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ORIGINAL ARTICLE

GH levels and insulin sensitivity are differently associated with biomarkers of cardiovascular disease in active acromegaly

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Summary

Context Acromegaly is characterized by GH excess and insulin resistance. It is not known which of these disorders is responsible for the increased atherogenic risk in these patients.

Objective To analyse the associations of GH and homoeostasis model assessment (HOMA) with biomarkers of cardiovascular disease and to compare the above-mentioned variables between patients with active acromegaly and controls.

Design and setting This open cross-sectional study was conducted at a University Hospital.

Patients Twenty-two outpatients were compared with sex- and age-matched control subjects.

Main outcomes Included clinical features, hormonal status, markers of insulin resistance, lipoprotein profile and biomarkers of cardiovascular disease.

Results Patients presented higher triglyceride (median [IQR])* (1·2[1·1–1·6] *vs* 0·9[0·6–1·1] mM, $P < 0·05$), low-density lipoprotein-cholesterol (LDL-C) (mean ± SD)* (3·5 ± 0·9 *vs* 3·0 ± 0·7mM, $P < 0·05$), apoB (0·98 ± 0·23 *vs* 0·77 ± 0·22 g/l, $P < 0·05$), free fatty acid (0·69 ± 0·2 *vs* 0·54 ± 0·2 mM, $P < 0·05$), oxidized-LDL (120 ± 22 *vs* 85 ± 19 U/l, $P < 0·05$) and endothelin-1 (0·90 ± 0·23 *vs* 0·72 ± 0·17 ng/l, $P < 0·05$) levels, increased cholesteryl ester transfer protein (CETP) activity (179 ± 27 *vs* 138 ± 30%/ml/h, $P < 0·01$) and lower C reactive protein (CRP) (0·25[0·1–0·9] *vs* 0·85[0·4–1·4] mg/l; $P < 0·05$) levels than control subjects. Vascular cell adhesion molecule (VCAM-1) concentration was not different. By multiple linear regression analyses, HOMA explained the variability of triglycerides (25%), high-density lipoprotein-cholesterol (HDL-C) (30%) and CETP activity (28%), while GH independently predicted LDL-C (18%), oxidized-LDL (40%) and endothelin-1 levels (19%).

Conclusions In patients with active acromegaly, GH excess contributes to the development of insulin resistance, and the interaction between both disturbances would be responsible for the appearance of atherogenic pro-oxidative and pro-inflammatory factors. Insulin resistance would be preferably associated with an atherogenic lipoprotein profile and to high CETP activity, while high GH levels would independently predict the increase in LDL-C, ox-LDL and endothelin-1.

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Introduction

It has been well established that active acromegaly, a condition defined by the presence of excessive secretion of growth hormone (GH), is associated with increased mortality from cardiovascular disease.[1] In several studies, different atherogenic risk factors and biomarkers of cardiovascular disease were detected to be altered in patients with acromegaly.[2,3] Most of the above-mentioned studies attributed these alterations to GH increment. However, it must be noted that acromegaly is also associated with insulin resistance, carbohydrate intolerance and, in about 40% of the patients, with type 2 diabetes, conditions known to be directly involved in chronic inflammation and atherogenesis.[4] In fact, many years ago, Moller *et al.*[5] evidenced the presence of profound disturbances not only in glucose but also in lipid metabolism.

Insulin resistance is considered to be a pivotal event in atherosclerosis through different pathways.[6] Therefore, it could be hypothesized that this disorder could play a pathophysiological role in the development of cardiovascular disease in patients with acromegaly beyond GH increment.

The specific consequences of insulin resistance in acromegaly are not completely known. In patients without acromegaly, lipid abnormalities, such as increased nonesterified fatty acid and triglyceride as well as low high-density lipoprotein-cholesterol (HDL-C) levels are frequently associated with insulin resistance, which is, in turn, closely related to hyperglycaemia.[7]

On the other hand, resistin, a peptide secreted by adipocytes and inflammatory cells, has been shown to be increased in

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*Median [IQR] and mean ± SD are presented for non-parametric and parametric distributed data, respectively.

patients with insulin resistance and early type 2 diabetes, populations at high risk of developing diffuse and extensive patterns of atherosclerosis. Nevertheless, the role played by this protein in insulin resistance in humans is controversial.[8,9]

The main objective of the present study was to analyse the associations of atherogenic risk factors and biomarkers of cardiovascular disease with markers of insulin resistance and GH in a cross-sectional study. Secondly, we aimed to compare the above-mentioned variables, including resistin levels, between a group of patients with active acromegaly and age- and sex-matched control subjects.

