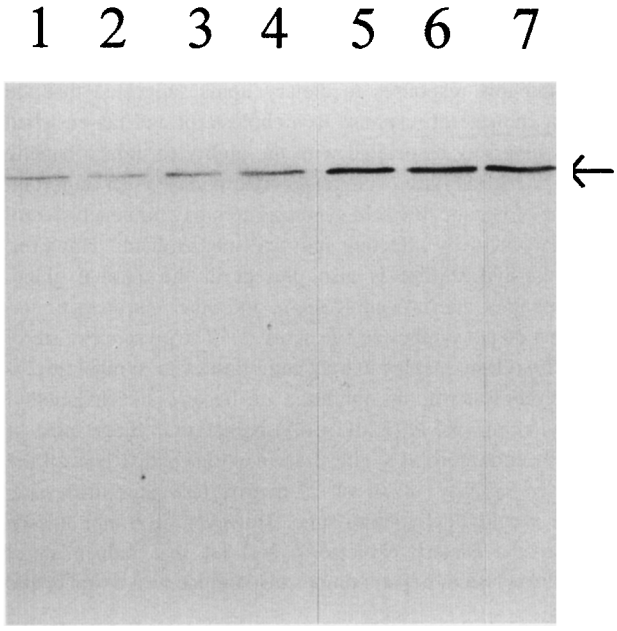


**Fig 1. Western blot analysis showing hepatic mitochondrial sterol 27-hydroxylase protein band from 3 baboons in duplicate. Lanes 1 and 2, a baboon with a low level of sterol 27-hydroxylase protein; lanes 3 and 4, a baboon with a medium level of sterol 27-hydroxylase protein; lanes 5 and 6, a baboon with a high level of sterol 27-hydroxylase protein; lane 7, a reference standard used for each Western blot. The primary antibody was raised in rabbits against sterol 27-hydroxylase peptide (residues 15 to 28), and detection was performed using the ECL Western blotting kit.**

**Table 1. Plasma and Lipoprotein Cholesterol Concentrations in Low- and High-Responding Baboons on Basal and HCHF Diet**

| | Low Responders | | | | High Responders | | | |
|---|---|---|---|---|---|---|---|---|
| Diet | Total Cholesterol | HDL | VLDL + LDL | VLDL + LDL/HDL Ratio | Total Cholesterol | HDL | VLDL + LDL | VLDL + LDL/HDL Ratio |
| Basal | 78 ± 5 | 48 ± 4 | 30 ± 8 | 0.771 ± 0.215 | 106 ± 13 | 63 ± 10 | 44 ± 13 | 0.822 ± 0.268 |
| HCHF | | | | | | | | |
| 3 wk | 133 ± 10* | 94 ± 1† | 40 ± 6 | 0.490 ± 0.133 | 240 ± 16*‡ | 103 ± 7* | 138 ± 14*‡ | 1.371 ± 0.166†§ |
| 6 wk | 150 ± 8* | 89 ± 10† | 62 ± 10 | 0.787 ± 0.174 | 235 ± 17*§ | 90 ± 8† | 145 ± 18*§ | 1.720 ± 0.328*‡ |
| 10 wk | 154 ± 9* | 93 ± 5† | 60 ± 6 | 0.655 ± 0.076 | 245 ± 9*‡ | 83 ± 4 | 162 ± 9*‡ | 1.992 ± 0.168*‡ |
| 18 wk | 145 ± 10* | 93 ± 6† | 52 ± 10 | 0.582 ± 0.134 | 264 ± 19*‡ | 101 ± 11* | 163 ± 17*§ | 1.748 ± 0.341*§ |

NOTE. Results are the mean ± SEM (n = 6 per group); units are mg/dL.
*$P < .01$ *v* basal diet.
†$P < .05$ *v* basal diet.
‡$P < .001$ *v* low responders.
§$P < .01$ *v* low responders.

measured after precipitation of VLDL and LDL by heparin–manganese chloride according to the method of the Lipid Research Clinics Program.[21] The VLDL + LDL cholesterol concentration was calculated as the difference between total plasma and HDL cholesterol concentrations.

