STIMULATION OF CHOLESTEROL SYNTHESIS AND HEPATIC LIPOGENESIS IN PATIENTS WITH SEVERE MALABSORPTION

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Short title : lipid synthesis in severe malabsorption

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ABSTRACT:
Patients with severe malabsorption have abnormal lipid metabolism with low plasma cholesterol and frequently high triglycerides 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 triglycerides (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 to hypertriglyceridemia. HMG-CoA reductase and LDL-receptor mRNA levels were decreased (p<0.05) in patients on long term parenteral nutrition. HMG-CoA reductase mRNA were normal in weaned patients. Conclusions: Severe malabsorption induces large increases of cholesterol synthesis and hepatic lipogenesis, independently of the presence of parenteral nutrition. These abnormalities are probably due to the malabsorption of bile acids.

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 to 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 triglycerides (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 increased utilization of cholesterol for bile acids 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 2 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 TG in very low density lipoproteins (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 triglycerides emulsion. It could also result from the intra-venous infusion of glucose since 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 lipids 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 metabolism (9), we measured in these cells the mRNA concentrations of HMG-CoA reductase, LDL-receptor and LRP. In order to determine to 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.

SUBJECTS 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 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 normal physical examination and normal plasma glucose and lipids concentrations (see Table 2). Subjects with unusual dietary habits were excluded.

The 22 patients with intestinal failure included 9 women and 13 men (aged 19 to 73 years, body mass index 11.8 to 27.1). Their characteristics are given in Table 1. Most of them (18/22) had a 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 or mucosal atrophy. 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 were on long-term parenteral nutrition for 47.5 ± 11.9 months (3.3 ± 0.4 all-in-one bags per week, one bag = 2181 ± 140 ml, 762 ± 36 kcal of glucose, 437 ± 26 kcal of lipids
and 10.4 ± 0.6 g of nitrogen); all consumed also 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 during the first ten days of the menstrual cycle in order to take in account the known variations of lipogenesis during 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 24h at least after the end of the last intra-venous infusion of nutriments. In the evening before the test the subjects drank a loading dose of deuterated water (Cambridge Isotope Laboratory, Andover, MA, USA) (3g/kg body water; 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 $^2$H$_2$O (4.5g $^2$H$_2$O/l of drinking water). The following morning at 07:30, in the post-absorptive 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 monocytes.

Analytical procedures

Concentrations and enrichments:

Metabolites were assayed with enzymatic methods on neutralized perchloric extracts of plasma (glucose and lactate) or on plasma (non esterified fatty acids -NEFA-, TG). Plasma insulin and glucagon concentrations were determined by radioimmunoassay. Total cholesterol was measured
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 very low density lipoproteins 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 the electronic impact ionization mode (70 eV). Carrier gas was helium. Ions 368 to 370 were selectively monitored for cholesterol and ions 270 to 272 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 per cent excess (MPE).

**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 described previously (18). LDL receptor 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 µg of total cellular RNA.

**Calculations**

The fractional contributions of cholesterol synthesis to 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 (12 hours 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 first as ASt = 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 (ASI), 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 plasma TG pool at the time of sampling (absolute lipogenesis, Alipo) was calculated as: Alipo = FS*Mtg with Mtg 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 standard error 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 (Table 2):
In the post-absorptive state, patients with intestinal failure had lower glucose (p<0.001) and higher NEFA (p<0.05) concentrations than control subjects. Lactate, glucagon 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 in subjects receiving or weaned of parenteral nutrition, no difference between these two groups were observed. Patients weaned of parenteral nutrition had always higher TG and lower cholesterol concentrations than the control group. They had also 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 figure 1. These fractional synthetis were largely, more than two folds, increased in the whole group of patients with intestinal failure (hepatic lipogenesis 15.54±1.36 vs 7.57±1.29%, p<0.01, cholesterol synthesis 12.99±0.88 vs 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 AS of cholesterol calculated with the pool of rapidly exchangeable total or free cholesterol were largely increased.
in patients receiving (ASl 2562±287 mg, ASL 572±64 mg p<0.001) or not (ASl 3241±304 mg, 
ASl 724±68 mg, p<0.01) parenteral nutrition compared to control subjects (ASl 750±113 mg, 
ASl 167±25 mg). Alipo was also largely increased in patient receiving (440±91 mg, p<0.01) or 
not (467±94 mg, p<0.05) parenteral nutrition (control subjects : 130±54 mg). These differences 
are still more marked when results are expressed per kg of body weight. No relation could be 
found between the amount of lipid synthesized (either hepatic lipogenesis or cholesterol 
synthesis) on 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 one. 
We found also no relation between hepatic lipogenesis and plasma TG concentrations. However 
cholesterol synthetic rate was inversely related with the remnant small bowell length (r = -0.47, 
p<0.05) and positively with steatorrhea (r = 0.50, p< 0.05).

