Macrophage 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Activity in Sitosterolemia: Effects of Increased Cellular Cholesterol and Sitosterol Concentrations

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Sitosterolemia is a rare, recessively inherited disease characterized clinically by accelerated atherosclerosis and xanthomas and biochemically by hyperabsorption and retention of sitosterol and other plant sterols in tissues. Decreased cholesterol biosynthesis and inhibition of 3-hydroxy-3-methylgluratyl coenzyme A (HMG-CoA) reductase and other enzymes in the biosynthetic pathway have been associated with enhanced low-density lipoprotein (LDL) receptor function. We examined the effects of cholesterol and sitosterol on sterol concentrations and composition and HMG-CoA reductase activity in monocyte-derived macrophages (MDM) from 12 control and 3 homozygous sitosterolemic subjects. The cells were cultured up to 7 days in media devoid of plant sterols, but containing increasing amounts of serum cholesterol. Before culture, MDM from the homozygous sitosterolemic subjects contained 22% more total sterols than cells from control subjects. Plant sterols and stanols represented 15.6% of MDM total sterols in sitosterolemic cells, but only 3.8% in control cells. After 7 days of culture in 10% delipidated serum (DLS) (20 μg/mL cholesterol, no sitosterol), all plant sterols were eliminated so that cells from both phenotypes contained only cholesterol. When DLS was replaced with fetal bovine serum (FBS) (300 μg/mL cholesterol), with and without addition of 200 μg/mL LDL, cholesterol levels in MDM from sitosterolemic subjects increased 108% ($P < .05$) compared with a 65% increase ($P < .04$) in control MDM cultured similarly. MDM HMG-CoA reductase activity from the 3 sitosterolemic subjects, which was significantly lower than controls at baseline (24 ± 3 *v* 60 ± 10 pmol/mg/min, $P < .05$), was not downregulated by increased cellular cholesterol levels, as observed in control cells. Control MDM were also cultured in medium that contained 10% DLS and was supplemented with 100 μg/mL cholesterol or sitosterol dissolved in ethanol or the ethanol vehicle alone. In contrast to cellular cholesterol accumulation, which significantly downregulated HMG-CoA reductase activity (-53%, $P < .05$), the increase in cellular sitosterol up to 25.1% of total sterols did not change MDM HMG-CoA reductase activity. Evidence of a normal HMG-CoA reductase protein in sitosterolemic cells, which was not derepressed upon removal of cellular sitosterol, and the failure of cellular sitosterol to inhibit normal HMG-CoA reductase activity argue against feedback inhibition by sitosterol as a mechanism for low reductase activity in this disease. The larger accumulation of sterols and inadequate downregulation of HMG-CoA reductase in MDM may be mechanisms for foam cell formation and explain, in part, the increased risk of atherosclerosis in sitosterolemia.

SITOSTEROLEMIA IS A recessive inherited disease with only about 40 cases reported worldwide. The disease is characterized biochemically by the accumulation of sterols other than cholesterol in all tissues, except brain, and is associated with premature atherosclerosis.[1-9] Sitosterol (which differs from cholesterol by an additional ethyl group at carbon 24), other plant sterols, their respective 5α-stanol derivatives, and cholestanol are detected in increased amounts in most tissues. The accumulation of plant sterols in tissues is due to enhanced intestinal absorption[10,11] coupled to decreased hepatic removal.[12,13] Except for plasma and mononuclear cells where high concentrations of cholesterol were reported,[2] most tissues from sitosterolemic subjects show lower levels of cholesterol.[5,6,9] In these subjects, cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity (rate-limiting enzyme of cholesterol biosynthesis)[4-8] and other early and intermediate enzymes in the cholesterol biosynthetic pathway[14] are suppressed, but low-density lipoprotein (LDL) receptor function is enhanced.[2,5,7,8] Several candidate genes for sitosterolemia (HMG-CoA reductase, HMG-CoA synthase, LDL receptor, and sterol regulatory element binding proteins [SREBP]-1 and –2) have been excluded.[15] The defective gene has been mapped to chromosome 2p21[16] and was recently suggested to relate to ABC transporters.[17] Because more than 1 enzyme in the cholesterol biosynthetic pathway is depressed, it has been suggested that a common inhibitor/modulator affects multiple enzymes in the cholesterol synthetic pathway and is responsible for deficient cholesterol biosynthesis in sitosterolemia. Increased concentrations of tissue plant sterols, particularly sitosterol, have pointed to this sterol as candidate for feedback inhibition of cholesterol biosynthesis.

