Gaucher disease type I: associated morbidities and long term efficacy of enzyme replacement therapy

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Citation for published version (APA):

Low HDL levels in Gaucher disease type I does not lead to an increased risk of cardiovascular disease as assessed by carotid intima-media thickness

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Abstract

Patients as well as carriers of the lysosomal storage disorder Gaucher disease type I are known to show abnormally low HDL, and to a lesser extend, LDL cholesterol levels. Whether this results in an increased risk of cardiovascular disease is unknown. Therefore, lipid profiles, apolipoproteins, and carotid artery intima-media thickness (cIMT), a biomarker for atherosclerosis and cardiovascular disease risk, were assessed in 42 Gaucher disease type I patients, 34 carriers and 41 family and non-family controls. Compared to controls, patients showed decreased HDL (1.6 ± 0.4 vs 1.1 ± 0.3 mmol/L) as well as mildly decreased LDL cholesterol levels (3.2 ± 1.1 vs 2.8 ± 0.7 mmol/L), with low levels of the major apolipoproteins related to these particles, apoA1 and apoB. In carriers, low LDL-cholesterol and apoB levels were found. Mean cIMT measurements did not differ among patients, carriers and controls (0.66 ± 0.12, 0.65 ± 0.13 and 0.64 ± 0.12mm, respectively). In conclusion, our data show that although lipid profiles in patients and carriers of Gaucher disease are altered, this does not result in an increased risk of cardiovascular disease. Increased reverse cholesterol transport may be involved, but further investigations are needed to fully elucidate this.
Introduction

Gaucher disease (GD) is the most common lysosomal storage disorder, with a prevalence of 1:50,000 in most countries. The disorder is characterized by a deficiency of the lysosomal enzyme glucocerebrosidase (OMIM #230800), resulting in accumulation of glucocerebroside in macrophages, so called Gaucher cells. Type 1 GD is the most prevalent non-neuronopathic form and can manifest itself at any age. Clinically, the storage of Gaucher cells in liver, spleen and bone marrow results in hepatosplenomegaly, skeletal disease and cytopenia. In addition to these classical symptoms, a number of co-morbidities is known to be associated with GD; increased prevalence of malignancies, hypergammaglobulinemias, pulmonary hypertension, polyneuropathies and abnormal cholesterol profiles have all been reported. Low levels of total plasma cholesterol (TC), low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) in patients with GD have been described. The reductions of LDL-c and HDL-c are associated with reduced levels of their respective major protein components apolipoprotein B100 (apoB) and apolipoprotein A1 (apoA-I), indicating a reduced level of these particles, while apolipoprotein E (apoE) levels are reported to be high. The levels of LDL-c and HDL-c are inversely correlated with parameters for disease severity and splenectomy is associated with a subsequent increase of LDL-c and HDL-c. It is hypothesized that enhanced fractional catabolism of LDL and HDL is induced by the Gaucher macrophages, possibly resulting in enhanced reverse cholesterol transport. Interestingly, although carriers of a glucocerebrosidase mutation (carriers) do not exhibit any Gaucher symptomatology, significantly lower HDL-c levels have been found in these subjects. Enzyme replacement therapy (ERT) with recombinant glucocerebrosidase for 1,5 years causes an increase in HDL-c and apo-A1 concentrations, but no normalization, while LDL-c and apoB levels remain unchanged.

In several epidemiologic studies it has been shown that low plasma HDL-c levels are associated with increased risk for coronary artery disease (CAD). As a consequence, GD patients as well as carriers could be considered at risk for premature atherosclerosis. A non-invasive validated biomarker for the status of atherosclerosis and present and future cardiovascular disease risk is ultrasonographically measured carotid intima-media thickness (cIMT). In order to evaluate whether the abnormal lipid profile in GD patients and carriers is associated with atherosclerosis and, potentially, an increased risk of cardiovascular disease, a cross sectional study was performed in GD patients, carriers and controls.

Methods

Study population

Subjects were recruited from the Dutch outpatient clinic for inherited metabolic diseases (Academic Medical Center, Amsterdam). In all patients, a diagnosis of GD was confirmed
by enzymatic assay as well as by mutation analysis. Patients that consented to the protocol were asked to recruit family members as well as non-related persons that shared the same environment and lifestyle. Subjects with a known familial dyslipidemia were excluded. In order to identify carriers and non-carriers, glucocerebrosidase mutation analysis was performed in all subjects.