### *Statistical Analysis*

Data in the tables are presented as the mean ± SEM. Plasma and liver variables between groups were compared by standard *t* test. The effect of the HCHF diet on plasma and liver variables was compared by paired *t* test. Activity and protein values for sterol 27-hydroxylase in the adrenal glands were analyzed by standard *t* test. Associations among the sterol 27-hydroxylase activity, 27-hydroxylase protein level, plasma 27-hydroxycholesterol, and VLDL + LDL cholesterol concentrations were determined using Pearson's correlation. Significance was set at *P* less than or equal to .05.

## RESULTS

### *Effects of HCHF Diet on Plasma and Lipoprotein Cholesterol Concentrations*

Plasma and lipoprotein cholesterol concentrations of baboons on the basal diet and after 3, 6, 10, and 18 weeks on the HCHF diet are presented in Table 1. There was a rapid increase in total plasma cholesterol in both high- and low-responding baboons. The increase in total plasma cholesterol was significant at 3 weeks. As expected, the major contributor to the plasma cholesterol of low-responding baboons was HDL cholesterol, whereas the major contributor to the plasma cholesterol of high-responding baboons was VLDL + LDL cholesterol. Plasma and VLDL + LDL cholesterol concentrations in high-responding baboons were higher than in low-responding baboons on the HCHF diet at each time. However, HDL cholesterol did not differ between high- and low-responding baboons at any time on the HCHF diet.

Due to the increase in HDL cholesterol and some increase in VLDL + LDL cholesterol in low-responding baboons, the VLDL + LDL/HDL cholesterol ratio remained the same (Table 1). However, due to the major increase in VLDL + LDL cholesterol in high-responding baboons, the VLDL + LDL/HDL cholesterol ratio increased significantly and was higher than the ratio in low-responding baboons on the HCHF diet at each time (Table 1).

### *Plasma 27-Hydroxycholesterol Concentration*

Plasma 27-hydroxycholesterol concentrations in both groups of baboons on the basal diet and after 3, 6, 10, and 18 weeks on the HCHF diet are presented in Table 2. When low-responding baboons began to consume the HCHF diet, plasma 27-hydroxycholesterol concentrations increased rapidly. The maximum increase occurred at 3 to 6 weeks and thereafter declined at 10 and 18 weeks; but, except at 10 weeks, HCHF values were significantly higher than basal diet values. However, when high-responding baboons began to consume the HCHF diet, plasma 27-hydroxycholesterol did not increase. On the HCHF diet, plasma 27-hydroxycholesterol in low-responding baboons was significantly higher than in high-responding baboons at 3 and 10 weeks.

### *Hepatic Sterol 27-Hydroxylase Activity and Protein Level*

Sterol 27-hydroxylase activities and protein levels measured at 3, 6, and 10 weeks on the HCHF diet are presented in Table 3. Hepatic sterol 27-hydroxylase activity in low-responding baboons increased significantly at 6 weeks after consuming the HCHF diet and remained elevated at 10 weeks; but in high-responding baboons, it did not increase significantly at 6 weeks after the HCHF diet. Sterol 27-hydroxylase activity in low-responding baboons was higher than in high-responding baboons after 10 weeks on the HCHF diet. Sterol 27-hydroxylase protein also increased in low-responding baboons and was

**Table 2. Plasma 27-Hydroxycholesterol Concentrations in Low- and High-Responding Baboons on Basal and HCHF Diets**

| Diet | Low Responders | High Responders |
|---|---|---|
| Basal | 5.23 ± 1.20 | 5.07 ± 0.53 |
| HCHF | | |
| 3 wk | 9.53 ± 0.77*‡ | 6.50 ± 0.44 |
| 6 wk | 10.10 ± 1.46†§ | 5.70 ± 0.31 |
| 10 wk | 7.22 ± 0.56 | 5.93 ± 0.76 |
| 18 wk | 8.12 ± 0.71* | 7.13 ± 0.35 |

NOTE. Results are the mean ± SEM (n = 6 per group); units are μg/dL.
*$P < .05$ *v* basal diet.
†$P < .01$ *v* basal diet.
‡$P < .005$ *v* high responders.
§$P < .05$ *v* high responders.

