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Glycogen storage disease type I

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Hyperlipidaemia and atherosclerosis in Glycogen Storage Disease type I

Chapter 5

5.1 Is Glycogen Storage Disease type Ia associated with atherosclerosis?

5.2 Are dyslipidaemia and microalbuminuria in adolescents with Glycogen Storage Disease type Ia associated with cardiovascular disease?

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5.1 Is Glycogen Storage Disease type Ia associated with atherosclerosis?

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Eur J Pediatr 2002;160:s62-s64

Summary

Deficiency of microsomal glucose-6-phosphatase in liver and kidney leads to glycogen storage disease type Ia (GSD Ia). Notwithstanding intensive dietary therapy, moderate to severe dyslipidaemia and microalbuminuria, both known atherosclerotic risk factors, remain present. Although more patients reach adult age, no information is still available about accelerated atherosclerosis. The aim of our study was to investigate whether GSD Ia was associated with premature atherosclerosis. In nine adolescent patients (mean age 22.7 ± 3.4 years) and nine matched healthy control subjects, lipid profile, blood pressure, ankle-brachial indices, aortic distensibility and intima-media thickness (IMT) of the carotid and femoral arteries were determined. As expected, lipid profiles were significantly unfavourable in the patient group compared with the control group. No differences were found in blood pressure, ankle brachial indices and aortic distensibility between both groups. IMT segments were comparable in both groups, with even thinner segments in the patient group. In different multivariate models, GSD Ia remained an independent predictor for a thinner IMT (R^2= 0.90; β= -0.69; P= 0.018).

In conclusion, glycogen storage disease type Ia is not associated with premature atherosclerosis, despite the existence of longstanding dyslipidaemia and microalbuminuria.

Introduction

Deficiency of glucose-6-phosphatase in liver and kidney leads to glycogen storage disease type Ia (GSD Ia) with dyslipidaemia and microalbuminuria as some of the metabolic changes[9,15]. Despite the introduction of intensive dietary therapy, these well known independent risk factors for atherosclerosis remain present[14,20]. More and more patients with GSD I a are reaching adult age, but data about the appearance of premature atherosclerosis in young adults remain scarce and conflicting (Table 5.1.1). Therefore, the aim of our study was to investigate whether GSD Ia was associated with premature atherosclerosis.

Table 5.1.1 Literature concerning (sub)clinical atherosclerosis in GSD Ia patients

reference	number of patients (n)	age (years)	results
[41]	1	10	small atheromatous plaques
[14]	4	10-16	normal exercise ECG in all patients
[55]	37	18-43	coronary artery disease in 2 patients
[32]	6	23-33	normal endothelial function in all patients
[48]	43	> 20	generalised atherosclerosis in 1 patient, with concomitant end-stage renal disease

Patients and methods

Nine adolescent patients (six male and three female) were recruited from our Out-patient Clinic for Metabolic Diseases. All nine patients shared the clinical and biochemical characteristics associated with GSD Ia, and were treated with intensive dietary therapy[13]. Patients were compared with nine healthy control subjects, matched for age, sex and body mass index (BMI, kg/m^2). No cardiovascular medication was taken by any of the control subjects.

Lipid profiles were determined using standard laboratory methods. Urinary albumin was measured by radioimmunoassay (Diagnostic Products Corporation, Apeldoorn, The Netherlands) and microalbuminuria was defined as an urinary albumin/creatinine ratio above 2.5 and 3.5 mg albumin/mmol creatinine in men and women, respectively.

Blood pressure (mm Hg) was measured using a calibrated automatic oscillometric manometer. The ankle-brachial index (%) was determined at rest and after standardised exercise[26]. Using pulse-wave velocity measurements[30], the aortic distensibility was calculated (in MPa^{-1}) from the time

delay between the start of the flow velocity waveforms recorded by two Doppler probes (5 MHz), placed on the right subclavian and the common femoral arteries, and the aortic length, defined as the distance between the top of the manubrium sterni and the right common femoral artery.

The intima-media thickness (IMT) of different segments of the carotid arteries and the right femoral artery was measured using high resolution B-mode ultrasound (Acuson XP128 duplex scanner). The IMT was defined as the distance between the intima and media double line pattern, expressed in mm. The mean-max IMT was determined as the arithmetic mean of the maximum values of all measured far wall segments in one patient.

Statistical analysis was performed with SPSS version 9.0. Differences between the two groups were determined with Mann-Whitney U-test and were considered statistically significant at P values < 0.05. Multiple regression analyses were performed to test for possible confounders.

Results

Mean age and BMI were 22.7 ± 3.4 years and 23.0 ± 2.7 kg/m^2 in the patient and 24.3 ± 2.4 years and 23.1 ± 2.2 kg/m^2 in the control group, respectively. Seven patients were treated with an angiotensin-converting enzyme (ACE) inhibitor because of (micro)albuminuria and one patient was using a fibrate preparation because of the risk of pancreatitis due to severe hypertriglyceridaemia.

Table 5.1.2 Biochemical and vascular characteristics of GSD Ia patients and control subjects

	GSD Ia patients	control subjects	p
lipid profile			
cholesterol (mmol/l)	8.33 ± 5.34	4.89 ± 0.61	< 0.05
triglycerides (mmol/l)	11.6 ± 11.1	1.10 ± 0.33	< 0.001
HDL-cholesterol (mmol/l)	0.62 ± 0.21	1.13 ± 0.35	< 0.01
cholesterol/HDL-cholesterol ratio	16.5 ± 12.0	4.55 ± 0.84	< 0.01
non-invasive vascular parameters			
blood pressure (mm Hg)	112/64 ± 10/6	118/68 ± 13/7	n.s
mean heart rate (bpm)	80 ± 7	65 ± 9	< 0.01
ankle-brachial index (%)	124 ± 15	118 ± 10	n.s
aortic distensibility (MPa^{-1})	24.3 ± 6.1	21.1 ± 5.4	n.s
IMT mean max (mm)	0.64 ± 0.03	0.71 ± 0.03	< 0.01

Data are given as mean ± SD
IMT mean max mean intima-media thickness of the maximum values of all measured segments; *n.s* not significant

In Table 5.1.2, the lipid profiles and results of the vascular measurements are given for both groups. The lipid profile in the patients was abnormal compared to control subjects. None of the control subjects had (micro)albuminuria. No differences were found in blood pressure, ankle-brachial indices and aortic distensibility between both groups. Mean heart rate was significantly higher in the patient group compared with the control group. In the patient group, no IMT segment appeared to be thicker compared with the control group, but some segments were significantly thinner in the patients compared with the control subjects. This was confirmed by the results of the multivariate models with the mean max IMT as dependent variable. Even after controlling for known cardiovascular risk factors (age, sex, BMI, dyslipidaemia and microalbuminuria), GSD Ia remained an independent predictor for a thinner mean max IMT (model R^2= 0.90; β= -0.69; P= 0.018).

