

Elevated Cholesterol and Bile Acid Synthesis in an Adult Patient with Homozygous Familial Hypercholesterolemia

REDUCTION BY A HIGH GLUCOSE DIET

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ABSTRACT Elevated levels of cholesterol synthesis are reported for several young children with homozygous familial hypercholesterolemia (HFH) and are considered to contribute directly to their hypercholesterolemia. In contrast, increased cholesterol production has not previously been found in adult patients with HFH. Using the fecal steroid balance technique, we studied rates of cholesterol and bile acid synthesis in a 24-yr-old man who had severe hypercholesterolemia typical of HFH and who lacked skin fibroblast low density lipoprotein (LDL) receptor activity.

On an average diet (45% carbohydrate, 40% fat, 15% protein) mean \pm SEM cholesterol (24.8 ± 1.4 mg/kg per d) and bile acid (11.1 ± 1.6 mg/kg per d) excretion were approximately threefold higher than normal. When an isocaloric high carbohydrate, low fat diet (90.5% glucose oligosaccharides, 1.3% safflower oil, 8.2% crystalline amino acids) was substituted, mean cholesterol (13.0 ± 0.5 mg/kg per d) and bile acid (8.6 ± 0.4 mg/kg per d) fell markedly. The decline in fecal steroid excretion was accompanied by modest reductions in plasma total and LDL cholesterol concentrations and by a softening of cutaneous xanthomata.

Although the patient phenotypically and biochemically resembled the HFH state, his family pedigree was not notable for hypercholesterolemia. While

the patient's father had premature cardiovascular disease, his mother had no evidence of heart disease, had normal plasma total and LDL cholesterol levels, and had normal fibroblast LDL receptor activity. Likewise, the plasma cholesterol levels of three other members of the patient's family were normal. Despite the unusual genotypic background of this individual, however, the fecal balance data shows that elevated cholesterol and bile acid synthesis may occur in adult, as well as juvenile, patients with HFH and may be responsive to dietary control.

INTRODUCTION

A major abnormality of cholesterol metabolism in patients with homozygous familial hypercholesterolemia (HFH)¹ is thought to be diminished binding of low density lipoproteins (LDL) to many cells (1). Skin fibroblasts from HFH patients cultured in the presence of LDL show an increased synthesis of cholesterol because of a lack of internalization of cholesterol that normally suppresses synthesis. If the same defect exists *in vivo*, an increase in total body synthesis of cholesterol might be expected. Indeed, an overproduction of cholesterol has been found in several young children with HFH (2-4). Older homozygous patients (5, 6), or those with heterozygous FH (2, 7-9), however, have not been found previously to have abnormal cholesterol synthesis. These observations led to the hypothesis (4) that excessive

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¹Abbreviations used in this paper: HFH, homozygous familial hypercholesterolemia; LDL, low density lipoproteins; VLDL, very low density lipoproteins.

production of cholesterol in HFH is manifested only in childhood and is offset later in life by other factors restoring cholesterol production to normal.

We report here studies in a 24-yr-old adult with LDL receptor-negative FH. This subject is unusual compared with previously studied HFH individuals in that at least one of his parents has normal circulating cholesterol levels and normal LDL receptor activity. The patient demonstrated increased synthesis of both cholesterol and bile acids on a normal diet. When he was placed on a diet high in glucose and low in fat, however, cholesterol and bile acid production diminished greatly. These results suggest that abnormal cholesterol synthesis may exist in adult patients with HFH, and may also be subject to dietary control.

METHODS

Patient. The clinical history of J.L. has been presented previously (10). This slender, 24-yr-old male developed xanthomata at age 10 yr and at age 16 his cholesterol was found to be 850 mg/dl. In his late teens he had lower extremity claudication and exertional chest pain, but in the past few years, these symptoms have abated. His response to conventional diet and drug therapy has been poor, and serum cholesterol has remained >600 mg/dl. Studies of his cultured fibroblasts obtained by skin biopsy were carried out by Dr. Joseph Goldstein, Dallas Tex., and the patient was found to be LDL receptor-negative.

