Stimulation of cholesterol synthesis and hepatic lipogenesis in patients with severe malabsorption

Ana Cachefo,* Philippe Boucher,* Eric Dusserre,† Paul Bouletreau,‡ Michel Beylot,§ and Cécile Chambrier§

INSERM U499* and U 449,† Faculté Laennec, Lyon, France; and Unité de Nutrition Artificielle,‡ Hopital Ed Herriot, 69008, Lyon, France

Abstract Patients with severe malabsorption have abnormal lipid metabolism with low plasma cholesterol and frequently high triglyceride (TG) levels. The mechanisms behind these abnormalities and the respective roles of malabsorption itself and of the parenteral nutrition given to these patients are unclear. We measured endogenous lipids synthesis (cholesterol synthesis and hepatic lipogenesis) and the expression (mRNA concentrations in circulating mononuclear cells) of regulatory genes of cholesterol metabolism in 10 control subjects and 22 patients with severe malabsorption receiving (n = 18) or weaned of parenteral nutrition (n = 4). Patients had low plasma cholesterol (P < 0.01) and raised TG (P < 0.05) levels. Both fractional and absolute cholesterol synthesis (P < 0.001) and hepatic lipogenesis (P < 0.01) were increased. These abnormalities are independent of parenteral nutrition since they were present in patients receiving or weaned of parenteral nutrition. No relation between hepatic lipogenesis and plasma TG levels was found, suggesting that other metabolic abnormalities participated in hypertriglyceridemia. HMG-CoA reductase and LDL receptor mRNA levels were decreased (P < 0.05) in patients on long-term parenteral nutrition. HMG-CoA reductase mRNAs were normal in weaned patients.


Supplementary key words stable isotopes • mRNA • parenteral nutrition • bile acids

Patients with chronic and severe intestinal failure due to extensive small bowel resection (short bowel syndrome) or long-term disease of the gut have profound alterations of lipids metabolism. They usually have a low plasma cholesterol level and often a rise in plasma triglyceride (TG) concentrations (1, 2). The decrease in plasma cholesterol has been ascribed to the interruption of the entero-hepatic cycle of bile acids (2–4), resulting (in addition to a possible decrease in the absorption of cholesterol) (2) in an enhanced synthesis of bile acids from cholesterol (1). This increase utilization of cholesterol for bile acid synthesis would itself lead then to a stimulation of the uptake by liver of cholesterol-rich lipoproteins and of hepatic cholesterol synthesis (1, 4, 5). However, to our knowledge, cholesterol synthesis has not been directly measured in humans with intestinal failure, except in one report showing an increased cholesterol turnover rate in two patients with total enterectomy and bile acid diversion (6).

The mechanisms responsible for the hypertriglyceridemia often observed in subjects with chronic intestinal failure are unclear. The rise in TG concentration could result from the malabsorption itself. This is supported by the finding of an increased hepatic synthesis and secretion of VLDL-TG during administration of cholestyramine in subjects with familial hypercholesterolemia (7). The hypertriglyceridemia could also be related to the parenteral nutrition often required to maintain the nutritional status of these patients. It could result merely from the infusion of exogenous TG emulsion. It could also result from the iv infusion of glucose because administration of large amounts of carbohydrates to normal subjects stimulates hepatic lipogenesis and increases the secretion rates and concentrations of TG (8).

Therefore, to clarify the mechanisms responsible for these abnormalities of lipid metabolism in patients with intestinal failure, we measured in normal subjects and, in such patients, lipid synthesis (hepatic lipogenesis and cholesterol synthesis). Since it has been proposed that circulating mononuclear cells could be representative of hepatocytes for the expression (as appreciated by mRNA levels) of genes involved in the regulation of cholesterol metabo-

To whom correspondence should be addressed.
e-mail: beylot@laennec.univ-lyon1.fr
lism (9), we measured in these cells the mRNA concentrations of HMG-CoA reductase, LDL receptor (LDLR), and LDLR-related protein (LRP). In order to determine the respective roles of intestinal failure and of parenteral nutrition in the abnormalities observed in patients with intestinal failure, these measurements were performed in patients receiving or weaned of parenteral nutrition.