The objective of this study was to test the hypothesis of inhibition of a normal HMG-CoA reductase protein in sitosterolemia via feedback inhibition by cellular sitosterol by (1) comparing HMG-CoA reductase activity in macrophages from sitosterolemic subjects, from which all plants sterols have been removed through culture, with similarly cultured cells from control subjects; (2) examining the effects of increasing cellular concentrations of cholesterol and sitosterol on normal HMG-CoA reductase activity; and (3) relating macrophage HMG-

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CoA reductase activity to cellular sterol concentrations and composition under various cell culture conditions.

MATERIALS AND METHODS

Subjects

Three homozygous sitosterolemic sisters, aged 30, 34, and 36 years and 12 healthy control subjects (4 females and 8 males, aged 20 to 58 years) were included in the study. The complete clinical and biochemical descriptions of the sitosterolemic homozygotes have been previously presented.[1-10] All 3 underwent ileal bypass surgery as treatment for premature atherosclerosis. Informed witnessed consent was obtained from all subjects, and the project was approved by the Institutional Review Board of the University of Medicine and Dentistry of New Jersey.

Cell Separation and Culture

Venous blood was collected from sitosterolemic and control subjects at the same time of the day (9:30 AM). Mononuclear leukocytes were isolated from 30 to 60 mL of venous blood according to Boyum.[18] Monocyte-derived macrophages (MDM) were isolated from mononuclear leukocytes (10^8 cells in 3 mL medium/25 mm^2 flask) by selecting adherent cells[19] after 2 hours of culture at 37°C under 5% carbon dioxide, in medium A (RPMI-1640 with L-glutamine, 25 mmol/L HEPES, and streptomycin/penicillin/fungizone in concentrations of 10,000 U/mL, 10,000 U/mL, and 250 μg/mL, respectively) that contained 10% human AB serum (GIBCO BRL, Long Island, NY). After removal of nonadherent cells, the adherent cells were washed 3 times with medium B (medium A without human AB serum) and cultured in experimental media up to 7 days with changes of fresh media every 2 to 3 days. The experimental media were: medium DLS (medium B supplemented with 10% delipidated serum, which was purchased from Sigma Chemical Co, St Louis, MO and contained 20 μg/mL cholesterol as determined by gas liquid chromatography), medium FBS (medium B supplemented with 10% fetal bovine serum from Gibco BRL that contained 300 μg/mL cholesterol), and medium FBS+LDL (medium B supplemented with 10% fetal bovine serum and 200 μg/mL LDL). LDL ($1.019 < d < 1.063$) was separated from venous blood of healthy volunteer donors by differential ultracentrifugation,[2] contained 737 $\pm$ 55 μg cholesterol/mg LDL protein, and provided about 150 μg/mL LDL cholesterol, thus bringing the total cholesterol concentration in medium FBS+LDL to 450 μg/mL.

At various time intervals (day 0, 2, 5, 7), the MDM were washed 3 times in phosphate-buffered saline (PBS) and recovered from the culture flasks with 0.05% Trypsin/0.53 mmol/L EDTA (Gibco BRL). After 7 days of culture, the cells were viable, as measured by the Trypan Blue exclusion test. After centrifugation at 2,000 rpm for 5 minutes at 4°C (Beckman centrifuge Model TJ-6, Palo Alto, CA), the cell pellets were resuspended and washed 3 times in 15 mL of PBS. The washed cell pellets were stored at -70°C for less than 2 months until used for sterol analysis and enzyme assays.

In a separate set of experiments, MDM from healthy volunteer subjects were cultured for 7 days in medium DLS (described above). For the last 48 hours, the cells were exposed to sitosterol or cholesterol (dissolved in ethanol and added to medium DLS at a concentration of 100 μg/mL sterol and 10 μL/mL ethanol) or control medium (same amount of ethanol, without sterol, in medium DLS). The cells were harvested and washed as described above. In additional experiments, following the 48-hour culture in sitosterol containing medium, control cells were cultured for 5 more days in control medium, then analyzed for tissue sterol concentrations.

