

## Prediction of premature atherosclerosis by endothelial dysfunction and increased intima-media thickness in glycogen storage disease types Ia and III

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**SUMMARY:** Yekeler E, Dursun M, Emeksiz E, Akkoyunlu M, Akyol Y, Demir F, Gökçay G, Demirkol M. Prediction of premature atherosclerosis by endothelial dysfunction and increased intima-media thickness in glycogen storage disease types Ia and III. Turk J Pediatr 2007; 49: 115-119.

The aim of this study was to investigate the endothelial dysfunction (ED) and carotid intima-media thickness (IMT) in patients with glycogen storage disease (GSD) types Ia and III.

In 22 patients with GSD (13, type Ia; 9, type III) and 18 healthy subjects, endothelial functions of the brachial artery and carotid IMT were evaluated by high-resolution ultrasound. Endothelial-dependent dilatation (EDD) was assessed by establishing reactive hyperemia. EDD and carotid IMTs were compared between the three groups.

Mean cholesterol level was slightly higher in GSD type III patients but the difference was not significant. Triglyceride levels and cholesterol to high density lipoprotein (HDL) ratio were significantly higher in GSD type Ia patients. EDD was significantly impaired in GSD type Ia ( $13\% \pm 8\%$ ,  $P=.001$ ) and type III ( $15\% \pm 6\%$ ,  $P=.005$ ) patients when compared with the healthy subjects ( $22\% \pm 4\%$ ). The carotid IMT was significantly higher in both GSD type Ia ( $0.23 \pm 0.03$  mm,  $P=.005$ ) and type III ( $0.26 \pm 0.05$  mm,  $P=.001$ ) patients when compared with the healthy subjects ( $0.20 \pm 0.02$  mm).

Both GSD type Ia and type III patients show significant ED and increased IMT, which are predictors of atherosclerosis.

**Key words:** glycogen storage diseases, endothelial dysfunction, endothelial-dependent dilatation, carotid intima-media thickness, ultrasound.

Glycogen storage diseases (GSD) are a heterogeneous group of inherited disorders of glycogen metabolism. GSD type Ia (von-Gierke disease) is an inborn error of metabolism caused by deficiency of glucose-6-phosphatase, the enzyme catalyzing the conversion of glucose-6-phosphate to glucose. The disease is characterized by hypoglycemia and hepatic glycogen and fat accumulation as well as severe hypertriglyceridemia, hypercholesterolemia, and hyperuricemia<sup>1-4</sup>. Another subtype, frequently causing hepatic involvement, is GSD type III with a deficiency of the glycogen debrancher enzyme. Hypercholesterolemia and hypertriglyceridemia have been reported in up to 40% of GSD III patients older than 10 years<sup>5,6</sup>.

Endothelial dysfunction (ED) is now recognized as a key early event in atherogenesis. Carotid intima-media thickness (IMT), measured noninvasively by ultrasonography, is a well-established index of atherosclerosis and is directly associated with an increased risk of cardiovascular disease<sup>7-9</sup>. The anti-atherogenic properties of intact endothelium and their loss prior to the development of detectable atherosclerotic plaque suggest that ED may not be simply a marker of early vascular wall disease but that it may signify the primary injury that initiates the atherosclerotic process<sup>10,11</sup>.

To our knowledge, GSD types Ia and III subgroups have not been included in the same study to depict and compare blood lipid levels,

endothelial functions, and carotid IMTs. Therefore, in the current study, endothelial function and carotid IMT of patients with GSD types Ia and III and a control group were investigated using high-resolution vascular ultrasound and the results were compared between groups. Blood lipid levels were also compared between GSD type Ia and III patients.

## Material and Methods

### Study Population

During a 10-month period, 22 patients diagnosed with GSD (19 months to 14 years; mean, 9.4 years) were enrolled in the study. Thirteen patients had GSD type Ia (7 boys, 6 girls; age range, 5 months-18 years; mean age, 8.6 years) and 9 patients had GSD type III (6 boys, 3 girls; age range, 26 months-21 years; mean age, 10.5 years). None of the patients with GSD types Ia and III had followed a regular diet program. Cholesterol, triglyceride, and high density lipoprotein (HDL) levels of each patient were recorded. The control group included 18 healthy volunteers (11 boys, 7 girls; age range, 2-14 years, mean age, 8.8 years). Examinations were performed after a fasting period (3 to 8 hours) between 8:00 and 9:30 a.m. The institutional ethics committee approved the study protocol and a parental informed consent was obtained from all patients and the control group.