**Table 3. Hepatic Sterol 27-Hydroxylase Activity (pmol/mg protein/min) and Protein Levels (relative units) in Low- and High-Responding Baboons on the HCHF Diet**

| HCHF Diet | Low Responders | | High Responders | |
|---|---|---|---|---|
| | Activity | Protein | Activity | Protein |
| 3 wk | 109 ± 10 | 0.633 ± 0.075 | 104 ± 12 | 0.520 ± 0.08 |
| 6 wk | 193 ± 34* | 0.824 ± 0.066* | 125 ± 9 | 0.649 ± 0.06 |
| 10 wk | 197 ± 12† | 0.921 ± 0.047† | 139 ± 16‡ | 0.674 ± 0.06 |

NOTE. Results are the mean ± SEM (n = 6 per group).
*$P < .05$ *v* 3 wk.
†$P < .01$ *v* 3 wk.
‡$P < .05$ *v* low-responders.

higher than in high-responding baboons at 10 weeks on the HCHF diet.

*Adrenal Sterol 27-Hydroxylase Activity and Protein Level*

Mitochondrial sterol 27-hydroxylase activity of the adrenal gland in low-responding baboons (37.03 ± 2.42 pmol/mg mitochondrial protein/min) was significantly higher ($P = .015$) than in high-responding baboons (25.73 ± 3.00 pmol/mg mitochondrial protein/min) maintained on the HCHF diet for 18 months. Similarly, mitochondrial sterol 27-hydroxylase protein levels in low-responding baboons (0.352 ± 0.040 relative units) were significantly higher ($P = .015$) than in high-responding baboons (0.219 ± 0.021 relative units) maintained on the HCHF diet for 18 months (Table 4).

*Relationship of Plasma 27-Hydroxycholesterol Concentration and Hepatic Sterol 27-Hydroxylase Activity With Plasma Lipoproteins*

There was no correlation between the plasma 27-hydroxycholesterol concentration and plasma lipoproteins on the basal diet. However, at 3 and 6 weeks on the HCHF diet, the plasma 27-hydroxycholesterol concentration was strongly negatively correlated with the plasma VLDL + LDL cholesterol concentration ($r = -.647$, $P = .004$ at week 3 and $r = -.635$, $P = .005$ at week 6; Fig 2) and VLDL + LDL/HDL cholesterol ratio ($r = -.581$, $P = .011$ at week 3 and $r = -.545$, $P = 0.019$ at week 6; Fig 3). Similarly, there was no correlation between hepatic sterol 27-hydroxylase activity and plasma lipoproteins on the chow diet. However, hepatic sterol 27-hydroxylase activity and protein levels were strongly negatively correlated with the plasma VLDL + LDL cholesterol concentration at week 6 ($r = -.582$, $P = .011$ for activity and $r = -.507$, $P = .032$ for protein) and week 10 ($r = -.493$, $P = .038$ for activity and $r = -.552$, $P = .018$ for protein).

**Table 4. Adrenal Gland Sterol 27-Hydroxylase Activity (pmol/mg protein/min) and Protein Levels (relative units) in Low- and High-Responding Baboons**

| Phenotype | Activity | Protein |
|---|---|---|
| Low responders | 37.03 ± 2.42* | 0.352 ± 0.040* |
| High responders | 25.73 ± 3.00 | 0.219 ± 0.021 |

NOTE. Results are the mean ± SEM (n = 6 per group).
*$P = .015$ *v* high-responders.

*Relationship of Hepatic Sterol 27-Hydroxylase Activity and Protein Levels With Plasma 27-Hydroxycholesterol*

After baboons consumed the HCHF diet for 6 weeks, the plasma 27-hydroxycholesterol concentration was strongly positively correlated with hepatic sterol 27-hydroxylase activity ($r = .899$, $P < .001$; Fig 4A) and protein levels ($r = .559$, $P = .016$; Fig 4B). On the HCHF diet, two baboons had high levels of plasma 27-hydroxycholesterol and may have distorted these correlations. However, when we removed data for the two animals with high plasma 27-hydroxycholesterol from the analysis, these correlations were still significant ($P < .05$).

