Increased lipogenesis and resistance of lipoproteins to oxidative modification in two patients with Glycogen Storage Disease type 1a

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Abstract

We describe two Glycogen Storage Disease 1a (GSD-1a) patients with severe hyperlipidemia without premature atherosclerosis. Susceptibility of low-density lipoproteins (LDL) to oxidation was decreased, possibly related to the ~40 fold increase in palmitate synthesis altering lipoprotein saturated fatty acid contents. These findings are potentially relevant for anti-hyperlipidemic treatment in GSD-1a patients.
Lipid metabolism in GSD-1a patients

Introduction

Glycogen storage disease type 1a (von Gierke disease) is an inborn error of metabolism caused by deficiency of G6Pase, the enzyme catalyzing the conversion of G6P to glucose. The disease is characterized by hypoglycemia and hepatic glycogen and fat accumulation as well as severe hypertriglyceridemia, hypercholesterolemia and hyperuricemia.\(^1\) The mechanistic relation between the primary abnormalities in glucose metabolism and hyperlipidemia are still speculative. Triglycerides and cholesterol are normally synthesized in the liver, incorporated into very-low density lipoprotein particles and secreted into the plasma. After lipolysis of the triglycerides, the fatty acids are removed from the blood and taken up by extrahepatic tissues, \textit{i.e.}, fat and muscle predominantly. Indications for decreased plasma lipid clearance as well as for increased lipid production have been reported in GSD-1 patients.\(^4\)\(^,\)\(^6\)\(^,\)\(^7\)

As the result of improved dietary management, patients with GSD-1a commonly reach adult age so that the potential contribution of hyperlipidemia to development of atherosclerosis becomes important. Conflicting reports have appeared on development of atherosclerosis in GSD-1 and the use of lipid lowering treatment.\(^8\)\(^,\)\(^9\)

We describe two young adult brothers with GSD-1a with severe hyperlipidemia without clinical signs of atherosclerosis. Susceptibility of low-density lipoproteins to oxidative modification, one of the primary steps in atherogenesis, was decreased in the patient compared to controls, related to an increased lipoprotein saturated fatty acid content. We hypothesized this might be related to increased production rates of (mainly saturated) fatty acids. By measuring the incorporation of \(^{13}\)C labeled precursors into cholesterol and palmitate strongly elevated synthesis of cholesterol and fatty acids was found. These observations indicate that lipid-lowering treatment in GSD-1a patients might not be beneficial.

Methods

Subjects

Two GSD-1a patients and 6 healthy volunteers (mean age: 27 years, range: 22-39 years; mean body mass index: 22.6 kg/m\(^2\), range: 19.5-25.5 kg/m\(^2\)) participated in this study. The patients were 25-year old, non-identical twin brothers A and B. All participants were non-smokers, had no familial history of hyperlipidemia or premature heart disease and none was taking any medication or special diet. Subjects were instructed to consume their regular diet until 22.00 h of the evening of the start of the study. Informed written consent was obtained in accordance with the University Hospital Groningen Ethical Committee.

In patient A the diagnosis was made by mutation analysis. At the age of 19 years, patient A was referred to our hospital, when physical examination showed a mildly mentally retarded boy, with stunted height (158 cm, -3.5 SD), normal weight (52 kg) and severe hepatomegaly. Numerous xantholasmata were present. Plasma cholesterol and
triglyceride concentrations were 26.2 mmol/l (1013 mg/dl) and 36.6 mmol/l (3242 mg/dl), respectively. Apolipoprotein A-I levels were 1.1 g/l (normal range: 1.35-2.35 g/l), apolipoprotein B levels were 2.1 g/l (normal range: 0.4-1.0 g/l) and the patient had an apo E phenotype E4/4. Dietary treatment was intensified and fat- (8 energy %), lactose-, and sodium restricted (protein: 13 energy %, carbohydrate: 78 energy %), with dietary triglycerides containing 27 % SFA, 15 % MUFA and 58 % PUFA. However, severe hyperlipidemia remained.

In patient B the diagnosis GSD-1a was also confirmed by mutation analysis. At the age of 23 years, patient B was referred to our hospital, when physical examination showed a mentally normal young man, with normal height (176 cm, -1.8 SD) and weight (68kg), and a mild hepatomegaly. Total cholesterol was 7.9 mmol/l (305 mg/dl), triglycerides 13.5 mmol/l (1196 mg/dl), apolipoprotein A-I 1.1 g/l and apolipoprotein B 1.1 g/l. Dietary treatment was adjusted by increasing the amount of slowly releasing carbohydrates and was fat-restricted (16.8 energy %; protein: 10 energy %, carbohydrate: 73 energy %) with a fatty acid composition of 26 % SFA, 26 % MUFA and 48 % PUFA.

