Increased Serum High-density Lipoprotein-Cholesterol Concentration in Celiac Disease After Gluten-free Diet Treatment Correlates With Body Fat Stores

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Background: Low high-density lipoprotein-cholesterol (HDL-C) concentration correlates with increased cardiovascular risk. A great prevalence of celiac disease (CD) was reported among patients with low HDL-C concentration, and gluten-free diet (GFD) treatment seems to normalize lipid profile. We evaluated blood lipids and body composition in 26 CD patients with low HDL-C level (< 1.0 mmol/L) at diagnosis and after GFD.

Study: A case-control study.

Methods: The diagnosis was based on histologic evidence of subtotal or total duodenal villous atrophy. Patients were studied before and after GFD treatment (14.2 ± 1.4 mo) with biopsy-proven return to normal of the duodenal mucosa. HDL-C was enzymatically assessed after precipitation of very low-density lipoprotein and low-density lipoprotein with heparin-magnesium. Apolipoprotein (Apo)-AI level was assessed by immunoturbidimetric assay; triglycerides by an enzymatic colorimetric method. Body composition was assessed by dual-energy x-ray absorptiometry.

Results: Body composition improved after GFD, with increasing body weight ($P < 0.05$) essentially owing to increased fat mass (FM) ($P < 0.01$), rather than fat-free mass ($P = 0.064$). Total cholesterol and HDL-C were lower in untreated compared with treated patients ($P < 0.001$ and $P < 0.0001$). Apo-AI level increased significantly after GFD (1.20 ± 0.22 vs. 1.46 ± 0.17 g/L; $P < 0.0001$). Apo-AI, sex, and FM were all significant determinants of HDL-C level; a positive correlation ($R^2 = 0.68$; $P < 0.0001$) was found between increase in HDL-C level and in FM after GFD treatment.

Conclusions: Restoration of lipid profile in CD patients after GFD treatment may be explained by an increase in both Apo-AI secretion by intestinal cells and body fat stores.

Key Words: apolipoprotein-AI, fat mass, fat-free mass, celiac disease, high-density lipoprotein cholesterol, lipids

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Celiac disease (CD) is an enteropathy determined by gluten ingestion in genetically predisposed subjects.[1] A life-long treatment with a gluten-free diet (GFD) is able to drastically improve or restore the intestinal mucosa and to decrease the risk of morbidity and mortality.[1,2]

An increasing prevalence of the subclinical and silent forms of CD, characterized by few or even absence of symptoms was reported,[3,4] thus the identification of risk groups could represent a key factor for an early diagnosis of CD with consequent decreased risk of developing malignant and nonmalignant complications.[1–3] In addition, patients with CD often show an impairment of nutritional status, either those with the classic or the subclinical form of the disease[4–6] and strict adherence to GFD was found to improve but not to completely normalize the body composition in these patients.[5,6]

A great prevalence of CD was recently reported among patients with low high-density lipoprotein-cholesterol (HDL-C) concentration,[7] probably as a consequence of decreased lipid absorption, reduction in cholesterol-transporting lipoproteins, and decreased apolipoprotein (Apo)-AI secretion from the altered small bowel mucosa.[7–12] In fact, as Apo-AI represents up to 70% of HDL-C particles, a defect in its secretion may determine a reduction in HDL-C in the bloodstream.[13]

Our group previously reported the restoration of blood lipid profile in a small series of CD patients with the normalization of the intestinal villi after GFD treatment,[7] whereas no longitudinal data on a large sample of patients or a possible association with changes in body composition is available in the literature.

The aim of this study was to evaluate the changes in blood lipid profile and body composition in a group of patients initially admitted to our metabolic unit for low HDL-C level (below 1 g/L), who then were found to be affected by CD, before and after GFD treatment.