Materials and methods

Subjects

Twenty-two adult patients with diagnosis of active acromegaly, who were not receiving any specific treatment for acromegaly, were consecutively recruited from the Endocrinology Division, Hospital de Clínicas 'José de San Martín', University of Buenos Aires, Argentina, during a period of 24 months. Patients were included in the present study when presenting (i) any typical clinical feature such as headache, acral growth, soft-tissue swelling, arthralgia, sweating and/or sleep apnoea, (ii) inadequate GH response (GH nadir > 1 μg/L) to the oral glucose tolerance test performed at 0, 30, 60, 90 and 120 min after oral administration of a solution containing 75 g of glucose in 375 ml of water and (iii) elevated insulin-like growth factor I (IGF-I) basal levels according to the patient age. Disease duration ranged between 2 and 10 years. Twenty-two healthy subjects, sex- and age-matched (as a whole) with the patients, agreed to participate in this study and were employed as controls. Both patients and controls had normal renal, hepatic and thyroid functions and did not present diagnosis of any other endocrine disorder; none of them presented history of any cardiovascular event, and they were not under treatment with antioxidants or any drug known to affect carbohydrates, lipids or biomarkers of cardiovascular disease. Only eight patients with acromegaly had altered glucose metabolism (evidenced by the oral glucose tolerance test). Five of them presented impaired fasting plasma glucose, and another three presented impaired glucose tolerance and no one was diabetic. Informed consent was obtained from all participants, and the protocol of this open transversal study was approved by the Ethical Committees from Faculty of Pharmacy and Biochemistry and from Hospital de Clínicas 'José de San Martín', University of Buenos Aires.

Study protocol and samples

Body weight, height and waist circumference were registered. The latter was measured midway between the lateral lower rib margin and the superior anterior iliac crest. This measurement was performed with the subject in a standing position and always by the same investigator.

After a 12-h overnight fast, venous blood was drawn from the antecubital vein. Aliquots were collected in clean tubes. Samples were centrifuged at 1500 ***g***, for 15 min at 4 °C. Serum was immediately employed for glucose determination and stored at 4 °C for lipid, lipoprotein and nonesterified fatty acid measurements within 24 h. Serum and plasma aliquots were also stored at −70 °C for determination of GH, IGF-I, insulin growth factor–binding protein-3 (IGFBP-3), insulin, resistin, cholesteryl ester transfer protein (CETP) activity, oxidized (ox) low-density lipoprotein (LDL), endothelin-1, vascular cell adhesion molecule (VCAM)-1 and C reactive protein (CRP).

Analytical procedures

Glucose, triglycerides and total cholesterol were quantified by standardized methods (Roche Diagnostics, Mannheim, Germany) in a Hitachi 917 autoanalyzer. Within-run precision (CV) were 2%, 1·3% and 1·1%, respectively. Between-day precision (CV) were 2·8%, 2·4% and 1·5%, respectively. Laboratory bias for triglycerides and total cholesterol was 1·1% and −1·7%, respectively. LDL-cholesterol (LDL-C) level was determined as the difference between total cholesterol and the cholesterol contained in the supernatant obtained after selective precipitation of LDL with 10 g/l polyvinylsulphate in polyethylenglycol (M.W. 600; 2·5% w/v; pH = 6·7).[10] Within-run and between-day precisions (CV) were 4·7% and 5·0%, respectively. HDL was isolated in the supernatant obtained following precipitation of apo B-containing lipoproteins with 40 g/l of phosphotungstic acid in the presence of magnesium ions.[11] Within-run and between-day precisions (CV) were 3·2% and 3·8%, respectively. Apo A-I and apo B were evaluated by immunoturbidimetry (Roche Diagnostics) in a Hitachi 917 autoanalyzer. Within-run and between-day precisions (CV) were 1·9% and 2·4% for apo A-I, and 1·2% and 2·1% for apo B, respectively. Nonesterified fatty acids were determined by a colorimetric method (Randox Laboratories Ltd, Crumlin, Co. Antrim, UK).