A thorough investigation of present and/or past CAD as well as a family history of CAD was performed in all subjects as follows; personal and family medical history of myocardial infarction, stroke, intermittent claudication, as well as the presence of cardiovascular risk factors (age, sex, smoking, diabetes, hypertension, chronic inflammation as assessed by C-reactive protein (CRP) and the presence of rheumatoid arthritis, vasculitis or systemic lupus erythematosus) and use of medication were assessed by questionnaires. Blood pressure, length and weight were measured. Body mass index (BMI) was calculated. Hypertension was defined as a blood pressure >140/90 mmHg. In addition, in GD patients, spleen status, severity score index (SSI, as described by Zimran\textsuperscript{15}), use of substrate deprivation or enzyme replacement therapy and genotype was recorded from patient files.

The study was approved by the institutional review board and all participants provided written informed consent to participate in the study.

**Laboratory analysis**

In all subjects, blood samples were drawn after an overnight fast. Leukocyte count, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were determined. TC, HDL-c and triglycerides (TG) were measured by enzymatic colorimetric procedure. LDL-c was calculated by means of the Friedewald formula. ApoA1 and ApoB were determined by immunonephelometry. Normal values were as follows; TC: 3.9-6.5 mmol/L; LDL: <4.49 mmol/L; HDL: male >1.1 mmol/L, female > 1.2 mmol/L; TG: 0.5-2.0 mmol/L; apoA-I: male 1.1-1.8 g/L, female 1.1-2.1 g/L; apoB: male 0.55-1.4 g/L, female 0.55-1.25 g/L. DNA was extracted from peripheral blood leukocytes. In non-Gaucher patients, the Gaucher mutations present in their family, as well as the six most prevalent Gaucher mutations in the Dutch population (N370S, L444P, R120W, 84GG, RecNciI, L324P) were determined. Together, these mutations are responsible for >82% of the abnormal alleles in the Dutch GD population\textsuperscript{16}.

**Carotid intima-media thickness (cIMT)**

B-mode ultrasound images were acquired using an Acuson Aspen (Siemens/Acuson Corporation, Erlangen, Germany and Mountainview, CA, USA) using an L7 5-12MHz broadband transducer. Bilaterally, DICOM still images of a total of six predefined arterial segments of the right and left common carotid artery, the carotid bulb and the internal carotid artery were acquired. cIMT of far arterial walls was measured by one image analyst,
blinded for the clinical and genetic status of patients. Carotid IMT was defined as the per subject average of the six far wall IMT measurements.

**Statistical analysis**

Descriptive statistics were used for exploration of the data. Differences in variables with a continuous or a dichotomous distribution between GD patients, carriers and controls were evaluated using linear or logistic regression analyses, respectively. These analyses were performed using the generalized estimating equations (GEE)-method in the SAS procedure GENMOD to account for correlations within families. The exchangeable correlation structure was used for these models. For differences in cIMT between the three groups, a stepwise backward multivariate regression analysis was used to adjust for potential confounders. Variables with a skewed distribution were log-transformed before statistical analyses. P-values < 0.05 were considered to indicate statistical significance. The analyses were performed with the SAS package version 9.1 (SAS Institute Inc, Cary, NC, USA).

**Indirect standardisation**

Indirect standardisation was used to compare the number of cerebrovascular events to that of the Dutch population. The files of all patients with GD type I followed at the national referral centre for GD at the Academic Medical Centre (AMC), Amsterdam, the Netherlands, (n=71) during the years 1991-2007, were reviewed. All patients had visited the clinic at least twice. Incidence of myocardial infarction (MI) and cerebral stroke in the GD cohort were compared to the age- and sex-specific incidence rates in the Dutch population as a whole in 2000\(^17\):\(^18\) using indirect standardisation for five-year intervals. Patients were considered at risk for MI or stroke starting at the date of the first visit to the clinic, making it possible to verify the presence or absence of these diseases in the medical files of the patients, thereby excluding the risk of overlooking a previous event. The period at risk ended at the closing date of the study (1-1-2007), the date of death or the date of the last hospital visit, whichever came first. For each of the five-year intervals, the expected number of events was calculated as the product of person years at risk and incidence rates. An event was included as an observed case only in patients who were diagnosed with stroke or MI during the period at risk. Standardized Rate Ratios (SRR) were calculated as the ratio of observed cases to expected numbers. 95% confidence intervals (95% CI) for the SRR presuming a Poisson distribution for the observed numbers were determined.

