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CONDITIONING FACTORS FOR HIGH CARDIOVASCULAR RISK IN PATIENTS WITH CUSHING'S SYNDROME

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Abstract

Objectives: To characterize the alterations in carbohydrate and lipoprotein metabolism, to evaluate markers of lipoprotein functionality and to identify the presence of novel atherogenic risk factors in patients with Cushing's Syndrome (CS) in comparison to sex and age-matched controls.

Patients and Methods: In an open cross-sectional study, thirty-two non-treated patients with active CS were consecutively recruited from the Endocrinology Service at "José de San Martín" Clinical Hospital, University of Buenos Aires, Argentina, between April 11, 2010 and December 11, 2012. The patients were compared with sex- and age-matched controls.

Results: Patients with CS *vs.* controls presented excess weight, central obesity and hypercortisolism. They also exhibited an insulin-resistant state, with high resistin levels [Median (interquartile range); 16(10-22)*vs*.6(5-9) ng/ml, p<0.0001], a more atherogenic lipoprotein profile, high oxidized low density lipoprotein (oxLDL; Mean±SD; 100±31 *vs*. 75±32 U/l, p<0.05) and high sensitive C reactive protein levels [1.2(0.6-3.1)*vs*.0.6(0.3-1.1) mg/l, p < 0.05], and increased leukocyte count (9.5±2.6 *vs.* 6.5±1.4.10^3 cells/µl, p<0.0001). Multivariate analyses showed that the increase in waist circumference was associated with both the diagnosis of CS and the degree of insulin resistance. Resistin concentration was related to a greater extent to the diagnosis of CS than to HOMA-IR. Triglyceride and oxLDL levels were only significantly associated with the diagnosis of CS.

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Conclusions: Hypercortisolism would be related to the increase observed in triglycerides and oxLDL levels, and, in combination with insulin resistance, would act to increase the waist circumference and to amplify the inflammatory process, key factors for the development of cardiovascular disease.

Abbreviations

Apo = apolipoproteins; **ARE =** arylesterase; **C =** cholesterol **; CETP =** cholesteryl ester transfer protein ; **CRP =** c-reactive protein; **CS =** Cushing's syndrome ; **CVD =** cardiovascular disease; **HDL =** high density lipoprotein; **HOMA =** homeostasis model assessment ; **LDL =** low density lipoprotein ; **LpPLA 2 =** lipoprotein associated phospholipase A2; **OxLDL =** oxidize LDL; **PON =** paraoxonase; **QUICKI =** quantitative sensitivity check index ; **TG =** triglycerides; **VLDL =** very low density lipoprotein.

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Introduction

Cushing's Syndrome (CS) is the result of a constant and inappropriate increase in circulating glucocorticoids. The mortality rate in patients with CS is four times higher than that in the general population. This higher mortality rate is mainly attributed to fatal cardiovascular events (1). This situation is similar to diabetes, which is directly considered a coronary heart disease equivalent (2). Nevertheless, conditioning factors for cardiovascular disease in CS patients have been incompletely explored.

Endothelial dysfunction, which is an early event in atherosclerosis, has been reported in patients with CS and it has been shown to reverse after pharmacological treatment or surgery (3,4). Although these results suggest a direct effect of hypercortisolism, the involvement of other atherogenic factors classically associated with CS, such as central obesity, hypertension, glucose intolerance, hyperlipemia, impaired adipocytokine secretion and a prothrombotic state, cannot be discarded (5,6). These clinical and metabolic disturbances are closely interconnected and intimately associated with insulin resistance and metabolic syndrome, which have been largely demonstrated to be a risk condition for diabetes and atherosclerotic cardiovascular disease (7).

Excessive adipose tissue of visceral localization, an important risk factor for cardiovascular disease in the general population (8), is able to secrete diverse hormones and adipocytokines, such as adiponectin, leptin and resistin (9,10). Adiponectin is an adipocytokine inversely related to abdominal obesity and insulin resistance. Interestingly, it has been shown that cortisol directly inhibits the secretion of adiponectin, the main antiatherogenic and anti-inflammatory adipocytokine (11). Accordingly, Barahona et al. (12) showed that patients with CS have low adiponectin levels, which were not corrected by

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surgery. However, these results are controversial given that other authors have reported similar adiponectin concentrations in patients and controls (11,13). Resistin, another adipocytokine initially associated with the development of insulin resistance in humans, is secreted not only by adipocytes, but also by circulating mononuclear cells and macrophages (14,15). This adipocytokine would be also susceptible to be modified in patients with CS.

