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Tissue-specific adaptive levels of glucocorticoid receptor alpha mRNA and their relationship with insulin resistance

R.B. Castro[1], C.A. Longui[1], C.D.C. Faria[1], T.S. Silva[1], F. Richeti[1], M.N. Rocha[1], M.R. Melo[1], W.L. Pereira[2], E.G. Chamlian[2] and L.A. Rivetti[2]

[1]Laboratório de Medicina Molecular, Departamento de Fisiologia, Faculdade de Ciências Médicas da Santa Casa de São Paulo, São Paulo, SP, Brasil
[2]Departamento de Cirurgia Cardíaca, Irmandade da Santa Casa de Misericórdia de São Paulo, São Paulo, SP, Brasil

Corresponding author: C.A. Longui
E-mail: carloslongui@msn.com

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ABSTRACT. Insulin resistance is an underlying cause of metabolic changes associated with cardiovascular diseases. Glucocorticoids are known determinant factors of insulin resistance. We quantified glucocorticoid receptor alpha (*GRα*) mRNA and 11 beta-hydroxysteroid dehydrogenase type 1 (*11β-HSD1*) mRNA in various tissues of 35 patients with previously established cardiovascular disease. This was a prospective study in a cardiac surgery patient setting. Samples of subcutaneous adipose tissue, epicardial fat, muscle, and peripheral blood mononuclear cells were examined. *GRα* and *11β-HSD1* mRNA were determined by real-time PCR. Mean age was 54.4 years. A significantly higher level of *GRα* mRNA was observed in muscle, with mean = 43.6 arbitrary units, median (p25-p75) = 39.4, compared to epicardial adipose tissue, with mean = 34.2, median (p25-p75) = 27.6, and to subcutaneous adipose tissue, with mean = 29.0, median (p25-p75) = 19.0, and lymphocytes, with mean = 17.5, median (p25-p75) = 14.02. When patients with diabetes mellitus were compared to patients

without insulin resistance, significantly lower levels of *GRα* mRNA were observed in epicardial fat. Lymphocytes had the lowest *11β-HSD1* mRNA concentration. We also observed significantly reduced *11β-HSD1* mRNA levels in visceral fat when compared with muscle tissue. *GRα* and *11β-HSD1* mRNA levels differed among tissues involved in the pathophysiology of metabolic syndrome. We conclude that epicardial adipose tissue has lower *GRα* mRNA levels in insulin-resistant patients; this seems to be an adaptive and protective mechanism.

Key words: Glucocorticoid receptor; Tissue-specific GR sensitivity; Insulin sensitivity; *GRα* mRNA

INTRODUCTION

Cardiovascular events are among the most common causes of death and are frequently related to coronary artery disease (CAD) (Rosenbaum and Ferreira, 2003). Insulin resistance (IR) and concomitant hyperinsulinemia are implicated in the pathophysiology of metabolic syndrome (MS) including endothelial dysfunction (Bansilal et al., 2007) and are associated with hypertension, dyslipidemia, visceral obesity, altered glucose metabolism, and inflammatory and prothrombotic states (Reaven, 2008). Hyperinsulinemia alone, even in the absence of other features of MS, is associated with CAD (Deveci et al., 2009).

IR also includes chronic and systemic inflammation leading to metabolic abnormalities (Bansilal et al., 2007) such as decreased peripheral glucose uptake and increased hepatic glucose synthesis. These abnormalities are related to the downregulation of muscular insulin receptors, decreased translocation of glucose transporter protein 4 (GLUT4) to the cell membrane, increased substrate bioavailability for gluconeogenesis, and alteration of the insulin post-receptor signaling pathway (Bansilal et al., 2007).

Glucocorticoids (GCs) are known determinants of IR (Wang, 2005). They act as potent insulin antagonists by blocking GLUT4 translocation and, consequently, decreasing glucose uptake (Whorwood et al., 2001; Wang, 2005). Other important effects of GCs include a deleterious influence on pancreatic β cells (Gesina et al., 2006), peripheral lipolysis, and amino acid release from the muscle, which in conjunction with increased phosphoenolpyruvate carboxykinase activity, enhances gluconeogenesis (Wang, 2005). GCs also induce adipocyte differentiation and proliferation (Anagnostis et al., 2009). Patients with Cushing syndrome present a spectrum of glucose intolerance, arterial hypertension, central obesity, and dyslipidemia (Stewart, 2005; Vegiopoulos and Herzig, 2007) similar in many ways to the features of MS.

Increased tissue cortisol concentration and increased tissue-specific GC sensitivity have been implicated in the pathophysiology of MS (Anagnostis et al., 2009). GC sensitivity is influenced by and directly related to the amount of GC receptor (*GR*) messenger RNA (mRNA) measured using *in vitro* methods (Melo et al., 2004; Gross et al., 2009; Longui and Faria, 2009; Faria et al., 2010; Sousa e Silva et al., 2010).