*Relationship of Sterol 27-Hydroxylase Activity With Protein in the Liver and Adrenal Gland*

Hepatic sterol 27-hydroxylase activity in baboons was highly positively correlated with protein levels at 3 ($r = .684$, $P = .002$), 6 ($r = .613$, $P = .007$), and 10 ($r = .852$, $P < .001$) weeks on the HCHF diet. Similarly, adrenal mitochondrial sterol 27-hydroxylase activity and protein levels were highly positively correlated ($r = .092$, $P < .001$).

*Effect of 27-Hydroxycholesterol on Cholesterol Synthesis in Lymphocytes*

Lymphocytes from low-responding baboons maintained on the HCHF diet for 6 weeks had a significantly higher concentration (75 ± 13 ng/dL) of 27-hydroxycholesterol than lymphocytes from high-responding baboons (45 ± 8 ng/dL, $P = .048$). After incubation for 72 hours in the presence of LPDS, blood lymphocytes from high-responding baboons had a 25.0% ± 1.7% decrease in cholesterol compared with baseline, whereas this decrease in low-responding baboons was much greater (36.3% ± 3.8%, $P < .05$). Addition of exogenous 27-hydroxycholesterol to the blood lymphocyte culture medium increased cellular 27-hydroxycholesterol ($r = .994$, $P = .001$; Fig 5A) linearly and decreased cellular cholesterol (Fig 5B) rapidly. Data for the decrease in the cholesterol concentration in response to exogenous 27-hydroxycholesterol were fitted to a biexponential regression curve. Initially, the decrease was rapid; however, with the increase in 27-hydroxycholesterol in the medium, the decrease in cellular cholesterol was slow.

## DISCUSSION

Our previous studies in selectively bred baboons suggested an important role for hepatic sterol 27-hydroxylase in attenuating the lipemic response to a dietary challenge.[7] Low-responding baboons compared with high-responding baboons had higher hepatic sterol 27-hydroxylase activity and plasma 27-hydroxycholesterol concentrations while consuming the HCHF diet.[8] After they began the HCHF diet, high-responding baboons had an increased plasma VLDL + LDL cholesterol concentration.[7] On the other hand, after they began the HCHF diet, low-responding baboons did not have increased plasma VLDL + LDL cholesterol concentrations.[8] The present results confirm previous findings and further suggest that in low-responding baboons the increase in plasma 27-hydroxycholes-

