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A PROSPECTIVE STUDY OF INSULIN RESISTANCE IN GAUCHER DISEASE TYPE 1 PATIENTS WITH NORMAL WEIGHT, UNDER ENZYME REPLACEMENT THERAPY

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Abstract

Context. A certain degree of insulin resistance in patients with Gaucher disease type 1 (GD) under enzyme replacement therapy (ERT) was reported. Data on insulin sensitivity in treatment naïve patients are inconsistent.

Objective. To analyse prospectively changes in parameters of insulin resistance under ERT and to estimate when they occur.

Design. prospective, controlled study; three years follow-up.

Patients and methods. 12 treatment naïve patients with GD type 1 (M/W 8/4), 29.5±12.9 years, without overweight, diagnosed enzymatically and by genotyping, without previous diabetes mellitus. Patients were evaluated before and every 6 months up to 3 years under ERT and compared at baseline and after 3 years with matched healthy controls. Fasting-glucose (FG), - insulin (FI), C-peptide, HOMA-IR, IRI, HOMA-B, blood count, hepatic and splenic volume, chitotriosidase, severity score index di Rocco (SSI) were assessed.

Results. Baseline glycemic parameters did not differ from controls. FG increased from baseline after two years of ERT (+16.4%,p<0.010), FI (+40.3%,p=0.030), HOMA-IR (+61.2%,p=0.007) and IRI (+9.1%,p=0.010) after 18 months, HOMA-B after 2.5 years (+51%, p=0.015. After 3 years of ERT patients were more insulin resistant compared to controls (p<0.001): FG (96.0±6.2 *vs.* 73.2±6.4 mg/dL), FI (11.2±2.4 *vs.* 5.6±1.3 µU/L), HOMA-IR (2.7±0.6 *vs.* 1.0+0.3), IRI (3.02±0.10 *vs.* 2.62±0.13). FG, FI, HOMA-IR, IRI, HOMA-B correlated with disease severity markers.

Conclusions. This is the first controlled study which evaluates prospectively insulin resistance in GD patients, finding significant differences compared to baseline starting with 18 months ERT.

Key words: Gaucher disease, insulin resistance, enzymatic replacement therapy.

INTRODUCTION

Gaucher disease type 1 (GD1) is the most frequent lysosomal storage disorder, caused by mutations in the glucocerebrosidase gene, and it is inherited in an autosomal recessive manner (1). The deficiency of acid ß-glucocerebrosidase (GBA) leads to the accumulation of the unmetabolised substrate glucosylceramide in the lysosomes of macrophages and explains the multiorgan damage with spleno-hepatomegaly, thrombocytopenia, anaemia and bone disease (2).

The prevalence of this rare disease varies according to literature data between 1/40.000 (3) and 1/100.000 (4).

The intravenous enzyme replacement therapy (ERT) with recombined glucocerebrosidase led to an important improvement in organomegaly, thrombocytopenia, anaemia and bone disease (5, 6).

Regarding insulin metabolism, only few studies investigate the issue in patients with GD1. Langeveld *et al.* found in six patients either naïve to treatment or with high disease burden a lower insulin mediated glucose uptake compared to controls during a hyperinsulinemic euglycemic clamp study and a tendency to less effective suppression of lipolysis by insulin (7). The same authors showed in a cross-sectional study including treated and untreated patients that long-term ERT induces a larger than average weight gain and a significant increase in the prevalence of type 2 diabetes mellitus, with similar prevalences as in the general population (8). A cross-sectional controlled study on fourteen patients with GD1 without overweight under ERT found a more insulin resistant state compared to healthy controls, expressed by higher fasting and postloading insulin concentrations and HOMA-IR-values (9). All these

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findings could be suggestive for a certain atherogenic risk under treatment, which would make necessary a more careful individual follow-up.

In line with this, literature data report on dyslipoproteinemia with marked reduction of high-density lipoprotein cholesterol (HDL-C) as well as increased concentrations of triglycerides (TG) (10-13). We could show in a previous prospective controlled study in initially treatment naïve patients with GD1 that TG and LDL-cholesterol (LDL-C) became comparable to healthy controls under ERT, while HDL-C increased but remained below that of controls (14).