Higher *GR* expression has been identified in visceral adipose tissue compared with that in subcutaneous fat (Rebuffé-Scrive et al., 1990; Anagnostis et al., 2009). Therefore, elevated local concentrations of cortisol or increased *GR* expression or sensitivity in visceral adipose

tissue can increase the predisposition to metabolic abnormalities including IR (Vegiopoulos and Herzig, 2007). The aim of the present study involving individuals with previously established cardiovascular diseases was to compare the severity of IR with the amount of GC receptor alpha (*GRa*) and 11 beta-hydroxysteroid dehydrogenase type 1 (*11β-HSD1*) mRNA present in insulin-targeted tissues, such as skeletal muscle, subcutaneous adipose tissue, epicardial visceral fat, and mononuclear cells.

MATERIAL AND METHODS

Thirty-five patients (males = 26; females = 9) aged 30 to 75 years who underwent cardiac surgery were evaluated. All patients included in the study signed a written consent approved by the Institutional Research Ethics Committee. The exclusion criteria were 1) use of GCs or any drug that interfered with steroidogenesis within the last 6 months, 2) thyroid hormone abnormalities, and 3) history of depression or alcoholism. Weight, height, abdominal circumference, and blood pressure were measured the day before surgery. The diagnosis of MS was established according to criteria proposed by the International Diabetes Federation (Benetos et al., 2008; Wassink et al., 2008). After excluding the interference of gender, body mass index (BMI), and associated drug intake and recognizing the influence of IR on the amount of *GRα* mRNA, the patient cohort was classified as IR or non-IR (NIR).

The diagnosis of IR was made based on the homeostatic model assessment-IR (HOMA-IR) proposed by Wallace et al. (2004). The cutoff value established by the Brazilian Metabolic Syndrome Study for the diagnosis of IR is ≥2.71 (Geloneze et al., 2006). According to these criteria, 22 of 35 patients were classified as NIR and 13 of 35 as IR. The latter group included 5 type 2 diabetes mellitus patients. Statins were administered for hypercholesterolemia in 16 of 22 NIR and 12 of 13 IR patients.

Blood samples for lymphocyte separation and biochemical measures were obtained after anesthetic induction and before thoracic incision. Subsequently, pectoral skeletal muscle, thoracic superficial subcutaneous adipose tissue, and epicardial visceral adipose tissue were collected.

Insulin and cortisol were measured through chemoluminescent assay (Immulite 2000, Siemens, Los Angeles, CA, USA). Free thyroxine, total thyroxine, triiodothyronine, and thyroid-stimulating hormone were determined through immunofluorometric assay (AutoDELFIA, PerkinElmer, Turku, Finland). Glucose, cholesterol, and triglycerides were measured by colorimetric/enzymatic assays (Bayer, São Paulo, SP, Brazil). The clinical features and biochemical findings are described in Tables 1 and 2, respectively.

Molecular study

Peripheral blood was collected in tubes containing sodium heparin. Mononuclear cells from peripheral blood were isolated via centrifugation against Histopaque (1:1 ratio; Sigma, St. Louis, MO, USA, Cat. #10771) at 800 *g* at room temperature for 30 min. RNA extraction from mononuclear cells was performed using the Trizol reagent (Invitrogen, Cat. #15596018) and chloroform. After extraction, RNA was eluted in pure DNase- and RNase-free water (Invitrogen, Cat. #10977023). Complementary DNA (cDNA) was synthesized using the TaqMan Reverse Transcription Reagents (Applied Biosystems, USA, Cat. #N8080234) according to

manufacturer recommendations. Muscular and adipose tissues were initially placed in a stabilization solution (RNAlater, Qiagen, Cat. #76106) and subsequently macerated in QIAzol (Qiagen, Cat. #79306) before chloroform extraction.

Table 1. Clinical features of the 35 patients submitted to cardiac surgery, separated into groups based on the presence of insulin resistance.

Variables	Non-insulin resistant (N = 22) mean (SD) median (p25-p75)	Insulin resistant (N = 13) mean (SD) median (p25-p75)	Total (N = 35)
Male:Female	17:5	9:4	26:9
Age (years)	55.4 (11.2)	52.7 (11.2)	54.4 (11.1)
	55.5 (45.0-65.0)	53.0 (44.2-57.7)	54.0 (45.2-64.5)
Weight (kg)	71.1 (12.4)	78.3 (12.3)	74.2 (12.7)
	69.0 (62.0-79.0)	75.0 (68.0-86.0)	75.0 (65.0-80.0)
Height (cm)	165.3 (9.6)	163.6 (11.0)	164.4 (10.0)
	165.0 (158.0-163.0)	166.5 (151.9-172.7)	165.0 (157.0-172.5)
BMI (kg/m^2)	26.0 (4.0)	29.3 (3.5)*	27.4 (4.3)
	26.5 (22.8-28.0)	30.3 (27.0-31.8)	27.5 (23.8-31.1)
WC (cm)	91.9 (8.7)	103.4 (8.7)*	96.2 (10.2)
	93.0 (86.0-96.0)	101.0 (97.2-109.50)	95.0 (91.0-101.7)
SBP (mmHg)	136.4 (30.6)	137.7 (24.2)	136.8 (28.0)
	140.0 (110.0-160.0)	130.0 (125.0-160.0)	140.0 (112.5-160.0)
DBP (mmHg)	81.8 (15.0)	87.0 (18.0)	83.7 (16.1)
	80.0 (70.0-90.0)	90.0 (70.0-90.0)	90.0 (70.0-90.0)

BMI = body mass index; WC = waist circumference; SBP = systolic blood pressure; DBP = diastolic blood pressure. SD = standard deviation. *Insulin resistant *vs* non-insulin resistant, *t*-test; $P < 0.05$.