It is remarkable that most clinical studies have investigated the high rate of cardiovascular events in patients with CS in relation to the disorder duration, the presence of hypertension and the patients´ age, and only a few have explored other traditional and emergent atherogenic risk factors (16-19). In patients with CS, it would be highly interesting to have information about lipoprotein-associated proteins and enzymes, such as: a) cholesteryl ester transfer protein (CETP), which interchanges triglycerides (TG) by cholesteryl esters among lipoproteins and is considered proatherogenic when it is increased; b) paraoxonase (PON), an antioxidant enzyme bound to high density lipoprotein (HDL); and lipoprotein-associated phospholipase A_2 (Lp-PLA_2), considered a specific marker of vascular inflammation (20).

The aim of the present study was to characterize the alterations in carbohydrate and lipoprotein metabolism, to evaluate markers of lipoprotein functionality and to identify the presence of novel atherogenic risk factors and biomarkers of cardiovascular disease in patients with active CS in comparison to sex- and age-matched controls.

Materials and methods

Forty-eight patients with active CS were consecutively recruited from the Department of Endocrinology at "José de San Martín" Clinical Hospital, University of Buenos Aires,

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Argentina, between April 11, 2010 and December 11, 2012. CS was diagnosed according to standard criteria previously published in a consensus statement (21). Briefly, these criteria included the presence of typical symptoms and signs, such as weight gain and central fat deposition, supraclavicular or cervical fat pad, fatigue, muscle wasting, hirsutism, acne, headache, purple striae distensae, easy bruisability, hypertension and/or diabetes mellitus. Endogenous hypercortisolism was demonstrated by high 24 h urinary free cortisol on at least two occasions [median (range), 210 (123-1020) µg/24 h; normal value < 90 µg/24 h] and failure of serum cortisol to decrease below 1.8 µg/dl after low-dose dexamethasone administration. ACTH levels in patients with Cushing disease were 75 (45-154) pg/ml. Exogenous intake of oral, parenteral, inhaled, or topical corticosteroids was discarded. Patients also fulfilled the following inclusion criteria: 1) absence of specific treatment for hypercortisolism; 2) absence of deficiency or overexpression of any other pituitary hormone; 3) normal thyroid function evaluated by plasma levels of thyroid-stimulating hormone and free thyroxine and clinical examination of the thyroid gland; 4) normal renal function evaluated by plasma levels of urea and creatinine, and proteinuria; 5) normal hepatic function evaluated by biochemical hepatic parameters and absence of hepatomegaly confirmed by clinical examination; and 6) lack of personal history of cardiovascular disease. Special care was taken to exclude subjects with additional causes of cardiovascular risk such as excessive tobacco (>10 cigarettes/day) or ethanol consumption (>30 g/day), therapy with antioxidants or with drugs that could affect lipoprotein or carbohydrate metabolism. Individuals presenting infectious processes or under acute stressing situations were also excluded. Of the 48 patients initially selected, 32 were finally included. Only one patient presented an adrenal tumor and the others pituitary tumors. Ten patients were newly diagnosed with type 2 diabetes according to the American Diabetes Association criteria. The patient group was compared with age- (± 3

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years) and sex-matched apparently healthy controls (n=32) recruited from the Department of Hemotherapy at "José de San Martín" Clinical Hospital, University of Buenos Aires, among voluntary blood donors who accepted to participate in the present study. Control subjects also fulfilled the inclusion criteria mentioned above. All participants in this study gave informed written consent and the protocol was approved by the Ethics Committee from the School of Pharmacy and Biochemistry, University of Buenos Aires (Res. Nº 2641).

Body weight, height, waist circumference and blood pressure were recorded. After a 12-hour overnight fast, venous blood was drawn between 8:00 and 9:00 am, after the subject remained sitting for at least 20 minutes. Whole blood was used for a complete blood count and the rest was centrifuged at 1500 x g, for 15 minutes, at 4º C. Serum was immediately used for glucose determination and stored at 4º C for lipid and lipoprotein measurements within 24 hours. Serum aliquots were also stored at -70º C for determination of cortisol, insulin, non-esterified fatty acids, resistin, adiponectin, apolipoproteins (apo) A-I and B, oxidized LDL (oxLDL) levels, and for CETP, PON 1M, arylesterase (ARE) and Lp-PLA_2 activities.