Cell Fractionation and Sterol Analysis

The cells were homogenized in 0.5 mL buffer (250 mmol/L sucrose, 10 mmol/L Tris, 0.1 mmol/L EDTA, pH 7.4) with a Potter-Elvehjem homogenizer (15 strokes at 500 rpm). Small aliquots of the whole homogenates were used for the determination of protein concentration.[20] Aliquots of plasma (0.5 mL) and whole cell homogenates (50 to 100 μL) were used for sterol analysis by gas liquid chromatography as previously described.[2] The remaining cell homogenates were used to prepare microsomal fractions by centrifugation and ultracentrifugation (12,000*g* to 100,000*g*). The microsomes were suspended in the homogenizing buffer at a protein concentration of about 5 mg/mL and stored at -70°C until assayed for total HMG-CoA reductase activity.

Assay for HMG-CoA Reductase Activity

MDM microsomal HMG-CoA reductase activity was assayed by a method modified from a previously published procedure.[5] Briefly, 2 to 10 μg MDM microsomal protein were preincubated for 2 minutes at 37°C with a reduced β-nicotinamide adenine dinucleotide phosphate (NADPH) generating system (34 mmol/L $NADP^+$, 30 mmol/L glucose-6-phosphate, and 0.3 IU glucose-6-phosphate dehydrogenase) in a total final volume of 100 μL buffer (50 mmol/L K_2HPO_4, 70 mmol/L KCl, 10 mmol/L dithiothreitol, 30 mmol/L EDTA, pH 7.4). The reaction was started with the addition of 15 μL ^{14}C-labeled substrate ([3-^{14}C]HMG-CoA, purchased from Amersham Corp, Arlington Heights, IL, diluted with unlabelled HMG-CoA to a specific activity of 50 dpm/pmol and a final concentration of 300 μmol/L). The mixture was incubated for 60 minutes at 37°C and the reaction stopped with 15 μL 6N HCl. About 10,000 cpm of [^{3}H]mevalonolactone and unlabeled mevalonolactone were added for recovery standard and product marker, respectively. After lactonization at 37°C for 15 minutes and adding 100 μL of water, the products were extracted twice with 1 mL of ether in the presence of 0.2 g of sodium sulfate and separated by thin layer chromatography on Silica Gel 60 plates (VWR Scientific, Bridgeport, NJ) with benzene/acetone (1:1, vol/vol) as solvent system. The immediate product (^{14}C-labeled mevalonolactone) was quantitated by scintillation spectrometry in Ecolume (ICN Radiochemicals, Irvine, CA), and total HMG-CoA reductase activity was calculated as picomole of mevalonolactone formed per milligram microsomal protein per minute.

Statistical Analysis

The statistical significance of differences between values of different groups were analyzed by the 1-way analysis of variance[21] and unpaired *t* test (Microsoft Excel 97, Redmond, WA).

RESULTS

The 3 sitosterolemic subjects, who had undergone ileal bypass surgery as therapy for premature atherosclerosis, had lower plasma sterol concentrations than the 12 control subjects (145 ± 8 *v* 189 ± 6 mg/dL, $P < .05$). Plant sterols represented 12% of total sterols in the sitosterolemic subjects, but less than 1% of total sterols in control subjects. Table 1 shows sterol concentrations and composition in MDM from control and sitosterolemic subjects before and after 7 days of culture in medium supplemented with 10% DLS or 10% FBS. Before culture, plant sterols and stanols constituted 15.6% and 3.8% of total sterols in sitosterolemic and control MDM, respectively. After 7 days of culture in media devoid of plant sterols (DLS or FBS media), essentially all plant sterols and stanols had been eliminated from the sitosterolemic cells, so that both control

Table 1. Sterol Concentrations in MDM From Control and Sitosterolemic Subjects

Cells	Culture Media*	MDM Sterol Concentrations* Total Sterols nmol/mg Protein	Cholesterol nmol/mg Protein	Plant Sterols and Stanols† % Total
Sitosterolemia				
(N = 3)	Baseline	144.6 ± 10.0	122.1 ± 7.7	15.6
(N = 3)	10% DLS	115.6 ± 29.3	115.6 ± 29.3	ND
(N = 3)	10% FBS	240.0 ± 27.0‡	240.0 ± 27.0§	ND
Control				
(N = 7)	Baseline	118.4 ± 8.1	113.9 ± 7.9	3.8
(N = 3)	10% DLS	97.3 ± 8.6	97.3 ± 8.6	ND
(N = 3)	10% FBS	160.7 ± 18.0‖	160.7 ± 18.0¶	ND

NOTE. N = number of subjects measured. Cells from each subject were collected at least twice (at 3-week intervals), and average values of all measurements from each subject were used in the reported mean ± SEM for each group.