The patient's father died at age 40 yr from a myocardial infarction. The remaining family history is noteworthy, however, for a paucity of other known cardiovascular disease extending over five generations (Fig. 1). Examination of J.L.'s mother and three other relatives revealed normal levels of plasma lipids and lipoproteins (Table I) and no evidence of xanthomata or symptomatic cardiac disease. Studies of the mother's fibroblasts (Dr. J. Goldstein) revealed normal LDL receptor activity.

Diets. The patient was studied at the Clinical Research Center at Vanderbilt University Hospital for 9 wk. During weeks 1-3 he received a basal diet consisting of 45% carbohydrate (as glucose), 40% fat (as lard), and 15% protein (as milk). The diet was divided into six portions and was taken

every 2 h from 9 a.m. to 9 p.m. The daily cholesterol intake was 109 mg. At the end of this period, a small-bore nasogastric tube (No. 8 French infant feeding tube) was passed and, during weeks 4-6, the patient received a 24-h constant nasogastric infusion of Vivonex (Eaton Laboratories). Vivonex contains 90.5% of calories as glucose or glucose oligosaccharides, 8.2% as crystalline amino acids, and 1.3% as safflower oil (containing 80% linoleic acid). The diet was supplemented with vitamins and minerals and contained no cholesterol. After this period, the daytime infusion was stopped and, during weeks 7-9, the patient received oral feedings of flavored Vivonex. Feedings were divided equally and spaced ~3 h apart during a 16-h waking period. The feedings were drunk over 30 min. The use of both Vivonex and the particular feeding schedule employed was based upon our earlier studies (11) of this regimen in the short-term management of hypercholesterolemia in another HFH patient.

Throughout the 9-wk study, daily intake of calories was 2,500 kcal and mean (\pm SD) body weight was 57.4 ± 0.7 kg.

Plasma lipids and lipoproteins. Blood was collected at the end of each week between 8 a.m. and 9 a.m., which was after an 11-12-h fast during the basal diet or immediately before the patient's morning feeding of Vivonex, when daytime feedings were given. Plasma lipoproteins were isolated by sequential preparative ultracentrifugation as described by Havel et al. (12) using a Beckman 40.3 rotor and model L 350 ultracentrifuge (Beckman Instruments, Inc., Spinco Division, Palo Alto, Calif.). Very low density lipoprotein (VLDL) was isolated at <1.006 g/ml by ultracentrifugation at 38,000 rpm for 18 h. LDL was isolated between densities 1.006 and 1.063 g/ml by centrifugation at 38,000 rpm for 22 h, and HDL was isolated between densities 1.063-1.210 g/ml by centrifugation at 38,000 rpm for 44 h. The cholesterol and triglyceride concentrations of each lipoprotein fraction were determined by the methods of Eggstein and Krentz (13) and Block et al. (14), respectively, as adapted to the autoanalyzer. Total plasma cholesterol or triglyceride was obtained by summing the lipid concentration in each lipoprotein fraction.

Cholesterol balance. Measurements of cholesterol balance were carried out as described previously (15). Stools were collected daily and aliquots were taken for analysis of neutral steroids (16) and acidic steroids (bile acids) (17). Beta-sitosterol (110 mg, twice daily) was used as an internal marker to correct for losses of neutral steroids that may have occurred during intestinal transit (18). Chromic oxide (60