Study design

Since there are no prospective controlled data regarding insulin resistance in this disease, we designed a prospective study in therapy-naïve patients with GD1 starting ERT, in comparison to healthy controls, with follow-up every six months up to three years. We aimed to better characterise the parameters of insulin resistance in untreated patients, their dynamics under ERT, the duration of treatment until a significant change from baseline occurs and to correlate the parameters of glucose metabolism with clinical and biochemical markers of disease severity as well as with parameters of lipid metabolism (previously published, 14).

PATIENTS AND METHODS

Patients

Twelve Caucasian patients with GD1 were included (10 adults, 2 children; 4 males, 8 females) from the 50 patients diagnosed, treated and followed up in the Center of Genetic Diseases of the Emergency Children's Hospital Cluj, Romania.

Inclusion criteria were: diagnosis of Gaucher disease, confirmed by demonstration of deficient activity of glucocerebrosidase in leukocytes and genotyping, no previous specific treatment (ERT, SRT). Exclusion criteria were: diabetes mellitus type 1 or 2, overweight or obesity, smoking, excess alcohol consumption (>20 g/day for women, > 30 g/day for men). Patients were followed up from baseline until three years after starting of ERT.

Two control groups were built up. Each included twelve healthy age-, gender- and BMI-matched subjects. The first control group (C1) was matched to the patient's characteristics before the start of ERT, the second was formed according to the traits of the patient's group three years after starting ERT (C7). Controls were asked about their exercise level (no sports at all, medium, active) and matched to the patients accordingly. The children in this group were selected from a General School in Cluj, Romania, in the setting of a routine auxologic control. The adults were healthy volunteers. All were non-smokers. Blood pressure levels were normal, no subject was on antihypertensive treatment or on any other medication.

Patients and controls were included in this observational prospective study after written informed consent of all participants or, if underage, of their parents. The study was approved by the local Medical Ethics Committee.

Methods

Confirmation of diagnosis

Enzymatic activity was measured according to Peters *et al.* (15). As a control, we determined the mean enzymatic activity in a group of age and gender-matched healthy subjects.

Five mutations (N 370S; L 444P; R 463C; 84 GG and V 394L) were screened for by PCR amplification and restriction enzyme digestion (16-18). The recombinant alleles recNCiI (including the mutations: L 444P; A 456P and V 460V) and rec TL (including the mutations: D 409H; L 444P; A 456P and V 460V) were analyzed by sequencing on an automated ABI 373A DNA sequencer according to the manufacturer's recommendations.

Patients follow-up

ERT was started after diagnosis with human recombinant glucocerebrosidase (imiglucerase) with 60 U/kg in four patients (the two children and two adults with severe forms of disease) and 30 U/Kg in the remaining eight adult patients, as an intravenous infusion every two weeks.

The patients were examined at baseline (P1) and every six months thereafter over three years (up to visit P7), recording the following data: medical history and physical examination (body height and weight were measured, BMI (kg/m^2) was calculated - Seca 702, Hamburg, Germany); haemoglobin (Hb g/dL); thrombocytes (T /mm^3); the volume of the liver and spleen, determined by ultrasonography (19) and expressed as multiples of normal values, accepted to be 2.5% and 0.2% of the patient's weight (kg), respectively (20); activity of serum chitotriosidase and lipid profile. Anaemia, thrombocytopenia, spleno- and hepatomegaly were defined according to published criteria (21). Serum chitotriosidase activity measurement was performed using the artificial substrate 4-methyllumbelliferyl-B-D-N, N', N''- triacetyl chitotriosidase (22) (normal

values: 170-5700 nmol/mL/h). The severity score index (SSI) was calculated according to di Rocco *et al.* (23). The control group was also evaluated by physical examination with assessment of auxologic parameters.

Carbohydrate metabolism

Fasting glucose, insulin, C-peptide and HbA1c were measured in every patient and healthy control after a minimum 12 hours overnight fast.