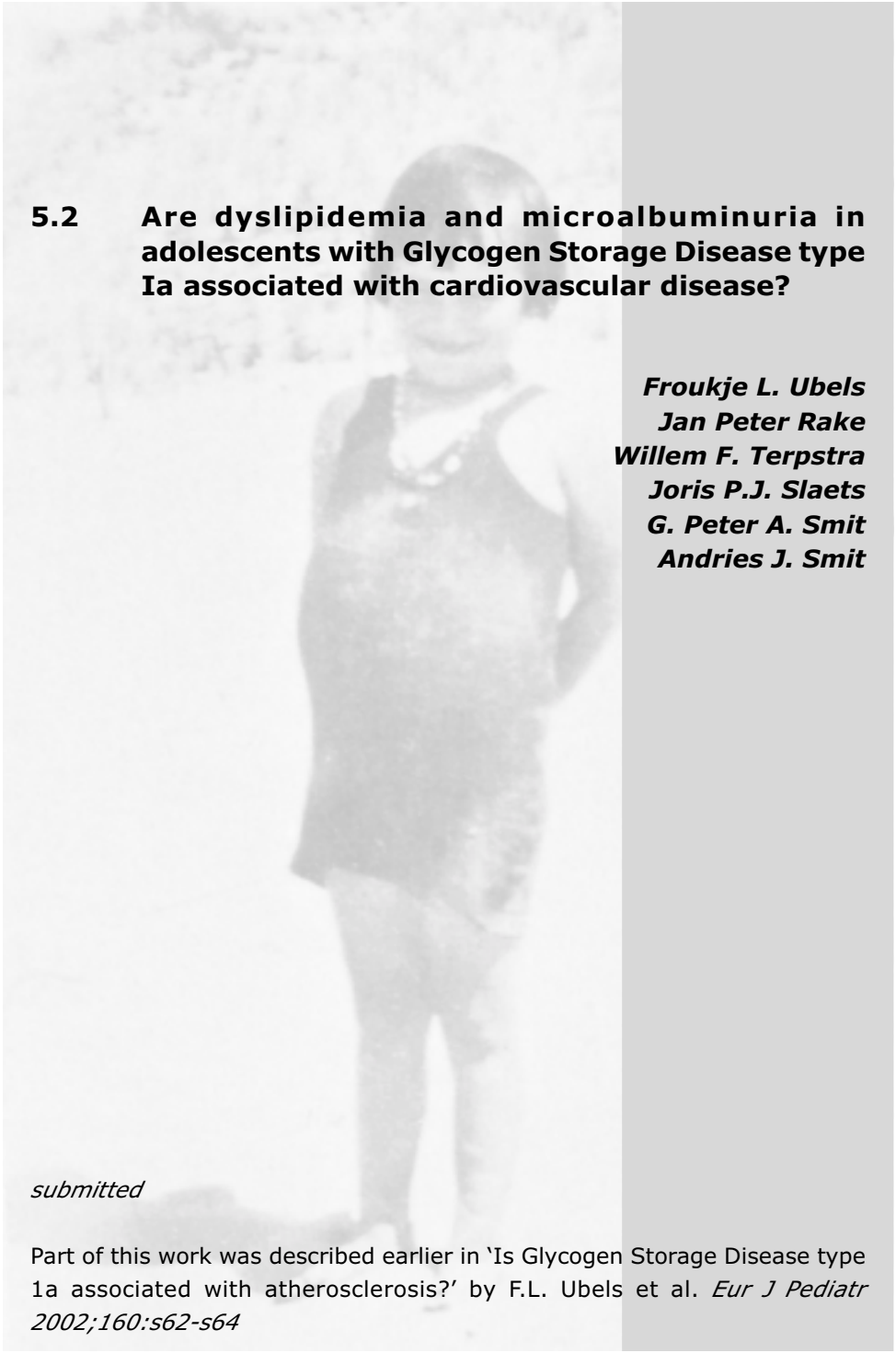
Discussion

In this study, no premature atherosclerosis was found in patients with GSD Ia, notwithstanding the existence of the atherosclerotic risk factors moderate to severe dyslipidaemia and (micro)albuminuria.

Formerly, from the few available reports in the literature, no convincing conclusions could be drawn about the presence of atherosclerosis. Besides the absence of atherosclerosis, GSD Ia appeared even to be an independent predictor for a thinner IMT, as indicated by the results of our multiple regression analyses. Little is known about possible vascular protective mechanisms against the dyslipidaemia in GSD Ia. The diminished platelet aggregation[40] can only be partly protective[50]. Recently, a decreased susceptibility of in vitro oxidation of (very) low-density lipoprotein cholesterol has been found[4]. Although most patients with GSD Ia were using an ACE inhibitor, the effects of these drugs on the IMT proved to be questionable[38,39].

Different non-invasive vascular measurement techniques were used in our study and these techniques have been able to detect subclinical premature atherosclerosis in adolescent patients with dyslipidaemia[34,56].

Our results provide no support for lipid-lowering therapy in patients with GSD Ia for the prevention of atherosclerosis. Although results are lacking, lipid-lowering drug treatment can be considered in preventing renal deterioration in case of dyslipidaemia[44,46].



5.2 Are dyslipidemia and microalbuminuria in adolescents with Glycogen Storage Disease type Ia associated with cardiovascular disease?

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Part of this work was described earlier in 'Is Glycogen Storage Disease type 1a associated with atherosclerosis?' by F.L. Ubels et al. *Eur J Pediatr 2002;160:s62-s64*

Summary

Microsomal glucose-6-phosphatase is important in regulation of blood glucose concentration. Deficiency of this enzyme leads to glycogen storage disease type Ia (GSD Ia). Dietary therapy has prolonged life expectancy, but dyslipidaemia and (micro)albuminuria remain present. The aim of our study was to investigate whether GSD Ia was associated with premature cardiovascular changes.

Lipid profiles were significantly unfavourable in 9 adolescent patients with GSD Ia compared with 9 matched healthy control subjects. Seven patients with GSD Ia were treated with an angiotensin-converting enzyme inhibitor because of (micro)albuminuria. Blood pressure was comparable in both groups. Intima-media thickness segments were comparable in both groups, but the relative myocardial wall thickness was significantly higher, and the early to atrial filling ratio lower in the patients with GSD Ia compared with the control subjects ($p= 0.002$ and $p= 0.03$, respectively). After controlling for known cardiovascular risk factors in different multivariate models, GSD Ia remained an independent predictor for a thinner intima-media thickness ($R^2= 0.90$; $ß= -0.69$; $p= 0.018$) and increase in relative myocardial thickness ($R^2= 0.83$; $ß= 1.09$; $p= 0.007$).

Despite the existence of longstanding dyslipidemia and microalbuminuria, GSD Ia is not associated with premature atherosclerosis, but with cardiac remodeling like an increased relative myocardial wall thickness and (incipient) diastolic dysfunction.

Introduction

Microsomal glucose-6-phosphatase (E.C.3.1.3.9) is the key enzyme in homeostatic regulation of blood glucose concentration by catalysing the terminal step in both glycogenolysis and gluconeogenesis. Deficiency of glucose-6-phosphatase in liver and kidney leads to glycogen storage disease type Ia (GSD Ia). This is an autosomal recessive inborn error of metabolism with an estimated incidence of 1 in 100.000 births[9]. Metabolic changes consist of severe fasting hypoglycaemia, hyperlactacidaemia, hyperuricaemia and combined hyperlipidaemia. These lead to complications such as growth delay, hepatomegaly and hepatic adenoma, (micro)albuminuria and progressive renal failure, gout, pancreatitis, anaemia, osteoporosis and ovarian cysts[9,15,48]. Life expectancy has been considerably prolonged after introduction of intensive dietary therapy. More and more patients are reaching adult age. However, insufficient data is available about the occurrence of premature clinical and subclinical atherosclerosis and cardiac changes in young adults, in the presence of not only the moderate to severe dyslipidaemia but also (micro)albuminuria. A comparable degree of hyperlipidaemia in familial hypercholesterolemia or familial combined hyperlipidemia, is a strong risk factor for cardiovascular morbidity and mortality at an early age[19,25]. Heterozygous familial hypercholesterolaemia is associated with increased intima-media thickness (IMT), transiently increased aortic distensibility and impaired flow-mediated dilation at adolescent age, suggesting subclinical atherosclerosis and endothelial dysfunction, followed by clinical manifestations before or at middle age[7,31,34,42,56]. Microalbuminuria has been found to be an independent risk factor for the development of cardiovascular disease, especially in insulin dependent diabetes mellitus (IDDM)[5,43].

One study has suggested conserved endothelial function in GSD Ia[32], but, with the exception of some case reports, further cardiovascular data are lacking. Therefore, the aim of our study was to investigate whether GSD Ia was associated with premature atherosclerosis and cardiac changes.