Abbreviation: ND, none detected.

*MDM were cultured for 7 days in RPMI medium containing 10% delipidated serum (DLS, 20 μg/mL cholesterol) or fetal bovine serum (FBS, 300 μg/mL cholesterol), and compared with cells before culture (baseline).

†Plant sterols and stanols include sitosterol (56%), campesterol (30%), and sitostanol (14%).

‡Significantly higher than total sterol level in sitosterolemia MDM at baseline ($P < .04$) and after culture in DLS ($P < .05$).

§Significantly higher than cholesterol level in sitosterolemia MDM at baseline ($P < .02$) and after culture in DLS ($P < .05$).

‖Significantly higher than total sterol level in control MDM after culture in DLS ($P < .04$).

¶Significantly higher than cholesterol level in control MDM at baseline ($P < .03$) and after culture in DLS ($P < .04$).

and sitosterolemic cells had no detectable noncholesterol sterols. Cellular cholesterol concentrations in both control and sitosterolemic MDM tended to decrease with culture in cholesterol-deficient medium, but the differences were not statistically significant. Cellular cholesterol concentrations in MDM from control subjects increased 41% and 65% after culture in cholesterol-containing FBS medium, compared with baseline and 7-day culture in delipidated medium, respectively. Cellular cholesterol concentrations in MDM from sitosterolemic subjects increased 97% and 108% after culture in cholesterol-containing FBS medium, compared with baseline and 7-day culture in delipidated medium, respectively. Addition of 200 μg/mL LDL to the FBS medium did not further increase cellular cholesterol concentrations in both control and sitosterolemic MDM (data not shown). Thus, in the presence of serum cholesterol (FBS *v* DLS), the net uptake of cellular cholesterol was about 2-fold greater in sitosterolemic than control cells (124.4 *v* 63.4 nmol/mg protein).

Figure 1 presents HMG-CoA reductase activity in MDM from healthy control and homozygous sitosterolemic subjects cultured up to 7 days in 3 media, which differed in amounts of cholesterol provided by serum and LDL. In medium deficient in cholesterol (DLS), HMG-CoA reductase activity in control MDM (Fig 1A) increased with culture time and reached a maximum after 5 days (to 87 ± 22 from 60 ± 10 pmol/mg/min). Enzyme activity stayed elevated after 7 days (83 ± 10 pmol/mg/min). In contrast, in the presence of 10% FBS, which contained 300 μg/mL cholesterol, control MDM reductase activity decreased to 34 ± 10 pmol/mg/min at day 7 ($P < .01$ *v* DLS at day 7). Supplementing the medium with 200 μg/mL LDL in addition to 10% FBS (FBS+LDL) further increased the cholesterol concentration in the medium, but did not reduce MDM HMG-CoA reductase activity further. Adding a higher concentration of LDL (1 mg/mL) to the FBS-containing medium (data not shown) also did not lower HMG-CoA reductase activity further than the value obtained with the FBS medium.

Baseline HMG-CoA activity of the sitosterolemic MDM (Fig 1B) was significantly lower than controls (24 ± 3 *v* 60 ± 10 pmol/mg/min, $P < .05$). HMG-CoA reductase activity in sitosterolemic cells tended to increase with culture in cholesterol-deficient medium, but the increase was not enough to bring it up to the level of control cells before and after culture in cholesterol-deficient medium. HMG-CoA reductase activity