Measurement of lipogenesis, cholesterogenesis and lipoprotein oxidation

Two healthy volunteers were studied without treatment and a second time after taking 8 g/day of cholestyramine for two weeks to also compare cholesterogenesis in controls to the patients after strong induction. They fasted from 22.00 h the day before the experiment till 10.00 h when they received an oral liquid diet replacement (Nutridrink, Nutricia BV, The Netherlands) at a rate of about 7 mg/kg/min of carbohydrates. This rate was similar to the amount of carbohydrates the two patients received through a nasogastric tube from 22.00 h until the end of the experiment to maintain normoglycemia (glucose levels 3-6 mmol/l). At midnight an infusion of [1-13C]acetate (Isotec, Miamisburg, OH, U.S.A) was started in volunteers and patients through a nasogastric tube at a rate of 0.12 mmol/kg/h for 16 hours. Blood samples were taken before, throughout and after the infusion. After 16 h the infusion was stopped and subjects were allowed to return to their regular diet.

Cholesterol was extracted from total plasma and derivatized according to Neese et al.10 VLDL from plasma samples was isolated11 and palmitate from VLDL fractions was methylated as described elsewhere.11 Lipids were analyzed by gas chromatography/mass spectrometry.11,12 De novo synthesis of cholesterol and palmitate in plasma and VLDL, respectively, were measured by MIDA, as described in detail previously.9,11 To obtain a semi-quantitative value for palmitate synthesis, we multiplied fractional synthesis de novo by the total amount of palmitate in VLDL at the end of the experiment. This reveals the total amount of newly synthesized palmitate present in VLDL after 16 hours of 13C-acetate infusion.

The oxidation of LDL and VLDL was measured according to the Esterbauer method with some modifications.13 Tocopherols (α and γ) and β-carotene were determined by high-performance liquid chromatography14 and ubiquinol levels were analyzed as described.15
Lipid metabolism in GSD-1a patients  

Results

At the time of the experiment, plasma triglyceride concentrations in patient A (18.2 mmol/l, 1612 mg/dl) and patient B (11.9 mmol/l, 1054 mg/dl) were more than ten times higher than in the control subjects (0.8 ± 0.4 mmol/l, 71 mg/dl). Likewise, plasma cholesterol concentrations in the patients were 15.0 mmol/l (580 mg/dl) and 10.8 mmol/l (418 mg/dl), respectively, which was markedly higher than in controls, i.e., 4.2 ± 0.4 mmol/l (162 ± 15 mg/dl). Increased lipid concentrations were almost solely due to increases in the VLDL fraction as determined by fast performance liquid chromatography (data not shown). Uric acid concentrations were normal with 0.26 mmol/l and 0.35 mmol/l in patient A and B, respectively. Mean glucose concentrations during the experiment.

Table 1. Oxidation characteristics of VLDL and LDL particles, fasting plasma antioxidant concentrations and VLDL and LDL fatty acid composition.