Glucose (mg/dL) was measured by a colorimetric method with a commercial kit from Diagnostikum (Hungary) on a Cobas Mira plus analyser (Roche, Switzerland). The intra- and interassay variations were less than 2.3 and 5.6%, respectively. The sensitivity was 0.04 mg/dL. Insulin was measured in serum by an electrochemoluminescence method (Insulin Elecsys; Roche Diagnostics, Boehringer Mannheim, Germany) on an Elecsys 1010 automatic analyser (Boehringer Mannheim). Sensitivity of the assay was 0.2μU/mL, within-run variation was less than 1.33%, between-run variation was less than 1.85%. C-peptide was measured in the serum using a similar electrochemiluminescence method (C-Peptide Elecsys; Roche Diagnostics, Boehringer Mannheim), on a 1010 automatic analyser. Sensitivity was 0.01ng/mL, intra- and interassay coefficients of variation were 1.6 and 2.6%, respectively.

HOMA-IR (homeostasis model assessment - insulin resistance) was calculated as: fasting insulin (μU/mL) × fasting glucose (mmol/L) divided by 22.5. IRI (insulin resistance index) was based on the formula: log10 [fasting insulin (μU/mL) × fasting glucose (mg/dL)]. As a cut-off value for insulin resistance as a predictor for cardio-metabolic risk in a general adult European population, a HOMA-IR value of 2.05 was chosen, according to Gayoso-Diz *et al.* (24). HOMA-B (homeostasis model assessment – insulin secretion β-cell, reflecting insulin secretion in basal conditions) was calculated as [20 × fasting insulin (μU/mL)/ fasting glucose (mmol/L) – 3.5] (25, 26).

Statistical analysis

Quantitative variables were expressed as means and standard deviations. For comparison between paired data (measurements before and under therapy), a Wilcoxon signed rank test was used. For comparison between groups (patients and controls), a Mann-Whitney test was used. The Pearson correlation was employed to measure the linear relationships between parameters of glucose metabolism and markers of disease severity. Parameters of glucose metabolism were also correlated to the corresponding parameters of lipid metabolism, previously and prospectively

Table 1. Characteristics of patients and controls

Characteristics	before ERT	after 3 years	p (P1, P7)
patients (P)	***P1***	***P7***	
Number	12	12	-
Gender (female/male)	8/4	8/4	-
Age (years) adults	34.1±8.0	37.1±8.1	-
Age (years) children	6; 7	9; 10	
BMI adults	21.0±3.4	22.2±4.4	n.s. (0.552)
children	11.8; 13.5	16.8; 17.2	
Hb (g/dL)	11.5±1.4	13.6±1.1	<0.001
Thrombocytes (/mmc)	94083±47559	162250±70508	<0.011
Spleen (xN)	14.7±8.7	4.6±2.2	<0.001
Liver (x N)	1.5±0.5	1.0±0.0	0.004
Chitotriosidase (mmol/mL/h)	30417±16810	5039±4983	<0.001
SSI	11.9±3.2	4.8±2.0	< 0.001
Controls (C)	***C1***	***C7***	
Number	12	12	-
Gender (female/male)	8/4	8/4	-
Age (years) adults	34.3±8.2	37.3±8.3	
Age (years) children	6;7	9;10	
	p(P1,C1) = ns (0.975)	p(P7,C7) = ns (0.974)	
BMI adults	21.9±3.8	21.8±4.6	
	p(P1,C1) = ns (0.552)	p(P7,C7) = ns (0.198)	
BMI children	12.4; 13.2	15.9; 16.3	

Hb-haemoglobin; x N – fold increase in comparison to normal; SSI – severity score; ns – not significant; P1-patient's group before the start of ERT; P7 – patient's group after three years of ERT (7th follow-up visit); C1 – control group matched to P1; C7 – control group matched to P7

measured by us in the same group (14). Statistical evaluation was performed with the statistical package IBM SPSS 21. Values for $p < 0.05$ were considered statistically significant.

RESULTS

Specific diagnosis of Gaucher disease was confirmed by measurement of β-glucocerebrosidase (with values between 1.6 and 28% of those measured in healthy subjects). Type 1 was diagnosed according to the clinical picture and the genotype with the presence of the N370S allele.

The characteristics of patients and controls before and three years after initiation of ERT are shown in Table 1. BMI is given separately for children and adults. The two children showed normal BMI-values for age and sex over the observation period. No patient was splenectomised.