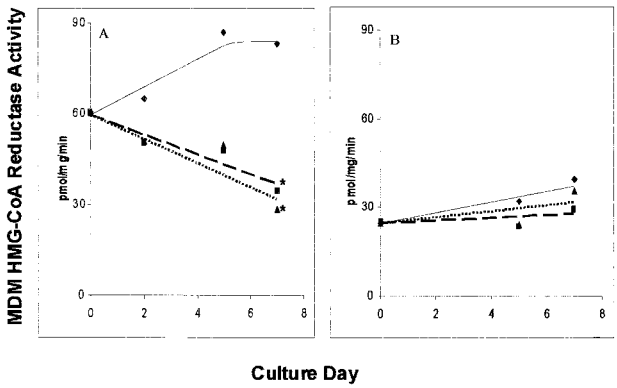

Fig 1. Effects of culture time and experimental media on HMG-CoA reductase activity in MDM from normal (A) and sitosterolemic (B) subjects. The ◆, ■, and ▲ represent medium DLS (10% DLS), medium FBS (10% FBS), and medium FBS+LDL (10% FBS plus 200 μg/mL LDL), respectively. Each symbol is the mean from 3 sitosterolemic subjects or 6 to 12 normal subjects. Cells from each subject were collected at least twice, and average values from each subject were used in the reported means. The coefficients of variation in the measurements of control and sitosterolemic subjects were 25% to 65% and 22% to 49%, respectively. *, Significantly lower than 7 days of culture in DLS, $P < .01$.

Table 2. Sterol Concentrations and Composition in MDM From Control Subjects Cultured in Lipid-Deficient Media in the Presence and Absence of Sitosterol and Cholesterol

	MDM Sterol Concentrations		
Culture Media*	Total Sterols nmol/mg Protein	Cholesterol nmol/mg Protein	Sitosterol % Total
Sitosterol (N = 5)	174.2 ± 15.3†	131.1 ± 17.6	25.1
Cholesterol (N = 5)	267.0 ± 15.6‡	267.0 ± 15.6§	ND
Control (N = 5)	140.4 ± 9.6	140.0 ± 9.6	ND

NOTE. N = number of subjects measured. Cells from each subject were collected at least twice (at 3-week intervals), and average values of all measurements from each subject were used in the reported mean ± SEM for each group.

Abbreviation: ND, none detected.

*MDM from healthy control subjects were cultured for 7 days in media containing 10% DLS supplemented the last 48 hours with 100 μg/mL sitosterol or cholesterol dissolved in ethanol, or the same amount of ethanol without sterol (control).

†Significantly higher than total sterol level after culture in control medium, $P < .05$.

‡Significantly higher than total sterol level after culture in control medium, $P < .01$.

§Significantly higher than cholesterol level after culture in control medium, $P < .01$.

in sitosterolemic MDM represented only 47% of the mean value for control cells after culture in delipidated media (39 ± 11 *v* 83 ± 10 pmol/mg/min, $P < .05$). In contrast to control MDM, in which HMG-CoA reductase was downregulated by cholesterol-containing media, sitosterolemic MDM showed no significant change in HMG-CoA reductase activity after 7 days of culture in either of the cholesterol-containing media (FBS and FBS+LDL).

Table 2 shows control MDM cultured in delipidated media containing 10 μg/mL ethanol with and without sitosterol or cholesterol. The presence of ethanol in cholesterol-deficient medium increased cellular total sterol concentrations (to 140.0 ± 9.6 from 97.3 ± 8.6 nmol/mg protein, $P < .02$). The addition of cholesterol to the medium further increased MDM total sterol concentrations (+91%, $P < .01$), and cholesterol was the only sterol present. Supplementing the medium with sitosterol also significantly increased cellular total sterol concentrations (from 140 ± 9.6 to 174.3 ± 15.3 nmol/mg protein, $P < .05$). However, the net sterol uptake consisted of mainly sitosterol, which represented 25.1% of total sterols. Some cellular cholesterol was apparently replaced by sitosterol so that cholesterol levels tended to decrease; however, the decrease in cholesterol concentrations in MDM cultured in sitosterol-containing media was not statistically significant. Sitosterol was not detected in control cells after a 48-hour exposure to sitosterol-containing medium, followed by culture in control medium (data not shown). This shows that sitosterol can be taken up and efflux from the control cells as sitosterolemic cells.

Figure 2 presents HMG-CoA reductase activity in control MDM cultured for 7 days in DLS medium and supplemented for the last 48 hours with ethanol-dissolved sitosterol or cholesterol (100 μg/mL) or the blank ethanol vehicle. MDM HMG-CoA reductase activity tended to increase after culture in the presence of sitosterol, even though a significant amount of sitosterol accumulated within the cells. In contrast, after culture in the presence of cholesterol and significant accumulation of cellular cholesterol, MDM HMG-CoA reductase activity was downregulated (-53%, $P < .05$).