**mRNA values in circulating mononuclear cells (table 3)**:

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 patient receiving parenteral nutrition. LDL receptors mRNA levels were 
also overall decreased whereas LRP mRNAs levels were unchanged.

**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 or not of parenteral nutrition. This excludes particularly the
possibility that the increased plasma TG levels and hepatic lipogenic rate resulted merely from the intra-venous infusion of triglyceride 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 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 bile acids malabortion by an increased synthesis from 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 results then 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, LDL-R 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 for estimating liver HMG-CoA reductase and LDL-R expression (9). However HMG-CoA reductase mRNA concentrations were decreased in patients receiving long term parenteral nutrition and normal in the weaned ones. LDL-R 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 7 alpha-hydroxylase and their cholesterol metabolism is not linked to the one 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. Actually infusion of a lipid emulsion decreased HMG-CoA reductase activity in rats (25).

Abnormalities of bile acids metabolism play also probably a role in the increased hepatic lipogenesis and plasma TG concentration of 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 increases and decreases plasma TG levels (28, 29). Moreover in vitro studies of human hepatocyte culture showed that addition of bile acids decreases VLDL secretion (30). Lastly the finding in the liver of mice lacking cholesterol 27-hydroxylase of increased expression and activity not only of CYP7A, SREBP-2 and cholesterol synthetic pathway, but also of SREBP-1c and the lipogenic pathway demonstrates the presence of links between bile acids and liver TG metabolism (31). This link in subjects with bile acids malabsorption could be the decreased activation by bile acids of liver FXR. This would result in an increased activity of LXRα (32). LXRα itself is an activator of the expression of the lipogenic pathway, both directly and through a stimulation of the transcription of SREBP-1c (33, 34). The increased hepatic lipogenesis may have participated to the hypertriglyceridemia of patients with intestinal failure. However it does not appear sufficient by itself since i) we found no relationship between hepatic lipogenesis and plasma TG levels, ii) some patients had normal TG levels despite enhanced hepatic lipogenesis. Although we measured only the contribution of hepatic lipogenesis to the plasma TG pool at the time of blood sampling, and not the true synthetic rates, this strongly suggests that another(s) defect(s) clearly participated to the hypertriglyceridemia of these patients. These defects could have been an enhanced hepatic reesterification of plasma NEFA, since their concentration were increased,
and/or a reduced clearance of plasma TG. FXR stimulates the transcription of apoprotein CII, an activator of lipoprotein-lipase (35). Thus, in addition to the stimulation of hepatic lipogenesis, a decreased activation of FXR in patients with intestinal failure could have contributed to their hypertriglyceridemia through a reduced clearance of plasma TG.

In conclusion our study shows that patients with severe malabsorption have a stimulation of cholesterol synthesis and hepatic lipogenesis. These abnormalities result both probably from bile acids malabsorption. The stimulation of hepatic lipogenesis participate to the hypertriglyceridemia of such patients but is probably not the only metabolic abnormality involved.