Patients and methods

Adolescent patients were recruited from our Out-patient clinic for Metabolic Diseases. All 9 patients shared the clinical and biochemical characteristics that are associated with GSD Ia. All patients were treated with intensive dietary therapy consisting of lactose- and saturated fat-restricted frequent meals with uncooked cornstarch during daytime and continuous gastric drip-feeding or uncooked cornstarch overnight. To classify the patients as responders and non-responders to dietary treatment, the standard deviation score for height was calculated by comparing with sex,

age and geographical/ethnical matched control values. Non-responders were defined as having a standard deviation score value below –2.0[14]. Two patients (numbers 1 and 2) were non-identical twin-brothers. Patients were compared with 9 healthy control subjects, matched for age, sex and body mass index (BMI, kg/m^2). All were in good health. No cardiovascular medication was taken by any of the controls. All participants performed normal physical activity and were non-smokers.

Creatinine, glucose and the lipid profile were measured using standard laboratory methods. Low-density lipoprotein-cholesterol could not be calculated or determined by the generally used kit in our laboratory because of the elevated triglyceride values. Bleeding time was measured according to Ivy, with normal values below 4 minutes. Urinary albumin was measured by RIA (Diagnostic Products Corporation, Apeldoorn, The Netherlands). Microalbuminuria was defined as an urinary albumin/creatinine ratio above 2.5 in men and 3.5 mg/mmol creatinine in women. Glomerular filtration rate was determined as described earlier[49] and hyperfiltration defined as a value above 145 ml/min/1.73m^2.

Blood pressure was measured using a calibrated automatic oscillometric manometer (in mmHg). The ankle-brachial index was determined at rest and after standardised exercise (in %), using the same reference values as in adult patients[26].

Aortic distensibility was determined with pulse-wave velocity measurements as extensively described elsewhere[30]. Two Doppler probes (of 5 MHz) were placed on the proximal part of the right subclavian artery and the right common femoral artery. Simultaneous registration of the Doppler pulses was made three times during 10 seconds. Aortic distensibility (in MPa^{-1}) was calculated from the time delay between the points of intersection of the systolic upstrokes of the maximum flow velocity waveforms recorded by each Doppler transducer and the distance between the top of the manubrium sterni and the right common femoral artery as measure of the aortic length.

The intima-media thickness (IMT) was measured using high resolution B-mode ultrasound (Acuson XP128 duplex scanner). In supine position, the far wall of three different predefined segments of both the carotid arteries (common and internal carotid artery and the carotid bifurcation) and the right femoral common and superficial artery were scanned and recorded on video. In the common carotid artery the last 1 cm before the carotid bifurcation, and in the internal carotid artery the first 1 cm after the flow divider were used for analysis. In the femoral artery the last 1 cm of the common femoral artery before the flow divider, and the first 1 cm of the superficial femoral artery after the flow divider were used for analysis. Off-

line measurement of the IMT was made using video image analysis, according to the method as described earlier[53], by a trained and certified technician unaware of patient characteristics. The IMT was defined as the distance between the intima and media double line pattern, expressed in mm. The mean-max IMT was determined as the arithmetic mean of the maximum values of all eight measured far wall segments in one patient.

Left ventricular mass (LVM) and diastolic function were determined three times using 2-D mode echocardiography (Acuson XP128) in the left lateral recumbent position. Left ventricular dimensions were recorded end-expiratory. The LVM[12] was indexed (LVMI) by height raised to a power of 2.7 ($g/m^{2.7}$)[11]. The relative wall thickness (RWT) of the left ventricle was determined by two times the left ventricular posterior wall thickness divided by the left ventricular end diastolic diameter (mm)[29]. Diastolic function parameters were determined with pulsed Doppler echocardiography in the standard apical view. Peak velocities of early (E) and atrial (A) filling (in m/sec) were measured and the E/A-ratio calculated by dividing the early by the atrial filling velocity.

Statistical analysis was performed with SPSS version 9.0. Results are given in mean ± standard deviation (SD) as appropriate. Differences between the two groups were determined with Mann-Whitney U-test because of small sample size. Multiple regression analyses were performed to test for possible confounding by known risk factors for cardiovascular disease. Differences were considered statistically significant at p-values <0.05.

Table 5.2.1 Clinical details of the patients with GSD Ia

patient number	1	2	3	4	5	6	7	8	9
sex (male/female)	M	M	F	M	M	F	M	M	F
age (years)	24	24	26	19	17	27	20	25	22
BMI (kg/m^2)	20.7	22.0	25.4	25.2	17.8	23.5	26.9	22.2	23.2
SDS height	-2.5	-0.6	0.0	-3.4	-2.5	-3.3	0.0	2.0	-0.4
GFR ($ml/min/1.73m^2$)	172	161	147	209	132	121	196	176	127
albuminuria	+	-	+	+	+	-	+	+	+
liver adenoma	-	+	+	+	-	+	-	-	-
osteopenia	+	+	-	+	+	+	+	+	+
anaemia	-	+	-	+	+	+	-	+	+
prolonged bleeding time	-	+	?	+	-	+	-	-	+

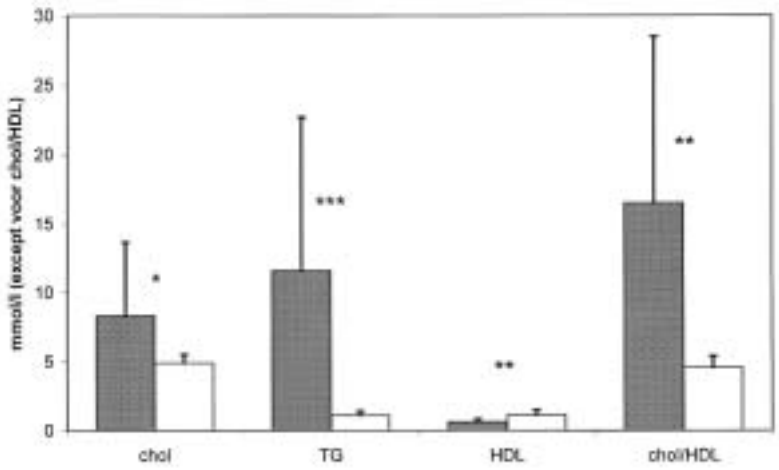
BMI body mass index; *SDS* standard deviation score; *GFR* glomerular filtration rate; *+* yes or present; - no or absent; *?* not known

Results

Each group consisted of 6 male and 3 female participants. Mean age was 22.7 ± 3.4 in the patient and 24.3 ± 2.4 years in the control group. Mean BMI in the patient and control group was 23.0 ± 2.7 and 23.1 ± 2.2 kg/m^2, respectively. Individual clinical characteristics of the patients are enumerated in Table 5.2.1. Overnight, seven patients were treated with nasogastric drip-feeding and the other two (patients 2 and 3) with uncooked cornstarch. Four patients were classified as non-responders to intensive dietary therapy. All patients were treated with allopurinol, a xanthine-oxidase inhibitor, because of hyperuricemia, 7 with an angiotensin-converting enzyme (ACE) inhibitor because of (micro)albuminuria, 6 with calcium, 6 with multivitamins and 3 with sodium bicarbonate. One patient was using a fibrate because the risk of pancreatitis due to severe hypertriglyceridemia (patient 1). In the control group, 2 of the 3 women were taking oral contraceptives.

For both groups, lipid profiles are represented in Figure 5.2.1. As expected, the lipid profile in the patient group was unfavourable in comparison to control group. Serum creatinine was 64.1 ± 8.6 and 91.0 ± 12.2 (reference value 62-106 μmol/l) in the patient and control group, respectively (p=0.001). Uric acid was not significantly different in both groups. None of the control subjects had (micro)albuminuria.