The dynamic changes in the parameters of the patient's glucose metabolism profiles, measured at baseline and every six months up to three years under ERT are shown in Table 2. Fasting glucose, insulin, C-peptide as well as the indices HOMA-IR, IRI, and HOMA-B before and three years after ERT were compared to the same parameters of the corresponding control group. The patient's results of glucose metabolism obtained at every six months follow-up visit were compared with the baseline patient's values (before ERT, P1), reporting the statistical significance and the percentage change of the dynamic alterations. As different dosage regimens were used according to disease severity (30 or 60 U/kg), absolute individual changes of the analyzed parameters in patients after three years of ERT are shown in Figure 1. The observed changes were not dependent on the treatment dosage, age or gender.

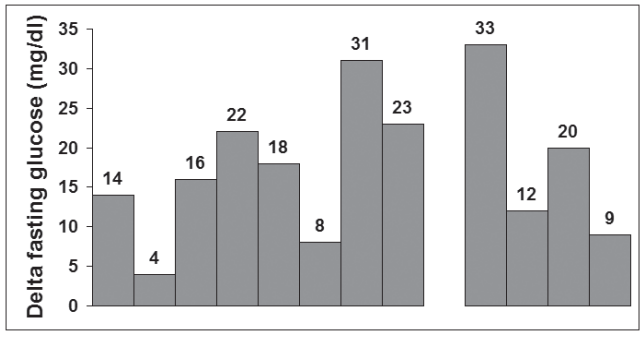
Mean and individual HbA1c values were normal at baseline and remained normal at all points under treatment. Fasting glucose and fasting insulin were subjected to obvious dynamic changes under ERT.

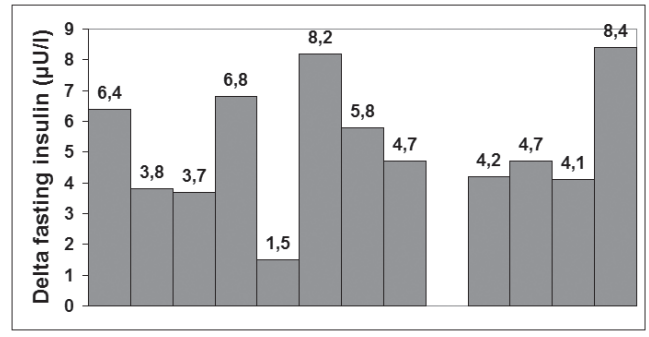
The fasting glucose levels in treatment of naïve patients were not different from the control group C1. The mean fasting glucose concentrations increased rapidly under ERT, with 16.4% after 24 months, when they became significantly different from baseline. Furthermore, fasting glucose increased up to 22.3% from baseline after 36 months, remaining significantly higher compared to baseline (P1) and healthy controls (C7). Fasting glucose values > 100 mg/dL have been registered under ERT in three adult patients after three

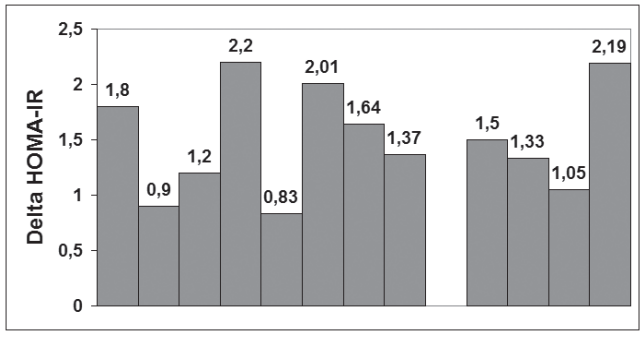
Table 2. Dynamic changes in the parameters of carbohydrate metabolism and HDL-cholesterol in patients with Gaucher disease type 1 before and under ERT compared to the corresponding control groups