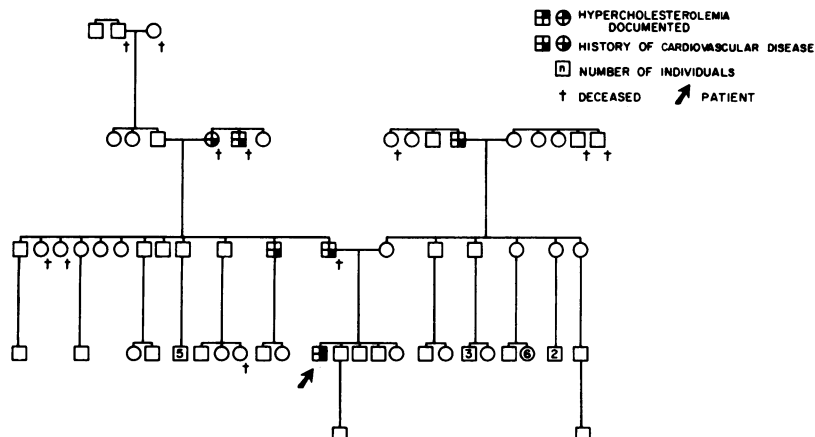


FIGURE 1 Pedigree of patient J.L.

TABLE I
Lipid and Lipoprotein Data on Relatives of Patient J.L.

Subject	Relation to patient	Total lipids		Plasma lipoprotein cholesterol		
		Cholesterol	Triglycerides	VLDL	LDL	HDL
		mg/dl		mg/dl		
1	Mother	143	69	6	96	41
2	Maternal uncle	140	175	19	82	39
3	Sister (age 8 yr)	136	173			
4	Brother (age 13 yr)	150	72			

mg, twice daily) was used to correct acidic steroid excretion for variations in fecal flow (19).

For comparison, 14 normal adult male subjects were studied on the same basal diet and by the same analytic techniques. Ages of subjects ranged from 29 to 63 yr (mean 51 yr); their weights were 95–115% of ideal.

RESULTS

As shown in Table II, plasma total- and LDL-cholesterol in the basal period averaged 618 and 555 mg/dl, respectively. Both declined modestly during the 6 wk of Vivonex administration, whereas plasma triglycerides and VLDL cholesterol increased. Plasma high-density lipoprotein (HDL) cholesterol fell from 44 to 33 mg/dl, while the ratio of LDL/HDL cholesterol rose slightly from 12.6 to 15. HDL triglyceride also decreased during Vivonex treatment from 17 to 12 mg/dl.

Table III summarizes cholesterol-balance studies during each diet period. The results are compared with those of 14 normal subjects. Basal outputs of neutral steroids and acidic steroids in J.L. were high, and net values for cholesterol balance were at least three times those of normal subjects (Table II) or adults with heterozygous FH studied previously (2, 7–9).

Fecal steroid excretion was affected markedly by administration of Vivonex. Outputs of neutral and acidic steroids fell by 48 and 23%, respectively, and

cholesterol balance decreased 37%, from 34.2 mg/kg per d to 21.6 mg/kg per d. No differences in steroid excretion between the periods of constant or intermittent feedings of Vivonex were noted, and the data for the two periods were pooled.

After beginning Vivonex infusions, the serum triglyceride levels rose transiently to maximum of 285 mg/dl, and this was associated with a rise in liver enzyme levels from normal values recorded during the basal-diet period. Serum alkaline phosphatase increased to a maximum of 240 IU/liter (40–110 IU/liter), glutamic pyruvic transaminase increased to 186 IU/liter (0–40 IU/liter) and glutamic oxaloacetic transaminase increased to 73 IU/liter (0–40 IU/liter). Serum bilirubin remained normal and a test for hepatitis-associated antigen was negative. During Vivonex administration, several of the patient's tuberous xanthomata softened and at least one planar xanthoma (~1 cm in greatest diameter) disappeared.