### Brachial Artery Measurements

Endothelium-dependent dilatation (EDD) of the brachial artery after transient ischemia, a non-invasive method for assessing endothelial function, was performed according to methods defined by Celermajer et al.<sup>12</sup>. The examinations were performed on a high-resolution ultrasound system (HDI 5000; Philips Medical Systems, Best, the Netherlands). In order to visualize the brachial artery properly, the arm was comfortably immobilized in the extended position and the brachial artery was scanned in the longitudinal section 2 to 5 cm above the antecubital fossa using a 10-MHz high-resolution linear-array transducer. After optimal transducer positioning, the skin was marked for reference for later measurements, and the arm was kept in the same position throughout the study. Brachial artery internal diameter was measured three times and the average was

accepted as baseline internal diameter of the brachial artery. After baseline measurement, the cuff was inflated to 200 mm Hg (or 50 mm Hg higher than systolic blood pressure) for 5 minutes to create forearm ischemia. Subsequently, the cuff was deflated and the arterial diameter was measured 60 seconds after deflation. The measurements were performed by agreement of two examiners (E.Y., M.D.) at the time of each examination. The examiners were blinded to the clinical and biochemical data. EDD was expressed as the percentage of change in brachial artery diameter from baseline to after reactive hyperemia.

### Carotid Intima-Media Thickness Measurements

Bilateral carotid ultrasound was carried out with the same high-resolution ultrasound system and the transducer used for brachial artery measurements. The common carotid arteries were scanned longitudinally. The bulb dilation served as a landmark to indicate the border between distal common carotid artery and the carotid bulb. Images were obtained from the distal portion of the common carotid artery, 1 to 2 cm proximal to the carotid bulb. The two bright echogenic lines in the arterial wall were identified as the intima and media lines. The intimal plus medial thickness (IMT) was measured as the distance from the main edge of the first to main edge of the second echogenic line. Each measurement was repeated three times, and the mean of the left and right common carotid artery measurements was taken and used for further analysis. All scans were made by agreement of the same examiners (E.Y., M.D.) who were blinded to the clinical and biochemical data. None of the subjects had atheromatous plaque, localized lesion of thickness greater than 2.0 mm or stenosis in this region.

### Statistical Analysis

All measurements are expressed as mean  $\pm$  standard deviation. Comparison of the measurements between the groups was performed using Mann-Whitney U test. The Mann-Whitney U test was applied to the groups in pairs, for all possible combinations. Categorical variables were compared with chi-square test. The relationship between the measurements (EDD and carotid IMT) and blood lipid levels was investigated by correlation analysis. A

P value of less than 0.05 was considered statistically significant. For data sets with more than two variables, a non-parametric analysis of variance (Kruskal-Wallis) was performed; a Bonferroni adjustment was applied for P value in those cases.

**Results**

There was no significant difference between the three groups (GSD type Ia, GSD type III, and the control group) regarding age and gender. Mean cholesterol and triglyceride levels, cholesterol to HDL ratio, and the significance of the differences between GSD types Ia and III subgroups are tabulated in Table I. Mean cholesterol level was slightly higher in GSD type III patients than GSD type Ia patients, but the difference was not significant statistically. Triglyceride levels and cholesterol to HDL ratios were markedly higher in GSD type Ia patients, and the differences were significant statistically (P=.006 and P=.007, respectively).

Brachial and carotid artery measurements and their significances are tabulated in Table II. Mean basal diameter of the brachial artery with SD was equal (2.4±0.4 mm) in the patient

subgroups and 2.4±0.3 mm in the control group. EDD was 13% ± 8% in GSD type Ia patients, 15% ± 6% in GSD type III patients, and 22% ± 4% in the control group. EDD differences were statistically significant between GSD type Ia and the control group (P=.001), and between GSD type III and the control group (P=.005). EDD difference was not significant between the patient subgroups. When the three groups were compared, the differences were significant statistically (P=.001).

Carotid IMT thickness was 0.23±0.03 mm in GSD type Ia patients, 0.26±0.05 mm in GSD type III patients, and 0.20±0.02 mm in the control group. Carotid IMT thickness differences were statistically significant between GSD type Ia and the control group (P=.005), and between GSD type III and the control group (P=.001). The difference was not significant between the GSD types Ia and III subgroups. When the three groups were compared regarding carotid IMT, the differences were significant statistically (P=.001).

The ultrasound measurements (EDD and carotid IMT) and blood lipid levels were not correlated in either the GSD type Ia or type III group.

**Table I.** Mean Cholesterol and Triglyceride Values and Cholesterol to HDL Ratios in GSD Types Ia and III

	GSD type Ia (n=13)	GSD type III (n=9)	P value
Cholesterol (mg/dl)	268±94	293±206	=.404
Triglyceride (mg/dl)	1108±628	445±329	=.006
Cholesterol/HDL	21.2±19.8	6.3±2.4	=.007

HDL: High density lipoprotein. GSD: Glycogen storage disease.

