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GH LEVELS AND INSULIN SENSITIVITY ARE DIFFERENTLY ASSOCIATED WITH BIOMARKERS OF CARDIOVASCULAR DISEASE IN ACTIVE ACROMEGALY.

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Abstract

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

Objective: to analyze the associations of GH and HOMA with biomarkers of cardiovascular disease and to compare the above mentioned variables between patients with active acromegaly and control subjects.

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 controls subjects.

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

Results: patients presented higher triglyceride (1.22[0.69-5.73]*vs*0.86[0.30-1.39]mmol/L, p<0.001), LDL-C (3.5±0.9*vs*3.0±0.7mmol/L, p<0.01), apoB (0.98±23*vs*0.77±22 g/L, p<0.01), free fatty acid (0.69±0.2*vs*0.54±0.2nmol/L, p<0.05), oxidized-LDL (120±22*vs*85±19 U/L, p<0.001), endothelin-1 (0.90±0.23*vs*0.72±0.17 ng/L, p<0.05) and lower HDL-C (1.2±0.4*vs*1.5±0.4mmol/L, p<0.01) levels, and higher CETP activity (179±27*vs*138±30 $\%ml^{-1}.h^{-1}$, p<0.001) than control subjects. VCAM-1 and hsCRP concentrations were not different. By multiple regression analyses, HOMA explained the variability of triglycerides (25%), HDL-C (30%), and CETP activity (28%), while GH independently predicted LDL-C (18%), oxidized-LDL (40%) and endothelin-1 levels (19%).

Conclusions: in active acromegalic patients, GH excess contributes to the development of an insulin resistant state and the interaction between both disturbances would be responsible for the appearance of atherogenic prooxidative and proinflammatory factors and markers. Insulin resistance would be preferably associated to 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 levels in acromegaly.

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 acromegalic patients [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 of not only glucose but also 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 acromegalic patients beyond GH increment.

The specific consequences of insulin resistance in acromegaly are not completely known. In non-acromegalic patients, lipid abnormalities, such as increased non-esterified 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 hyperglycemia [7].

On the other hand, resistin, a peptide secreted by adipocytes and inflammatory cells, has been shown to be increased in patients with insulin resistance and early type 2 diabetes, populations at high risk for the development of 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 analyze the associations of atherogenic risk factors and biomarkers of cardiovascular disease with markers of insulin resistance and parameters of the GH axis in a cross-sectional study. Secondly, we aimed to compare the above mentioned variables, including resistin levels, between a group of active acromegalic patients 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 Service, Hospital de Clínicas "José de San Martín", Buenos Aires, Argentina, during a period of 24 months. Patients were included in the present study when presenting typical clinical features, lack of suppression of GH during an oral glucose tolerance test (OGTT) and elevated insulin-like growth factor-1 (IGF-1) level for corresponding sex and age. Disease duration ranged between 2 and 10 years. Twenty two healthy subjects, sex- and age-matched 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. None of the patients or control subjects was diabetic and only 3 acromegalic patients presented impaired glucose tolerance, evidenced by the OGTT. 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-hour overnight fast, venous blood was drawn from the antecubital vein. Aliquots were collected in clean tubes. Samples were centrifuged at 1500 x g, for 15 minutes; at 4º C. Serum was immediately employed for glucose and stored at 4º C for lipid, lipoprotein and non-esterified fatty acids measurements within 24 hours. Serum and plasma aliquots were also stored at -70º C for determination of GH, IGF-1, insulin growth factor binding protein-3 (IGFBP-3), insulin, resistin,

cholesteryl ester transfer protein (CETP), oxidixed (ox) low density lipoprotein (LDL), endothelin-1, vascular cell adhesion molecule (VCAM)-1and 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 phosphotungstic acid in the presence of magnesium ions [11]. Within-run and between-day precisions (CV) were 3.2 % and 3.8 %, respectively. Laboratory bias was -2.0 %. Apo A-I and apo B were evaluated by immunoturbidimetry (Roche Diagnostics Mannheim, Germany) in a Hitachi 917 autoanalyzer. Within-run and between-day precisions (CV) for apo A were 1.9% and 2.4% and for apo B were 1.2 % and 2.1 %, respectively. Non-esterified fatty acids were determined by a colorimetric method (Randox Laboratories Ltd.UK).