**Results**

**General characteristics**

A total of 117 subjects (42 patients, 34 carriers and 41 controls) were studied. Age, gender, BMI, cigarette and alcohol use, CRP and the prevalence of hypertension and diabetes were
comparable in the three study groups (Table 1). The presence of a history of cardiovascular events was also similar. Thirty-four of the patients had received enzyme replacement therapy (Cerezyme, imiglucerase, Genzyme Corp., Mass., USA) for a median of 13 years (range 2-16). Two patients used substrate deprivation therapy (Miglustat, Zavesca\textsuperscript{TM}, Actelion Pharmaceuticals, Switzerland), both for 9 years.

**Lipids and lipoproteins**

Levels of HDL-c and apoa-I were significantly lower, and TG levels significantly higher, in patients as compared to controls and carriers (Figure 1). In patients compared to controls TC levels were significantly lower, while LDL-c, but not apoB levels showed a trend towards lower levels. In carriers, no differences in HDL-c and apoa-I levels were detected versus controls, while the trend towards a lower TC was explained by significantly decreased LDL-c levels, with a slighter decrease in apoB levels (p=0.07). In fact the decrease in LDL-c was more pronounced in carriers than in the GD patients.

<table>
<thead>
<tr>
<th></th>
<th>controls</th>
<th>carriers</th>
<th>patients</th>
<th>p-value controls versus carriers</th>
<th>p-value patients versus carriers</th>
<th>p-value carriers versus patients</th>
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<tr>
<td>N</td>
<td>41</td>
<td>34</td>
<td>42</td>
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<tr>
<td>Age (years)(mean+/SD)</td>
<td>47.7 +/- 16.6</td>
<td>48 +/- 16.5</td>
<td>51.4 +/- 11.4</td>
<td>0.93</td>
<td>0.22</td>
<td>0.37</td>
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<td>Sex (m/f)</td>
<td>19/22</td>
<td>dec-22</td>
<td>24/18</td>
<td>0.24</td>
<td>0.65</td>
<td>0.07</td>
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<td>BMI (kg/m2)(mean+/SD)</td>
<td>25.1 +/- 3.5</td>
<td>25 +/- 2.6</td>
<td>24.4 +/- 3.0</td>
<td>0.88</td>
<td>0.30</td>
<td>0.33</td>
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<td>Hypertension (y/n)</td>
<td>15/26</td>
<td>13/21</td>
<td>13/29</td>
<td>0.91</td>
<td>0.60</td>
<td>0.46</td>
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<td>CRP (mg/L)(mean+/SD)</td>
<td>2.3 +/- 2.6</td>
<td>2.1 +/- 1.7</td>
<td>2.2 +/- 2.2</td>
<td>0.77</td>
<td>0.93</td>
<td>0.78</td>
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<td>Alcohol (U/wk)(mean+/SD)</td>
<td>7.6 +/- 7.7</td>
<td>5.8 +/- 7.1</td>
<td>5.0 +/- 6.3</td>
<td>0.31</td>
<td>0.06</td>
<td>0.47</td>
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<td>Smoking(y/n)</td>
<td>12/29</td>
<td>9/25</td>
<td>6/36</td>
<td>0.96</td>
<td>0.11</td>
<td>0.17</td>
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<td>History of MI (y/n)</td>
<td>0/41</td>
<td>3/31</td>
<td>0/42</td>
<td>0.09</td>
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<td>History of stroke (y/n)</td>
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<td>0/34</td>
<td>0/42</td>
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**Plasma lipoprotein**

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<th>patients</th>
<th>p-value controls versus carriers</th>
<th>p-value patients versus carriers</th>
<th>p-value carriers versus patients</th>
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<tbody>
<tr>
<td>TC (mmol/L)(mean+/SD)</td>
<td>5.2 ± 1.2</td>
<td>4.7 ± 0.9</td>
<td>4.5 ± 0.9</td>
<td>0.07</td>
<td>0.003</td>
<td>0.30</td>
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<td>LDL (mmol/L)(mean+/SD)</td>
<td>3.2± 1.1</td>
<td>2.7± 0.8</td>
<td>2.8 ± 0.7</td>
<td>0.03</td>
<td>0.06</td>
<td>0.54</td>
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<tr>
<td>HDL-c (mmol/L)(mean+/SD)</td>
<td>1.6 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>1.1 ± 0.3</td>
<td>0.90</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<td>TG (mmol/L)(median (25th-75th percentile))</td>
<td>0.8 (0.5-1.1)</td>
<td>0.7 (0.6-1.8)</td>
<td>1.0 (0.7-1.5)</td>
<td>0.79</td>
<td>0.01</td>
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**Plasma apolipoprotein**