MATERIALS AND METHODS

Subjects

After full explanation of the nature, purpose, and possible risks of the study, informed written consent was obtained from 10 healthy volunteers and 22 patients with intestinal failure who were regularly followed up in an authorized French Home Parenteral Nutrition Center for adults. The control group consisted of six women and four men (aged 20 to 51 years, body mass index 18 to 25). No control subject had a personal or familial history of diabetes, dyslipidemia, or obesity, or was taking any medication. All had a normal physical examination and normal plasma glucose and lipids concentrations (see Table 1). Subjects with unusual dietary habits were excluded.

The 22 patients with intestinal failure included nine women and 13 men (aged 19 to 73 years, body mass index 11.8 to 27.1). Their characteristics are given in Table 2. Most of them (18/22) had short bowel syndrome due to mesenteric infarction (10/18), Crohn’s disease (7/18), or radiation enteritis (1/18). The others (4/22) had medical malabsorption due to Crohn’s disease (7/18), or radiation enteritis (1/18). The others had short bowel syndrome due to mesenteric infarction (10/18), or jejunal atrophy (4/22). The presence of malabsorption was established by measurement of steatorrhea and/or demonstration of abnormal xylose absorption (data not shown). All the patients were free of other disease. Except for one patient, all had normal plasma C-reactive protein and cortisol levels. Eighteen patients were on long-term parenteral nutrition for 47.5 ± 11.9 months (3.3 ± 0.4 all-in-one bags per week, one bag = 2,181 ± 140 ml, 762 ± 36 kcal of glucose, 437 ± 26 kcal of lipids, and 10.4 ± 0.6 g of nitrogen); all also consumed some oral alimentation. Four had been weaned from parenteral nutrition for several weeks before the study.

Protocols

The protocol of the study was approved by the Ethical Committee of Lyon, and the study was conducted according to the Hurriet law. When appropriate, the tests in women were performed at the end of the menstrual cycle (there are no menstrual variations for cholesterol synthesis) (10). The control subjects and the four patients weaned from parenteral nutrition consumed their usual diet during the days preceding the study. Patients on long-term parenteral nutrition were studied at least 24 h after the end of the last iv infusion of nutrients. In the evening before the test, the subjects drank a loading dose of deuterated water (Cambridge Isotope Laboratory, Andover, MA) (3 g/kg body weight; one-half after the evening meal and one-half at 10:00 PM). Then until the end of the study, they drank only water enriched with 2H2O (4.5 g 2H2O/1 of drinking water). The following morning at 07:30, in the postabsorptive state after an overnight fast, an indwelling catheter was placed in a forearm vein and blood samples were drawn for the measurements of various concentrations and enrichments and for the separation of circulating mononuclear cells.

Analytical procedures

Concentrations and enrichments. Metabolites were assayed with enzymatic methods on neutralized perchloric extracts of plasma (glucose and lactate) or on plasma [nonesterified fatty acid (NEFA), TG]. Plasma insulin and glucagon concentrations were determined by enzymatic assay. Plasma lipids were extracted by the method of Folch et al. (11). Free cholesterol and TG were separated by thin-layer chromatography and scraped off the silica plates. Cholesterol was eluted from silica with ether before preparing its trimethylsilyl derivative (12). The methylated derivative of the palmitate of TG was prepared according to Morrison and Smith (13). Deuterium enrichments were also measured in the TG part of VLDLs purified by ultracentrifugation (14). Deuterium enrichment determinations were performed as previously described (12, 15) on a gas chromatograph interfaced with a mass spectrometer (HP5971A, Hewlett-Packard) operating in

| TABLE 1. Hormones and metabolites concentrations in the postabsorptive state |
|---------------------------------------------|----------------|------------------|
|                                | Controls (n = 10) | All (n = 22) | With Parenteral Nutrition (n = 18) | Weaned Patients (n = 4) |
| Glucose mM                      | 4.51 ± 0.13     | 3.96 ± 0.09a   | 3.85 ± 0.11a   | 4.10 ± 0.27   |
| Lactate µM                      | 701 ± 54        | 759 ± 87       | 743 ± 99       | 708 ± 22      |
| NEFA µM                         | 434 ± 48        | 654 ± 36a      | 654 ± 71a      | 608 ± 145     |
| TG mM                           | 0.76 ± 0.07     | 1.64 ± 0.17a   | 1.65 ± 0.07a   | 1.59 ± 0.49a  |
| Cholesterol mM                  | 4.87 ± 0.24     | 3.52 ± 0.24a   | 3.54 ± 0.27a   | 3.44 ± 0.59a  |
| Insulin mU/l                    | 7.2 ± 1.0       | 8.3 ± 1.9      | 8.1 ± 2.1      | 10.5 ± 5.1    |
| Glucagon ng/l                   | 169 ± 21        | 170 ± 17       | 165 ± 20       | 227 ± 50      |