Hormonal Parameters:

Serum GH was measured by the ultrasensitive immunochemiluminometric assay (Access®, Beckman Coulter TM, 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-1 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 20 ng/ml and 0.1 µg/ml. Within-run and between-day precisions (CV) for IGF-1 were below 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 (MEIA) (ABBOTT, Japan). Within-run and between-day precisions (CV) were 2.9 % and 4.4%, respectively. Homeostasis model assessment (HOMA) was calculated as [Glucose (mmol/L)x Insulin (U/ml)]/22.5.

Resistin:

Resistin plasma levels were determined by monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) following the manufacturer` s instructions (R & D Systems, USA). Sample levels were calculated by analyzing standards with known concentrations of recombinant molecules coincident with samples and plotting of signal *vs.* concentration. Within-run and between-day precisions (CV) were less than 5.3 % and less than 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 labeled HDL_3 (^{3}H-CE-HDL_3) (NEN Life Science Products, Boston, USA) to serum apo B-containing lipoproteins was evaluated. Samples were incubated with ^{3}H-CE-HDL_3 (50 µmol/L cholesterol) and 1.5 mmol/L iodoacetate for 3h, at 37° C. After incubation, lipoproteins were separated by a selective precipitation method employing 40 g/L 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). 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 which employs the monoclonal antibody 4E6 (Mercodia AB, Uppsala, Sweden). Measurements of ox LDL were all carried out 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, USA). 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, USA). 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 Mannheim, Germany) in a Hitachi 917 autoanalyzer. Within-run and between-day precisions (CV) were 0.4 % and 3.4 %, respectively.

Data and Statistical Analysis:

The sample size required to detect significant differences between groups was estimated for the main parameters at a significance level of 0.05 and 80% power. Data distribution was tested employing the Shapiro-Wilk test. Results were expressed as mean ± standard deviation (SD) for normally distributed data and as median [range] for skewed data. In this last case, data were normalized by log-transformation. Then, analysis of covariance was used including body mass index (BMI) as a covariate. Partial correlations were performed between different parameters including BMI as a fixed variable. Multiple linear regression analysis was performed to examine the variables independently associated with atherogenic risk factors and biomarkers of cardiovascular disease. In these analyses, GH and HOMA were always included as independent variables while triglycerides, HDL-C, LDL-C, non-esterified fatty acids, CETP, ox LDL, endothelin-1, resistin, VCAM-1 and CRP were alternatively included as the dependent variable and the rest as independent ones. Differences were considered significant at $p< 0{\cdot}05$ in the bilateral situation. For statistical analysis, INFOSTAT software was 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 acromegalic patients and control subjects are shown in table I. 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 BMI as a covariate. Deriving from the inclusion criteria, mean or median GH, IGF-1 and IGFBP-3 concentrations were also significantly elevated in the group of patients with active acromegaly. Furthermore, all the markers of insulin resistance, including non-esterified fatty acids, were significantly higher in acromegalic patients than in control subjects, except for resistin which showed no difference.

Acromegalic patients presented a more atherogenic lipoprotein profile than control subjects, consisting of significantly higher triglyceride (1.22 [0.69-5.73] *vs*. 0.86 [0.30-1.39] mmol/L, p <0.001), LDL-C (3.5±0.9 *vs*. 3.0±0.7 mmol/L, p <0.01) and apo B (0.98±0.23 *vs*. 0.77±0.22 g/L, p <0.01) levels, and lower HDL-C (1.2±0.4 *vs*. 1.5±0.4 mmol/L, p <0.01) concentration (Figure 1). Moreover, CETP activity, responsible for modulating lipoprotein composition in plasma, was significantly increased in acromegalic patients (179±27 *vs*. 138±30 %.ml^{-1}.h^{-1}, p <0.001).

Table II shows different biomarkers of cardiovascular disease from acromegalic patients 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, and CRP, which has been implicated in multiple aspects of atherogenesis and plaque vulnerability, showed no differences between both groups.