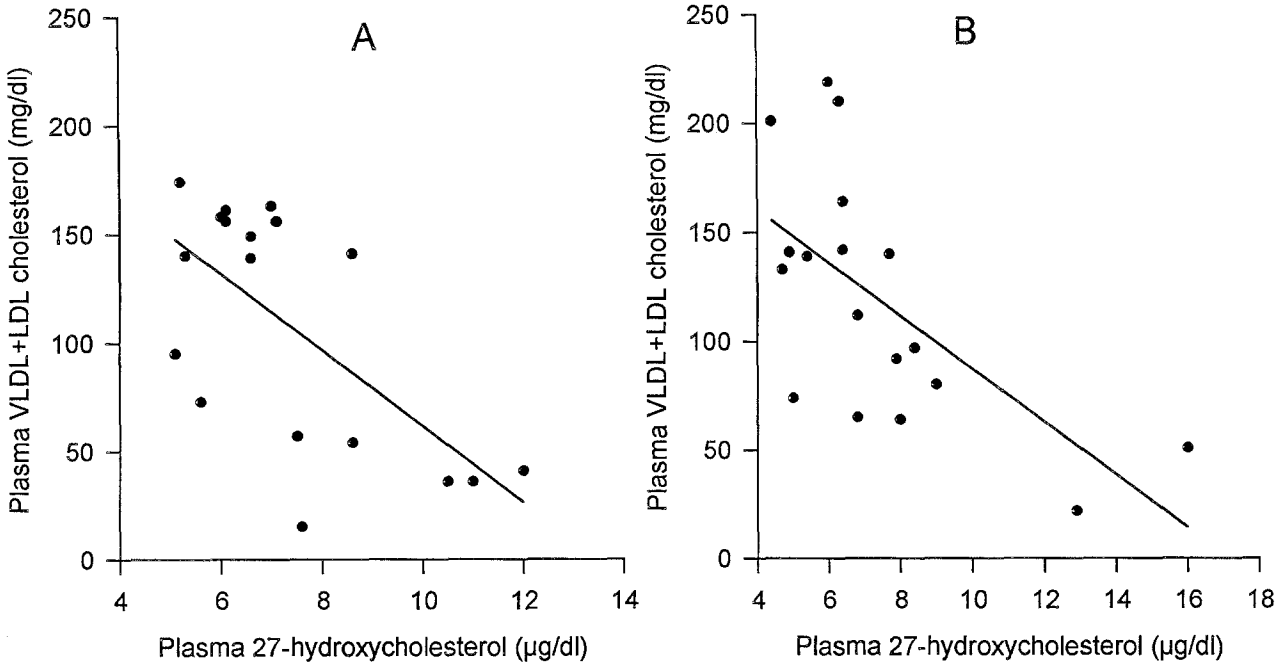


**Fig 2. Relationship of plasma 27-hydroxycholesterol concentration with plasma VLDL + LDL cholesterol concentration in baboons at 3 (A) and 6 (B) weeks after consuming the HCHF diet. Plasma 27-hydroxycholesterol was negatively associated with VLDL + LDL cholesterol at 3 ($r = -.581$, $P = .011$) and 6 ($r = -.545$, $P = .019$) weeks.**

terol is associated with an increase of sterol 27-hydroxylase activity in both hepatic and extrahepatic tissues. At the maximum induction, hepatic sterol 27-hydroxylase activity was negatively associated with plasma VLDL + LDL cholesterol. These results support the hypothesis that induction of sterol 27-hydroxylase activity in hepatic and extrahepatic tissues mediates the lipemic responsiveness to the diet.

Sterol 27-hydroxylase is an important enzyme of hepatic bile acid synthesis, and it participates in both pathways of bile acid synthesis.[9,22] A lack of hepatic activity of this enzyme causes a