DISCUSSION

This study demonstrated that (1) baseline HMG-CoA reductase activity in MDM from sitosterolemic homozygotes was significantly lower than from control MDM; (2) 7-day culture in cholesterol-deficient medium eliminates all plant sterols from sitosterolemic cells, yet HMG-CoA reductase was still only half the level in control cells cultured similarly; (3) cells from sitoterolemic subjects accumulated 2-fold more cellular cholesterol than control cells after culture in cholesterol-containing medium; (4) increasing cellular cholesterol in cells from sitosterolemic subjects via culture failed to downregulate HMG-CoA reductase activity; (5) a culture-mediated increase in cellular sitosterol in control MDM up to 25.1% of total sterols did not inhibit the activity of HMG-CoA reductase, whereas an increase in cellular cholesterol significantly downregulated it.

It was previously demonstrated that HMG-CoA reductase was markedly inhibited, and more importantly, could not be upregulated in the liver and mononuclear leukocytes when bile acid synthesis was stimulated by treatment with bile acid malabsorption.[4,5,7,8,22] As a result, plasma cholesterol levels declined markedly in sitosterolemic subjects treated with cholestyramine or ileal bypass surgery. The recent demonstration that enzymes in the cholesterol biosynthetic pathway other than

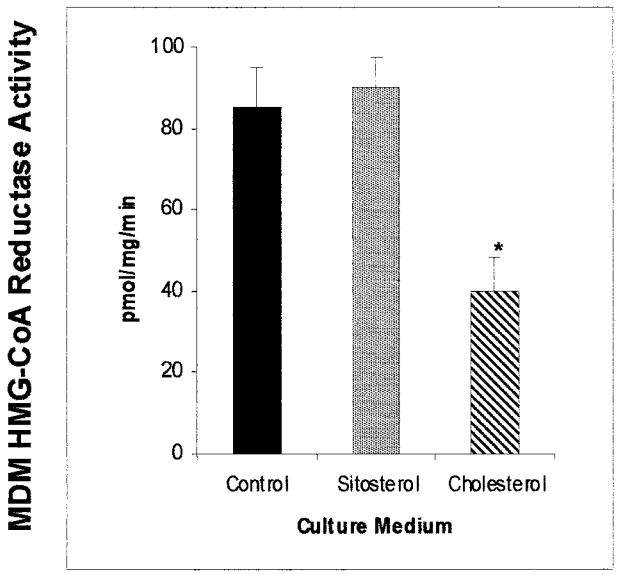

Fig 2. HMG-CoA reductase activity in MDM from normal subjects (N = 5) after culture for 7 days in 10% DLS and exposed for the last 48 hours to 100 μg/mL sitosterol, 100 μg/mL cholesterol, or the ethanol solvent alone (control).*, $P < .05$ compared with culture in control medium.

HMG-CoA reductase are deficient in sitosterolemia[14] suggests that the inherited defect is not located at the gene of the rate-limiting enzyme. Recent reports[15,17] have excluded the HMG-CoA reductase gene as the defect in sitosterolemia, and previous studies[5] have suggested that the reductase protein in cells from sitosterolemic subjects is normal (ie, similar immunologic reaction and catalytic efficiency compared with control cells). The mechanism for inhibition of cholesterol biosynthetic enzymes in sitosterolemia could relate to the accumulation of sitosterol in tissues rather than cholesterol feedback inhibition since, except for monocytes/macrophages, cholesterol levels are not elevated in most tissues of sitosterolemic subjects. However, the experiments described in this report argue against this possible mechanism.