NEFA, nonesterified fatty acid; TG, triglyceride. Results are shown as mean and SEM.

a P < 0.05.

b P < 0.01.

c P < 0.001 versus control subjects.

| TABLE 2. Patient characteristics |
|----------------------------------|----------------|----------------|
|                                   | Patients with | Weaned Patients |
|                                   | Parenteral Nutrition (n = 18) | (n = 4) |
| Sex                               | 8 F, 10 M     | 3 F, 1 M       |
| Age (years)                       | 50 ± 4       | 56 ± 8        |
| Weight (kg)                       | 55 ± 3       | 48 ± 12       |
| Body mass index                   | 19.7 ± 0.8   | 21.5 ± 1.5    |
| Resected patients                | 14/18        | 4/4           |
| Remnant small bowel length       | 101 ± 25 cm  | 201 ± 38 cm   |
| Steatorrhea (g/day)               | 36 ± 6       | 78 ± 40       |

Results are expressed as mean ± SEM.
the electronic impact ionization mode (70 eV). The carrier gas was helium. Ions 368 to 370 were selectively monitored for cholesterol, and ions 270 to 272 were maintained for palmitate. Deuterium enrichment in plasma water was measured by the method of Yang et al. (16). Special care was taken to obtain comparable ion peak areas between standard and biological samples, adjusting the volume injected or diluting the sample when necessary. Enrichment values are expressed as mole percent excess.

Measurement of mRNA concentrations. Mononuclear cells were immediately isolated by centrifugation of whole venous blood on a Ficoll gradient at 4°C as described (17) and stored at −80°C. Total RNA was prepared from frozen samples as previously described (18). LDLR and HMG-CoA reductase mRNA copy numbers were determined by competitive RT-PCR. LRP mRNA concentrations were measured by RT-competitive PCR. The detailed procedure has been published previously (18, 19). The results were expressed as copy number per microgram of total cellular RNA.

Calculations. The fractional contributions of cholesterol synthesis to the plasma free cholesterol pool and of hepatic lipogenesis to the plasma TG-fatty acids pool were calculated from the deuterium enrichments in free cholesterol in the palmitate of plasma TG and in plasma water, as previously described (12, 15). In short, the deuterium enrichments that would have been obtained if endogenous synthesis were the only source of plasma cholesterol and TG-fatty acids pool were calculated from plasma water enrichment. The comparison of the actual enrichments observed with these theoretical values gives the percent contributions, expressed as fractional synthesis (FS), of endogenous synthesis to the pool of rapidly exchangeable free cholesterol and of plasma TG at the time of blood sampling (2 h since the ingestion of the loading dose of deuterated water). These values were then transformed in absolute contributions or absolute synthesis (AS) (19). For cholesterol, we calculated first the total pool of rapidly exchangeable cholesterol (M1, which comprises cholesterol in plasma, liver, intestine, and blood cells) according to the equation of Goodman et al. (20). Cholesterol AS (mg) was then calculated as AS1 = FS × M1. Since M1 comprises esterified and free cholesterol and we found deuterium enrichment only in free cholesterol, we calculated the AS in the rapidly exchangeable free cholesterol pool (AS1), estimating that the ratio in plasma of free over total cholesterol concentrations (mean value 0.22) is representative of this ratio in the whole pool. For TG, the contribution of hepatic lipogenesis to the plasma TG pool at the time of sampling [absolute lipogenesis (Alipo)] was calculated as: Alipo = FS × M2, with M2 being the pool of TG obtained by multiplying the TG concentration by the plasma volume estimated to 37 ml/kg (21).