After reverse transcription for cDNA synthesis, the absolute quantitation of *GRα* mRNA was determined using quantitative real-time polymerase chain reaction (qRT-PCR) as previously described (Melo et al., 2004; Longui and Faria, 2009; Faria et al., 2010). This method uses the breakpoint cluster region gene (*BCR*) as a normalizing gene and calibration with standard curves constructed with cDNA from synchronized lymphoblastic cells (Jurkat, ATCC Clone E6-1). Briefly, qRT-PCRs for *GR* and *BCR* amplification were performed using the TaqMan PCR Core kit (Applied Biosystems): 2.5 µL buffer TaqMan A10X (with ROX as a passive reference), 4.5 µL 25 mM $MgCl_2$, 2.0 µL deoxynucleoside triphosphate, 0.13 µL 5 U/µL AmpliTaq Gold, 0.5 µL primer (sense), 0.5 µL primer (antisense), 0.5 µL probe, 9.37 µL DNase- and RNase-free water, and 5 µL cDNA in a total volume of 25 mL. Cycle conditions on an ABI 7500 (Applied Biosystems) were 95°C for 10 min (AmpliTaq Gold activation) followed by 45 cycles of 95°C for 15 s (denaturation) and 60°C for 90 s (annealing and extension). The primers and probes used were *GRα* sense primer (5'-GAAGGAAACTCCAGCCAGAA-3'), *GRα* antisense primer (5'-CAGCTAACATCTCGGGG AAT-3'), *GRα* probe (6-FAM-CTTCCAAACATTTTTGGATAAGACCAT-TAMRA), *BCR* sense primer (5'-CCTTCGACGTCAATAACAAGGAT-3'), *BCR* antisense primer (5'-CCTGCGATGGC GTTCAC-3'), and *BCR* probe (6-FAM-TCCATCTCGCTCATCATCACCGACA-TAMRA).

Quantitation of *11β-HSD1* was carried out against calibration with standard curves constructed with mRNA from normal liver tissue. Normalization with *BCR* mRNA was also performed. qRT-PCR was performed according to manufacturer recommendations with a Platinum® SYBR® Green qPCR SuperMix-UDG Kit (Invitrogen, Cat. #11733038). Each reaction contained 12.5µL Platinum SYBR mix, 0.5 µL ROX reference dye, 0.5 µL of each primer, 9.0 µL water, and 2 µL cDNA. Cycle conditions on an ABI 7500 (Applied Biosystems) were 95°C for 10 min (Taq Platinum activation) followed by 45 cycles of 95°C for 15

s (denaturation) and 60°C for 90 s (annealing and extension). A final dissociation stage was included, and melting curves were analyzed to ensure specific amplification. The primers used were *11β-HSD1* sense primer (5'-TTGCCCAAGCAGGAAAGC-3') and *11β-HSD1* antisense primer (5'-TTGGTGATGTGGTTGAGAATGAG-3').

Statistical analyses were performed with SigmaStat 3.5 for Windows (SPSS, Richmond, CA, USA). The Mann-Whitney rank sum test or the Student *t*-test were used to compare the same variable in 2 groups according to non-parametric or parametric sample distributions, respectively. When the same variable was compared across 3 groups, analysis of variance on ranks (Kruskal-Wallis test) or for repeated measures was used. Pairwise analyses used the Student-Newman-Keuls test. Correlations between variables were made with linear regression analyses, and Pearson's coefficient of correlation was determined. Multiple regression analysis was used to evaluate the influence of variables such as BMI, gender, and drug intake on *GRα* and *11β-HSD1* gene expression. Significance was defined at the level $P < 0.05$.

RESULTS

Of the 35 patients included in this study, a diagnosis of CAD was established in 27 (77.2%). In the remaining patients, aortic insufficiency was detected in 4 cases, congenital cardiopathy in 2 cases, and aortic dissection in 2 cases. Patients were separated into 2 groups according to the presence of IR: the first (NIR group) comprised 22 NIR patients (HOMA-IR <2.71). The second (IR group) contained 8 patients with a HOMA-IR value of ≥2.71 and 5 patients previously diagnosed with type 2 diabetes mellitus. The hormone evaluation results of both groups are listed in Table 2. The absolute quantitation of *GRα* and *11β-HSD1* mRNA was expressed in arbitrary units as mean ± SD and median (interquartile range). The *GRα* and *11β-HSD1* mRNA levels determined for each tissue type are shown in Table 3.

