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ORIGINAL PAPER

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Disturbed lipid metabolism in glycogen storage disease type 1

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Abstract Glycogen storage disease type 1 (GSD1) is an inborn error of metabolism caused by deficiency of glucose-6-phosphatase, the enzyme catalysing the conversion of glucose-6-phosphate (G6P) to glucose. GSD1 is associated with severe hyperlipidaemia and hepatic steatosis. The underlying mechanisms responsible for these abnormalities in lipid metabolism are only partly known. This review summarises data available on hyperlipidaemia and steatosis in GSD1 and postulates new hypotheses for unresolved issues. Evidence indicates that lipid clearance from the blood compartment is decreased in GSD1. Furthermore, in two GSD1a patients synthesis of palmitate, an indicator of de novo lipogenesis, and cholesterol were found to be increased 40-fold and 7-fold, respectively. Elevated hepatic G6P levels may play a regulatory role in lipid synthesis via activation of transcription of lipogenic genes. In addition, accelerated glycolysis will supply acetyl-CoA molecules required for lipogenesis. It is as yet unclear whether hepatic secretion of lipids in the form of very low density lipoprotein-triglycerides (VLDL-TG) is altered in GSD1 patients: we recently found unaffected VLDL-TG secretion rates in an acute animal model of GSD1b. Hepatic steatosis, which is invariably present in GSD1 is probably mainly caused by an increased free fatty acid flux from adipose tissue to the liver and, to a limited extent, by increased de novo lipogenesis. *Conclusion:* future studies, using novel stable isotope methodologies, are warranted to further clarify the disturbances in lipid and lipoprotein metabolism in glycogen storage disease type 1 and the role of glucose-6-phosphate herein.

Keywords Lipogenesis · Stable isotopes · Glucose-6-phosphate · Glycogen storage disease · Very low density lipoprotein

Abbreviations *G6P* glucose-6-phosphate · *G6Pase* glucose-6-phosphatase · *GSD1* glycogen storage disease type 1 · *LDL* low density lipoprotein · *LPL* lipoprotein lipase · *SREBP* sterol regulatory element-binding protein · *VLDL* very low density lipoprotein · *VLDL-TG* very low density lipoprotein-triglycerides · *FFA* face fatty acids · *ER* endoplasmic reticulum

Introduction

Glycogen storage disease type 1 (GSD1) is caused by deficiency of the glucose-6-phosphatase (G6Pase) enzyme complex. G6Pase catalyses the conversion of glucose-6-phosphate (G6P) into glucose and represents the final step in glucose production from either glycogen breakdown or gluconeogenesis. The enzyme complex is mainly active in liver, but is also expressed in kidney and intestine and might be present in other tissues [22]. GSD1 has been separated into at least two distinct types of diseases, i.e. types 1a and 1b, on the basis of the underlying gene defects. The catalytic subunit of the G6Pase complex is deficient in GSD1a [36], whereas the G6P translocase, responsible for transport of G6P from cytosol into the lumen of the endoplasmic reticulum, is deficient in GSD1b [12,20]. Apart from the abnormalities found in carbohydrate metabolism (severe hypoglycaemia, hyperlactacidaemia, hepatic glycogen deposition), GSD1 is also associated with distinct hyperlipidaemia. Both plasma triglyceride and cholesterol concentrations are usually increased in GSD1 [6, 19,21] and only partially respond to therapeutic interventions [7, 24]. Furthermore, severe lipid accumulation in the

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liver is a characteristic hallmark of GSD1 [18]. Research in our laboratory is focussed on elucidating the mechanisms underlying the development of hyperlipidaemia and steatosis in GSD1. This review aims at summarising the current knowledge of lipid metabolism in GSD1 and provides hypotheses concerning some of the unresolved questions.