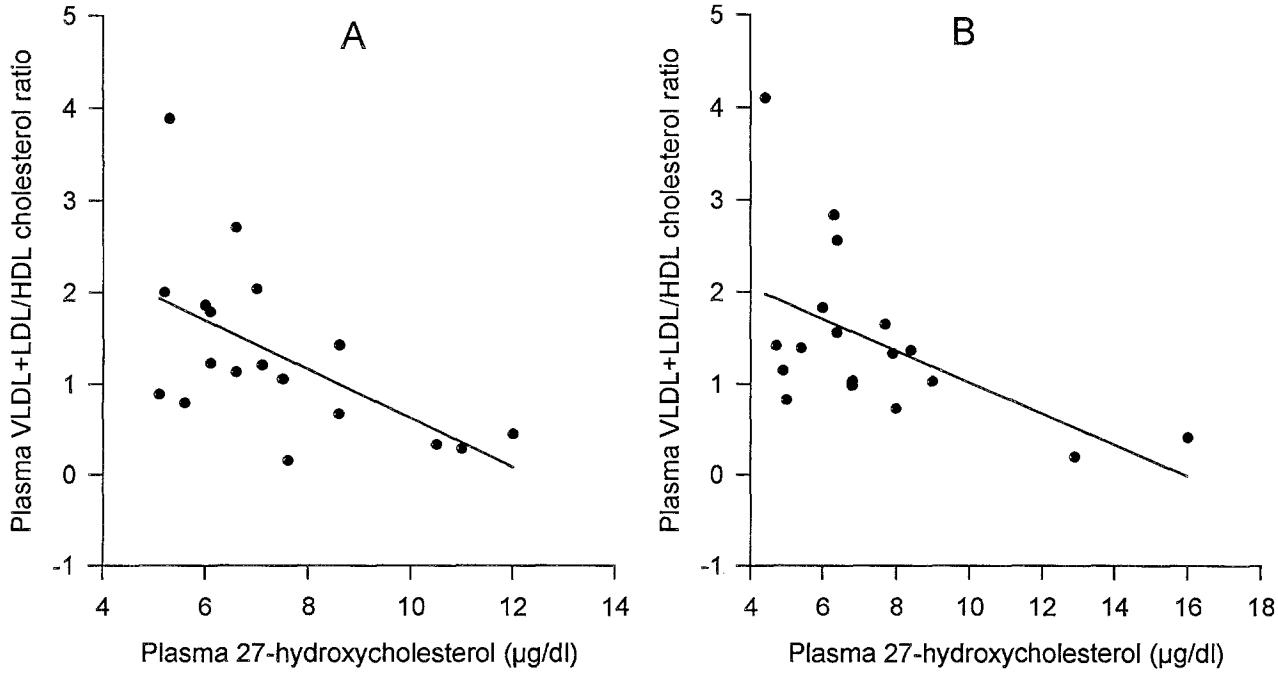


**Fig 3. Relationship of plasma 27-hydroxycholesterol concentration with plasma VLDL + HDL cholesterol ratio at 3 (A) and 6 (B) weeks after consuming the HCHF diet. Plasma 27-hydroxycholesterol was negatively associated with the VLDL + LDL/HDL cholesterol ratio at 3 ($r = -.647$, $P = .004$) and 6 ($r = -.635$, $P = .005$) weeks after consuming the HCHF diet.**