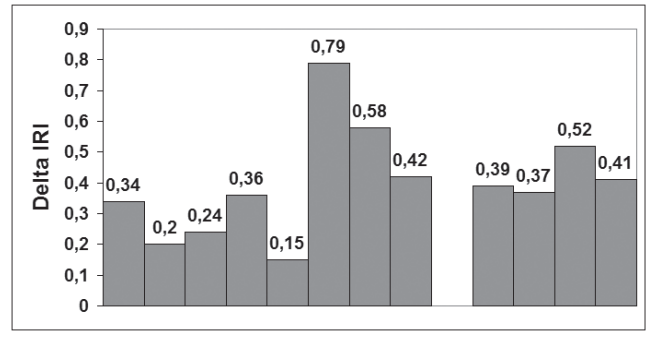
No.		FG (mg/dL)	FI (μU/mL)	C-peptide (mg/L)	HOMA-IR	IRI	HOMA-B	HDL-C (mg/dL)
	Basal: P1	78.5±12.2	6.0±2.7	1.2±0.7	1.2±0.5	2.62±0.23	24.7±13.4	23.6±5.4
1.	C1	75.8±7.2	5.4±1.4	1.4±0.7	1.1±0.3	2.58±0.14	22.0±7.8	52.9±8.3
	$p_{(P1,C1)}$	0.817	0.488	0.750	0.563	0.355	0.564	**<0.001**
	6 mo: P2	81.0±11.2	6.9±2.9	1.4±0.9	1.4±0.6	2.51±0.71	28.4±13.5	29.2±5.7
2.	%change	+3.1	+14.9	+15.2	+23.2	-4.2	+14.9	+23.7
	$p_{(P2,P1)}$	0.644	0.435	0.772	0.259	0.583	0.386	**0.023**
	12mo: P3	88.0±9.3	7.8±2.4	1.3±0.7	1.7±0.6	2.81±0.15	28.8±10.4	31.9±4.3
3.	%change	+12.1	+29.6	+6.7	+46.5	+7.2	+16.6	+35.1
	$p_{(P3,P1)}$	0.060	0.141	0.685	0.063	0.094	0.371	**<0.001**
	18mo: P4	89.3±8.4	8.5±2.3	0.9±0.5	1.9±0.6	2.86±0.13	31.0±9.5	35.7±8.5
4.	%change	+13.7	+40.3	-21.2	+61.2	+9.1	+25.5	+51.2
	$p_{(P4,P1)}$	0.060	**0.030**	0.224	**0.007**	**0.010**	0.126	**<0.001**
	24mo: P5	91.4±8.9	9.4±2.6	0.9±0.6	2.1±0.6	2.91±0.15	34.1±10.2	38.3±9.5
5.	%change	+16.4	55.4	-21.2	+81.0	+11.0	+38.1	+62.2
	$p_{(P5,P1)}$	**<0.010**	**0.007**	0.434	**0.001**	**0.001**	0.065	**<0.001**
	30mo: P6	92.6±5.9	10.3±2.4	0.9±0.6	2.4±0.6	2.95±0.11	37.3±10.2	41.2±6.9
6.	%change	+17.9	+71.3	-18.7	+103.4	+12.5	+51	+74.5
	$p_{(P6,P1)}$	**0.003**	**<0.001**	0.385	**<0.001**	**<0.001**	**0.015**	**<0.001**
7.	36mo: P7	96.0±6.2	11.2±2.4	1.1±0.9	2.7±0.6	3.02±0.10	38.7±8.7	42.9±8.3
	%change	+22.3	+85.9	-4.3	+125	+15.4	+56.7	+81.7
	C7	73.2±6.4	5.6±1.3	0.8±0.4	1.0+0.3	2.62±0.13	24.4±6.5	57.7±9.4
	$p_{(P7,P1)}$	**0.001**	**<0.001**	0.664	**<0.001**	**<0.001**	**0.006**	**<0.001**
	$p_{(P7,C7)}$	**<0.001**	**<0.001**	0.541	**<0.001**	**<0.001**	**0.001**	**<0.001**

No-number of visit; Mo-months; P1-7 – patient's visit before and every six months until 3 years after ERT; C1- control group matched to the patient's group before the start of ERT; C7 – control group matched to the patient's group at the visit three years after the start of ERT; FG – fasting glucose, FI – fasting insulin









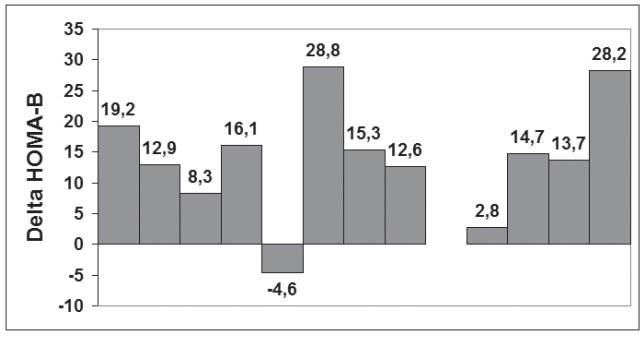


Figure 1. Absolute individual changes of fasting glucose (mg/dL), fasting insulin (µu/mL), HOMA-IR, IRI and HOMA-B (delta) in patients after 3 years of enzyme replacement therapy (ERT) *versus* the used dose of imiglucerase.