<table>
<thead>
<tr>
<th></th>
<th>Patient A</th>
<th>Patient B</th>
<th>Controls *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma α-tocopherol (µmol/l)</td>
<td>57.5</td>
<td>81.7</td>
<td>21.7 ± 4.9</td>
</tr>
<tr>
<td>Relative (µmol/mmol)</td>
<td>3.1</td>
<td>2.7</td>
<td>4.6 ± 0.7</td>
</tr>
<tr>
<td>β-carotene (µmol/l)</td>
<td>0.17</td>
<td>0.76</td>
<td>0.74 ± 0.39</td>
</tr>
<tr>
<td>Relative (µmol/mmol)</td>
<td>0.01</td>
<td>0.03</td>
<td>0.16 ± 0.10</td>
</tr>
<tr>
<td>Ubiquinol (µmol/l)</td>
<td>1.21</td>
<td>1.44</td>
<td>0.92 ± 0.37</td>
</tr>
<tr>
<td>Relative (µmol/mmol)</td>
<td>0.06</td>
<td>0.05</td>
<td>0.20 ± 0.07</td>
</tr>
<tr>
<td>VLDL Lag time (min)</td>
<td>359</td>
<td>372</td>
<td>135 ± 12</td>
</tr>
<tr>
<td>Propagation speed (nmol/mg/min)</td>
<td>4.5</td>
<td>3.5</td>
<td>13.1 ± 2.3</td>
</tr>
<tr>
<td>SFA (%)</td>
<td>51.8</td>
<td>50.0</td>
<td>45.6 ± 4.6</td>
</tr>
<tr>
<td>MUFA (%)</td>
<td>28.5</td>
<td>36.8</td>
<td>26.1 ± 2.2</td>
</tr>
<tr>
<td>PUFA (%)</td>
<td>19.7</td>
<td>13.2</td>
<td>28.3 ± 3.2</td>
</tr>
<tr>
<td>LDL Lag time (min)</td>
<td>97</td>
<td>107</td>
<td>84 ± 6</td>
</tr>
<tr>
<td>Propagation speed (nmol/mg/min)</td>
<td>7.0</td>
<td>6.7</td>
<td>10.9 ± 0.9</td>
</tr>
<tr>
<td>SFA (%)</td>
<td>43.7</td>
<td>40.8</td>
<td>30.8 ± 1.0</td>
</tr>
<tr>
<td>MUFA (%)</td>
<td>28.4</td>
<td>25.4</td>
<td>20.1 ± 3.3</td>
</tr>
<tr>
<td>PUFA (%)</td>
<td>27.9</td>
<td>33.8</td>
<td>49.1 ± 4.2</td>
</tr>
</tbody>
</table>

Lag time denotes the time after administration of copper until oxidation starts and propagation speed the actual rate of oxidation. SFA denotes saturated fatty acid, MUFA monounsaturated fatty acid, and PUFA polyunsaturated fatty acid. * Values were obtained from five control subjects and are displayed as means ± SD. ‘Relative’ values for α-tocopherol, β-carotene, and ubiquinol concentrations refer to antioxidant concentrations divided by cholesterol plus triglyceride concentrations.
were 4.45 mmol/l in patient A and 4.50 mmol/l in patient B and mean lactate levels were 3.2 and 3.4 mmol/liter (normal upper value: 2.2 mmol/l), respectively.

Susceptibility of both LDL and VLDL to oxidative modification was markedly lower in the GSD patients (Table 1), as indicated by an increased lag time and a decreased propagation rate. Plasma concentrations of α-tocopherol, β-carotene and ubiquinol showed no major differences when expressed relative to total lipid content: if anything, the relative content of β-carotene and ubiquinol appeared to be decreased (Table 1).

Fatty acid composition of LDL particles and, to a lesser extent, of VLDL particles showed increased SFA contents in the patients. The high relative amount of SFA was markedly different from the composition of their dietary fat intake, which consisted mainly of PUFA. In contrast, the lipoprotein fatty acid composition of the healthy volunteers matched the estimated fatty acid composition of their diet, which, based on a recent regional survey, contained 41 %, 21 % and 38 % SFA, MUFA and PUFA, respectively. Significant correlations were observed between propagation speed in LDL and SFA, MUFA and PUFA content (Figure 1). Significant, but less pronounced, correlations were also found for VLDL lag time and propagation speed and lipoprotein fatty acid composition.

A more than 40-fold increase in the amount of newly synthesized VLDL-palmitate in the two GSD patients compared to controls was calculated (Table 2). Calculation of absolute cholesterol synthesis rates revealed a 7-fold increase in the two patients which was even higher than in the volunteers after cholestyramine treatment to induce this process. Calculation of precursor pool enrichments, which were at steady state after 6 hours of [1-13C]acetate infusion, revealed a much lower acetyl-CoA pool enrichment in the patients compared to the control subjects.

**Table 2.** Cholesterol and palmitate synthesis in two GSD-1a patients and control subjects, the latter before and after cholestyramine treatment.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Absolute cholesterol production (mg/day)</th>
<th>Newly synthesized VLDL palmitate (mmol)</th>
<th>Precursor pool enrichment † (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient A</td>
<td>4419</td>
<td>119.7</td>
<td>8</td>
</tr>
<tr>
<td>Patient B</td>
<td>2212</td>
<td>209.0</td>
<td>9</td>
</tr>
<tr>
<td>Subject A</td>
<td>505</td>
<td>7.5</td>
<td>16</td>
</tr>
<tr>
<td>Subject B</td>
<td>426</td>
<td>0.3</td>
<td>15</td>
</tr>
<tr>
<td>Subject A*</td>
<td>1256</td>
<td>25.4</td>
<td>16</td>
</tr>
<tr>
<td>Subject B*</td>
<td>1477</td>
<td>2.6</td>
<td>15</td>
</tr>
</tbody>
</table>