Hormonal parameters

Serum GH was measured by the ultrasensitive immunochemiluminometric assay (Access®; Beckman Coulter TM, Fullerton, CA USA) with analytical sensitivity of 0·003 μg/l. Within-run and between-day precisions (CV) were 12·3% and 15·5%, respectively. Serum IGF-I and IGFBP-3 levels were measured by solid-phase chemiluminiscent enzyme immunoassay (Diagnostics Products Corp., Los Angeles, CA, USA) in an Immulite 2000 with analytical sensitivity of 2·6 nmol/L and 0·1 mg/L, respectively. Within-run and between-day precisions (CV) for IGF-I were 5·4% and 11·9%, respectively. Measurements of IGFBP-3 were all carried out within the same assay. Within-run precision (CV) was 4·8%. Insulin concentration was measured by microparticle enzyme immunoassay (ABBOTT, Minato-ku, Tokyo, Japan). Within-run and between-day precisions (CV) were 2·9% and 4·4%, respectively. Homoeostasis model assessment (HOMA) was using the formulae:

$$[\text{glucose}\,(\text{mM}) \times \text{INSULIN}(\mu\text{U/ml})]/22{\cdot}5.$$

Resistin

Resistin plasma levels were determined by monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) following

the manufacturer' s instructions (R & D Systems, Mineapolis, MN, USA). Sample levels were calculated by analysing standards with known concentrations of recombinant molecules coincident with samples and plotting of signal *vs* concentration. Within-run and between-day precisions (CV) were < 5·3% and < 9·2%, respectively.

CETP activity

CETP activity was determined in serum samples following the general procedure previously described[12] with a few modifications. Briefly, the ability of serum to promote the transfer of tritiated cholesteryl esters from a tracer amount of biosynthetically labelled HDL_3 (^{3}H-CE-HDL_3) (NEN Life Science Products, Boston, MA, USA) to serum apo B-containing lipoproteins was evaluated. Samples were incubated with ^{3}H-CE-HDL_3 (50 μM cholesterol) and 1·5 mM iodoacetate for 3h at 37 °C. After incubation, lipoproteins were separated by a selective precipitation method employing 40 g/l of phosphotungstic acid in the presence of magnesium ions.[10] Radioactivity was measured both in the incubation mixture and in the supernatant containing the HDL fraction in a Liquid Scintillation Analyzer (Packard 210TR; Packard Instruments, Meridian, CT, USA). Measurements were all carried out in duplicate within the same assay. Within-run precision (CV) was 4·9%.

Biomarkers of cardiovascular disease

Ox-LDL was measured by a competitive enzyme-linked immunosorbent assay that employs the monoclonal antibody 4E6 (Mercodia AB, Uppsala, Sweden). Measurements of ox-LDL were all taken within the same assay. Within-run precision (CV) was 6·1%. Endothelin-1 levels were determined by monoclonal antibody-based enzyme-linked immunosorbent assay following the manufacturer's instructions, with few modifications (ELISA) (R & D Systems). Within-run and between-day precisions (CV) were 4·5% and 5·5%, respectively. VCAM-1 plasma levels were determined by the monoclonal antibody-based enzyme-linked immunosorbent assay following the manufacturer's instructions (ELISA) (R & D Systems). Within-run and between-day precisions (CV) were 3·5% and 7·7%, respectively. CRP concentration was determined by Tina-quant CRP (Latex) high sensitive immunoturbidimetric assay (Roche Diagnostics) in a Hitachi 917 autoanalyzer. Within-run and between-day precisions (CV) were 0·4% and 3·4%, respectively.