Significant differences for *GRα* mRNA content were found among tissues (Kruskal-Wallis test, $P < 0.01$). The comparison between the values observed in each pair of tissues (Student-Newman-Keuls test) confirmed that *GRα* mRNA was higher in the muscle [mean ± SD = 43.6 ± 38.3, median (p25-p75) = 39.4 (17.2-52.9)] than in the epicardial visceral fat [mean ± SD = 34.2 ± 37.8, median (p25-p75) = 27.6 (11.8-44.9), $P = 0.04$], subcutaneous adipose tissue [mean ± SD = 29.0 ± 38.1, median (p25-p75) = 19.0 (8.5-27.7), $P < 0.001$], and lymphocytes [mean ± SD = 17.5 ± 11.2, median (p25-p75) = 14.02 (7.6-25.0), $P < 0.001$, Figure 1]. A significant reduction in the amount of *GRα* mRNA was also detected in epicardial adipose tissue from patients with IR (see Table 3), specifically those with overt diabetes mellitus [mean ± SD = 12.4 ± 10.7, median (p25-p75) = 12.0 (5.1-16.5); see Figure 2].

A positive correlation was found between lymphocyte *GRα* mRNA and the *GRα* mRNA levels measured in subcutaneous adipose tissue ($r = 0.41$, $P < 0.01$), muscle ($r = 0.47$, $P < 0.01$), and epicardial visceral fat ($r = 0.60$, $P < 0.01$). A positive correlation was also observed between subcutaneous and visceral adipose tissue ($r = 0.541$, $P < 0.01$). The strongest correlation was observed between epicardial adipose tissue and muscular tissue ($r = 0.654$, $P < 0.01$). HOMA-IR index had a significant negative correlation with serum cortisol ($r = -0.44$, $P \leq 0.01$), with lymphocyte *GRα* mRNA ($r = -0.45$, $P \leq 0.01$), and with muscular *GRα* mRNA ($r = -0.44$, $P \leq 0.01$) levels in NIR patients only.

We measured *11β-HSD1* mRNA levels in all patients. Significant differences were detected among the tissues studied (Kruskal-Wallis test, $P < 0.01$). The results are reported as

Table 2. Laboratory findings in non-insulin resistant (NIR) and insulin resistant (IR) patients.

Variables	NIR (N = 22)	IR (N = 13)
Total cholesterol (mM)	4.6 (1.4)	5.0 (1.5)
	4.3 (3.5-4.9)	4.3 (4.0-6.3)
HDL-C (mM)	1.1 (0.2)	1.0 (0.2)
	1.0 (0.9-1.1)	1.1 (0.9-1.2)
LDL-C (mM)	2.8 (0.9)	3.1 (1.1)
	2.8 (2.3-3.4)	2.9 (2.2-3.7)
Triglyceride (mM)	1.8 (0.8)	2.6 (2.0)
	1.6 (1.1-2.1)	1.8 (1.4-2.6)
Glucose (mM)[a]	5.2 (0.6)	5.1 (0.4)
	5.2 (4.8-5.6)	4.9 (4.8-5.4)
Insulin (pM)[a]	40.4 (19.1)	117.2 (49.2)*
	41.7 (20.8-55.6)	97.2 (83.3-138.9)
HOMA-IR[a]	1.5 (0.6)	5.2 (2.4)
	1.5 (1.1-1.9)	4.9 (3.1-6.4)**
Cortisol (nM)	250.8 (103.6)	203.5 (48.2)
	246.9 (173.8-281.4)	212.4 (182.8-240.0)
TSH (µU/mL)	3.1 (2.7)	2.5 (1.2)
	2.3 (1.4-4.0)	2.4 (1.5-2.6)
Thyroxine (nM)	111.8 (29.6)	103.9 (22.2)
	112.0 (84.9-124.8)	103.0 (89.7-118.4)
Free thyroxine (pM)	16.7 (2.6)	14.8 (2.8)
	16.1 (15.5-19.3)	14.1 (12.9-16.7)
Triiodothyronine (nM)	1.3 (0.3)	1.4 (0.2)
	1.3 (1.1-1.5)	1.4 (1.3-1.4)

Data are reported as means (SD) and median (p25-p75). Cholesterol = mM x 38.7 = mg/dL; triglyceride = mM x 88.49 = mg/dL; glucose = mM x 18.01 = mg/dL; insulin = pM x 0.139 = µU/mL; cortisol = nM x 0.0362 = µg/dL; thyroxine = nM x 0.0777 = µg/dL; free thyroxine = pM x 0.0777 = ng/dL; triiodothyronine = nM x 65.1 = ng/dL. *IR *vs* NIR, *t*-test; $P < 0.01$. **IR *vs* NIR, Mann-Whitney rank sum test; $P < 0.01$. [a]Values of diabetes mellitus patients (N = 5) were not included. In these patients mean [standard deviation (SD)] and median (IQR) were: glucose = 8.56 (2.89) and 8.49 (6.88-9.56); insulin = 84.7 (45.9) and 86.1 (35.9-121.9). Homeostatic model assessment-insulin resistance was not calculated for diabetic patients.