MATERIALS AND METHODS

Subjects

Out of the whole patient population referred to our outpatient Clinic from June 1996 to September 2007, consisting of 2984 subjects, 212 patients with serum HDL-C concentration below 36 mg/dL were consecutively enrolled in the study. Twenty-six of them (17 women and 9 men) showed positive immunologic tests for CD, both antiendomysium (EMA) and antitransglutaminase (TGA) antibodies. Thus, after having obtained an informed written consent, they underwent an endoscopic and bioptic examination, and resulted to be affected by CD. The diagnosis was based on histologic evidence of subtotal or total duodenal villous atrophy and increased intraepithelial lymphocytes and crypt hyperplasia.[1] The duodenal

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histologic findings were evaluated according to modified Marsh's criteria.[14] All patients were evaluated at diagnosis and after 1-year treatment with a GFD (14.2 ± 1.4 mo).

Exclusion criteria were represented by secondary causes of intestinal atrophy, endocrine disorders, consumption of drugs able to influence data collection, hepatic, renal or cardiovascular disease, more than 10 cigarettes smoked daily, excessive alcohol consumption (higher than 35 g/die for men and 25 g/die for women), fever, and intense physical activity. All the women were examined during the follicular phase of the menstrual cycle, as determined by medical questionnaire, ultrasound examination, and hormonal assessment. All subjects were on a free diet, containing at least 300 mg of cholesterol daily.

The study was approved by the Ethics Committee of the Catholic University of Rome and all subjects gave their informed consent before enrolment.

Analytical Measurements

A blood sample was collected after an overnight fast in an ice bath, immediately centrifuged at 1500*g* for 15 minutes at 25°C, and stored at −20°C until analyzed. Samples were collected at baseline and at the follow-up visit after GFD treatment. Anti-endomysium antibodies (EMA) were detected by using an indirect immunofluorescence technique and antitransglutaminase antibodies were assessed by enzyme-linked immunosorbent assay (Eurospital, Trieste, Italy) and titers above 7 arbitrary units were considered positive. Total protein, albumin, glucose, hemoglobin, iron, ferritin, transferrin, iron-binding capacity, vitamin B_{12}, folic acid, hematocrit, and white and red blood cell counts were measured by using standard laboratory techniques. Total cholesterol and triglycerides were assayed by an enzymatic colorimetric method (Boehringer Mannheim, Mannheim, Germany). HDL-C was enzymatically measured after precipitation of very low-density lipoproteins and low-density lipoproteins (LDLs) with heparin-magnesium and plasma Apo-AI concentration was measured by an immunoturbidimetric assay (Boehringer Mannheim, Mannheim, Germany).[15]

Body Composition Assessment

On the same morning of the blood sample collection, body weight was measured to the nearest 0.1 kg with a beam scale, and height was measured to the nearest 0.5 cm with a wall-mounted stadiometer while the subjects were wearing light clothes and no shoes. Body mass index was computed as the ratio between body weight (kg) and height (m^2). Body composition, that is, fat mass (FM) and fat-free mass (FFM), was assessed by dual-energy x-ray absorptiometry, using a whole body densitometer (Lunar Prodigy Advance, General Electric Healthcare, UK; software Encore 2005).

Statistical Analysis

All results are expressed as mean ± SD. A 2-tailed *P* value < 0.05 was considered significant. The Wilcoxon test was used for comparisons of values at the beginning of the study and after GFD in each patient. Multiple linear regression analysis (method stepwise) was performed to identify the best predictors of HDL-C level values using the independent variables, Apo-AI, sex, body mass index, FFM, FM, presence of the disease, and degree of villous atrophy. Spearman correlation coefficients were calculated for the estimates of the level of association between 2 variables.

RESULTS

EMA and antitransglutaminase antibodies were detected in all the 26 patients with CD at baseline but were absent in patients after GFD treatment. None of the subjects enrolled had an immunoglobulin A deficiency. The duodenal histologic findings in CD patients were as follows: out of the untreated patients, 17 (65%) had a score of IIIB and 9 (35%) of IIIC, whereas after GFD treatment, 16 (62%) patients had a score of 0 and 10 patients (38%) of I.