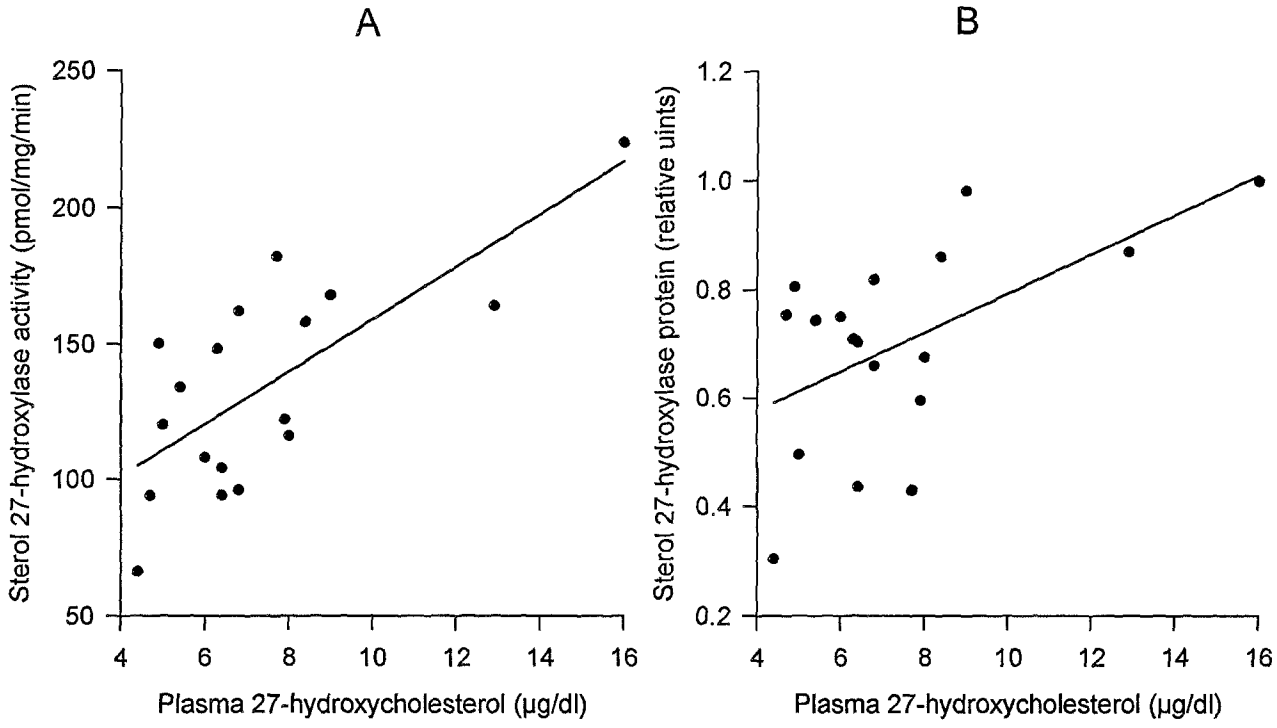


**Fig 4. Relationship of plasma 27-hydroxycholesterol concentration with hepatic sterol 27-hydroxylase activity (A) and hepatic sterol 27-hydroxylase protein level (B) in baboons at 6 weeks after consuming the HCHF diet. Plasma 27-hydroxycholesterol concentrations were positively correlated with the hepatic sterol 27-hydroxylase activity ($r = .899$, $P < .001$) and hepatic sterol 27-hydroxylase protein level ($r = .559$, $P = .016$).**

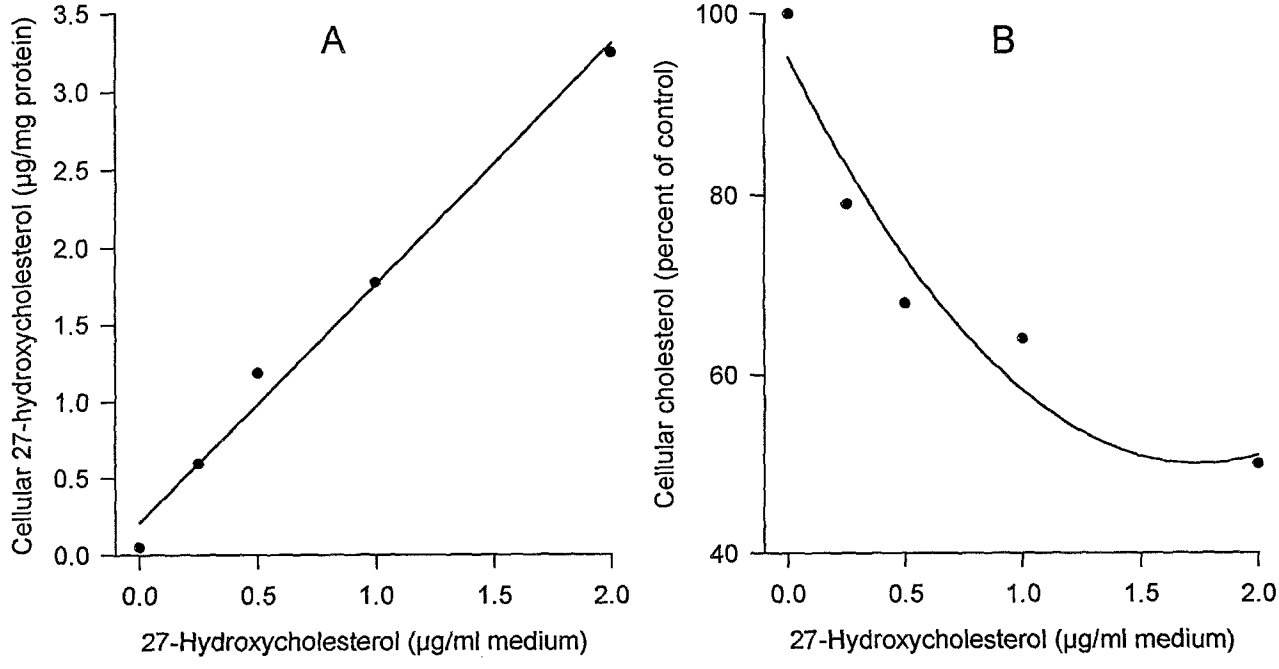


**Fig 5. Effect of exogenous 27-hydroxycholesterol on cellular 27-hydroxycholesterol concentration (A) and cholesterol concentration of blood lymphocytes expressed as a percent of the control where no 27-hydroxycholesterol was added (B). Each point represents the average of 2 observations. There was a linear increase in the cellular 27-hydroxycholesterol concentration ($r = .994$, $P = .001$) but a biphasic decrease in cellular cholesterol with an increase in exogenous 27-hydroxycholesterol in the culture medium.**