Table 3. Quantitation of *GRα* and *11b-HSD1* mRNA (expressed as arbitrary units).

Tissues	NIR (N = 22)		IR (N = 13)	
	GRα	*11β-HSD1*	*GRα*	*11β-HSD1*
Lymphocytes	18.5 (12.3)	7.40 (24.6)	15.7 (9.4)	0.40 (1.2)
	15.3 (7.6-26.6)	0.14 (0.03-2.8)	14.0 (7.2-22.5)	0.02 (0.01-0.1)
Subcutaneous fat	36.8 (46.1)	12.70 (38.9)	15.8 (9.8)	0.90 (0.7)
	20.9 (8.4-38.9)	0.70 (0.5-3.4)	16.1 (8.5-20.5)	0.70 (0.4-1.4)
Muscle	50.1 (45.1)	11.30 (27.5)	31.1 (17.9)	3.40 (6.1)
	44.5 (20.7-54.7)	0.90 (0.6-10.6)	34.4 (14.8-45.5)	1.10 (0.6-1.7)
Epicardial visceral fat	42.8 (44.0)	1.70 (3.1)	19.7 (17.1)	1.10 (1.5)
	30.9 (16.6-48.2)	0.70 (0.4-1.2)	12.1 (8.3-30.2)*	0.60 (0.3-1.2)

Mean [standard deviation (SD)] and median (p25-p75) are shown in non-insulin resistant (NIR) and insulin resistant (IR) patients submitted to cardiac surgery. *IR *vs* NIR, Mann-Whitney rank sum test; $P < 0.05$.

means ± SD, median (p25-p75) for lymphocytes [4.8 ± 19.6, 0.09 (0.01-1.0)], subcutaneous fat [8.3 ± 31.0, 0.7 (0.5-2.0)], muscle [8.4 ± 22.3, 1.0 (0.6-8.2)], and epicardial visceral fat [1.5 ± 2.6, 0.7 (0.3-1.2)]. Muscle tissue displayed the highest *11β-HSD1* mRNA concentration (Student-Newman-Keuls test, $P < 0.05$). A reduced *11β-HSD1* mRNA level was observed in lymphocytes compared with that in adipose subcutaneous tissue, muscle, and epicardial visceral fat (Student-Newman-Keuls test; $P < 0.05$). No significant differences occurred between subcutaneous adipose tissue and epicardial adipose tissue (Student-Newman-Keuls test, P =

0.06). A positive correlation between lymphocyte *11β-HSD1* mRNA and *11β-HSD1* levels was observed in subcutaneous fat (r = 0.96, P < 0.01).

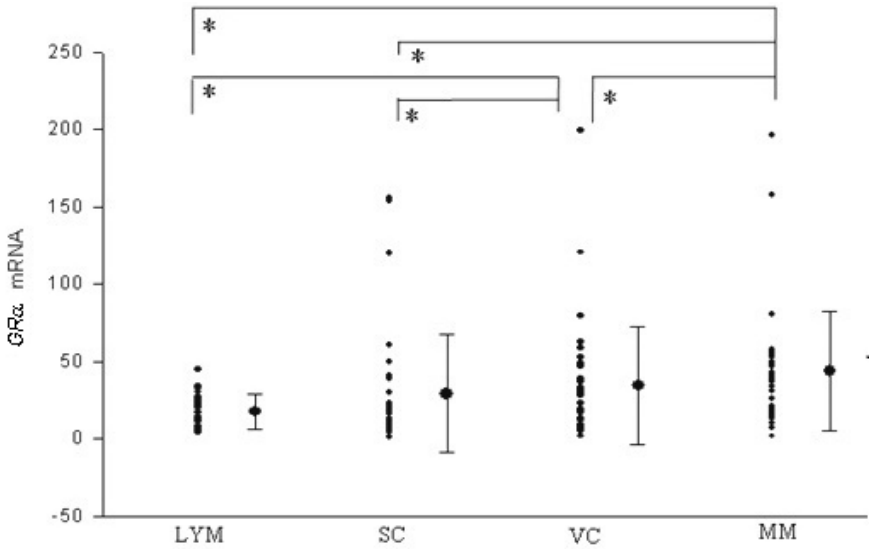

Figure 1. Comparison of the amount of *GRα* mRNA (expressed in arbitrary units) from the same individual across different tissues: lymphocytes (LYM); subcutaneous adipose tissue (SC); epicardial visceral fat (VC); muscle (MM). *Pairwise analysis (Student-Newman-Keuls), P < 0.05. Vertical bars represent the median value and the interquartile range.