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References:


**Figure legend:**

**Figure 1:** Fractional values for cholesterol synthesis (upper panel) and hepatic lipogenesis (lower panel) in control subjects (C) and in patients with severe intestinal malabsorption receiving (NP) or weaned (W) of parenretal nutrition. * p<0.05, ** p<0.01, *** p<0.001 vs control subjects.
Table 1: Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Patients with parenteral nutrition</th>
<th>Weaned patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 18</td>
<td>n = 4</td>
</tr>
<tr>
<td>Sex</td>
<td>8 F, 10 M</td>
<td>3F, 1 M</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50 ± 4</td>
<td>56 ± 8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>53 ± 3</td>
<td>48 ± 12</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>19.7 ± 0.8</td>
<td>21.5 ± 1.5</td>
</tr>
<tr>
<td>Resected patients</td>
<td>14/18</td>
<td>4/4</td>
</tr>
<tr>
<td>Remnant small bowel length</td>
<td>101 ± 25 cm</td>
<td>201 ± 38 cm</td>
</tr>
<tr>
<td>Steatorrhea (g/day)</td>
<td>36 ± 6</td>
<td>78 ± 40</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± sem.
Table 2: Hormones and metabolites concentrations in the post-absorptive state

<table>
<thead>
<tr>
<th></th>
<th>Controls n = 10</th>
<th>Patients with intestinal failure</th>
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<tbody>
<tr>
<td></td>
<td>All n = 22</td>
<td>With parenteral nutrition n = 18</td>
<td>Weaned patients n = 4</td>
<td></td>
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<tr>
<td>Glucose mM</td>
<td>4.51±0.13</td>
<td>3.90±0.09 ***</td>
<td>3.85±0.11 **</td>
<td>4.10±0.27</td>
</tr>
<tr>
<td>Lactate µM</td>
<td>701±54</td>
<td>759±87</td>
<td>743±99</td>
<td>708±22</td>
</tr>
<tr>
<td>NEFA µM</td>
<td>434±48</td>
<td>654±36 *</td>
<td>654±71 *</td>
<td>608±145</td>
</tr>
<tr>
<td>TG mM</td>
<td>0.76±0.07</td>
<td>1.64±0.17 **</td>
<td>1.65±0.07 **</td>
<td>1.59±0.49 *</td>
</tr>
<tr>
<td>Cholesterol mM</td>
<td>4.87±0.24</td>
<td>3.52±0.24 **</td>
<td>3.54±0.27 **</td>
<td>3.44±0.59 *</td>
</tr>
<tr>
<td>Insulin mU/L</td>
<td>7.2±1.0</td>
<td>8.3±1.9</td>
<td>8.1±2.1</td>
<td>10.5±5.1</td>
</tr>
<tr>
<td>Glucagon ng/L</td>
<td>169±21</td>
<td>170±17</td>
<td>165±20</td>
<td>227±50</td>
</tr>
</tbody>
</table>

Results are shown as mean and sem. * p<0.05, ** p<0.01, *** p<0.001 vs control subjects
Table 3: mRNA levels in circulating mononuclear cells

<table>
<thead>
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<th>Controls n = 10</th>
<th>Patients with intestinal failure</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>All n = 2</td>
<td>With parenteral nutrition n = 18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weaned patients n = 4</td>
</tr>
<tr>
<td>HMG-CoA reductase</td>
<td>973±174</td>
<td>450±64 **</td>
<td>385±66 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>728±88 a</td>
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<tr>
<td>LDL-R</td>
<td>38±7</td>
<td>22±3 **</td>
<td>23±3 *</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>18±1</td>
</tr>
<tr>
<td>LRP</td>
<td>114±18</td>
<td>136±26</td>
<td>150±30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>43±24</td>
</tr>
</tbody>
</table>

Results are shown as number of copy \(10^4\) per \(\mu g\) of total RNA. * \(p<0.05\), ** \(p<0.01\) vs control subjects, a \(p<0.05\) vs patients receiving parenteral nutrition.