DISCUSSION

Patient J.L. is the oldest patient with HFH in whom synthesis of cholesterol has been found to be distinctly increased. This finding supports the hypothesis that an absence of LDL receptors in vivo can release feedback inhibition on cholesterol synthesis, as it does

TABLE II
Lipid and Lipoprotein Response to High Glucose Diet

Diet treatment	Total lipids*		Plasma lipoprotein cholesterol			LDL/HDL cholesterol ratio
	Cholesterol	Triglycerides	VLDL	LDL	HDL	
		mg/dl		mg/dl		
Basal	618±36	136±25	19±7.0	555±39	44±2.6	12.6
Vivonex-CI‡	575±39	158±79	36±1.2	507±38	33±1.7	15.4
Vivonex-D + I	561±66	177±11	30±0.9	499±61	33±6.8	15.1

* Total cholesterol and triglycerides represent the sum of the individual cholesterol and triglyceride levels obtained in the VLDL, LDL, and HDL fractions. Values reported are mean±SEM of three determinations.

‡ CI refers to the period of constant Vivonex infusion and D + I to the period of daytime Vivonex drinks and nighttime infusion.

TABLE III
Cholesterol Balance Data*

Subject	Period	Dietary cholesterol intake (a)	Fecal steroid excretion						Cholesterol balance	
			Neutral steroids		Acidic steroids		Total steroids (b)		(b) - (a)	
		mg/d	mg/d	mg/kg/d	mg/d	mg/kg/d	mg/d	mg/kg/d	mg/d	mg/kg/d
J.L.	Basal	109	1,407±81	24.8±1.4	631±35	11.1±0.6	2,039±91	36.1±1.6	1,929±91	34.2±1.6
J.L.	Vivonex	0	753±28	13.0±0.5	500±24	8.6±0.4	1,170±87	21.6±0.8	1,254±45	21.6±0.8
Normal† (14 subjects)				6.5±0.5		4.9±0.5		11.4±0.7		9.6±0.6†

* Values are mean±SEM based on daily stool collections during each dietary period. The number of determinations in each period was 15 during the basal period and 13 during Vivonex administration. The data from the periods of constant and intermittent Vivonex infusions were analyzed together.

† The cholesterol intake for this group averaged 1.8±0.5 mg/kg per d.

in tissue culture. It also indicates that cholesterol overproduction is not restricted to young patients with HFH, even though it may be more common in the younger age group.

Although from his clinical presentation, plasma and total and LDL cholesterol levels, and LDL receptor negativity, patient J.L. is typical for HFH. His pedigree does not suggest increased prevalence of hypercholesterolemia or cardiovascular disease. Although his father died at an early age from heart disease and may have been heterozygous for FH, his circulating lipid levels and fibroblast LDL receptor status are unknown. In addition, the patient's alleged biological mother has normal plasma total and LDL cholesterol concentrations and normal fibroblast LDL receptor activity and at least three other relatives also have normal plasma cholesterol levels. In the absence of blood typing or other detailed genetic information, we cannot speculate about the cause of LDL receptor negativity in this patient, in whose family the FH genotype does not appear to be strongly expressed. Whether the unusual genetic background apparent in J.L. contributed in some way to his high basal rate of cholesterol and bile acid synthesis in adult life or to his response to high carbohydrate intake is also unknown. However, the phenotypic expression in J.L. clearly is that of HFH. Furthermore, as discussed below, his response to carbohydrate feeding is congruent with the response observed in other HFH individuals. Thus, we believe the results obtained in this study of J.L. are applicable to HFH patients in general.

The finding of an increased synthesis of cholesterol in an adult patient with HFH is important because it firmly establishes the existence of this abnormality in HFH. A concern from previous studies in young HFH patients was that an elevated synthesis of cholesterol might be spurious, since it was possible that a relatively high rate of cholesterol synthesis might

be common in both normal and HFH children, but had gone undetected in healthy children because of insufficient studies of this age group. Since the normal range for adults is well established, the results obtained in the present patient remove any doubt that HFH can be associated with a marked overproduction of cholesterol.