Data and statistical analysis

Power analysis was carried out employing our database on patients with acromegaly who have been attending the Endocrinology Division and the Department of Clinical Biochemistry since 1990. The variables selected for the sample size calculation were triglycerides, apo B, insulin, ox-LDL, endothelin-1 and CETP activity. Having defined a significance level of 0·05, 80% power and an effect size of 1·0, supported by our previous studies,[2,13] the power analysis revealed that the sample size should not be lower than 22 patients and controls.

Data distribution was analysed employing the Shapiro–Wilk test. Results were expressed as mean ± standard deviation (SD) for normally distributed data and as median (interquartile range [IQR]) for skewed data. For further analyses, skewed data were log transformed, and normal distribution was verified again by the Shapiro–Wilk test. These transformed variables were always employed in the analysis of covariance (ANCOVA) and in the multiple linear regression analyses. ANCOVA was carried out including body mass index (BMI) as a covariate to analyse differences between patients with acromegaly and control subjects. Forward stepwise multiple linear regression analyses were performed to examine the variables independently associated with atherogenic risk factors and biomarkers of cardiovascular disease. In these analyses, GH and HOMA were always regarded as independent variables while triglycerides, HDL-C, LDL-C, nonesterified fatty acids, CETP activity, ox-LDL, endothelin-1, resistin, VCAM-1 and CRP were alternatively considered as the dependent variable and the rest as independent ones. Residuals of every regression model were normally distributed as evaluated by the Shapiro–Wilk test. Differences were significant at $P < 0{\cdot}05$ in the bilateral situation. For statistical analysis, INFOSTAT (Grupo INFOSTAT, Universidad Nacional de Córdoba, Argentina) and SPSS 17·0 (Chicago, IL, USA) statistic software were used.

Results

In the present study, 22 patients with active acromegaly were studied in comparison with 22 sex- and age-well-matched control subjects. Clinical characteristics, hormonal parameters and biomarkers of insulin resistance from patients with acromegaly and control subjects are shown in Table 1. In accordance with the well-known physical features of subjects with acromegaly, BMI and waist circumference were significantly increased in the patient group. Given this difference between both studied groups, all the results obtained were compared performing analysis of covariance, including log-transformed BMI as a covariate. Deriving from the inclusion criteria, mean or median GH, IGF-I and IGFBP-3 concentrations were also significantly elevated in the group of patients with active acromegaly. Furthermore, all the markers of insulin resistance, including nonesterified fatty acids, were significantly higher in patients with acromegaly than in control subjects, except for resistin that showed no difference.

Patients with acromegaly presented a more atherogenic lipoprotein profile than control subjects, consistent of significantly higher triglyceride, LDL-C and apo B levels (Table 2). Moreover, CETP activity, responsible for modulating lipoprotein composition in plasma, was significantly increased in patients with acromegaly (179 ± 27 *vs* 138 ± 30%/ml/h, $P < 0{\cdot}01$).

Table 3 shows different biomarkers of cardiovascular disease from patients with acromegaly and control subjects. Oxidized-LDL, a pro-inflammatory and pro-atherogenic biomarker, and endothelin-1, the most potent constrictor of human vessels, were significantly increased in acromegaly. On the other hand, VCAM-1, a cell adhesion molecule of endothelial location that actively participates in the firm adhesion and extravasation of circulating leucocytes into the artery wall, showed no differences

Table 1. Clinical characteristics, hormonal parameters and biomarkers of insulin resistance from patients with acromegaly and control subjects

	Patients with acromegaly	Control subjects	*P*
n	22	22	–
Women/men	16/6	16/6	–
Age (years)	44 ± 14	44 ± 13	0·3066
BMI (kg/m^2)	28 (27–31)	22 (20–24)	<0·0001
Waist (cm)	95 ± 10	86 ± 8	0·0025
GH (μg/l)	7·9 (5·8–35)	1·1 (0·2–5·3)	<0·0001
IGF-I (nM)	93 (81–122)	20 (18–24)	<0·0001
IGFBP-3 (mg/l)	7·6 ± 1·63	4·4 ± 0·9	<0·0001
Glucose (mM)	5·4 ± 0·6	4·8 ± 0·6	0·8818
Insulin (pM)	132 (68–197)	42 (29–60)	0·0008
HOMA	4·4 (2·1–6·7)	1·3 (0·8–1·8)	0·0014
NEFA (mM)	0·69 ± 0·2	0·54 ± 0·2	0·0197
Resistin (μg/l)	7·9 (4·6–10)	5·4 (4·6–9·6)	0·6164