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<th>patients</th>
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<th>p-value patients versus carriers</th>
<th>p-value carriers versus patients</th>
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<tbody>
<tr>
<td>ApoA-I (g/L)(mean+/SD)</td>
<td>1.6 ± 0.3</td>
<td>1.6 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>0.40</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<td>ApoB (g/L)(mean+/SD)</td>
<td>0.9 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>0.9 ± 1.2</td>
<td>0.07</td>
<td>0.64</td>
<td>0.12</td>
</tr>
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Table 1. Characteristics and levels of cholesterol, triglycerides, apolipoproteins and c-IMT measurements of Gaucher disease patients, carriers of a glucocerebrosidase mutation and controls. Abbreviations: m, male; f, female; BMI, body mass index; y, yes; n, no; CRP, C-reactive protein; MI, myocardial infarction; TC, total cholesterol; LDL, low density lipoprotein; HDL-c, high density lipoprotein cholesterol; TG, triglycerides.
Figure 1. Levels of cholesterol, triglycerides, apolipoproteins and c-IMT measurements of Gaucher disease patients, carriers of a glucocerebrosidase mutation and controls. Abbreviations: TC, total cholesterol; LDL, low density lipoprotein; HDL-c, high density lipoprotein cholesterol; TG, triglycerides; c-IMT, carotid intima media thickness.
Intima media thickness

The mean cIMT was 0.66 ± 0.12 mm in GD patients, compared to 0.65 ± 0.13 mm and 0.64 ± 0.12 mm in the carrier and control group, respectively. Univariate analyses revealed no differences in cIMT between the three groups. Also by multivariate analysis, after adjustment for gender and age, no significant difference between the three groups could be detected.

Indirect standardisation

The number of observed and expected cases of MI were 1 and 1.05 respectively, with a SRR of 0.95 (95% CI: 0.024-5.3). During the observation period, no strokes were reported. The expected number was 0.74. No evidence for an increased risk of MI or cerebral stroke was thus found in GD patients.

Discussion

In this study we have established that low levels of HDL-c and apoA-I, as seen in GD type I, do not confer an additional risk of CAD. The importance of HDL-c levels as a risk factor for the development of cardiovascular disease is supported by many studies. In the Framingham Heart Study, HDL-c was a more potent risk factor for subsequent CAD than was LDL-c, TC, or plasma TG. In this study, the risk for myocardial infarction has been reported to increase by about 25% for every 5 mg/dL (0.13 mmol/l) decrease in serum HDL below median values for men and women11. Carotid IMT measurement is widely accepted as a modality that can reliably predict the risk for cardiovascular disease and as such is used as a non-invasive surrogate marker in lipid-lowering drug trials19. In the current study, as demonstrated in previous studies1,8, low levels of HDL-c cholesterol and apoA-I protein were demonstrated in GD patients, even after years of ERT, as compared to healthy controls and carriers of a glucocerebrosidase mutation. Using cIMT measurements and calculating the risk for cardiovascular disease by indirect standardization of historic data, we established that there is no indication that the low levels of HDL-c in GD are associated with an increased risk of cardiovascular disease.

Our results are in line with an earlier study, showing no increase in coronary artery disease in 67 GD patients as compared to 132 carriers for a glucocerebrosidase mutation and 59 controls20. The reason why GD patients seem protected from the atherogenic effects of low HDL-c is unclear. In other genetic disorders that lead to low HDL-c levels, different risks for the development of atherosclerosis, as assessed by cIMT, have been established. For example, heterozygosity for an apoA-I mutation resulting in 50% loss of apoA-I concentration and a concomitant decrease in HDL-c levels, yields a more pronounced effect on atherosclerosis progression than similar reductions in HDL-c due to loss of either ABCA1
or LCAT activity (for review see21). As a consequence, HDL-c levels per se do not necessarily reflect the atheroprotective potential of HDL.