Figure 5.2.1 Lipid profile of the patient (black bar) and control group (white bar). *chol* total cholesterol; *TG* triglycerides; *HDL* high-density lipoprotein cholesterol; c*hol/HDL* cholesterol/HDL-cholesterol ratio
significance levels denote differences between both groups:
* p<0.05; ** p<0.01; *** p<0.001



No differences were found in blood pressure and ankle-brachial indices between both groups. Blood pressure was 112/64 ± 10/6 in the patient and 118/68 ± 13/7 mmHg in the control group, ankle-brachial indices in rest 124 ± 15% and 118 ± 10% and after exercise 119 ± 15% and 119 ± 14%, in the patient and control group, respectively. Mean heart rate was 80 ± 7 in the patient and 65 ± 9 beats/minute in the control group (p= 0.003). In the patients, aortic distensibility was comparable with the controls (24.3 ± 6.1 and 21.1 ± 5.4 MPa^{-1}).

In Table 5.2.2 the results of the cardiovascular measurements are summarised. In the patient group, no IMT segment appeared to be thicker compared with the control group. The IMT of the mean max, the common carotid artery and the carotid bifurcation was even significantly thinner in the patients compared with the control subjects, with all values in the normal ranges. In the GSD Ia group, no differences in IMT were found between responders and non-responders to dietary treatment.

Table 5.2.2 Intima-media thickness and echocardiographic findings of GSD Ia patients and control subjects

	GSD Ia n = 9	Controls n = 9	p
intima-media thickness			
mean max (mm)	0.64 ± 0.03	0.71 ± 0.03	0.002
echocardiography			
LV-mass index ($g/m^{2.7}$)	31.5 ± 4.17	30.8 ± 4.67	n.s
relative wall thickness	0.40 ± 0.04	0.35 ± 0.03	0.002
early/atrial ratio	1.50 ± 0.28	1.84 ± 0.33	0.03

mean ± SD;
n.s not significant; *mean max* mean of the maximum values of all measured segments; *LV* left ventricular

The LVMI was comparable in both groups, with a higher RWT in the patients. The E/A ratio was significantly lower in the patients compared with the control subjects, due to a higher atrial contributing to left ventricular filling. Although these differences were observed, all echocardiographic parameters were within normal ranges. In the GSD Ia group, no differences in RWT and E/A-ratio were found between responders and non-responders to dietary treatment.

The results of the multivariate models with the mean max IMT, RWT and E/A-ratio as dependent variables and adjusting for cardiovascular risk factors are given in Table 5.2.3. After controlling for known risk factors for cardiovascular disease: age, sex, BMI, dyslipidaemia and microalbuminuria, GSD Ia remained an independent predictor for a thinner mean max IMT (model R^2= 0.90; ß= -0.69; p= 0.018, Table 5.2.3). This is in agreement with the above-mentioned results of the non-parametric tests in which the IMT of the GSD Ia patients appeared unaltered or even thinner compared to the control subjects. After controlling for the cardiovascular confounders, GSD Ia remained an independent predictor for an increase in RWT, also (R^2= 0.83; ß= 1.09; p= 0.007, Table 5.2.3). However, in the models with the E/A-ratio as dependent variable, after additional adjusting for dyslipidemia and microalbuminuria, GSD Ia lost statistical significance (complete adjusting model R^2= 0.44; ß= -0.56; p= 0.33, Table 5.2.3).

Table 5.2.3 Regression analysis with adjusting for possible confounding factors

dependent variable	IMT mean max		RWT		E/A ratio	
	ß	p	ß	p	ß	p
GSD Ia	-0.73	0.001	0.65	0.004	-0.51	0.032
adjusting for age / sex / BMI	-0.72	0.001	0.77	0.001	-0.58	0.026
adjusting for chol/HDL	-0.43	0.036	0.62	0.037	-0.66	0.10
adjusting for ma	-1.08	0.001	1.18	0.001	-0.55	0.19
adjusting for chol/HDL and ma	-0.69	0.018	1.09	0.007	-0.56	0.33

IMT mean max mean intima-media thickness of the maximum values of all measured segments; *RWT* relative wall thickness; *E/A* early/atrial ratio; *ß* standardized coefficient; *p*, significance level; *BMI* body mass index; *chol/HDL* cholesterol/high-density lipoprotein cholesterol ratio; ma microalbuminuria; controls coded 0 and GSD Ia coded 1; men coded 0 and women coded 1; normoalbuminuria coded 0 and micro- or macroalbuminuria coded 1

Discussion

Our study in adolescents with GSD Ia shows no early signs of atherosclerosis, but the presence of incipient myocardial changes in a condition associated with longstanding moderate to severe dyslipidaemia and (micro)albuminuria. This lack of signs of vascular damage is surprising, because the life long dyslipidaemia and the microalbuminuria would have suggested early atherosclerosis[5,43,50].

The dyslipidaemia in GSD Ia is thought to be due to a combination of increased synthesis of fatty acids and cholesterol from the excess of pyruvate and lactate, decreased lipoprotein lipase activity and the effects of hypoglycemia counter-regulation with low insulin levels and high glucagon and cortisol levels[3,6,15,17,36]. But despite all dietary efforts, in most patients with GSD Ia, total and low-density lipoprotein cholesterol and triglyceride

values remain elevated and high-density lipoprotein cholesterol decreased[14].

In our multivariate models with cardiovascular risk factors, GSD Ia appears to be an independent predictor for a thinner IMT. In literature, in a case report (45 years ago) of a girl with GSD Ia who died at the age of 10 years of progressive right-sided heart failure, small amounts of atheromatous plaque were found[41]. After the introduction of dietary treatment[13], data about the appearance of atherosclerosis or its clinical sequela in patients with GSD Ia remain scarce. In 4 children, no abnormalities were found at exercise electrocardiography[14]. Among 37 adult GSD Ia patients in the United States of America (18-43 years), two 35-year old men were known with coronary heart disease[55]. Among 43 adult GSD Ia patients in Europe of 20 years and over, atherosclerotic lesions were found at autopsy in just one 46-year-old woman who died after a second renal transplantation[48]. In this case, it is well possible that the accelerated atherosclerosis is mainly due to the progressive renal failure and not to GSD Ia or dyslipidaemia. In a previous study in 6 adult patients with GSD Ia, endothelial function seemed to be normal[32]. This is in agreement with and complementary to our results. Furthermore, the non-invasive vascular measurement techniques used in our study, are suitable for detecting premature atherosclerosis in adolescents, as shown by others[31,33,34,56,60].

In the apparent absence of atherosclerosis, little is known about a vascular protective mechanisms against the moderate to severe dyslipidemia in GSD Ia. The diminished platelet aggregation, expressed as a prolonged bleeding time and dependent of the metabolic control[22,40] can only be partly protective[50]. Recently, a decreased susceptibility of in vitro oxidation of (very)low-density lipoprotein cholesterol was found[4]. The small, non-significant difference in blood pressure, perhaps due to and complemented by the use of ACE-inhibitors in most patients, might be proposed as explanation for the observed lack of difference in IMT. However, the evidence for a specific effect of ACE-inhibitors on IMT is at best equivocal[38,39].