BMI, body nass; GH, growth hormone; IGF-I, insulin-like growth factor I; IGFBP-3, IGF binding protein-3; HOMA, homoeostasis model assessment. NEFA: nonesterified fatty acids Results were expressed as mean ± SD or as median (IQR), depending on data distribution. Differences were tested by ANCOVA with log-transformed BMI as fixed variable, except for age and waist that were analysed by Student's t-test with no fixed variable and for BMI for which Mann–Whitney U-test was employed.

Table 2. Lipoprotein profile from patients with acromegaly and control subjects

	Patients with acromegaly (n = 22)	Control subjects (n = 22)	*P*
TG (mM)	1·2 (1·1–1·6)	0·9 (0·6–1·1)	0·0307
TC (mM)	5·2 ± 1·0	5·0 ± 1·0	0·5210
LDL-C (mM)	3·5 ± 0·9	3·0 ± 0·7	0·0179
HDL-C (mM)	1·2 ± 0·4	1·5 ± 0·4	0·084
Apo A-I (g/l)	1·4 ± 0·3	1·5 ± 0·2	0·3967
Apo B (g/l)	0·98 ± 0·23	0·77 ± 0·22	0·0184

TG, triglycerides; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; apo, apolipoprotein. Results were expressed as mean ± SD or as median (IQR), depending on data distribution. Differences were tested by ANCOVA with log-transformed BMI as fixed variable.

Table 3. Biomarkers of cardiovascular disease from patients with acromegaly and control subjects

	Patients with acromegaly (n = 22)	Control subjects (n = 22)	*P*
Oxidized-LDL (U/l)	120 ± 22	85 ± 19	0·0268
Endothelin-1 (ng/l)	0·90 ± 0·23	0·72 ± 0·17	0·0489
VCAM-1 (μg/l)	37·8 ± 13·3	38·0 ± 7·4	0·5165
CRP (mg/l)	0·25 (0·10–0·92)	0·85 (0·36–1·40)	0·0251

VCAM-1, vascular cell adhesion molecule 1; CRP, C reactive protein. Results were expressed as mean ± SD or as median (IQR), depending on data distribution. Differences were tested by ANCOVA with log-transformed body mass index as fixed variable.

Table 4. Multiple linear regression analysis for the association of TG, HDL-C, NEFA, CETP activity, LDL-C, ox-LDL and Endothelin-1 as dependent variables

Dependent variable	Significant independent variable	*B*	*t*	Significance <	R^2
TG	Model 1: HOMA	0·52	3·8	0·01	0·25
	Model 2: HOMA	0·36	2·4	0·05	0·32
	GH	0·33	2·2	0·05	
LDL-C	GH	0·44	3·1	0·01	0·18
HDL-C	HOMA	−0·59	−4·55	0·0001	0·34
NEFA	Model 1: TG	0·48	3·30	0·005	0·21
	Model 2: TG	0·54	3·86	0·0001	0·29
	HDL-C	0·31	2·27	0·05	
CETP Activity	Model 1: HOMA	0·55	3·6	0·001	0·28
	Model 2: HOMA	0·57	4·0	0·0001	0·37
	Resistin	0·34	2·4	0·05	
Ox-LDL	Model 1: GH	0·69	4·1	0·0001	0·45
	Model 2: GH	0·58	4·5	0·0001	0·70
	BMI	0·52	4·1	0·001	
Endothelin-1	GH	0·46	2·7	0·01	0·19

TG, triglycerides; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; NEFA, nonesterified fatty acids; CETP, cholesteryl ester transfer protein; ox-LDL, oxidized-LDL; GH, growth hormone; HOMA, homoeostasis model assessment; BMI, body mass index. In each regression model, the candidate-independent variables, except when considered as dependent variable, were BMI, HOMA, GH, TG, NEFA, LDL-C, HDL-C, CETP activity, oxLDL, endothelin-1, resistin, VCAM-1 and CRP. Analyses carried out considering resistin, VCAM-1 and CRP as dependent variables were not shown given that results obtained were not statistically significant.

between both groups, while CRP levels were significantly reduced in acromegalic patient with acromegaly.