Hyperlipidaemia and glycogen storage disease type 1

Hyperlipidemia is present in both GSD1a and GSD1b [6, 19, 21] but GSD1a is usually associated with much more severe lipid abnormalities than GSD1b [39]. Hyperlipidaemia in GSD1 is characterised by a combined hypercholesterolaemia and hypertriglyceridaemia [18]. Increased concentrations of cholesterol are found in both very low density lipoprotein (VLDL) and low density lipoprotein (LDL) fractions whereas high density lipoprotein cholesterol and apolipoprotein A-1 concentrations are usually decreased [7, 24, 25]. VLDL and LDL particles are not only increased in number, as is evident from increased levels of apolipoprotein B [7, 24], but also in size due to the accumulation of triglycerides in these fractions [24]. An example of the cholesterol and triglyceride lipoprotein distribution in two GSD1a patients is shown in Fig. 1. The introduction of nocturnal gastric drip feeding and resistant cornstarch for maintenance of normoglycaemia at night time was found to lower plasma cholesterol and triglyceride levels [4, 13, 24, 32] but generally not to normal values [4, 7, 24, 32, 38]. Treatment with fibrates [13, 14] and/or fish oil [14, 27] has also been shown to improve hyperlipidaemia, although the effects of these therapies were found to diminish again over time in a number of patients [14, 27].

In GSD1, evidence for increased synthesis and release of lipids into the blood compartment as well as decreased lipid clearance from the blood have been reported. Both processes may contribute to the development of hyperlipidaemia. In a recent study, we infused two severely hyperlipidaemic GSD1a patients and two healthy control subjects with [1-^{13}C]acetate and determined de novo lipogenesis and cholesterogenesis by analysing the ^{13}C label incorporation in free cholesterol and VLDL-palmitate by mass isotopomer distribution analysis [16]. This study showed a 40-fold increase in de novo lipogenesis in the GSD1a patients. Cholesterogenesis was about 7-fold higher in patients than in controls [1]. Furthermore, we found indications for an increased flux through the acetyl-CoA pool, consistent with an increased glycolytic rate. Lipogenesis and cholesterogenesis are, at least in part, regulated by a family of transcription factors called sterol regulatory element-binding proteins (SREBPs) [17]. Three isoforms of SREBPs have been identified. SREBP-1a and -1c are derived from a single gene via the use of alternative transcription start sites and are mainly involved in regulation of lipogenesis and fatty acid metabolism. SREBP-2 regulates intracellular cholesterol homeostasis via transcriptional control of genes involved in cholesterol synthesis, including HMG-CoA reductase, and cellular cholesterol uptake, i.e. LDL receptor [17]. SREBP-1c expression is induced by insulin [8, 37] and very recently it has been reported that both glucose and insulin are separately able to simulate de novo