In Gaucher disease, several mechanisms may play a role in the protection from the development of atherosclerosis. Firstly, it has been suggested that the presence of loads of macrophages results in increased clearance of LDL-c and HDL-c particles from the circulation. Indeed, in a study using radio-isotope labelled particles, enhanced catabolism was established, pointing towards the macrophages as the responsible cells8. As has been suggested in previous studies, a concurrent increase in apoE may be responsible for a macrophage induced counter-reaction to enhance reverse cholesterol transport, leading eventually to increased clearance and lower HDL-c levels8. Evidence for enhanced cholesterol efflux by macrophage-derived apoE-containing lipoproteins comes from experiments showing that lipid loading of macrophages stimulates synthesis and secretion of apoE, subsequently facilitating cholesterol release from these cells (For review see22). Although earlier studies have shown elevated apoE levels in untreated GD patients9,10 no investigations so far have shown that the Gaucher macrophage is capable of producing and secreting excessive amounts of apoE. Secondly, a natural anticoagulant status in Gaucher disease, caused by either thrombocytopenia, thrombocytopathy and/or clotting factor deficiencies, may have a protective effect on the occurrence of cardiovascular events (for review see23). Thirdly, subfractions of HDL may have different properties with respect to their ability for reverse cholesterol transport. The apoA-I HDL particles are strongly associated with the cholesterol efflux-promoting effects of HDL, while particles containing both apoA-I and A-II (HDL-2) are less effective at mobilizing cholesterol from peripheral stores24. It is therefore suggested that the plasma apoA-I or the apoA-I associated HDL subfraction may serve as a better predictor of coronary atherosclerosis than total HDL25. Although the HDL sub-fractions have not been determined in GD in the current study, the decreased concentration of apoA-I points does not suggest that the low levels are mainly caused by a decrease in HDL-2. Fourthly, the increased concentration of glucosylceramide and GM3 in plasma may have an as yet unknown effect on lipoprotein metabolism31:32. In the context of the current study, it is of interest to emphasize the association between low HDL-c levels and insulin resistance26,27. Low plasma concentrations of HDL-c, in combination with qualitative changes in LDL as well as hypertriglyceridemia comprise the typical dyslipidemia of insulin resistant states28. In addition, significant negative correlations between fasting and postprandial plasma TG levels and HDL-c and apoA-I concentrations26 suggest a close link between TG and HDL metabolism. Interestingly, we recently established that untreated Gaucher patients are relatively insulin resistant29, which might be related to increased levels of glycosphingolipids, for which glucocerebroside, the stored substrate in GD, is the precursor. Since we also established in the current study that triglyceride concentrations were higher in GD patients as compared to controls, insulin resistance may contribute to the observed lipid abnormalities.
It is worth mentioning that although we found significant decreased LDL-c levels in carriers of a glucocerebrosidase mutation, this was not the case for HDL-c. This is in contrast to an earlier publication, showing decreases in HDL-c, but not in LDL-c. It may be no surprise that we found that cIMT measurements were normal and no increased risk for cardiovascular disease was established. In fact, the cholesterol profile seen in our study may point towards a protective effect, reviving an old discussion as to why the prevalence of Gaucher disease carriers is so high. Suggestions that carriehip may protect against infectious diseases have been made in the past, but it could be that a survival advantage is also created by the favourable lipid profile in these patients.

Some limitations of our study warrant further discussion. For example, the study sample was relatively small, although the earlier mentioned studies using cIMT in rare genetic diseases did show significant differences in even smaller cohorts (see). Furthermore, most patients had received ERT for several years, which may have influenced the levels of HDL. However, this explanation is unlikely, since in the period prior to the initiation of ERT also no increase in the occurrence of cardiovascular disease was established. In addition, 27 of 42 patients (64%) still had a HDL level below the lower limit of normal, consistent with a previous study showing only a partial correction of the low HDL levels.

In conclusion, the results from our study show that in Gaucher disease type I the low HDL-c and apoA-I levels are not associated with an increased risk for cardiovascular disease. The mechanisms involved deserve further study.

Acknowledgements

We greatly acknowledge the cooperation of the Gaucher patients and their family members and relatives, as well as the assistance of Patrick Rol, Elsa Rijff, Johan Gort, Wilma Donker, Saskia Schei, Maaike Wiersma, Anouk Vedder, Gabor Linthorst, Els Ormel and Marijke Biegraaten in the collection of the data.

Reference List