Multiple linear regression analyses were carried out to identify independent predictors of the atherogenic risk factors and biomarkers of cardiovascular disease (Table 4). When triglyceride levels were evaluated, HOMA explained a 25% of its variability, while HOMA and GH together explained a 32%. In the case of HDL-C and nonesterified fatty acids, only HOMA and triglycerides appeared to be independently associated, respectively. The variability of CETP activity was attributed in a 28% to HOMA and in a 37% to both HOMA and resistin. GH was the only independent predictor of LDL-C and endothelin-1 (18% and 19%, respectively). Finally, in ox-LDL analysis, GH alone and GH with BMI were identified as the independent predictors explaining 45% and 70%, respectively, while no significant associations were detected for VCAM-1 or CRP.

Discussion

Acromegaly is a progressive chronic disease associated with high risk of cardiovascular disease[4] whose severity, according to our point of view, could be mainly attributed to the complex interaction between hormonal and metabolic disturbances present in affected patients. In fact, alterations in GH axis are most frequently associated with insulin resistance,[14] two situations

independently related to the development of cardiovascular disease. Our main findings point out that patients with acromegaly show an increase in triglyceride levels and CETP activity and a tendency towards lower values in HDL-C concentration mainly predicted by the insulin-resistant marker HOMA. Moreover, the increment observed in LDL-C, ox-LDL and endothelin-1 levels would be mostly associated with GH excess, independent of other metabolic parameters.

It has been well documented that GH hypersecretion and insulin resistance are closely interconnected.[15] Accordingly, in the present study, patients with acromegaly showed significantly higher insulin levels, as well as HOMA index than healthy controls. GH is known to have different metabolic effects on the adipose tissue, the liver and the skeletal muscle with focus on lipid and carbohydrate metabolism, which are attributed to a direct mechanism carried out by GH. The effects of GH on carbohydrate metabolism are supposed to be mediated indirectly via the antagonism of insulin action, while GH action on somatic growth is mainly mediated by IGF-I. Nevertheless, the exact underlying mechanisms are not completely understood.[16,17]

Given the controversial data available on the role of resistin in the development of insulin resistance, this parameter was evaluated in the studied population, and neither statistically significant difference nor a relationship with GH was found in this study. Accordingly, Silha *et al.*[18] also observed similar resistin levels in patients with acromegaly and control subjects. Although some studies showed an increase in resistin concentration in obesity and type 2 diabetes, most reports did not detect any correlation between resistin and BMI or markers of insulin resistance.[19]

The causal coexistence of GH excess and insulin resistance has been clearly established.[20–22] However, it is still ignored whether the multiple metabolic abnormalities present in patients with acromegaly are attributed to GH hypersecretion, to insulin resistance or to a combination of both of them.

As it has been previously shown,[2] the group of patients with acromegaly evaluated in this study showed significantly increased triglyceride and apo B levels and lower HDL-C concentration, the so called 'atherogenic dyslipidemia' characteristic of insulin-resistant states.[23] Actually, in multivariate analysis, HOMA index was able to independently predict the variations in triglyceride and HDL-C levels, as well as in CETP activity, which was also higher in patients than in controls.

The increment detected in triglyceride concentration could be due to the increased flux of free fatty acids from adipose tissue, mainly of visceral localization, to the liver which may be, in turn, attributed to the high activity of hormone-sensitive lipase in insulin resistance.[17] It is very well-known that in a frame of insulin resistance, the liver employs these free fatty acids for triglyceride synthesis and that high activity of microsomal transfer protein enables their assembly in triglyceride-rich very low-density lipoprotein (VLDL) particles, afterwards poured into the circulation.[24] Even if multivariate analysis pointed out HOMA as the most powerful predictor of triglyceride levels, GH effects cannot be discarded. In adipose tissue, GH activates hormone-sensitive lipase.[25] In the liver, GH stimulates free fatty acid uptake by inducing lipoprotein lipase/hepatic lipase expression, it promotes lipogenesis and it inhibits both lipolysis and fatty acid oxidation.[17] Overall, these actions facilitate the intrahepatic storage of triglycerides. Then, GH could also contribute to VLDL assembly by upregulating the expression of microsomal transfer protein.[26]