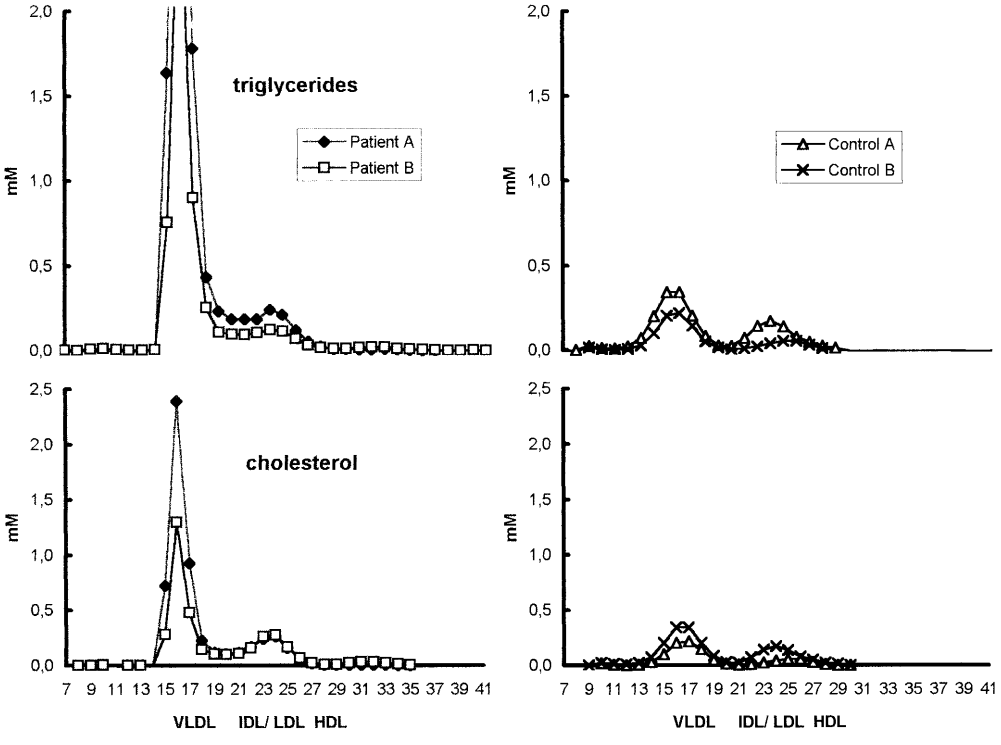

Fig. 1. Triglyceride and cholesterol concentrations in lipoprotein fractions of two GSDIa patients and two healthy volunteers after fast performance liquid chromatography (FPLC) separation. Treatment in the patients consisted of fat- and lactose-restricted and slowly releasing carbohydrate containing diets

lipogenesis through activation of a novel carbohydrate response factor and SREBP-1c, respectively [23]. Both factors are then able to bind independently on promotor sites of genes encoding lipogenic enzymes. In GSD1, with generally low insulin and glucose levels, one would thus not expect increased lipogenesis through activation via these mechanisms. Possibly, the increase in glycolytic flux in itself, leading to a higher production of acetyl-CoA precursor, is able to increase lipid synthesis. An alternative option is that one or more of the glycolytic intermediates possesses metabolic regulatory functions. G6P levels are increased in GSD1 patients, as has been shown using phosphorus magnetic resonance spectroscopy [34]. During the last few years, several studies have indicated a strong metabolic regulatory function for G6P and it has been postulated that G6P is able to influence lipogenesis [10, 33]. Doiron et al. [5] have suggested a similar role for xylulose-5-phosphate, an intermediate in the pentose-phosphate pathway. Both G6P and xylulose-5-phosphate may be involved in the upregulation of de novo lipogenesis in GSD1, but the molecular mechanism of this putative regulatory system remains to be established.

Insulin is a well-known inhibitor of VLDL secretion [28, 29], especially of triglyceride-rich VLDL1 particles. Lipogenesis and cholesterogenesis have been also implicated in regulation of VLDL secretion [35, 40, 41] and in GSD1 patients, with generally low insulin concentrations, one might therefore expect increased hepatic VLDL secretion of mainly triglyceride-rich particles. In a recent study in rats treated with an acute inhibitor of G6P-translocase activity, however, we found no increase in VLDL-triglyceride (VLDL-TG) secretion rates [42]. There is also evidence to suggest that, also with respect to VLDL secretion, G6P might have a regulatory role. Making use of mannoheptulose as an inhibitor of the glucokinase reaction, Brown et al. [3] recently showed that glucose needs to be phosphorylated before it is able to stimulate VLDL-TG secretion in cultured rat hepatocytes. It might be that compartmentalisation of G6P plays a role in its regulatory capacity. In GSD1b patients and in our rat model of GSD1b, G6P concentrations are expected to be decreased in the lumen of the endoplasmic reticulum and increased in the cytoplasm, whereas G6P concentrations are probably increased in both compartments in GSD1a. If G6P in the endoplasmic reticulum lumen does indeed play a role in formation and/or secretion of VLDL particles, this might lead to differences in VLDL secretion in GSD1a and 1b patients. No studies have been published so far to asses whether or not increased VLDL-TG secretion is present in GSD1 patients nor is it known whether differences in VLDL metabolism exist between GSD1a and 1b patients. Studies to reveal these issues are in progress in our laboratory.