* Values after 2-week treatment with cholestyramine. † Calculated from isotopomer distribution of plasma cholesterol at 12 and 16 hours after start of the 13C-acetate infusion.
Figure 1. Correlation between SFA, MUFA and PUFA content of LDL particles and the oxidation propagation speed, in healthy volunteers (closed symbols) ($n = 5$) and in patients (open symbols).
Discussion

A crucial and at present unanswered question is whether the severe hyperlipidemia in GSD-1a patients will ultimately lead to an increased risk for atherosclerosis, especially since patients have a better life expectancy due to improved dietary treatment. A few GSD-1 patients who were in their thirties have been described with atherosclerosis. However, Lee et al. using ultrasound techniques to investigate endothelial function, found no indication for premature atherosclerosis in GSD-1 patients. We chose to study two adult GSD-1a patients with severe hyperlipidemia and atherogenic lipid profile who were expected to show signs of premature atherosclerosis. Surprisingly, determination of ankle-brachial indices, aortic distensibility and intima-media thickness of the carotid and femoral arteries showed no signs of atherosclerotic lesions (data not shown). Ex vivo Cu-induced oxidation of lipoprotein particles, an important indicator of their atherogenicity, revealed a much lower oxidation susceptibility of patient LDL compared to controls. Antioxidants and fatty acid composition are known to influence the oxidizability of lipoprotein particles. Our data do not support a role for antioxidants in decreasing lipoprotein oxidizability in GSD-1a, since Tocopherols, β-carotene and uric acid concentrations were not increased, although hyperuricemia is a common phenomenon in GSD-1a patients. It is well known that PUFA display a higher susceptibility to oxidation than MUFA and SFA. Although we have only obtained data from two patients, our data suggests that the relatively high lipoprotein SFA content in GSD-1a patients plays a role in protection of plasma lipoproteins against oxidative modification. An important question is why patients have a relatively high SFA lipoprotein content compared to healthy control subjects, since patients have a high relative PUFA intake. Application of stable isotopes revealed that synthesis of saturated fatty acids, i.e. palmitate, as well as cholesterogenesis were severely increased compared to healthy controls. Control values found in this study were similar to values previously reported. With respect to the values found for de novo lipogenesis a number of factors must be taken into account. Similar amounts of carbohydrates were given to patients and controls, which led, however, to higher insulin concentrations in control subjects compared to the patients (data not shown). This might be partly attributable to hepatic glucose uptake, which is transformed to G6P and then unable to be released again as glucose. Lockwood et al. have shown decreased insulin secretion to a carbohydrate load in adult GSD-1 patients. Insulin and glucose are both separate stimulators of de novo lipogenesis. Differences in lipogenesis between control subjects and patients are therefore probably underestimated. A second factor potentially influencing the calculated synthesis values is the possible decreased clearance of VLDL triglycerides. The values for lipogenesis are a combination of formation and clearance. Decreased clearance is expected to lower the fraction of newly synthesized palmitate found at the end of the experiment, since it increases palmitate pool size leading to a higher dilution with unenriched palmitate molecules. Finally, the increases in lipid synthesis found in the patients studied here might be more pronounced than in GSD patients in better metabolic control and with less severe hyperlipidemia.
Decreased acetyl-CoA pool enrichments observed in the GSD-1a patients indicates that labeled acetyl-CoA is diluted to a larger extent with endogenous acetyl-CoA, reflecting a higher glycolytic flux towards the acetyl-CoA pool. This increased flux may contribute to higher fatty acid synthesis in GSD-1a patients by stimulating acetyl CoA carboxylase. Furthermore, data suggests that G6P itself might act as a mediator of carbohydrate-induced lipogenic activity.\textsuperscript{20}

In conclusion we hypothesize that absence of G6Pase activity together with a low fat diet increases lipogenesis, somewhat paradoxically in view of the well-known association of dietary SFA intake with atherosclerosis incidence, decreases the degree of oxidative modification of LDL by altering lipoprotein fatty acid profile. The use of fish oil might not be helpful to prevent premature atherosclerosis in GSD-1a patients, since normolipidemia is usually not achieved and fish oil could lead to increased lipoprotein oxidizability by increasing the lipoprotein PUFA content.

**Acknowledgements**

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References


Ik daalde tussen de cellen neer
en sinds ik in het plasma drijf
zie ik die bron dat hele lijf
niet helder meer niet meer