In nonacromegalic subjects, insulin resistance has been largely shown to be implicated not only in triglyceride increase but also in the induction of CETP activity and in the reduction in HDL-C levels.[27] Beyond insulin resistance, it is noteworthy that these three parameters, 'triglycerides', 'CETP activity' and 'HDL-C', are closely interconnected. Triglyceride levels are known to upregulate CETP activity that is responsible for interchanging triglycerides and cholesteryl esters between apo B-containing lipoproteins and HDL, thus adding to HDL-C diminution. Furthermore, in hypertriglyceridemia, HDL particles have been shown to be triglyceride enriched and less efficient in the promotion of cell cholesterol efflux, which finally contributes to reduce HDL cholesterol content.[28]

Among the different atherogenic risk factors and biomarkers of cardiovascular disease evaluated in this study, LDL-C, ox-LDL and endothelin-1 levels were significantly elevated in patients with acromegaly in comparison with healthy controls. Interestingly, these three parameters were independently predicted by GH levels and not by HOMA. On the other hand, CRP levels were significantly lower in patients with acromegaly, finding that was previously reported by Andreassen *et al.*[29]

The increase in ox-LDL levels has been already reported in patients with acromegaly,[13] and we now report that this increase is independently predicted by GH concentration. In the literature, there is weak support for GH to stimulate oxidative stress directly. Nevertheless, it must be taken into consideration that patients with acromegaly showed hypercholesterolaemia, which is known to be associated with oxidative stress.[30] Then, Yarman *et al.*[31] found higher thiobarbituric acid reactive substances (TBARS) levels in a group of newly diagnosed patients with acromegaly, and Andersson *et al.*[32] associated GH overexpression with a time- and vessel-specific deterioration in endothelial function, initially caused by increased oxidative stress in GH transgenic mice. Moreover, biochemical studies showed that ceruloplasmin, which was found to be elevated in patients with acromegaly,[13] is a potent catalyst of LDL oxidation *in vitro*.

The other novel biomarker of cardiovascular disease, endothelin-1, which is the most potent vasoconstrictor in humans, has been implicated in atherosclerotic and ischaemic heart disease.[33] Both, our previous results[2] and those from Kirilov *et al.*[34] showed an increase in endothelin-1 in active acromegaly. In the present study, an association between endothelin-1 and GH excess was evidenced independently of other metabolic parameters such as HOMA. Then, it could be assumed that in active acromegaly the GH secretory status would be an important determinant of plasma endothelin-1 level.[2,34]

Besides contributing to the characterization of high risk of cardiovascular disease in patients with acromegaly, results from the present study could also have a great impact on potential

therapeutic management of acromegaly by employing not only specific inhibitors of GH secretion/action, but in combination with insulin sensitizer agents. Nevertheless, confirmation of the above-mentioned independent associations would be necessary by studying models in which GH excess would not be accompanied by insulin resistance. However, up to our knowledge, given the role played by GH in the genesis of insulin resistance, there are no available models to be explored. Another limitation of the present study is the number of patients with acromegaly evaluated, although this is related to the low prevalence and incidence of active acromegaly with absence of other endocrine pathologies, specific therapy or treatment with other drugs known to affect carbohydrate or lipid metabolism.

In conclusion, in patients with active acromegaly, GH excess clearly contributes to the development of an insulin-resistant state and the complex interaction between both disturbances would be responsible for the appearance of an atherogenic cluster containing pro-oxidative and pro-inflammatory factors and markers. From our results, insulin resistance would be preferably associated with an atherogenic lipoprotein profile (high triglyceride and low high-density lipoprotein-cholesterol levels) and to high cholesteryl ester transfer protein activity, while high GH levels would independently predict the increase in low-density lipoprotein-cholesterol, ox- low-density lipoprotein and endothelin-1 levels in patients with acromegaly.

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Competing interests/financial disclosure

The authors have nothing to disclose.

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