Results are shown as mean and SE of the mean. Comparisons between groups were performed using the Mann-Whitney U test. Correlations were established using Spearman’s test.

RESULTS

Hormonal and metabolic parameters

In the postabsorptive state, patients with intestinal failure had lower glucose (P < 0.001) and higher NEFA (P < 0.05) concentrations than control subjects. Lactate, glucose, and insulin concentrations were comparable. Cholesterol concentrations were decreased (P < 0.01) in patients, whereas TG levels were increased (P < 0.01). When the patients with intestinal failure were separated into subjects receiving or weaned of parenteral nutrition, no difference between these two groups was observed. Patients weaned of parenteral nutrition always had higher TG and lower cholesterol concentrations than the control group. They also had lower glucose and higher NEFA concentrations than control subjects, but these modifications did not reach significance in the weaned group, probably because of the small number of subjects in this group.

Hepatic lipogenesis and cholesterol synthesis

Deuterium enrichments in plasma water were 0.28 ± 0.01%, 0.34 ± 0.02%, and 0.37 ± 0.01% in control subjects and in patients receiving or weaned of parenteral nutrition, respectively. The corresponding enrichments in plasma free cholesterol were 0.26 ± 0.01%, 1.12 ± 0.09%, and 1.49 ± 0.05%. Enrichments in the palmitate of plasma TG were 0.44 ± 0.01%, 1.04 ± 0.10%, and 1.48 ± 0.19% (these enrichments can be higher than in plasma water since there are multiple possible incorporation sites of deuterium in the molecules of cholesterol and of palmitate during their synthesis). The percent contributions of hepatic lipogenesis and cholesterol synthesis to the plasma pools of TG and free cholesterol in the different groups of subjects are shown in Fig. 1. These FS were largely, more than 2-fold, increased in the whole group of patients with intestinal failure (hepatic lipogenesis, 15.1 ± 1.36% versus 7.57 ± 1.29%, P < 0.01; cholesterol synthesis, 12.99 ± 0.88% versus 3.45 ± 0.44%, P < 0.001). These increases in lipid synthesis were comparable in the groups with and without parenteral nutrition (Fig. 1). Hepatic lipogenesis was also calculated using the enrichment of palmitate in the TG fraction of VLDL purified by ultracentrifugation. Comparable results were obtained (data not shown). The ASs of cholesterol calculated with the pool of rapidly exchangeable total or free cholesterol were largely

![Fig. 1](https://www.jlr.org) Fractional values for cholesterol synthesis (A) and hepatic lipogenesis (B) in control subjects (C) and in patients with severe intestinal malabsorption receiving (NP) or weaned (W) of parenteral nutrition. *P < 0.05, **P < 0.01, ***P < 0.001 versus control subjects.
increased in patients receiving parenteral nutrition (AST, 2562 ± 287 mg, ASL, 572 ± 64 mg, P < 0.001) or not (AST, 3241 ± 904 mg, ASL, 724 ± 68 mg, P < 0.01) compared with control subjects (AST, 750 ± 113 mg, ASL, 167 ± 25 mg). Alipo was also largely increased in patients receiving parenteral nutrition (440 ± 91 mg, P < 0.01) or not (467 ± 94 mg, P < 0.05) (control subjects: 130 ± 54 mg). These differences are still more marked when results are expressed per kilogram of body weight. No relation could be found between the amount of lipid synthesized (either hepatic lipogenesis or cholesterol synthesis) on the one hand and either the total energy or carbohydrate provided by parenteral nutrition (patients receiving parenteral nutrition) or plasma insulin (all patients) on the other. We also found no relation between hepatic lipogenesis and plasma TG concentrations. However, cholesterol synthetic rate was inversely related to the remnant small bowel length (r = −0.47, P < 0.05) and positively related to steatorrhea (r = 0.50, P < 0.05).

**mRNA values in circulating mononuclear cells**

Despite the large stimulation of cholesterol synthesis, HMG-CoA reductase mRNA levels were not increased, but decreased (P < 0.05) in patients with intestinal failure. However, in the weaned patients, HMG-CoA reductase mRNA levels were comparable to those of control subjects and higher (P < 0.05) than in patients receiving parenteral nutrition. LDLR mRNA levels were also decreased overall, whereas LRP mRNA levels were unchanged (Table 3).