1-8: patients receiving 30 U/kg imiglucerase, 9-12: patients receiving 60 U/kg imiglucerase; 1-5, 10,12: female patients, adults; 6-8: male patients adults; 9: male, child, age at start of ERT 6 yrs; 11: female, child, age at start of ERT 7 yrs.

years of ERT (106, 104 and 101 mg/dL) with BMI-values of 24.0, 22.6 and 15.9 kg/m^2 and the following genotypes: N370S/ unidentified mutation, N370S/ N370S, N370S/ unidentified mutation.

Fasting insulin showed also an increasing pattern. In treatment naïve patients, the concentrations of fasting insulin did not differ from the appropriate controls. They increased steadily under ERT, with 40.3% after 18 months, becoming significantly higher compared to baseline. After three years of ERT, fasting mean insulin concentrations were significantly higher compared to baseline (+85.9%) and to the appropriate control group (C7).

C-peptide did not show a clear tendency to change during ERT and did not differ from controls before and after three years of ERT.

HOMA-IR was comparable to the healthy control group C1 at baseline and increased continuously under ERT, becoming significantly higher compared to baseline after 18 months (+ 61.2%). Mean HOMA-IR values steadily increased and were 125% higher than before ERT after 36 months of treatment, significantly higher from baseline and compared to the C7-control group (2.7±0.6 in patients *vs.* 1.0±0.3 in controls). Regarding individual HOMA-IR values, all but one was > 2.05 after 36 months of ERT. The highest value (3.9) was seen in a 38 years old female patient with the genotype N370S/ unidentified allele and a BMI of 24 kg/m^2, who also had the highest fasting glucose level after three years of ERT (106 mg/dL).

IRI at baseline was also comparable to controls and showed a similar raising dynamics under ERT, with a significant increase of the mean value compared to baseline already after 18 months of ERT (9.1%) and an increase with 15.4% after 36 months of ERT.

HOMA-B was similar to the C1 control group

before ERT with a significant increase of 51% after 30 months. After 36 months of ERT HOMA-B was 56.7% higher than before ERT and also significantly higher compared to the C7-control group.

The correlations between the parameters of glucose metabolism and the markers of disease severity are presented in Table 3.

The correlations between the parameters of glucose and lipid metabolism are presented in Table 4. Total cholesterol, LDL-cholesterol and triglycerides (with initial values for total and LDL-cholesterol in patients before treatment lower than in controls and higher triglycerides concentrations) were comparable to controls after 36 months of ERT (data not shown; (14). HDL-cholesterol increased from baseline (23.6±5.4 mg/dL) to 42.9±8.3 mg/dL after three years of ERT but remained significantly lower compared to the appropriate controls (57.7±9.4mg/dL; $p<0.001$); (see Table 2).

No patient was overweight before or under ERT. No patient developed diabetes mellitus under ERT.

DISCUSSION

Our study provides first evidence for discrete but progressive alterations of insulin sensitivity under ERT in patients with GD1 investigated in a prospective and controlled manner.

Fasting glucose and insulin as well as HOMA-IR, IRI and HOMA-B did not differ from controls before the start of ERT, in our patients with GD1 without overweight.

Few literature data investigate treatment naïve GD patients regarding insulin resistance status. Langeveld *et al.* pointed out to a certain degree of insulin resistance in a small group of six patients, of whom three were untreated and three had a poor treatment response. The authors hypothesize that this finding can be due to increased levels of glucosylceramides in specialized cell membrane domains called rafts, which negatively influence insulin signaling (7). Increased concentrations of plasma ganglioside GM3 have been reported in patients with GD1 (27) and inhibition of glycosphingolipid synthesis increased hepatic insulin action in diet induced obese mice (28) and ameliorated hepatic steatosis in obese mice (29). Whether ganglioside levels are also elevated in muscle and fat tissue of Gaucher patients, which account for the majority of the insulin mediated glucose uptake, is unknown. However, among the six patients investigated by Langeveld *et al.*, some were overweight (mean BMI was 24 kg/m^2, ranging between 20-29 kg/m^2) (7).