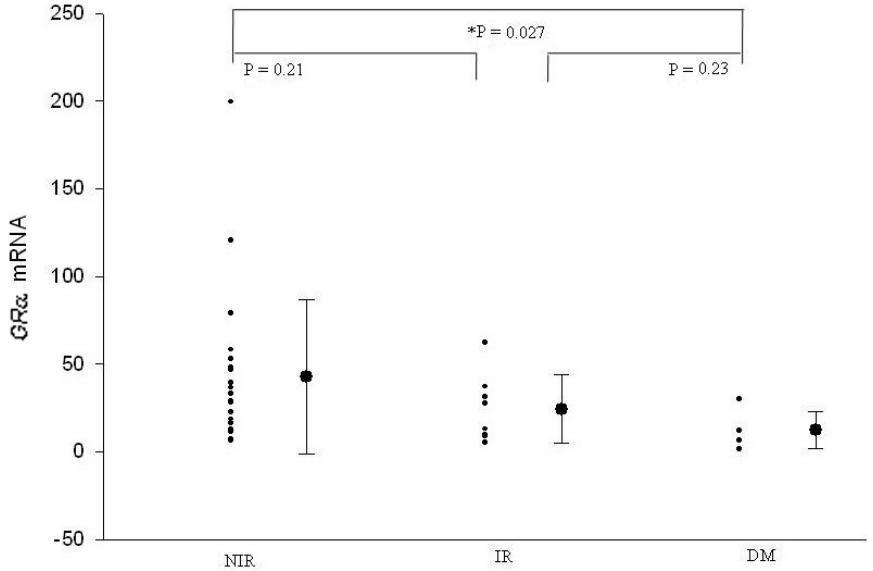

Figure 2. Comparison of epicardial visceral fat *GRα* mRNA (expressed in arbitrary units) among non-insulin resistant patients (NIR; N = 22), insulin resistant non-diabetic patients (IR; N = 8) and patients with type 2 diabetes mellitus (DM; N = 5). *Mann-Whitney test; P < 0.05. Vertical bars represent the median value and the interquartile range.

No significant difference in *11β-HSD1* mRNA level was observed when patients were categorized according to the presence or absence of IR (see Table 3). Conversely, obese patients with a BMI >25 (N = 25) displayed higher levels of *11β-HSD1* mRNA in adipose subcutaneous tissue [mean ± SD = 11.4 ± 36.6, median (p25-p75) = 1.0 (0.7-3.0)] compared with that of patients with a BMI of <25 [N = 10; mean ± SD = 0.6 ± 0.5, median (p25-p75) = 0.5 (0.4-0.7); Mann-Whitney test, P = 0.02].

To minimize potential confounding factors such as gender, obesity, and drug intake in establishing the relationship between insulin sensitivity and *GRα* or *11β-HSD1* mRNA expression, we performed a multiple regression analysis. The main conclusions of the multivariate test were that 1) visceral *GRα* mRNA level is significantly correlated to HOMA-IR index (r = 0.50, P = 0.05), and 2) subcutaneous *GRα* mRNA level is predominantly correlated to subcutaneous *11β-HSD1* mRNA level (r = 0.95, P < 0.01) and BMI (r = 0.94, P = 0.03).

Clinical criteria confirming the diagnosis of MS were present in 25 patients, 13 of which were classified into the IR group. No significant differences were observed in tissue *GRα* and *11β-HSD1* mRNA levels between patients with or without MS, suggesting that the presence of IR, but not MS, was the determining factor for the varying amounts of *GRα* mRNA.

DISCUSSION

Tissue-specific *GRα* expression has been reported in previous studies (Rebuffé-Scrive et al., 1990; Goedecke et al., 2006; Anagnostis et al., 2009; Gross et al., 2009). *GRα* is highly expressed in skeletal muscle (Whorwood et al., 2001), reinforcing the possibility of interactions of GCs and insulin at the muscular level. Considering the influence of GCs on the induction of reduced muscular insulin sensitivity, *GRα* overexpression in muscle has been suggested as an associated feature in the mechanism of MS (Whorwood et al., 2001). Additionally, a positive correlation has been found between human myoblast *GRα* expression and BMI, percent fat mass, and arterial blood pressure (Whorwood et al., 2002; Reynolds et al., 2002). *GRα* expression is negatively correlated with glucose uptake during euglycemic clamping (Whorwood et al., 2002), demonstrating the correlation between increased *GRα* and reduced insulin sensitivity.

In this study, the tissue specificity of *GRα* expression was also observed. The quantitation of *GR* mRNA from various tissues in the same individual revealed that the highest amount of *GRα* mRNA was found in muscle, followed by a progressive decrease in *GRα* levels in epicardial visceral fat, subcutaneous adipose tissue, and lymphocytes. Interestingly, muscle and visceral fat are tissues strongly involved in the pathophysiology of MS. Visceral obesity and chronic inflammation have been associated with MS. Inflammatory cytokines, such as interleukin 1β and tumor necrosis factor α, increase *11β-HSD1* levels and therefore can increase the intracellular bioavailability of cortisol (Escher et al., 1997; Whorwood et al., 2002). In the patients of our study, both chronic inflammation and a certain degree of acute inflammation were expected related to the ischemic process and surgical procedures. In these patients, increased cortisol secretion and increased tissue cortisol bioavailability represented a protective mechanism against tissue injury.