Glucose levels and complete blood count were determined by standardized methods. Specific immunoassays were used to evaluate cortisol (Access®, Beckman Coulter™, USA) and insulin (RIA, Diagnostics Products Corp., Los Angeles, CA, USA) plasma concentrations. The HOMA-IR (Homeostasis Model Assessment) [glucose (mmol/l)×insulin (μU/ml)/22.5] and QUICKI (Quantitative Sensitivity Check Index) [1/[ln glucose (mmol/l)+ln insulin (mU/l)]] indexes were calculated. Non-esterified fatty acid levels were determined by a colorimetric method (Randox Laboratories Ltd., UK).

Triglycerides and total cholesterol were quantified by standardized methods (Roche Diagnostics, Mannheim, Germany) in a Hitachi 917 autoanalyzer. Within-run precision (CV_W) for triglycerides and cholesterol was 1.3 % and 1.1 %, respectively, whereas between-

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day precision (CV_B) was 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). CV_W and CV_B for LDL-C 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. CV_W and CV_B for HDL-C were 3.2 % and 3.8 %, respectively. Very low density lipoprotein-cholesterol (VLDL-C) was calculated as the difference between the cholesterol contained in the supernatant after LDL precipitation and the cholesterol contained in the supernatant after precipitation of all apo B-containing lipoproteins, while non-HDL-C was estimated as the difference between total cholesterol and HDL-C. Apo A-I and apo B were evaluated by immunoturbidimetry (Roche Diagnostics Mannheim, Germany) in a Hitachi 917 autoanalyzer. CV_W and CV_B were 1.9% and 2.4% for apo A-I, and 1.2 % and 2.1 % for apo B, respectively. The following ratios were calculated: TG / HDL-C, total cholesterol / HDL-C, LDL-C / apo B, and HDL-C / apo A-I.

CETP activity was determined in serum samples following the general procedure previously described (22). All measurements were carried out in duplicate within the same assay. CV_W for CETP activity was 4.9 %.

Lp-PLA_2 activity was measured following the radiometric assay described by Blank *et al*. (23). All measurements were carried out within the same assay. CV_W for LpPLA_2 activity was 5.1 %.

PON 1 and ARE activities were evaluated using two different substrates: paraoxon (Sigma Chemical Co., St Louis, USA; PON 1 activity) and phenylacetate (Sigma Chemical

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Co., St Louis, USA; ARE activity). Both activities were measured in serum samples following the method of Furlong *et al*. (24). All measurements were carried out within the same assay. CV_W for PON 1 and ARE activities was 4.8 %.

Resistin, adiponectin (R&D, Systems, USA) and oxLDL (Mercodia, Sweden) levels were measured by specific enzymoimmunoassays. C-reactive protein concentration was determined by a Tina-quant CRP (Latex) high sensitive immunoturbidimetric assay, hsCRP, (Roche Diagnostics Mannheim, Germany) in a Hitachi 917 autoanalyzer.

Statistical analysis

Before the study, power analysis was done setting an estimated effect size = 0.7 (based on previous lipid profile results from our laboratory), a chosen power of 80% and a significance level (α) of 0.05. The sample size estimated from such calculations indicated that each group should be constituted by at least 21 subjects. A number of 32 patients achieved a power of 90% and an effect size of 0.9. Parameters following Gaussian distribution were presented as the mean±standard deviation and Student parametric test was used to compare the different groups. For skewed distributed data, the median (interquartile range) expression and the Mann-Whitney non-parametric test were used. The associations between the different parameters were analyzed using Pearson and Spearman tests, depending on the data distribution. Multiple regression tests were also applied and the model tested included age, presence of CS and HOMA-IR as independent variables and waist circumference, triglycerides, resistin, and oxLDL levels as dependent variables. Differences were considered significant at $p<0.05$ in the bilateral situation. The statistical software INFOSTAT® (Córdoba University, Argentina) was used.

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Results

The clinical characteristics, plasma cortisol levels and markers of insulin resistance from the 32 patients with CS and from age- and sex-matched controls are shown in table 1. Most of the patients studied were young (78% were younger than 45 years old), women (70%) and presented abdominal obesity (92%), evidenced by high waist circumference according to the cut off values recently published for the South-American population (25). As expected, patients with active CS exhibited increased cortisol plasma levels and alterations in every marker of insulin resistance, as well as significantly higher resistin concentration (Fig. 1). By contrast, plasma levels of adiponectin showed no differences ($p>0.05$) between groups [10 (7-14) *vs.* 11 (7-15) mg/l].