Lipolysis of circulating lipoproteins has been found to be impaired in GSD1 [9, 24]. Forget et al. [9] reported a two-fold decrease of lipoprotein lipase (LPL) activity in children with GSD1 leading to a decreased triglyceride clearance from the blood compartment when compared to control children. Havel et al. [15] also reported a decrease in lipolytic activity which was confirmed by Levy et al. [24], describing a four-fold decrease in LPL activity as well as a ten-fold decrease in hepatic lipase activity in patients with GSD1. Levy et al. [26] also showed a decreased uptake of LDL particles in vitro by fibroblasts from GSD1 patients. Decreased LDL uptake might thus contribute to the hypercholesterolaemia observed in these patients. However, it must be realised that measurements mentioned in the studies above were performed during relative fasting with low insulin and glucose concentrations. It is well known that insulin stimulates LPL activity. Increases in plasma free fatty acid levels, which are present in GSD1 patients, indicate increased lipolysis in adipose tissue, which is a normal response during fasting and is probably more pronounced in GSD1. However, in order for lipids to be released from adipose tissue, it must first be taken up from the blood compartment. This means that although plasma lipolytic activity is probably decreased over a longer period of time due to a prolonged 'fasting' state in GSD1 patients, sufficient lipolysis and uptake by the adipose tissue must be present during the absorptive period.

Steatosis and glycogen storage disease type 1

GSD1 is associated with massive storage of neutral lipids in the liver [30]. Steatosis is an often-described phenomenon in many diseases, including diabetes, but the underlying mechanisms are often not clear and may be different in various disease states. Generally speaking, steatosis is the result of either increased hepatic uptake, increased synthesis, decreased secretion, impaired oxidation of fat or a combination thereof.

It is assumed that because of the elevated plasma free fatty acid levels, more fatty acids are taken up by the liver and converted to triglycerides and cholesterylester in GSD1 patients. Havel et al. [15] found increased splanchnic free fatty acid uptake in three GSD1 patients to be a major cause of steatosis in GSD1 [15]. We found that the absolute amount of de novo lipogenesis is massively increased in GSD1 patients but its contribution to total fat content remains limited in these patients and can only account for a minor part of steatosis [1]. Impaired secretion of VLDL-TG has not been reported in GSD1 patients and, as previously described, we recently found similar amounts of VDLD-TG secretion in an acute rat model of GSD1b [42]. The increased de novo lipogenesis is expected to inhibit fatty acid oxidation by generating malonyl-CoA, an inhibitor of carnitine palmitoyltransferase 1 [31]. Indeed, decreased ketone body concentrations have been reported [2], indicating decreased fatty acid oxidation although one study did not confirm this finding [15]. Furthermore, in an acute GSD1b model in rats, the plasma concentrations of β-hydroxybutyrate were identical to those in

control rats [42], indicating no impairment in hepatic fatty acid oxidation in this situation. The fact that lower ketone body concentrations are usually found in GSD1 patients does not imply decreased ketogenesis by definition. In fact, it may reflect an increased ketone body flux through more rapid uptake by the brain. Furthermore, although hepatic fatty acid oxidation might be inhibited, fatty acid oxidation is probably very active in muscle as indicated by respiratory quotient values of 0.79–0.85 measured using indirect calorimetry in three GSD1 patients (unpublished observations). Indeed, data available so far indicate that elevated free fatty acid flux is probably the major contributor to the development of hepatic steatosis in GSD1.