Regarding the dynamics of the parameters of insulin resistance in our group, we found fasting glucose values > 100 mg/dL only in three patients after three years of ERT. However, mean fasting glucose levels were significantly higher compared to healthy controls after 24 months of treatment and remained significantly higher after three years of ERT (Table 2). The observed

Table 3. Correlations between parameters of carbohydrate metabolism and markers of the disease severity

	Hb		Tr		Chito		Hep		Spl		SSI	
	r	p	r	p	r	p	r	p	r	p	r	p
Fasting glucose (FG) (mg/dL)	0.723	0.067	0.942	**0.001**	-0.969	**<0.001**	-0.951	**0.001**	-0.854	**0.014**	-0.962	**0.001**
Fasting insulin (FI) (µU/L)	0.707	0.076	0.897	**0.006**	-0.937	**0.002**	-0.925	**0.003**	-0.853	**0.015**	-0.930	**0.002**
HOMA-IR	0.681	0.092	0.889	**0.007**	-0.924	**0.003**	-0.911	**0.004**	-0.840	**0.018**	-0.919	**0.003**
IRI	0.516	0.236	0.864	**0.012**	-0.873	**0.010**	-0.846	**0.016**	-0.679	0.099	-0.865	**0.012**
HOMA-B	0.732	0.061	0.873	**0.010**	-0.917	**0.004**	-0.910	**0.004**	-0.858	**0.013**	-0.910	**0.004**

Hb-haemoglobin; Tr-thrombocytes; Chito – chitotriosidase; Hep – hepatomegaly; Spl – splenomegaly; SSI – severity score; r – coefficient of correlation; p – value of statistical significance (significant values - p<0.05 - are presented in bold).

Table 4. Correlations between parameters of carbohydrate and lipid metabolism

	HDL-C (mg/dL)		LDL-C (mg/dL)		TC (mg/dL)		LDL/HDL-C		TG (mg/dL)	
	r	p	r	p	r	p	r	p	r	p
Fasting glucose (mg/dL)	0.973	**<0.001**	0.969	**0.001**	0.966	**<0.001**	-0.871	**0.011**	-0.786	**0.036**
Fasting insulin (µU/l)	0.987	**<0.001**	0.991	**<0.001**	0.977	**<0.001**	-0.862	**0.013**	-0.875	**0.010**
HOMA-IR	0.980	**<0.001**	0.985	**<0.001**	0.969	**<0.001**	-0.845	**0.017**	-0.854	**0.014**
IRI	0.896	**0.006**	0.911	**0.004**	0.926	**0.003**	-0.709	0.075	-0.795	**0.033**
HOMA-B	0.980	**<0.001**	0.981	**<0.001**	0.966	**<0.001**	-0.870	**0.011**	-0.868	**0.011**

HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol; TC: total cholesterol; TG: triglycerides; p: value of statistical significance (significant values - p<0.05 - are presented in bold)

significant increase in fasting insulin concentrations occurs most probably as a compensatory reaction of the pancreatic β-cells, in an attempt to maintain normal plasma glucose concentrations. Mean fasting insulin concentrations became significantly higher compared to baseline after 18 months of ERT and almost doubled from baseline (11.2±2.4 µU/L *vs.* 6.0±2.7 µU/mL) after three years of ERT. This finding is in line with previous data from Ucar *et al.*, who reported in patients with GD1 without overweight mean fasting insulin levels almost twice as high as those of controls after a median treatment duration of four years (9).