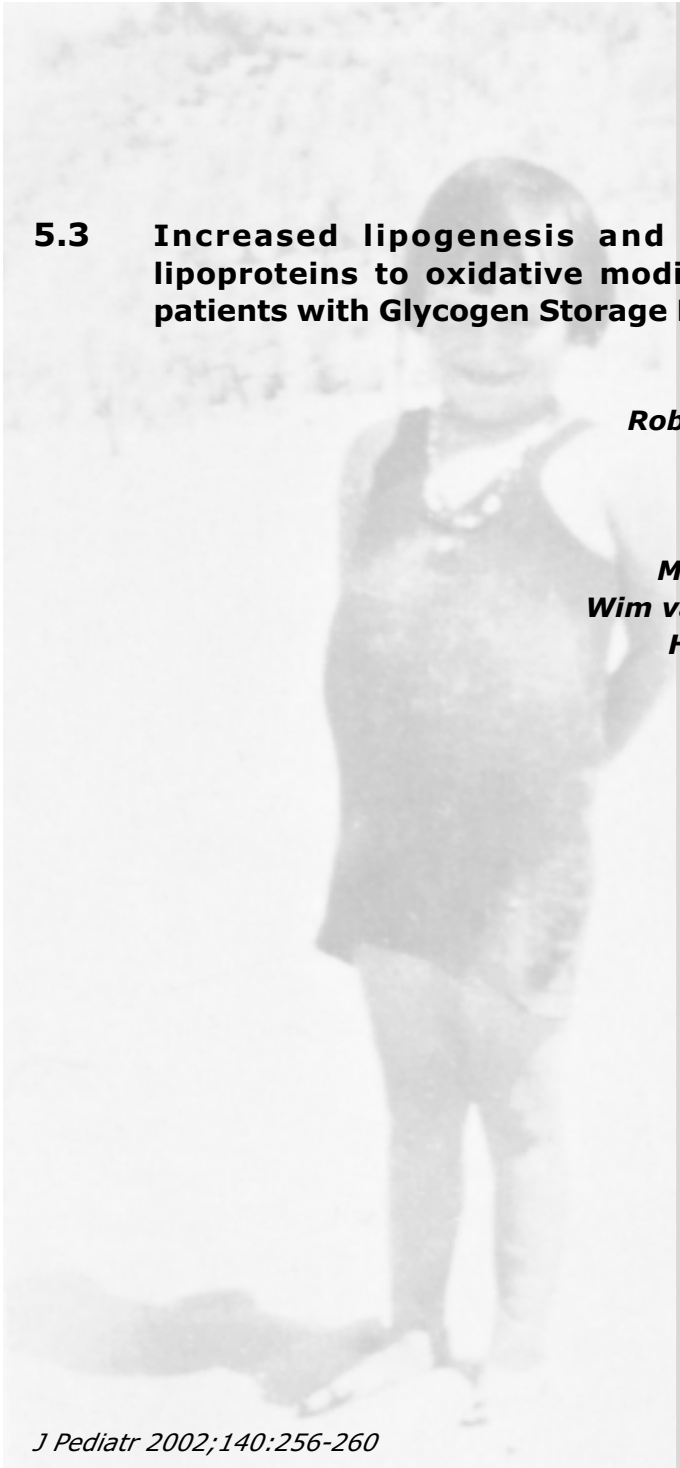
Besides a difference in lipid profile between the two groups, the serum creatinine value in the patient group was lower compared to the controls. This can be the result of a smaller muscle mass in the GSD Ia patients and the higher glomerular filtration rate (see also below). The comparable uric acid values in both groups can be explained by the use of allopurinol.

In our patients, LVMI was not significantly different compared with the control subjects, but the RWT of the left ventricle was thicker and the E/A ratio lower suggesting concentric remodeling of the left ventricle in patients with GSD Ia[29]. Our echocardiographic findings are in agreement with a study in children with several metabolic storage diseases by Senocak et al[54] who

found in 4 of the 12 included GSD Ia patients increased myocardial wall segments. The mean E/A ratio in their patient group, including 8 children with GSD I, was significantly lower compared to the mean of the controls, in accordance to our results. In the formerly mentioned 4 children, no abnormalities were found at echocardiography[14]. In the autopsy of the 10 year old girl, the right ventricle was found to be hypertrophied and moderately dilated. At microscopy, only a few glycogen granules were seen without evidence of glycogen storage in the myocardium[41]. In one of the 37 adult patients from the United States of America, decreased left ventricular function was described detected by echocardiography[55].

We only can speculate about the pathophysiological mechanims behind these cardiac changes in the absence of atherosclerosis. Glycogen storage is not a plausible explanation, because in GSD I, glycogen does not accumulates in the myocardium[15,41]. The normal aortic distensibility virtually excludes functional vascular changes as a contributing factor. An elevated blood pressure, frequently seen in GSD Ia[48,55], would have been a good explanation[24]. However, blood pressure is similar to control subjects in our patients, many of whom are using long-term ACE-inhibition. In fact, it is remarkable that the use of ACE-inhibitors in a majority of the patients apparently did not correct the myocardial functional abnormalities. One wonders of course whether more outspoken abnormalities might have been present without ACE-inhibitors[51]. Comparable cardiac changes are often found in young adults with well controlled IDDM and microalbuminuria[27]. A hyperdynamic circulation might be a common provoking factor[10]. Hyperfiltration of glomeruli is another common factor in GSD Ia and IDDM, resulting in microalbuminuria[2,8,43,49]. Most of our patients had glomerular hyperfiltration and a variable degree of microalbuminuria. However, the glomerular filtration rate appeared not to be of statistical significance, possible due to various disturbing factors (ACE-inhibitors and drip-feeding). Microalbuminuria is an early manifestation of damage of the kidney and the cardiovascular system, but not necessarily related to atherosclerosis[43]. In our multivariate models, microalbuminuria only contributed to the variance in the RWT. It is reasonable to assume that the cardiac changes have to be related to metabolic factors, for example the hypoglycemia counterregulation, as the degree of renal damage is related to metabolic control[8,59].

In conclusion, GSD Ia is not associated with premature atherosclerosis, despite the existence of dyslipidaemia and microalbuminuria. However, an increased relative myocardial wall thickness and (incipient) diastolic dysfunction are found in GSD Ia, suggesting cardiac remodeling. About the pathogenetic mechanisms we only can speculate.



5.3 Increased lipogenesis and resistance of lipoproteins to oxidative modification in two patients with Glycogen Storage Disease type Ia.

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Summary

We describe two Glycogen Storage Disease Ia (GSD Ia) patients with severe hyperlipidaemia 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-hyperlipidaemic treatment in patients with GSD Ia.

Introduction

Glycogen storage disease type Ia (GSD Ia, von Gierke disease) is an inborn error of metabolism caused by deficiency of glucose-6-phosphatase (G6Pase), the enzyme catalysing the conversion of glucose-6-phosphate (G6P) to glucose. The disease is characterised by hypoglycaemia and hepatic glycogen and fat accumulation as well as severe hypertriglyceridaemia, hypercholesterolaemia and hyperuricaemia[1,15,17,20,35]. The mechanistic relation between the primary abnormalities in glucose metabolism and hyperlipidaemia is still speculative. Triglycerides and cholesterol are normally synthesized in the liver, incorporated into very-low density lipoprotein (VLDL) particles and secreted into plasma. After lipolysis of the triglycerides, the fatty acids are taken up by extrahepatic tissues (fat and muscle predominantly). Indications for decreased plasma lipid clearance as well as for increased lipid production have been reported in GSD Ia patients[6,17,36].

As the result of improved dietary management, patients with GSD Ia commonly reach adult age, so the potential contribution of hyperlipidaemia to the development of atherosclerosis becomes important. Conflicting reports have appeared on development of atherosclerosis in GSD Ia and the use of lipid-lowering treatment[32,55].

We describe two young adult brothers with GSD Ia with severe hyperlipidaemia without clinical signs of atherosclerosis. In these patients, we studied the incorpororation of ^{13}C labeled precursors into cholesterol and palmitate to determine the synthesis of cholesterol and fatty acids. Furthermore, we determined in vitro the susceptibility of low-density lipoproteins (LDL) to oxidative modification, one of the primary steps in atherogenesis.

Patients and methods

Two patients with GSD Ia 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, nonidentical twin brothers A and B. All participants were non-smokers, had no familial history of hyperlipidaemia or premature heart disease, and none was taking any medication or special diet. Informed written consent was obtained in accordance with the University Hospital Groningen Ethical Committee.