When analyzing the associations between GH with different atherogenic risk factors and biomarkers of cardiovascular disease evaluated in the present study, direct correlations were observed with triglycerides, LDL-C, oxLDL, endothelin-1 and CETP activity, though the latter did not reach

statistical significance ($p < 0.06$) (Table III). On the other hand, HOMA was positively associated with triglycerides, CETP activity and showed a non-significant tendency with oxLDL ($p < 0.06$), while it also presented a negative correlation with HDL-C.

Multiple regression analyses were carried out in order to identify independent predictors of the atherogenic risk factors and biomarkers of cardiovascular disease (Table IV). 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 non-esterified 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 to insulin resistance [13], two situations independently related to the development of cardiovascular disease. Our main findings point out that acromegalic patients show an increase in triglyceride levels and CETP activity and a reduction 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 [14]. Accordingly, in the present study, acromegalic patients showed significantly higher glucose and insulin levels, as well as HOMA index than healthy controls. In fact, GH has potent effects on intermediary metabolism and, in particular, it has the capacity to antagonize insulin actions. However, the underlying mechanisms are not completely understood. One possible explanation could be the increase in free fatty acid flux from the adipose tissue, also observed in acromegalic subjects evaluated in this study, which could, in turn, impair insulin action at target tissues [15,16]. Given the controversial data available on resistin role in the development of insulin resistance, this parameter was evaluated in the studied population and neither statistically significant difference nor a relationship with GH were found in this study. Accordingly, Silha et al. also observed similar resistin levels in acomegalic patients and control subjects [17]. 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 body mass index or insulin resistance [18].

The causal coexistence of GH excess and insulin resistance has been clearly established [19-21].However, it is still ignored whether the multiple metabolic abnormalities present in acromegalic patients are due to GH hypersecretion, to insulin resistance or to a combination of both of them.

As it has been previously shown [2], the group of acromegalic patients evaluated in this study showed significantly increased triglyceride and apo B levels, and decreased HDL-C concentration, the so called "atherogenic dyslipidemia" characteristic of insulin-resistant states [22]. 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 (HSL) in insulin resistance [23]. 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 HSL [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 [23]. 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 non-acromegalic 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 of HDL-C levels [27]. Beyond insulin resistance, it is noteworthy that these three parameters, "triglycerides", "CETP" and "HDL-C", are closely interconnected. Triglyceride levels are known to upregulate CETP which 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, oxidized LDL and endothelin-1 levels were significantly elevated in

acromegalic patients in comparison to healthy controls. Interestingly, these three parameters were independently predicted by GH levels and not by HOMA.

The increase in oxLDL levels has been already reported in acromegalic patients [29] 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 acromegalic patients showed hypercholesterolemia, which is known to be associated to oxidative stress [30]. Then, Yarman et al. [31] found higher thiobarbituric acid reactive substances (TBARS) levels in a group of newly diagnosed acromegalic patients 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 acromegalic patients [29], 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 ischemic heart disease [33]. Both, our previous results [2] and those from Kirilov et al. [34] showed and 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 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 acromegalic patients, 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 is the number of acromegalic patients studied, though this is related with the low prevalence 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 active acromegalic patients, 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 prooxidative and proinflammatory factors and markers. From our results, insulin resistance would be preferably associated to an atherogenic lipoprotein profile (high triglyceride and low HDL-C levels) and to high CETP activity, while high GH levels would independently predict the increase in LDL-C, ox LDL and endothelin-1 levels in acromegalic patients.

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Figure Legends

Fig. 1: Lipid, lipoprotein and apolipoprotein profile from acromegalic patients (n = 22) and control subjects (n = 22). Results were expressed as Mean±SD. TG, triglycerides; TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein.

[a] $p<0.0001$; [b] $p<0.01$; [c] $p<0.05$ *vs.* acromegalic patients.