Conclusions

Taking into consideration the evidence available today, we propose the following disturbances in lipid metabolism in GSD1 (Fig. 2): due to deficiency of the G6Pase complex, increased glycolysis and production of acetyl-CoA occurs. This leads directly or indirectly, possibly through signalling actions of G6P, to stimulation of de novo lipogenesis and cholesterogenesis and, via production of malonyl-CoA, to inhibition of hepatic fatty acid oxidation. Increased lipid synthesis, however, cannot solely explain the severity of the hyperlipidaemia observed in GSD1 and decreased lipolysis must be a major contributor. However, sufficient lipolysis must occur probably during a shortened "absorptive phase", in order to explain the increased free fatty acid concentrations present. This implies that probably a more active adipocyte compartment is present in GSD1 or, alternatively, that fatty acids are released directly after lipolysis by LPL and transported to the liver as proposed by Frayn et al. [11]. Free fatty acids are partially oxidised but for a major part used for synthesis of triglycerides in the liver. Steatosis occurs due to a discrepancy between free fatty acid uptake and synthesis on the one hand, and fatty acid oxidation and release as VLDL-triglycerides on the other. Increased VLDL secretion of especially large triglyceride-rich particles, however, might occur due to the low insulin levels, increased flux and uptake of free fatty acids and increased lipogenesis and cholesterogenesis contributing to the hyperlipidaemia. However, data obtained in an acute GSD1b rat model does not support this latter sequence of events in this particular model.

Combined results suggest that increased production by the liver and especially decreased clearance of cholesterol and triglyceride containing lipoproteins contribute to the hyperlipidaemia observed in GSD1. A detailed study examining both lipid production and clearance simultaneously in vivo using specially designed stable isotope methodology is warranted to determine the extent to which both processes are responsible. The role of G6P and intracellular G6P compartmentalisation in the regulation of lipogenesis and VLDL metabolism needs to be clarified by studying the differences between GSD1a and 1b patients and using specific pharmacological G6Pase and G6P translocase inhibitors.

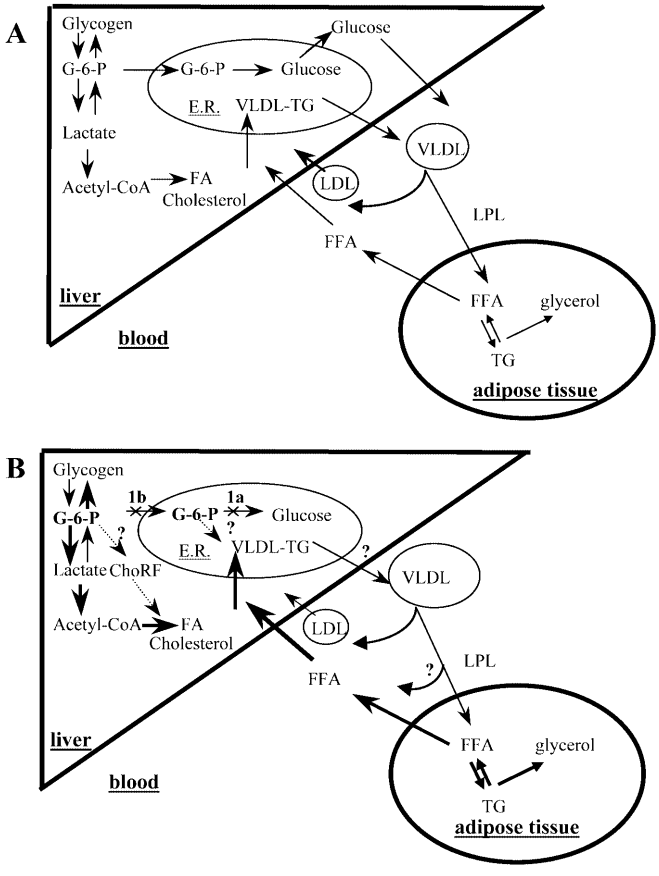

Fig. 2. Overall metabolic effects in GSD1 patients. **Panel A** represents the metabolic situations in normal healthy individuals. **Panel B** displays the situation in patients with GSD1

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