Table 2 exhibits the lipid, lipoprotein and apolipoprotein profiles. The main differences between groups consisted of higher levels of triglycerides, total cholesterol, non-HDL-C, apo B, and apo A-I, as well as the TG/HDL-C ratio. The HDL-C/apo A-I ratio was significantly lower in patients with CS than in control subjects.

On the other hand, no statistically significant differences (all $p>0.05$) were observed in the activities of the lipoprotein-associated transfer proteins and enzymes studied: CETP (170 ± 35 *vs.* 175 ± 52 %/ml.h, respectively), Lp-PLA_2 (8.2 ± 1.5 *vs.* 8.0 ± 1.9 µmol/ml.h, respectively), PON 1 [302 (151-571) *vs.* 209 (157-453) nmol//ml.min, respectively] and ARE (158 ± 47 *vs.* 148 ± 35 µmol/ml.min, respectively).

Evaluation of oxLDL plasma levels revealed that patients with CS presented significantly higher concentration than control subjects (Fig. 2). Regarding the different inflammatory biomarkers measured, significantly higher values were only detected in hsCRP

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concentration [1.2 (0.6-3.1) *vs.* 0.6 (0.3-1.1) mg/l, $p<0.05$] and leukocyte count (9.5 ± 2.6 *vs.* 6.5 ± 1.4.10^3 cells/µl, $p<0.0001$) in patients relative to the control group.

The associations of cortisol levels and the degree of insulin resistance, evaluated through the HOMA-IR index, with different parameters were analyzed. Cortisol showed significantly positive correlations with triglycerides ($r = 0.32$, $p<0.05$), resistin ($r = 0.52$, $p<0.001$) and oxLDL ($r = 0.51$, $p<0.01$). On the other hand, HOMA-IR was directly associated with waist circumference ($r = 0.43$, $p<0.01$), and resistin ($r = 0.40$, $p<0.01$). Based on these results, multivariate analyses were carried out to identify whether diagnosis of CS and/or the degree of insulin resistance were able to independently predict the alterations in atherogenic risk factors and biomarkers of cardiovascular disease (Table 3).

Discussion

In the present study, CS was shown to be associated not only to an insulin-resistant sate, characterized by the presence of higher levels of the novel marker resistin, but also by an atherogenic lipoprotein profile and a proinflammatory status. Interestingly, hypercortisolism was shown to be involved in the increase observed in triglycerides and oxLDL levels, and, in combination with insulin resistance, would act to increase the waist circumference and to amplify the inflammatory process, key factors for the development of cardiovascular disease.

The insulin-resistant state present in the group of patients with active CS was evidenced by high glucose and insulin levels, increased HOMA-IR, and reduced QUICKI index. Accordingly, the TG / HDL-C ratio, which has been proposed as a marker of insulin resistance and of the proportion of small and dense LDL particles, was significantly higher in patients with CS than in control subjects. Insulin resistance is considered an important risk

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factor for cardiovascular disease and not only obesity is closely related to its development but also glucocorticoids play a crucial role. In agreement, Fallo et al. (10) described insulin-resistant states both in obese and non-obese CS patients and in obese subjects in comparison with non-obese controls. Moreover, it is noteworthy that patients with CS, considered already cured and followed up for 5 years, presented high prevalence of atherosclerosis probably due to residual abdominal obesity and/or underlying insulin resistance (26).

Adipose tissue modulates energetic metabolism through the secretion of different adipocytokines and, among them, adiponectin is the only one described as an insulin-sensitizing agent (27). Adiponectin levels were similar in both studied groups, which agrees with previous reports (13,28) and supports the hypothesis that hypercortisolism-associated insulin resistance is not related to adiponectin reduction (29). Another adipocytokine which has also been associated with insulin resistance is resistin, though results obtained in humans are highly controversial. While some authors have described increased resistin levels in obese and in diabetic subjects, others have not been able to confirm those results (30, 31). Sheng et al. (32) showed that human resistin is able to induce insulin resistance and that metformin treatment could reverse this effect on hepatocytes, thus suggesting a relevant role for this adipocytokine in liver insulin resistance. In the present study, the increase observed in resistin concentrations in CS patients is in agreement with the results obtained by Krsek et al. (28), with the difference that these authors only studied women with CS. Moreover, high resistin expression by monocytes and macrophages suggests other pathophysiological roles in humans (33). Resistin has also been implicated in the induction of endothelial dysfunction and proliferation of smooth muscle cells, thus facilitating atherogenesis (34).