In NIR patients, a negative correlation was found between muscle *GRα* mRNA levels and HOMA-IR, suggesting that reduced *GRα* expression in muscle could be one of the adaptive mechanisms reducing GC action and allowing partial preservation of insulin sensitivity.

With the progression of IR, the correlation of *GRα* and HOMA-IR is no longer observed. *In vitro* studies have shown that increased insulin concentration under serum-free conditions induces elevated 11β-HSD1 activity in myoblasts, in turn increasing intracellular free cortisol and possibly downregulating *GRα* (Whorwood et al., 2001). In our study, IR patients displayed no differences in muscle expression of *GRα* or *11β-HSD1*.

GRα mRNA has been described previously as being expressed predominantly in omental fat versus subcutaneous adipose tissue (Rebuffé-Scrive et al., 1990). This finding is concordant with our finding of higher *GRα* mRNA levels in epicardial visceral fat compared to those in subcutaneous adipose tissue. Other reports from studies evaluating various cohorts have identified no difference in *GRα* mRNA levels among visceral, superficial, or deep subcutaneous fat (Goedecke et al., 2006) but detected a negative correlation between superficial subcutaneous fat *GRα* mRNA and insulinemia and a positive correlation with glycemia.

Lower *GRα* mRNA levels in the epicardial adipose tissue of patients with type 2 diabetes mellitus is an important finding of our study and suggests that increased muscular IR is associated with lower *GRα* triggered by excessive GC effects in visceral fat. This down-regulatory effect could represent a partial protective mechanism activated by the progression of IR.

Conversely, GCs are well-known determinants of IR (Gathercole et al., 2007), acting by reducing insulin receptor substrate 1 expression and GLUT4 in muscle and differentiated adipocytes (Löwenberg et al., 2006) and consequently reducing glucose uptake (Lundgren et al., 2004).

In rodents, the overexpression of *11β-HSD1* causes central obesity, dyslipidemia, and IR (Masuzaki et al., 2001). In humans, the expression of *11β-HSD1* in superficial subcutaneous adipose tissue has been correlated with central obesity and IR, but no difference has been found in *11β-HSD1* mRNA among adipose tissue compartments and suggesting that enzyme activity, but not *11β-HSD1* mRNA level, was higher in visceral fat (Wake et al., 2003; Goedecke et al., 2006). Additionally, superficial subcutaneous fat *11β-HSD1* mRNA levels were associated with MS (Goedecke et al., 2006). Superficial subcutaneous adipose tissue and visceral fat also present similar *11β-HSD1* mRNA levels, except in obese patients, who display higher *11β-HSD1* mRNA expression in subcutaneous adipose tissue (Goedecke et al., 2006). Increased *11β-HSD1* expression in visceral fat was also found in prepubertal children with normal birth weight and normal BMI despite the presence of similar *GRα* mRNA expression in subcutaneous and visceral adipose tissues (Mericq et al., 2009).

Insulin seems to increase *11β-HSD1* mRNA and enzymatic activity (Balachandran et al., 2008). Considering that muscle has high IR, we expected that neither elevated *11β-HSD1* mRNA level nor increased enzymatic activity would be present in this tissue; therefore, we expected no substantial increase in muscular cortisol level with any adaptive downregulation of *GR* gene expression. In visceral fat, IR is not as severe, and the increased activity of *11β-HSD1*, despite the presence of normal *11β-HSD1* mRNA levels, can be expected. At least for gene expression, this expectation was confirmed by our findings showing that *11β-HSD1* remained unchanged despite the presence of IR. Thus, in visceral fat, the direct and excessive effect of insulin can combine increased *GRα* phosphorylation and GC sensitivity (Georgakopoulos and Tsawdaroglou, 1996) with increased *11β-HSD1* activity, resulting in *GRα* mRNA downregulation. This finding agrees with our determination of higher *GRα* mRNA levels in muscle and lower levels in visceral adipose tissue.

Taking together our findings and the data reported in the literature, we suggest an

additional relationship between GC action and insulin sensitivity as follows: patients with IR usually display differences in insulin sensitivity in specific tissues. Under these conditions, IR is more severe in muscle than in visceral fat. GCs can aggravate IR and increase hyperinsulinemia. Our findings of persistent high levels of *GRα* mRNA in muscle suggest that in this tissue, the worsening of IR should be more intense. At the visceral fat level, relative protection against cortisol effects is determined by *GRα* mRNA downregulation, and a less severe IR is observed, allowing progressive adipogenesis (Figure 3). This protective effect of *GRα* mRNA downregulation seems to be partially lost with the progression of IR. At the final stages of IR, severe hyperinsulinemia alone is an activating modulator of GC action through induction of *GRα* phosphorylation, which consequently increases GC sensitivity.