rare disorder known as cerebrotendinous xanthomatosis characterized by tendon xanthomas, premature atherosclerosis, and cataracts. The role of sterol 27-hydroxylase in cholesterol metabolism in extrahepatic tissues is being revealed slowly. Axelson et al[15] reported that 27-hydroxycholesterol inhibits HMG-CoA reductase. As in the case of other oxysterols, 27-hydroxycholesterol decreases HMG-CoA gene transcription by preventing the proteolysis of sterol regulatory element binding proteins.[23] In the present studies, the increased activity of sterol 27-hydroxylase in low-responding baboons was associated with increased 27-hydroxycholesterol in the plasma and blood lymphocytes. Blood lymphocytes from low-responding baboons had a greater decrease in cellular cholesterol than blood lymphocytes from high-responding baboons. These observations suggest that a higher concentration of endogenous 27-hydroxycholesterol in blood lymphocytes from low-responding baboons retards cholesterol synthesis. These findings were confirmed by the experiment in which we added exogenous 27-hydroxycholesterol in the incubation medium of lymphocytes and found a rapid decrease in the cellular cholesterol concentration of blood lymphocytes with an increasing concentration of 27-hydroxycholesterol. Thus, the induction of sterol 27-hydroxylase in both hepatic and extrahepatic tissues of low-responding baboons by dietary cholesterol and fat plays a role in attenuating the dietary responsiveness. Because most body cholesterol is synthesized in extrahepatic tissues,[16] the major effect of sterol 27-hydroxylase on the responsiveness may be mediated through its effects on cholesterol synthesis in extrahepatic tissues.

The decrease in the cellular cholesterol concentration was biphasic (Fig 5B). Initially, the decrease in cellular cholesterol was rapid, but with an increase of 27-hydroxycholesterol in the medium, the decrease began to slow. The reason for the slow decrease in cellular cholesterol during the second phase may be due to the decrease in cellular cholesterol synthesis. After the maximum downregulation of HMG-CoA synthase is achieved by exogenous 27-hydroxycholesterol, a further decrease in the cellular cholesterol concentration will be slow.

Our previous studies demonstrated that the increased activity of sterol 27-hydroxylase in response to dietary lipids is due to increased hepatic transcription of the gene for this enzyme.[7] In the present study, activity and protein levels of hepatic sterol 27-hydroxylase were highly correlated with plasma VLDL + LDL cholesterol and the VLDL + LDL/HDL cholesterol ratio on the HCHF diet. The present studies also demonstrated a positive association of hepatic and extrahepatic sterol 27-hydroxylase activity with protein levels, and thus support the conclusion of previous studies that the regulation of this enzyme occurs at the transcriptional level and not at the protein structure level. Further studies are needed to detect polymorphisms that affect transcription of the sterol 27-hydroxylase gene in low-responding baboons.

As in previous studies,[8] upon consuming the HCHF diet, plasma 27-hydroxycholesterol concentrations in low-responding baboons increased rapidly and peaked at 6 weeks, after which they decreased but remained higher than the levels during basal conditions. Our hypothesis was that the decrease in the plasma 27-hydroxycholesterol concentration after 10 weeks on the HCHF in low-responding baboons was due to an increase in bile acid synthesis. However, preliminary results suggest that the bile acid synthetic rate does not increase at any time on the HCHF diet in high- and low-responding baboons (G..T. Everson, R.S. Kushwaha, L.-D. Chen, et al, unpublished data, March 1997). Thus, the decrease in plasma 27-hydroxycholesterol concentrations at 10 weeks is not related to bile acid synthetic rates. A likely explanation is that most plasma 27-hydroxycholesterol is derived from extrahepatic tissues and, due to increased sterol 27-hydroxylase activity and 27-hydroxycholesterol concentrations in extrahepatic tissues, cholesterol synthesis decreases, and in turn, the cellular cholesterol concentration also decreases. At 10 weeks after consuming the HCHF diet, there is less cholesterol available for the synthesis of 27-hydroxycholesterol in extrahepatic tissues, and thus plasma 27-hydroxycholesterol decreases.

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