Figure. 1

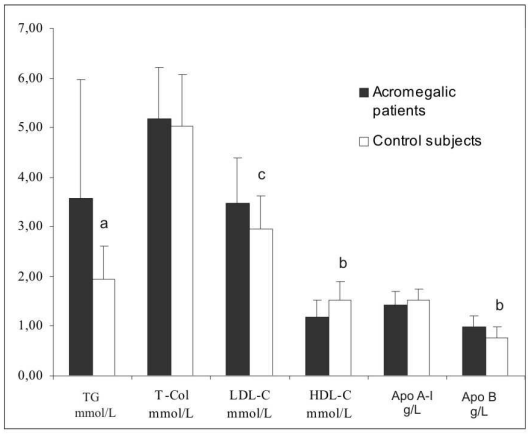

TABLE I: Clinical characteristics, hormonal parameters and biomarkers of insulin resistance from acromegalic patients and control subjects.

	Acromegalic Patients	Control Subjects
N	22	22
Women/Men	16/6	16/6
Age (years)	44±14	44±13
B.M.I. (kg/m^2)	28 (21-43)	22 (19-28)[a]
Waist (cm)	95±10	86±8[a]
GH (µg/L)	7.9 (1.9-55.0)	1.07 (0.05-8.6)[a]
IGF-1 (nmol/L)	92.8 (35.6-142.3)	20.5 (10.0-52.0)[a]
IGFBP-3 (mg/L)	7.6±1.63	4.4 (2.2-6.2)[a]
Glucose (mmol/L)	5.4±0.6	4.8±0.6 [b]
Insulin (pmol/L)	132.4 (40.2-391.8)	42.3 (13.6-122.7)[a]
HOMA	4.4 (1.1-13.5)	1.3 (0.5-3.8)[a]
NEFA (nmol/L)	0.69±0.2	0.54±0.2 [c]
Resistin (µg/L)	7.9 (2.7-15.8)	5.4 (2.7-33.6)

B.M.I, Body Mass; GH, Growth Hormone; IGF-1, Insulin-like Growth Factor I; IGFBP-3, IGF Binding Protein-3; HOMA, Homeostasis Model Assessment. NEFA: Non-Esterified Fatty Acids Results were expressed as mean±S.D. or as median (range), depending on data distribution.

[a] $p<0.0001$ [b] $p<0.01$ and [c] $p<0.05$ *vs.* acromegalic patients.

TABLE II: Biomarkers of cardiovascular disease from acromegalic patients and control subjects.

	Acromegalic patients (n =22)	Control subjects (n =22)
Oxidized LDL (U/L)	120±22	85±19[a]
Endothelin-1 (ng/L)	0.90±0.23	0.72±0.17[b]
VCAM-1 (µg/L)	37.8±13.3	38.0±7.4
CRP (mg/L)	0.25 (0.10-22.70)	0.85 (0.30-10.20)

VCAM-1, vascular cell adhesion molecule 1; CRP, C reactive protein. Results were expressed as mean±S.D. or as median (range), depending on data distribution.

[a] $p<0.001$; [b] $p<0.05$ *vs.* acromegalic patients.

Table III: Correlations of GH and HOMA with different parameters in acromegalic patients and controls subjects (n=44).

	GH r (p)	HOMA r (p)
TG	0.49 (<0.001)	0.35 (<0.05)
LDL-C	0.44 (<0.01)	0.04 (0.78)
HDL-C	-0.12 (0.45)	-0.54 (<0.001)
NEFA	0.13 (0.40)	0.13 (0.39)
CETP	0.33 (0.06)	0.44 (<0.01)
Ox LDL	0.72 (<0.0001)	0.41 (0.06)
Endothelin-1	0.43 (<0.05)	0.31 (0.09)

GH, Growth Hormone; HOMA, Homeostasis Model Assessment; TG, triglycerides; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; NEFA, non-esterified fatty acids; CETP, cholesteryl ester transfer protein; ox LDL, oxidized LDL. Correlations were performed including body mass index as fixed variable.

Table IV: Multiple regression analysis for the association of TG, HDL-C, NEFA, CETP, LDL-C, ox LDL and Endothelin-1 as dependent variable.

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**	-0.56	-4.28	0.0001	0.30
NEFA	**TG**	0.31	2.04	0.05	0.07
CETP	**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, non-esterified fatty acids; CETP, cholesteryl ester transfer protein; ox LDL, oxidized LDL; GH, Growth Hormone; HOMA, Homeostasis Model Assessment; BMI, body mass index.

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