On the other hand, the impact of insulin resistance on lipoprotein metabolism in individuals not affected by CS is widely known. In our study, patients with CS exhibited

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significantly higher triglyceride and total cholesterol levels than control subjects, probably due to VLDL accumulation, apart from increased apo B concentration, which is considered the parameter with the highest predictive value for cardiovascular disease. Besides, patients with CS presented higher TG / HDL-C ratio, a useful marker of the proportion of small and dense LDL particles, which is consistent with conserved LDL-C levels and increased apo B levels in the patient group. It is interesting to note that small and dense LDL particles are known to be highly susceptible to undergo oxidative processes and, in fact, oxidized LDL levels were significantly higher in patients with CS than in control subjects. These findings might reflect the sequence of events by which, once in the subendothelial space, small and dense LDL would be easily oxidized and then return to circulation as minimally oxidized LDL particles. Moreover, this pathological process could be facilitated by the lack of increase in PON 1 activity, an enzyme partially responsible for HDL capacity to inhibit LDL oxidation (35) and by the oxidative stress characteristic of insulin-resistant states (36). Nonetheless, CS was the main predictor of the increase in oxLDL levels in the multivariate analysis; thus, a role of CS over oxidative stress beyond insulin resistance should be considered.

HDL-C levels were not altered in this group of patients with CS, which is in contrast with that found in other studies and with data from patients with metabolic syndrome, who share many characteristics with CS patients (16). Among the multiple factors that may modulate HDL-C levels, it is remarkable that CETP was similar in both groups. Nevertheless, it is well known that HDL-C levels not always reflect their antiatherogenic capacity (37). In this respect, it is important to note that the HDL-C / apo A-I ratio was significantly reduced in patients with CS, which could suggest the existence of normal particle number but deficient capacity to promote cellular cholesterol efflux from peripheral cells.

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Among the wide spectrum of alterations described above in relation with CS, hypercortisolism and insulin resistance are the most closely related between them, and could be independently responsible for the alterations in the inflammatory biomarkers and risk factors of cardiovascular disease observed in patients with CS. The multivariate analysis showed that diagnosis of CS was significantly associated with the increase in waist circumference, and in triglycerides, oxLDL and resistin levels and that insulin resistance was able to predict the increase in waist circumference, and, to a lesser extent, in resistin levels.

The hypercortisolism of CS leads to disturbances in other endocrine axes and systems, and its link with insulin resistance is of particular intrest. This relationship rules out the possibility of counting with *in vivo* models of hypercortisolism without the presence of insulin resistance. Nevertheless, the multivariate analysis of the data allowed identifying whether diagnosis of CS and/or the degree of insulin resistance were able to independently predict the alterations in atherogenic risk factors and biomarkers of cardiovascular disease. The evaluation of insulin resistance through different markers and not through the hyperinsulinemic-euglycemic clamp could be considered a limitation of the present study, but it is worth noting that the presence of insulin resistance in patients with CS is well-known and that indexes such as HOMA-IR have been widely shown to be excellent surrogates of insulin-resistant states.

In conclusion, the findings here reported contribute to the identification of factors responsible for the increased cardiovascular disease in patients with CS, as well as to a better understanding of the underlying pathophysiological mechanism involved. A more atherogenic lipoprotein profile and a proinflammatory state might be crucial in the induction of endothelial dysfunction in CS patients, the formation of fatty streaks, and the definitive

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formation of atherosclerotic plaques, the sequence of events that leads to the clinical manifestations of cardiovascular disease.

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Figure legends

Figure 1: Resistin levels in patients with Cushing's Syndrome and control subjects ([a] $p < 0.0001$).

Figure 2: Oxidized LDL levels in patients with Cushing's Syndrome and control subjects ([a] $p < 0.05$).

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Table 1. Clinical characteristics and plasma levels of cortisol and biomarkers of insulin resistance in patients with Cushing's Syndrome and control subjects.

	Patients with Cushing's Síndrome	Control Subjects
N	32	32
Women / Men	23 / 9	23 / 9
Age (years)	34 (27-42)	33 (27-45)
BMI (kg/m^2)	29 (26-33)	22 (21-24)[a]
WC (cm)	96 (91-113)	85 (78-91)[b]
Hypertension (%)	86	0
Cortisol (nmol/l)	772±331	331±137[a]
Glucose (mmol/l)	5.1(4.7-6.8)	4.7(4.3-5.3)[d]
Insulin (mU/l)	6.5 (4.8-12.0)	4.8 (2.4-7.0)[a]
HOMA	2.0 (1.0-3.0)	1.0 (0.5-1.5)[c]
QUICKI	0.26 (0.24-0.32)	0.32 (0.29-0.41)[c]
NEFA (nmol/l)	0.7 (0.6-1.1)	0.6 (0.5-0.8)

BMI, body mass index; WC, waist circumference; HOMA-IR, Homeostasis Model Assessment; QUICKI, Quantitative insulin sensitivity check index; NEFA, non-esterified fatty acids. Results are expressed as mean±SD when data are normally distributed and as median (interquartile range) with non-parametric distribution. [a]$p<0.0001$; [b]$p<0.001$; [c]$p<0.01$; [d]$p<0.05$ *vs.* patients with Cushing's Syndrome.