**Table II.** Brachial Artery Responses to Endothelium-Dependent Stimuli and Carotid Intima-Media Thickness in the Patient and Control Groups

	GSD type Ia (n=13)	GSD type III (n=9)	Controls (n=18)	P value
Baseline diameter (mm)	2.4±0.4	2.4±0.4	2.4±0.3	NS
% EDD-diameter (%)	13%±8%	15%±6%	22%±4%	P=.001
Carotid IMT	0.23±0.03	0.26±0.05	0.20±0.02	P=.001

GSD: Glycogen storage disease. % EDD-diameter (%): Percent increase in diameter induced by endothelium-dependent stimuli (reactive hyperemia). IMT: Intima-media thickness. NS: Not significant.

## Discussion

Glycogen storage disease type Ia (von Gierke disease) is an inborn error of metabolism caused by deficiency of glucose-6-phosphatase, the enzyme catalyzing the conversion of glucose-6-phosphate to glucose. The disease is characterized by hypoglycemia and hepatic glycogen and fat accumulation as well as severe hypertriglyceridemia, hypercholesterolemia, and hyperuricemia<sup>1,2,4,13,14</sup>. Hyperlipidemia is caused by an increase in syntheses of very low density lipoprotein (VLDL), low density lipoprotein (LDL), and triglyceride, a decrease in peripheral lipolysis, and hypoinsulinism. Blood triglyceride and cholesterol levels can increase up to 4000-6000 mg/dl and 400-600 mg/dl, respectively. Numerous complications have been observed in GSD type Ia patients, such as kidneys stones, proteinuria, progressive renal failure, gout, xanthomas, pancreatitis, anemia, osteoporosis, and atherosclerosis<sup>2</sup>.

Glycogen storage disease type III (Cori-Forbes disease or limit dextrinosis) is characterized by deposition of structurally abnormal glycogen in muscles and liver of the patients with debranching enzyme deficiency. Although degradation of glycogen to glucose is defective, there is no problem in gluconeogenesis. Symptoms are related to muscle and liver involvement. Hypoglycemia, in contrast to GSD I, can be compensated to a certain extent by gluconeogenesis and ketogenesis<sup>2</sup>. Patients often have ketosis at fasting, high cholesterol levels, and hyperbetalipoproteinemia without excessive triglycerides. Over 40% of patients with GSD type III have hypercholesterolemia and hypertriglyceridemia after 10 years of age.

Endothelial dysfunction is considered to play an important role in the pathogenesis of vascular disease<sup>15</sup>. An imbalance characterized by reduced production of nitric oxide or increased production of reactive oxygen species may promote ED<sup>16,17</sup>. It has been shown that angiotensin II contributes to ED by stimulating the production of reactive oxygen species, such as superoxide, through the activation of membrane-bound NADH (reduced nicotinamide adenine dinucleotide)/NADPH (reduced nicotinamide adenine dinucleotide phosphate) oxidase<sup>18</sup>.

Hyperlipidemia is a recognized risk factor for atherosclerosis and ischemic heart disease. Improved dietary treatment has resulted

in better life expectancy in GSD patients. Therefore, potential risk for atherosclerosis should be decreased with regular dietary treatment in these patients. Dietary treatment is less demanding in GSD type III than in GSD type Ia.

The relationship between hyperlipidemia and premature atherosclerosis has been investigated in several studies. In a study by Talente et al.<sup>6</sup>, a few patients with GSD type Ia who were in their thirties were described with atherosclerosis. Lee et al.<sup>19</sup> found no endothelial dysfunction predicting premature atherosclerosis in hyperlipidemic patients with GSD type Ia. Hershkovitz et al.<sup>10</sup> found no significant differences in EDD and the lipid and lipoprotein concentrations between 11 GSD type III patients and the matched control group. Similarly, in the current study, the ultrasound measurements (EDD and carotid IMT) and blood lipid levels were not correlated in either GSD type Ia or type III groups. In contrast to previous studies, we found significant differences in EDD and the carotid IMT between the GSD subgroups (type Ia and III) and control subjects. These significant differences might be due to lack of a regular dietary treatment in our patient population. EDD was worse in GSD type Ia ( $P=.001$ ) than GSD type III patients ( $P=.005$ ); in contrast, the carotid IMT was increased more in GSD type III ( $P=.001$ ) than GSD type Ia ( $P=.005$ ) patients when compared with healthy subjects.

We concluded that EDD and the carotid IMT, predictors for premature atherosclerosis, were impaired in both GSD type Ia and type III patients. Persistent hyperlipidemia as a result of insufficient treatment may lead to this. Impairment in EDD was more evident in GSD type Ia patients; conversely, carotid IMT was higher in those patients with GSD type III.

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