In patient A, the diagnosis was made in childhood by enzyme studies in fresh liver tissue and later on confirmed by mutation analysis. At the age of 19 years, he was referred to our hospital and physical examination showed a young man with mild mental retardation, with stunted height (-2.5 SDS), 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) and apolipoprotein B levels 2.1 g/l (normal range: 0.4-1.0 g/l). He had an apolipoprotein E phenotype E4/4. Dietary treatment was intensified and lactose-, sodium- and fat-restricted (fat 8 energy %; protein 13 energy %, carbohydrate 78 energy %), with a fatty acid composition of 27% saturated fatty acid (SFA), 15 % monounsaturated fatty acid (MUFA) and 58 % polyunsaturated fatty acid (PUFA). However, severe hyperlipidaemia remained.

In patient B, the diagnosis GSD Ia was also confirmed by mutation analysis. At the age of 23 years, he was referred to our hospital and physical examination showed a mentally normal young man with normal height (-0.6 SDS) and weight (68kg), and a mild hepatomegaly. Plasma cholesterol and triglyceride concentrations were 7.9 mmol/l (305 mg/dl) and 13.5 mmol/l (1196 mg/dl), respectively. Apolipoprotein A-I levels were 1.1 g/l and apolipoprotein B levels 1.1 g/l. Dietary treatment was adjusted by increasing the amount of slowly releasing carbohydrates and was fat-restricted (fat 17 energy %; protein 10 energy %, carbohydrate 73 energy %) with a fatty acid composition of 26 % SFA, 26 % MUFA and 48 % PUFA.

Two healthy volunteers were studied without treatment and a second time after taking 8 g per day of cholestyramine for two weeks to also compare cholesterogenesis in controls to the patients after strong induction. The controls 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 glucose rate of 7 mg per kilogram bodyweight per minute. This rate was similar to the amount of carbohydrates that the two patients received through a nasogastric tube from 22.00 h until the end of the experiment to maintain normoglycaemia (glucose levels 3-6 mmol/l). At midnight, an infusion of [1-^{13}C]acetate (Isotec, Miamisburg, OH, U.S.A) was started in volunteers and patients through a nasogastric tube at a rate of 0.12 mmol per kilogram bodyweight per hour for 16 hours. Blood samples were taken before, throughout and after the infusion. After 16 hours, the infusion was stopped and subjects were allowed to return to their regular diet.

Cholesterol was extracted from total plasma and derivatised according to Neese et al[45]. VLDL from plasma samples was isolated and palmitate from VLDL fractions was methylated as described elsewhere[21]. Lipids were analysed by gas chromatography/mass spectrometry[3,21]. De novo synthesis of cholesterol and palmitate in plasma and VLDL, respectively, were measured by MIDA, as described in detail previously[3,21,45]. To obtain a semiquantitative

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 synthesised palmitate present in VLDL after 16 hours of ^{13}C-acetate infusion.

The oxidation of LDL and VLDL was measured according to the Esterbauer method with some modifications[47]. Tocopherols (α and γ) and β-carotene were determined by high-performance liquid chromatography[23] and ubiquinol levels were analyzed as described earlier[16].

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 ± 35 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 the control subjects (4.2 ± 0.4 mmol/l; 162 ± 15 mg/dl). Increased lipid concentrations were almost solely caused by 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 were 4.4 mmol/l in patient A and 4.5 mmol/l in patient B, and mean lactate levels were 3.2 and 3.4 mmol/l (normal upper value: 2.2 mmol/l), respectively.

Susceptibility of both LDL and VLDL to oxidative modification was markedly lower in the patients with GSD Ia (Table 5.3.1), 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.

Fatty acid composition of LDL particles and, to a lesser extent, of VLDL particles showed increased SFA contents in the patients with GSD Ia (Table 5.3.1). 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 control subjects 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 5.3.1). Significant, but less pronounced, correlations were also found for VLDL lagtime and propagation speed and lipoprotein fatty acid composition.

Table 5.3.1 Oxidation characteristics of VLDL and LDL particles, fasting plasma antioxidant concentrations, and VLDL and LDL fatty acid composition

		patient A	patient B	controls*
plasma	α-tocopherol (μmol/l)	57.5	81.7	21.7 ± 4.9
	relative (μmol/mmol)	3.1	2.7	4.6 ± 0.7
	β-carotene (μmol/l)	0.17	0.76	0.74 ± 0.39
	relative (μmol/mmol)	0.01	0.03	0.16 ± 0.10
	ubiquinol (μmol/l)	1.21	1.44	0.92 ± 0.37
	relative (μmol/mmol)	0.06	0.05	0.20 ± 0.07
VLDL	lagtime (min)	359	372	135 ± 12
	propagation speed (nmol/mg/min)	4.5	3.5	13.1 ± 2.3
	SFA (%)	51.8	50.0	45.6 ± 4.6
	MUFA (%)	28.5	36.8	26.1 ± 2.2
	PUFA (%)	19.7	13.2	28.3 ± 3.2
LDL	lagtime (min)	97	107	84 ± 6
	propagation speed (nmol/mg/min)	7.0	6.7	10.9 ± 0.9
	SFA (%)	43.7	40.8	30.8 ± 1.0
	MUFA (%)	28.4	25.4	20.1 ± 3.3
	PUFA (%)	27.9	33.8	49.1 ± 4.2

lagtime denotes time after administration of copper until oxidation starts and *propagation speed* the actual rate of oxidation
'relative' values for α-tocopherol, β-carotene and ubiquinol concentrations refer to antioxidant concentrations divided by cholesterol plus triglyceride concentrations
*values were obtained from five healthy control subjects and are displayed as mean ± SD

A more than 40-fold increase in the amount of newly synthesised VLDL-palmitate in the two patients with GSD Ia compared with the control subjects was calculated (Table 5.3.2). 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-^{13}C]acetate infusion, revealed a much lower acetyl-CoA pool enrichment in the patients compared to the control subjects.

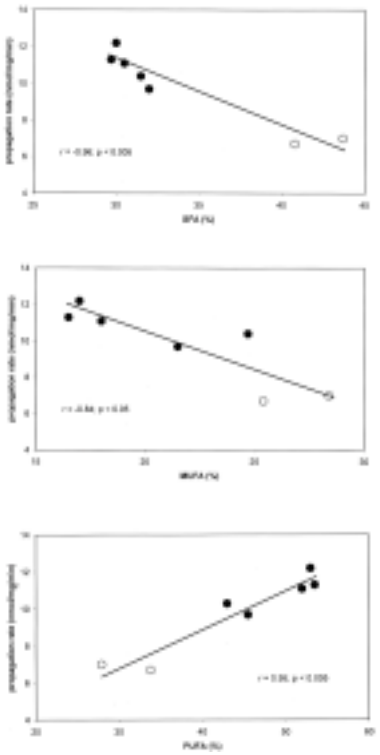

Figure 5.3.1 Correlation between SFA, MUFA and PUFA content of LDL particles and oxidation propagation speed. Values from healthy control subjects (n= 5) are represented by the closed symbols and those from both GSD Ia patients by the open symbols

Table 5.3.2 Cholesterol and palmitate synthesis in two GSD-1a patients and control subjects, the latter before and after cholestyramine treatment

subject	absolute cholesterol production (mg/d)	newly synthesized VLDL palmitate (mmol)	precursor pool enrichment # (%)
patient A	4419	119.7	8
patient B	2212	209.0	9
control subject A	505	7.5	16
control subject B	426	0.3	15
control subject A*	1256	25.4	16
control subject B*	1477	2.6	15

* represents values after two week treatment with cholestyramine.
calculated from isotopomer distribution of plasma cholesterol at 12 and 16 hours after start of the ^{13}C-acetate infusion