At diagnosis, 9 CD patients reported abdominal bloating, 7 had low blood iron and ferritin, 4 had low blood folate concentration, and 4 patients reported asthenia. None of the patients had diarrhea. At the 1-year follow-up visit, only 2 patients still had abdominal bloating and 1 had low iron and ferritin concentration.

Table 1 reports the anthropometric and body composition variables of the subjects examined. CD patients at diagnosis had lower body weight ($P < 0.01$) and FM ($P < 0.01$) compared with values after GFD. The increase in body weight after GFD was mostly determined by an increase in FM ($P < 0.01$), as the increase in FFM did not reach statistical significance ($P = 0.072$).

Table 2 reports the lipid profile of overall CD patients, with patients at diagnosis showing reduced total cholesterol ($P < 0.001$), HDL-C ($P < 0.001$), and Apo-AI ($P < 0.001$) but no difference in LDL-C ($P = 0.068$), compared with posttreatment values, after GFD, HDL-C, and Apo-AI levels returned within the normal range in all patients.

Table 3 shows the changes in lipid profile and body composition in CD patients after GFD separate by sex. No sex difference in increase in HDL-C, Apo-AI, or FM was found in our series.

A forward stepwise multiple regression analysis identified Apo-AI ($P < 0.0001$), sex ($P < 0.0001$), FM ($P < 0.001$), body weight ($P < 0.001$), and presence of the disease ($P < 0.001$) as the best predictors of HDL-C concentration before and after GFD treatment, whereas no association with lipid variables was found with FFM changes or degree of villous atrophy. A positive correlation ($P < 0.001$) was found between HDL-C and increase in FM after GFD (Fig. 1).

DISCUSSION

In the present study, the normalization of blood lipid profile after GFD treatment in CD patients with initially low HDL-C level was reported, and a strong correlation between HDL-C concentration and increased body fat stores was showed for the first time.

TABLE 1. Anthropometric and Body Composition Data of the Celiac Disease Patients

	Before GFD (n = 26)	After GFD (n = 26)	*P*
Sex (W/M)	17/9	17/9	
Height (cm)	165 ± 6.0	165 ± 6.0	NS
Weight (kg)	60.3 ± 3.8	63.0 ± 3.5	< 0.05
BMI (kg/m^2)	22.1 ± 1.2	23.1 ± 1.3	< 0.01
FM (kg)	14.6 ± 2.6	16.7 ± 2.7	< 0.01
FFM (kg)	45.7 ± 4.0	46.2 ± 3.9	NS

BMI indicates body mass index; FM, fat mass; FFM, fat-free mass; GFD, gluten-free diet treatment; M, men; NS, not significant; W, women.

TABLE 2. Lipid Profile and Albumin Values in Celiac Disease Patients Before and After Gluten-free Diet Treatment

	Before GFD (n = 26)	After GFD (n = 26)	*P*
TC (mmol/L)	4.45 ± 0.41	4.81 ± 0.28	< 0.001
HDL-C (mmol/L)	0.80 ± 0.08	1.19 ± 0.11	< 0.0001
LDL-C (mmol/L)	3.37 ± 0.31	3.24 ± 0.24	NS
VLDL-C (mmol/L)	0.28 ± 0.06	0.36 ± 0.07	< 0.01
LDL-C/HDL-C ratio	4.2 ± 0.3	2.7 ± 0.3	< 0.0001
Apo-AI (g/L)	1.20 ± 0.22	1.46 ± 0.17	< 0.0001
TGL (mmol/L)	1.53 ± 0.44	1.59 ± 0.34	NS
Albumin (g/L)	41 ± 4	42 ± 3	NS

Apo-AI indicates apolipoprotein-AI; GFD, gluten-free diet treatment; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; TC, total cholesterol; TGL, triglycerides; VLDL-C, very low-density lipoprotein cholesterol.