Patient J.L. presented a pattern of excretion of fecal steroids similar to another HFH subject reported recently by Schwarz et al. (4). Both patients had approximately three times normal production rates for cholesterol and bile acids. The observation of an excess production of bile acids eliminates the possibility that the elevated cholesterol synthesis was secondary to an isolated malabsorption of cholesterol; if this were the case, synthesis of bile acids should be in the normal range, since experimentally-induced inhibition of cholesterol absorption in human subjects occurs despite continued normal bile acid secretion (20). Also, for the reasons cited by Schwarz et al. (4), an isolated malabsorption of bile acids also is unlikely. Essentially, these reasons include the doubtful association of a second defect in absorption of bile acids in the extremely rare HFH patient, a normal fraction conversion (32%) of newly synthesized cholesterol into bile acids that would not occur with bile acid malabsorption, and the absence of any evidence of gastrointestinal dysfunction. The finding of a similar pattern of excretion of bile acids in these two patients with HFH further reduces the probability of a defect in absorption of bile acids.

It is well established that the hypercholesterolemia in HFH is due to both a decreased fractional catabolic rate (5, 21, 22) and an increased synthetic rate (22, 23) of LDL. It is unknown, however, precisely how cholesterol synthesis and LDL synthesis are linked. In situations where both LDL and total cholesterol production occur, it is unclear if LDL overproduction in some way drives cholesterol overproduction or if

increased absolute rates of LDL catabolism result in cholesterol overproduction. Several HFH patients have been reported to have normal synthesis of cholesterol by fecal sterol balance criteria and yet still have marked hypercholesterolemia (5, 6). Therefore, it seems likely that, when overproduction of cholesterol occurs in HFH, it probably accentuates hypercholesterolemia to only a minor degree.

In the present study, the ingestion of a high-carbohydrate, low-fat diet (Vivonex) reduced circulating LDL-cholesterol levels, and this change was associated with a reduction in the synthesis of cholesterol and bile acids. This observation confirms our recent report that continuous or intermittent administration of intragastric Vivonex to a young HFH patient induced a rapid and marked fall in plasma total and LDL cholesterol (up to 57% below untreated levels) (11). Although total and LDL-cholesterol concentrations responded only modestly to Vivonex in J.L., cholesterol synthesis decreased substantially. In both patients, xanthomatous lesions regressed, indicating that the effect of Vivonex was not due to deposition of excess plasma cholesterol in peripheral tissues.

Our finding that a high-carbohydrate diet low in fat can inhibit synthesis of cholesterol confirms and extends results obtained in several previous studies. Thus, several HFH patients undergoing portacaval shunt operations have been treated for 1–3 wk before surgery with total parenteral alimentation and have shown striking reductions in serum total and LDL-cholesterol levels (5, 24–26). Glucose contributed a major portion of the calories contained in their parenteral solutions. In addition, Winitz and co-workers (27) observed marked decreases in serum cholesterol in healthy, normocholesterolemic men fed a liquid diet composed of glucose, essential amino acids and small amounts of essential fatty acids. Finally, Den Bestin et al. (28) administered intravenously or orally a liquid diet comprised of 80% glucose and 20% amino acids to healthy men. Serum cholesterol fell rapidly in all subjects, regardless of the route of administration, although the hypocholesterolemic effect was more pronounced during continuous, intravenous feeding. In these subjects, fecal cholesterol and bile acid excretion diminished greatly during intravenous alimentation.

In summary, we have confirmed that HFH can be associated with overproduction of cholesterol and bile acids even in adults. Since the LDL receptor pathway that functions in most peripheral tissues (1) also appears to exist in hepatic parenchymal and intestinal epithelial cells (29), then the observed increase in synthesis of cholesterol in HFH may be related to a decrease in hepatic and intestinal LDL receptors with a release of feedback control. In addition, the excess cholesterol synthesis may be curtailed by a high carbo-

hydrate, low-fat diet. Although this diet can reduce circulating levels of LDL-cholesterol, the magnitude of the cholesterol-lowering effect appears to vary among patients. Nevertheless, a high-carbohydrate, low-fat diet may prove to be an adjunct in the treatment of patients with HFH.

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