Discussion

A crucial and at present unanswered question is whether the severe hyperlipidaemia in GSD Ia patients will ultimately lead to an increased risk for atherosclerosis, especially because patients have a better life expectancy as the result of improved dietary treatment. A few GSD Ia patients who were in their thirties have been described with atherosclerosis[55]. However, using ultrasound techniques to investigate endothelial function, Lee et al[32] found no indication for premature atherosclerosis in young adult patients with GSD Ia. We chose to study two adult patients with GSD Ia and severe hyperlipidaemia and atherogenic lipid profile who were expected to show signs of premature atherosclerosis. However, determination of ankle-brachial indices, aortic distensibility and intima-media thickness of the carotid and femoral arteries in these two patients (and 7 other adult GSD Ia patients) showed no signs of premature atherosclerosis[57]. Ex vivo copper-induced oxidation of lipoprotein particles, an important indicator of atherogenicity[58], revealed a much lower oxidation susceptibility of LDL of GSD Ia patients compared with that in controls. Antioxidants and fatty acid composition are known to influence the oxidizability of lipoprotein particles[58]. Our data do not support a role for antioxidants in decreasing lipoprotein oxidizability in GSD Ia because tocopherols, β-carotene, and uric acid concentrations were not increased, although hyperuricaemia is a common phenomenon in patients with GSD Ia. It is well known that PUFA display a higher susceptibility to oxidation than MUFA and SFA[58]. Although we have only obtained data from two patients, our data suggests that the relatively high lipoprotein SFA content in patients with GSD Ia plays a role in the 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, as patients have a high relative PUFA intake. Application of stable isotopes revealed that synthesis of saturated fatty acids, for example, palmitate, as well as cholesterogenesis were severely increased compared with healthy control subjects. Control values found in this study were similar to values previously reported[52]. 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 control subjects, which led, however, to higher insulin concentrations in control subjects compared with 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 as glucose again. Decreased insulin secretion to a carbohydrate load has been demonstrated in adult GSD Ia patients before[37]. Insulin and glucose are both separate stimulators of de novo lipogenesis[28]. 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[17]. The values for lipogenesis are a combination of formation and clearance. Decreased clearance is expected to lower the fraction of newly synthesised palmitate found at the end of the experiment because 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 patients with GSD Ia in better metabolic control and with less severe hyperlipidaemia.

Decreased acetyl-CoA pool enrichments observed in the GSD Ia 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 Ia patients by stimulating acetyl CoA carboxylase. Furthermore, data suggests that G6P itself might act as a mediator of carbohydrate-induced lipogenic activity[18].

In conclusion, we hypothesize that the absence of G6Pase activity, together with a low fat diet, increases lipogenesis, and, 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 the lipoprotein fatty acid profile. The use of fish oil might not be helpful to prevent premature atherosclerosis in patients with GSD Ia because normolipidemia usually is not achieved, and fish oil could lead to increased lipoprotein oxidizability by increasing the lipoprotein PUFA content.

References

1 Alaupovic P, Fernandes J (1985) The serum apolipoprotein profile of patients with glucose-6-phosphatase-deficiency. Pediatr Res 19:380-384

2 Baker L, Dahlem S, Goldfarb S, Kern EF, Stanley CA, Egler J, Olshan JS, Heyman S (1989) Hyperfiltration and renal disease in glycogen storage disease, type I. Kidney Int 35:1345-1350

3 Bandsma RHJ, Stellaard F, Vonk RJ, Nagel GT, Neese RA, Hellerstein MK, Kuipers F (1998) Contribution of newly synthesized cholesterol to rat plasma and bile determined by mass isotopomer distribution analysis: bile-salt flux promotes secretion of newly synthesized cholesterol into bile. Biochem J 329:699-703

4 Bandsma RHJ, Rake JP, Visser G, Neese RA, Hellerstein MK, van Duyvenvoorde W, Princen HM, Stellaard F, Smit GP, Kuipers F (2002) Increased lipogenesis and resistance of lipoproteins to oxidative modification in two patients with glycogen storage disease type 1a. J Pediatr 140:256-260

5 Bianchi S, Bigazzi R, Campese VM (1999) Microalbuminuria in essential hypertension: significance, pathophysiology, and therapeutic implications. Am J Kidney Dis 34:973-995

6 Binkiewicz A, Senior B (1973) Decreased ketogenesis in von Gierke's disease (type I glycogenosis). J Pediatr 83:973-978

7 Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE (1992) Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 340:1111-1115

8 Chen Y-T, Coleman RA, Scheinman JI, Kolbeck PC, Sidbury JB (1988) Renal disease in type I glycogen storage disease. N Engl J Med 318:7-11

9 Chen YT (2001) Glycogen Storage Diseases. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease. 8th ed. McGraw-Hill, New York, pp 1521-1551

10 Christiansen EH, Mølgaard H, Christensen PD, Sørensen KE, Christensen CK, Mogensen CE (1998) Increased left ventricular systolic function in insulin dependent diabetic patients with normal albumin excretion. Eur Heart J 19:1735 1739

11 De Simone G, Daniels SR, Devereux RB, et al (1992) Left ventricular mass and body size in normotensive children and adults: assessment of allometric relations and impact of overweight. J Am Coll Cardiol 20:1251-1260

12 Devereux RB, Reichek N (1977) Echocardiographic determination of left ventricular mass in man. Circulation 55:613-618

13 Fernandes J, Leonard JV, Moses SW, Odievre M, di Rocco M, Schaub J, Smit GPA, Ullrich K, Durand P (1988) Glycogen storage disease: recommendations for treatment. Eur J Pediatr 147:226-228

14 Fernandes J, Alaupovic P, Wit JM (1989) Gastric drip feeding in patients with glycogen storage disease type I: its effects on growth and plasma lipids and apolipoproteins. Pediatr Res 25:327-331

15 Fernandes J, Smit GPA (2000) The Glycogen-storage diseases. In Fernandes J, Saudubray JM, Van den Berghe G (eds) Inborn metabolic diseases. 3rd ed. Springer Verlag, Berlin, pp 85-101

16 Finckh B, Kontush A, Commentz J, Hubner C, Burdelski M, Kohlschutter A (1995) Monitoring of ubiquinol-10, ubiquinone-10, carotenoids, and tocopherols in neonatal plasma microsamples using high-performance liquid chromatography with coulometric electrochemical detection. Anal Biochem 232:210-216

17 Forget PP, Fernandes J, Haverkamp Begemann P (1974) Triglyceride clearing in glycogen storage disease. Pediatr Res 8:114-119

18 Foufelle F, Girard J, Ferre P (1996) Regulation of lipogenic enzyme expression by glucose in liver and adipose tissue: is glucose-6-phosphate the signaling metabolite? Biochem Soc Trans 24:372-378

19 Goldstein JL, Hobbs HH, Brown MS (1994) Familial hypercholesterolemia. In Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease. 7th ed. McGraw-Hill Inc, New York, pp 1981-2030

20 Greene HL, Swift LL, Knapp HR (1991) Hyperlipidemia and fatty acid composition in patients treated for type IA glycogen storage disease. J Pediatr 119:398-403

21 Hellerstein MK, Neese RA, Schwarz JM (1993) Model for measuring absolute rates of hepatic de novo lipogenesis and reesterification of free fatty acids. Am J Physiol. 265:E814-E820

22 Hutton RA, Macnab AJ, Rivers RPA (1976) Defect of platelet function associated with chronic hypoglycaemia. Arch Dis Child 51:49-55

23 Jansson L, Nilsson B, Lindgren R (1980) Quantitation of serum tocopherols by high-performance liquid chromatography with fluorescence detection. J Chromatogr 181:242-247

24 Johnson MC, Bergersen LJ, Beck A, Dick G, Cole BR (1999) Diastolic function and tachycardia in hypertensive children. Am J Hypertens 12:1009-1014

25 Kane JP, Havel RJ (1994) Disorders of the biogenesis and secretion of lipoproteins containing the B Apolipoproteins. In Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease. 7th ed. McGraw-Hill Inc, New York, pp 1872-1885