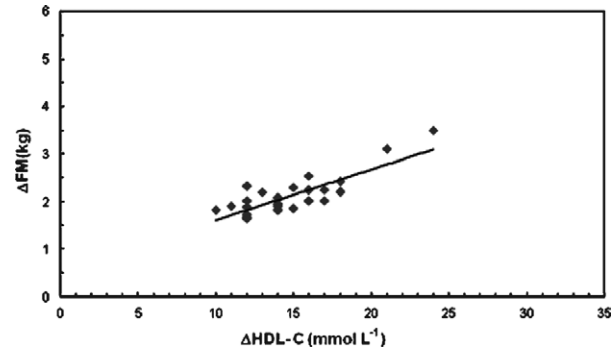

FIGURE 1. Correlation between increase in fat mass and in high-density lipoprotein-cholesterol level in celiac disease patients after gluten-free diet ($R^2 = 0.68$; $P < 0.0001$).

Low HDL-C level is widely reported among the major risk factors in the progression of atherosclerosis and in increased risk of developing coronary artery disease.[16] A large amount of clinical trials on the potential benefit of different therapeutic approaches to increase HDL-C, along with reducing LDL-C level, is constantly produced by researchers, in the attempt of reducing the incidence of cardiovascular diseases.[17] As far as the cardiovascular risk in CD was concerned, an adjusted relative risk of developing pathologic cardiovascular events was found to be 1.9 in CD patients with respect to the normal population,[18] and an increased mortality rate for both malignant and nonmalignant causes was reported in patients compared with the unaffected population.[1,2] Moreover, a high prevalence of CD was found in patients with dilated cardiomyopathy (up to 5.7%) and with autoimmune myocarditis (4.4%),[19,20] suggesting that patients with CD, even in absence of signs and/or symptoms, should be screened for cardiovascular risk factors, including blood lipid profile.

Several studies reported lipid abnormalities in CD, and low HDL-C concentration should be considered as a feature of both the classic and subclinical form of the disease.[6–9] The mechanisms at the basis of low HDL-C concentration in CD may involve different factors. First, decreased lipid absorption could determine a reduction in cholesterol-transporting lipoproteins; second, the alteration of the small bowel mucosa could be responsible for decreased Apo-AI secretion, and as it represents up to 70% of HDL-C particles, for reduced HDL-C synthesis.[7] On the other hand, the improvement of nutritional status after GFD may be related to reduced malabsorption, increased absorptive capacity, and to larger amounts of calories ingested by the patient owing to decreased intestinal symptoms.[6,11,21] The restoration of blood lipid profile after the normalization of the intestinal villi with GFD and the correlation with the increase in FM strongly support these hypotheses.

All patients at diagnosis had a Marsh score of IIIb and IIIc, and as the increase in HDL-C and Apo-AI concentration did not correlate with the severity of villous atrophy in our series, as well as in previous reports,[11,12] it is conceivable that even in presence of subtotal duodenal mucosa atrophy the impairment of lipid profile may occur, strongly suggesting that all CD patients, independently of the presentation of the disease, should be accurately screened for blood lipid alterations.

In conclusion, the normalization of lipid profile in CD patients after GFD treatment, and in particular the increase in circulating HDL-C level, may be explained by increased Apo-AI secretion by intestinal cells and increased nutrient absorption with greater body fat storage. Further studies on a larger sample population are warranted to better ascertain the role of increased HDL-C in CD after GFD as a protective factor in preventing the cardiovascular disease.

TABLE 3. Sex Differences in Changes (Δ) in Body Composition and Lipid Variables in Celiac Disease Patients After Gluten-free Diet Treatment

	Women (n = 17)	Men (n = 9)	*P*
Δ Weight (kg)	2.8 ± 1.0	2.4 ± 0.5	NS
Δ FM (kg)	2.2 ± 0.5	2.0 ± 0.2	NS
Δ FFM (kg)	0.5 ± 0.4	0.4 ± 0.3	NS
Δ TC (mmol/L)	0.31 ± 0.11	0.43 ± 0.22	NS
Δ HDL-C (mmol/L)	15.1 ± 3.7	14.9 ± 2.2	NS

FM indicates fat mass; FFM, fat-free mass; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol.

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