**DISCUSSION**

This study shows that subjects with severe intestinal failure have, in addition to the previously described modifications of plasma cholesterol and TG concentrations, a large stimulation of both endogenous cholesterol synthesis and hepatic lipogenesis, expressed either as fractional contributions or quantitative values. These modifications of lipid metabolism were observed irrespective of the presence of parenteral nutrition. This particularly excludes the possibility that the increased plasma TG levels and hepatic lipogenic rate resulted merely from the iv infusion of TG emulsion and/or glucose, and shows that they result from intestinal failure itself.

The large increase in cholesterol synthesis is consistent with previous reports of raised concentrations in the plasma of cholesterol precursors (1, 4, 5) and of increased activity of hepatic HMG-CoA reductase (4) in patients with ileum resection. The initial stimulus is considered to be the malabsorption of bile acids resulting in the loss of repression by Farnesoid X receptor (FXR) of the expression of CYP7A1 (22, 23) in order to compensate for bile acids malabsorption by an increased synthesis of cholesterol. This increased use of cholesterol for bile acids synthesis induces an increase in hepatic cholesterol synthesis (1, 4, 5) and also in the uptake of cholesterol-rich lipoproteins by the liver (4), which then results in a decrease of plasma cholesterol concentration. This picture is overall comparable to the one observed in mice overexpressing CYP7A1 in the liver (24). We measured HMG-CoA reductase, LDLR, and LRP mRNA levels in circulating mononuclear cells since it had been initially proposed that these cells could be used as a substitute for hepatocytes, at least in estimating liver HMG-CoA reductase and LDLR expression (9). However, HMG-CoA reductase mRNA concentrations were decreased in patients receiving long-term parenteral nutrition and normal in the weaned ones. LDLR and LRP mRNAs concentrations were not modified. These results are inconsistent with the observed increase of cholesterol synthesis and postulated enhanced liver uptake of cholesterol. Mononuclear cells do not express 7α-hydroxylase, and their cholesterol metabolism is not linked to that of bile acids. Therefore, they do not appear as representative of hepatocytes, at least in this situation. With respect to HMG-CoA reductase mRNA levels, an interesting point is the fact that these levels were decreased in patients receiving parenteral nutrition and comparable to values of the control group in weaned subjects. This suggests that parenteral nutrition down-regulated the expression of HMG-CoA reductase. Infusion of a lipid emulsion actually decreased HMG-CoA reductase activity in rats (25).

Abnormalities of bile acid metabolism probably also play a role in the increased hepatic lipogenesis and plasma TG concentration in patients with intestinal failure. This link is suggested by the observation that subjects with familial hypertriglyceridemia have an impaired intestinal absorption and an increased hepatic synthesis of bile acids (26, 27). The presence of a cause-and-effect relationship is supported by in vivo studies showing that administration of bile acid-binding resins and of chenodeoxycholic acid respectively increase and decrease plasma TG levels (28, 29). Moreover, in vitro studies of human hepato-

### TABLE 3. mRNA levels in circulating mononuclear cells

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 10)</th>
<th>All (n = 2)</th>
<th>With Parenteral Nutrition (n = 18)</th>
<th>Weaned Patients (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMG-CoA reductase</td>
<td>973 ± 174</td>
<td>450 ± 64a</td>
<td>385 ± 66a</td>
<td>728 ± 88a</td>
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<tr>
<td>LDL receptor</td>
<td>38 ± 7</td>
<td>22 ± 3b</td>
<td>23 ± 3b</td>
<td>18 ± 1</td>
</tr>
<tr>
<td>LDL receptor-related protein</td>
<td>114 ± 18</td>
<td>136 ± 26</td>
<td>150 ± 30</td>
<td>43 ± 24</td>
</tr>
</tbody>
</table>

Results are shown as number of copy; 10⁴ per μg of total RNA.
* P < 0.05.
* P < 0.01 versus control subjects, a P < 0.05 versus patients receiving parenteral nutrition.
This work was supported by a grant from the Hospices Civils de Lyon and by a grant from Danone Institut to A.C. The authors wish to thank all the subjects who participated in this study.

REFERENCES