26 Katz S, Globerman A, Avitzour M, Dolfin T (1997) The ankle-brachial index in normal neonates and infants is significantly lower than in older children and adults. J Pediatr Surg 32:269-271

27 Kelbæk H, Jensen T, Feldt-Rasmussen B, Christensen NJ, Richter EA, Deckert T, Nielsen SL (1991) Impaired left-ventricular function in insulin-dependent diabetic patients with increased urinary albumin excretion. Scand J Clin Lab Invest 51:467-473

28 Koo SH, Dutcher AK, Towle HC (2001) Glucose and insulin function through two distinct transcription factors to stimulate expression of lipogenic enzyme genes in liver. J Biol Chem 276:9437-9445

29 Koren MJ, Devereux RB, Casale PN, Savage DD, Laragh JH (1991) Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension. Ann Intern Med 114:345-352

30 Laogun AA, Gosling RG (1982) In vivo arterial compliance in man. Clin Phys Physiol Meas 3:201-212

31 Lavrencic A, Kosmina B, Keber I, Videcnik V, Keber D (1996) Carotid intima-media thickness in young patients with familial hypercholesterolaemia. Heart 76:321-325

32 Lee PJ, Celermajer DS, Robinson J, McCarthy SN, Betteridge DJ, Leonard JV (1994) Hyperlipidaemia does not impair vascular endothelial function in glycogen storage disease type Ia. Atherosclerosis 110:95-100

33 Lehmann ED, Gosling RG, Sönksen PH (1992) Arterial wall compliance in diabetes. Diabetic Med 9:114-119

34 Lehmann ED, Watts GF, Fatemi-Langroudi B, Gosling RG (1992) Aortic compliance in young patients with heterozygous familial hypercholesterolaemia. Clinical Science 83:717-721

35 Levy E, Thibault LA, Roy CC, Bendayan M, Lepage G, Letarte J (1988) Circulating lipids and lipoproteins in glycogen storage disease type I with nocturnal intragastric feeding. J Lipid Res 29:215-226

36 Levy E, Thibault L, Roy CC, Letarte J, Lambert M, Seidman EG (1990) Mechanisms of hypercholesterolaemia in glycogen storage disease type I: defective metabolism of low density lipoprotein in cultured skin fibroblasts. Eur J Clin Invest 20:253-260

37 Lockwood DH, Merimee TJ, Edgar PJ, Greene ML, Fujimoto WY, Seegmiller JE, Howell RR (1969) Insulin secretion in type I glycogen storage disease. Diabetes 18:755-758

38 Lonn EM, Yusuf S, Dzavik V, Doris CI, Yi Q, Smith S, Moore-Cox A, Bosch J, Riley WA, Teo KK (2001) Effects of ramipril and vitamin E on atherosclerosis. The study to evaluate carotid ultrasound changes in patients treated with ramipril and vitamin E (SECURE). Circulation 103:919-925

39 MacMahon S, Sharpe N, Gamble G, et al (2000) Randomized, placebo-controlled trial of the angiotensin-converting enzyme inhibitor, ramipril, in patients with coronary or other occlusive arterial disease. PART-2 collaborative Research Group. Prevention of Atherosclerosis with Ramipril. J Am Coll Cardiol 36:438-443

40 Marti GE, Rick ME, Sidbury J, Gralnick HR (1986) DDAVP infusion in five patients with type Ia glycogen storage disease and associated correction of prolonged bleeding times. Blood 68:180-184

41 Mason HH, Andersen DH (1955) Glycogen disease of the liver (Von Gierke's disease) with hepatomata. Pediatrics 16:785-800

42 Mietus-Snijder M, Malloy MJ (1998) Endothelial dysfunction occurs in children with two genetic hyperlipidemias: improvement with antioxidant vitamin therapy. J Pediatr 133:35-40

43 Mogensen CE (1999) Microalbuminuria, blood pressure and diabetic renal disease: origin and development of ideas. Diabetologia 42:263-285

44 Moorhead JF, El-Nahas M, Chan MK, Varghese Z (1982) Lipid nephrotoxicity in chronic progressive glomerular and tubulo-interstitial disease. Lancet 2:1309-1311

45 Neese RA, Faix D, Kletke C, Wu K, Wang AC, Shackleton CH, Hellerstein MK (1993) Measurement of endogenous synthesis of plasma cholesterol in rats and humans using MIDA. Am J Physiol. 264:E136-E147

46 Obara K, Saito T, Sato H, Ogawa M, Igarashi Y, Yoshinaga K (1993) Renal histology in two adult patients with type 1 glycogen storage disease. Clin Nephrol 39:59-64

47 Princen HM, van Duyvenvoorde W, Buytenhek R, Blonk C, Tijburg LB, Langius JA, Meinders AE, Pijl H (1998) No effect of consumption of green and black tea on plasma lipid and antioxidant levels and on LDL oxidation in smokers. Arterioscler Thromb Vasc Biol 18:833-41

48 Rake JP, Visser G, Labrune Ph, Leonard JV, Ullrich K, Smit GPA (2002) Glycogen storage disease type I: diagnosis, management, clinical course and outcome. Results of European study on glycogen storage disease type I (ESGSD I). Eur J Pediatr 161[suppl1]:s20-s34

49 Reitsma-Bierens WCC, Smit GPA, Troelstra JA (1992) Renal function and kidney size in glycogen storage disease type I. Pediatr Nephrol 6:236-238

50 Ross R (1999) Atherosclerosis: an inflammatory disease. N Engl J Med 340:115-126

51 Schmieder RE, Martus P, Klingbeil A (1996) Reversal of left ventricular hypertrophy in essential hypertension. A meta-analysis of randomized double-blind studies. JAMA 275:1507-1513

52 Schwarz JM, Neese RA, Turner S, Dare D, Hellerstein MK (1995) Short-term alterations in carbohydrate intake in humans -Striking effects on hepatic glucose production, de novo lipogenesis, lipolysis, and whole body fuel selection. J Clin Invest 96:2735-2743

53 Selzer RH, Hodis HN, Kwong-Fu H, Mack WJ, Lee PL, Liu CR, Liu CH (1994) Evaluation of computerized edge tracking for quantifying intima-media thickness of the common carotid artery from B-mode ultrasound images. Atherosclerosis 111:1-11

54 Senocak F, Sarçlar M, özkutlu S (1994) Echocardiographic findings in some metabolic storage diseases. Jpn Heart J 35:635-643

55 Talente GM, Coleman RA, Alter C, Baker L, Brown BI, Cannon RA, Chen YT, Crigler JF Jr, Ferreira P, Haworth JC, Herman GE, Issenman RM, Keating JP, Linde R, Roe TF, Senior B, Wolfsdorf JI (1994) Glycogen storage disease in adults. Ann Intern Med 120:218-226

56 Tonstad S, Joakimsen O, Stensland-Bugge E, Leren TP, Ose L, Rusell D, Bonaa KH (1996) Risk factors related to carotid intima-media thickness and plaque in children with familial hypercholesterolemia and control subjects. Arterioscler Thromb Vasc Biol 16:984-991

57 Ubels FL, Rake JP, Slaets JPJ, Smit GPA, Smit AJ (2002) Is Glycogen Storage Disease type 1a associated with atherosclerosis? Eur J Pediatr 160:s62-s64

58 Witztum JL, Steinberg D (1991) Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest 88:1785-1792

59 Wolfsdorf JI, Laffel LMB, Crigler JF Jr (1997) Metabolic control and renal dysfunction in type I glycogen storage disease. J Inher Metab Dis 20:559-568

60 Yamasaki Y, Kawamori R, Matsushima H, Nishizawa H, Kodama M, Kajimoto Y, Morishima T, Kamada T (1994) Atherosclerosis in carotid artery of young IDDM patients monitored by ultrasound high-resolution B-mode imaging. Diabetes 43:634-639