According to literature data, HOMA-IR was also significantly higher under treatment. Ucar *et al.* found in the above cited study median HOMA-IR values of 1.9 ± 1.3 compared to controls (0.9 ± 0.2) in his group consisting of 14 patients with GD1, with normal weight and investigated in a cross sectional manner under ERT (9). Langeveld *et al*. described in a group of 42 patients with GD type 1, of whom seven were untreated and 35 had median duration of ERT of 11 years, that insulin resistance, defined in his work as HOMA-IR values >4.65, was detected in 6% of the treated patients and in none of the untreated (8). The same authors report a significant increase of overweight prevalence over 11 years of ERT from 16 to 56%, without correlation to the duration of therapy, nor to the response to treatment as measured by the relative decrease in chitotriosidase; four patients developed diabetes mellitus type 2 during ERT, increasing the prevalence of diabetes from 0% before treatment to 8.2% after a median treatment duration of 11 years (8). In our patients, none reached a HOMA-IR value > 4.65 during three years of ERT and none developed diabetes mellitus. Only two patients had HOMA-IR values > 3 after three years of ERT (3.2 and 3.9, respectively), but all except one had at this time point HOMA-IR values > 2.05, defined according to recent literature data as a predictive cut-off value for cardio-metabolic risk in an adult general Caucasian population (24). Even if there is no consensus in literature about a firm cut-off value for HOMA-IR in order to reflect a clinically relevant insulin resistant status and healthy controls displayed values of 1.2±0.5 (C1) and 1.0±0.3 (C7), we additionally observed that the mean HOMA-IR values increased compared to baseline already after 18 months of ERT, with mean values more than double from baseline after three years of ERT. This finding, in line with the similar increases in IRI and the increases in fasting insulin, probably as a measure of compensatory response, are clinically relevant observations, when placed in the light of their dynamic progress. Moreover, the parameters of glucose metabolism under ERT, indicating a progress towards an insulin resistant status potentially relevant for cardio-metabolic risk, showed significant correlations with the improvement of all parameters of disease severity under ERT (direct correlations with hemoglobin and platelet count and indirect correlations with hepato- and splenomegaly, chitotriosidase and SSI). The same changes towards insulin resistance correlated directly with the increasing concentrations of TC, LDL-C and HDL-C and with the decreasing levels of TG under ERT (Table 4). The direct correlation between insulin resistance and HDL-C dynamics, which is known to increase from very low to almost normal values under ERT (14), seems to be a specific constellation for GD1 under treatment, resulting in a combination between traits of cardiovascular risk and protective effect. This is in line with the finding of de Fost *et al.*, who reported that the marked reduction in HDL-C before treatment is not accompanied by an increased carotid artery intima-media thickness (12).

The main limitation of the study is the low number of patients. This is due to the strict inclusion and exclusion criteria with selection of only therapy naïve patients and to the reduced prevalence of the disease. The only previous data on insulin resistance including only seven therapy naïve patients with GD1 together with patients under ERT were reported by Langeveld *et al.* [8].

Learning points:

Patients with GD1 without overweight show a tendency towards insulin resistance under ERT.

Significant differences compared to healthy controls occur 18 months after treatment start and progress until three years of ERT.

The cardiovascular relevance of this finding is unclear in the setting of a specific dyslipidemic pattern.

This finding suggests the clinical need for individual follow-up, including assessment of the parameters of insulin resistance under ERT and, whenever necessary, preventive life-style intervention.

In conclusion, we analyzed prospectively dynamic changes in parameters of glucose metabolism in patients with Gaucher disease type 1 over three years of ERT. To the best of our knowledge, this is the first study to evaluate systematically only therapy naïve patients under ERT, with biannual follow-up investigations, with comparison to appropriate control groups. This was done in order to further characterize metabolic changes with potential atherogenic risk, in an effort to investigate if and when ERT is accompanied

by the development of a certain degree of insulin resistance.

We found at baseline in therapy naïve patients without overweight parameters of glucose metabolism comparable to healthy controls, in the setting of severely reduced HDL-cholesterol concentrations. Significant increases from baseline were registered for fasting glucose after 24 months, fasting insulin, HOMA-IR and IRI after 18 months and for HOMA-B after 30 months of ERT. All these parameters were significantly higher compared to the appropriate matched healthy control group after three years of ERT, while HDL-cholesterol increased, remaining however below healthy controls. These observations, in a group of patients with GD type 1 with normal weight, define a metabolic profile of potential atherogenic risk. The above observations of the progress towards insulin resistance under ERT, in the setting of subnormal HDL-cholesterol values, are important for the clinical practice, in order to monitor glucose and lipid profile under treatment, identify individual risk constellations, prevent excessive weight gain and offer whenever appropriate dietary and psychological counselling to keep or restore normal weight.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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