When cellular sitosterol levels in normal macrophages were increased via culture to levels found in some sitosterolemic subjects, HMG-CoA reductase activity was not inhibited. Similar results were reported by Boberg et al[23] and Shefer et al[13] in liver from rats made sitosterolemic by intravenous infusion of sitosterol. In both of these studies, the increase in cellular sitosterol concentrations clearly did not inhibit hepatic HMG-CoA reductase activity. Field et al[24] reported that human intestinal CaCo-2 cells showed decreased HMG-CoA reductase activity after culture in sitosterol-containing medium, whereas Brown and Goldstein[25] reported no significant effect of sitosterol on HMG-CoA reductase activity in human fibroblast culture. In this study, we examined the effect of sitosterol on HMG-CoA reductase activity in culture of macrophages (cells implicated in the atherosclerotic process) from healthy control and sitosterolemic subjects. As previously demonstrated in other tissues, HMG-CoA reductase activity in sitosterolemic macrophages was significantly lower than macrophages from healthy controls. After sitosterolemic cells were cultured in the absence of plant sterols, virtually no plant sterols were detected within the cells; yet, HMG-CoA reductase activity was still only about half the activity of control cells after similar culture. Recently, Honda et al[26] showed that increasing the sitosterol concentration in both control and sitosterolemic fibroblasts via culture increased HMG-CoA reductase activity in both cell types. Our contention that decreased HMG-CoA reductase activity and cholesterol biosynthesis in this disease is not due to feedback inhibition by elevated cellular sitosterol is supported by the following observations: (1) HMG-CoA reductase activity was still lower in cells from sitosterolemic cells than control cells after removal of all cellular sitosterol; (2) the reductase protein in sitosterolemic cells has previously been shown to be present in low level, but appears to be normal; and (3) HMG-CoA reductase activity is not normally inhibited by high levels of cellular sitosterol. However, there may be other regulatory factors in cells from sitosterolemic subjects, yet to be defined, that may differ from the sitosterol-loaded normal cells.

An important observation in this study was the relatively high cellular sterol concentrations in sitosterolemic cells at baseline despite low plasma sterol concentrations and the markedly greater increase in cholesterol concentrations after a 7-day incubation in cholesterol-containing media, as compared with control cells cultured similarly. This greater increase from baseline occurs probably because of the enhanced LDL receptor function that is characteristic of sitosterolemic cells.[2,5] The failure of sitosterolemic cells to downregulate HMG-CoA reductase activity in culture with cholesterol-containing media may be due to the low enzyme activity at baseline that partly results from inhibition by increased cellular cholesterol levels. However, the finding that HMG-CoA reductase activity in sitosterolemic cells after culture in cholesterol-containing media was at the same level as control cells with only 2 thirds of the cholesterol content suggests altered feedback regulation of HMG-CoA reductase by cholesterol. It is unlikely that this inability of sitosterolemic cells to downregulate HMG-CoA reductase activity is due to a sterol secretory process, because both sitosterolemic and sitosterol-loaded normal cells could release sitosterol into the medium.

The failure of sitosterolemic cells to show coordinate regulation between HMG-CoA reductase and LDL receptors is an important, but as yet unexplained, abnormality in sitosterolemia. It is well established that the sterol regulatory element binding protein (SREBP) contains a transcription factor (an NH_2 terminal segment) that can be cleaved and released when cellular sterol levels are low. When released, this transcription factor serves as feedback information to the nucleus where it activates transcription of genes encoding several enzymes in the cholesterol biosynthetic pathway, including HMG-CoA reductase, and the gene encoding the LDL receptor.[27,28] The decoupling of LDL receptor and HMG-CoA reductase, but coordinate downregulation of several enzymes in the cholesterol biosynthetic pathway in sitosterolemia[14] suggests that a regulatory factor other than SREBP is involved. Recently, mutations in 2 genes that encode members of the adenosine triphosphate-binding cassette transporter family were detected in 9 patients with sitosterolemia.[17] Expression of the genes of these transporters was upregulated in the intestine and liver of cholesterol-fed mice, which suggests an important role of these transporters in the overabsorption and decreased removal of sterols in sitosterolemia.

The continued accumulation of sterols in MDM converts these cells to foam cells and is associated with the accelerated atherosclerosis seen in sitosterolemia. The therapy with bile acid malabsorption (ileal bypass) that markedly decreases plasma cholesterol concentrations would be expected to also reduce MDM sterol concentrations and, in turn, reduce the risk of atherosclerosis. We have noted marked clinical improvement with such therapy, yet these cells still contained high levels of sterols (both cholesterol and plant sterols). The increased sterol uptake by sitosterolemic MDM is consistent with previously reported enhanced cellular LDL receptor function[2,5,7] and higher fractional catabolic rate of apolipoprotein B.[29] Although the mechanisms that cause elevated LDL receptor function, but a general downregulation of cholesterol biosynthesis are still unclear, what is clear from this and earlier studies is that (1) increased atherosclerosis in this disease is associated with more rapid accumulation of cholesterol in macrophages and possibly altered feedback regulation of HMG-CoA reductase by cholesterol and (2) low HMG-CoA reductase activity in sitosterolemia (observed so far in liver, intestine, mononuclear leukocytes, and macrophages) is not due to the accumulation of tissue plant sterols.

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