Table 2. Lipid, lipoprotein and apolipoprotein profile in patients with Cushing's Syndrome and control subjects.

	Patients with Cushing's Syndrome (n=32)	Control Subjects (n=32)
TG (mmol/l)	1.50(0.98-2.30)	0.84(0.58-1.02)[a]
TC (mmol/l)	5.6(4.2-6.5)	4.6(4.2-5.4)[c]
VLDL-C (mmol/l)	0.59 (0.41-0.83)	0.36(-0.28-0.49)[b]
LDL-C (mmol/l)	3.1(2.2-4.1)	2.7(2.4-3.5)
HDL-C (mmol/l)	1.4 (1.1-1.7)	1.4(1.2-1.6)
Non-HDL-C (mmol/l)	3.9 (2.8-5.2)	3.2(2.8-3.9)[a]
Apo B (g/l)	1.02±0.37	0.78± 0.18 [b]
Apo A-I (g/l)	1.75±0.39	1.52±0.32 [b]
TG / HDL-C	2.7 (1.3-4.6)	1.3 (1.1-1.9)[b]
TC / HDL-C	4.0 (2.7-5.5)	3.4 (2.8-4.2)
LDL-C / apo B	1.3±0.31	1.5±0.2[b]
HDL-C / apo A-I	0.32±0.06	0.37±0.08[b]

TG, triglycerides; TC, total cholesterol; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoproteína; Apo, apolipoprotein. Results are expressed as mean±SD when data are normally distributed and as median (interquartile range) with non-parametric distribution. [a]$p<0.0001$; [b]$p<0.01$; [c]$p<0.05$ *vs.* patients with Cushing's Syndrome.

Table 3. Multivariate analysis including diagnosis of Cushing's Syndrome and the degree of insulin resistance with waist circumference, resistin levels, triglyceride concentrations and leukocyte count in patients with Cushing's Syndrome and control subjects.

Depedent Variable	**Indepedent Variable**	**Bst**	**t**	**P=**	**R^2**
Log WC	Age	0.04	0.36	0.724	0.37
	Log HOMA-IR	0.39	3.05	0.004	
	Cushing Diagnosis	0.39	3.07	0.004	
Log TG	Age	0.06	0.51	0.614	0.31
	Log HOMA-IR	0.21	1.76	0.083	
	Cushing Diagnosis	0.47	4.01	0.001	
Log resistin	Age	0.01	0.10	0.920	0.45
	Log HOMA-IR	0.32	2.63	0.012	
	Cushing Diagnosis	0.54	4.41	0.001	
oxLDL	Age	0.20	1.34	0.188	0.20
	Log HOMA-IR	0.24	1.51	0.140	
	Cushing Diagnosis	0.36	2.29	0.028	

Log: logaritm_{10}; WC: waist circumference; TG: triglycerydes; HOMA-IR: Homeostasis Model Assessment; oxLDL: oxidized LDL.

Figure 1

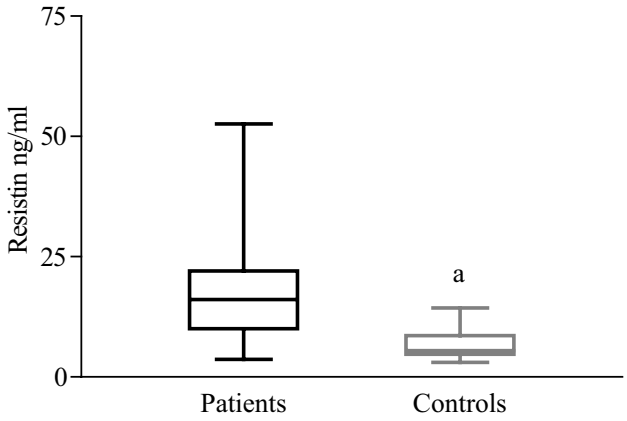

Figure 2