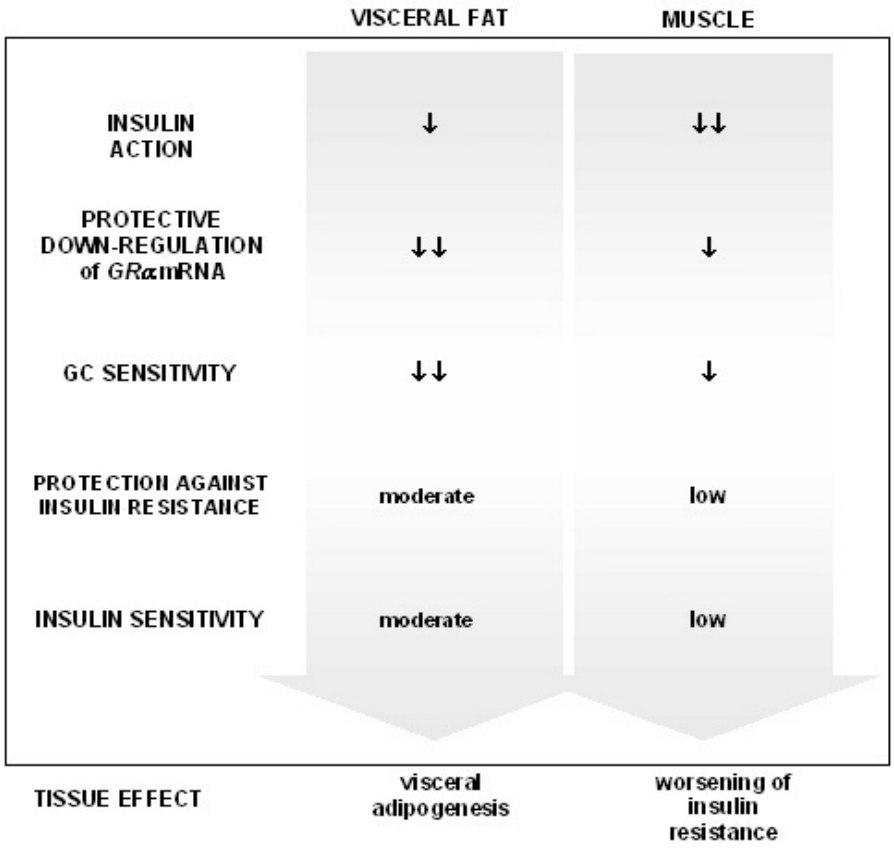

Figure 3. Adaptive levels of *GRα* mRNA and glucocorticoid (GC) sensitivity during insulin resistance. In patients with insulin resistance, tissue-specific insulin sensitivity is observed. Failure in insulin action is predominant in muscle compared to visceral fat. We identified more intense *GRα* down-regulation in visceral fat, which could represent a protective effect by decreasing GC sensitivity in this tissue. Decreased GC action induces minor aggravation in insulin sensitivity, and therefore maintains visceral adipogenesis. Additionally, insulin is able to increase *GRα* phosphorylation and *11β-HSD1* activity, consequently down-regulating *GRα* gene expression and reducing GC sensitivity. Individuals with polymorphic variants conferring higher GC sensitivity (C3; *Bcl*I; N363S) can present more severe progression of metabolic syndrome. In muscle, *GRα* down-regulation is less severe, allowing higher GC action with a consequent worsening of insulin resistance.

Future investigations should address other modulating factors involved in adaptive *GRα* downregulation. Activating polymorphic variants of *GRα* are also implicated in variable tissue GC sensitivity in patients with IR. The *Bcl*I and N363S GR variants are associated with increased GC sensitivity and metabolic abnormalities (Di Blasio et al., 2003). The proportion of several *GRα* isoforms is also related to tissue sensitivity to GC (Gross et al., 2009). *GRα*-C3 is considered the most active isoform (Lu and Cidlowski, 2005), and therefore a higher proportion of C3 isoform increases GC sensitivity, even in the presence of a lower number of GRs. Another area of future research is the expression of tissue-specific co-activators, which also influence tissue-specific GC sensitivity (Duma et al., 2006) and can explain the variable amount of *GRα* mRNA in visceral fat and muscular tissue. Another source of influence is represented by GR polymorphisms in residues involved in GR phosphorylation (Webster et al., 1997) and ubiquitination (Wallace and Cidlowski, 2001), which reduce the half-life of the receptor.

Another aspect to be explored is the alternative action of GC through the mineralocorticoid receptor (MR) in tissues with low or absent *11β-HSD2*, such as the adipose tissue. The GC effect through MR signaling can also activate adipogenesis (Zennaro et al., 2009) and inflammatory pathways (Hoppmann et al., 2010). These effects can be relevant in IR patients. MR polymorphisms, such as p.I180V, are also reportedly associated with known cardiovascular risk factors, such as elevated BMI and low-density lipoprotein level (Fernandes-Rosa et al., 2010), through modulation of GC effects in adipose tissue (Zennaro et al., 2009).

In conclusion, our findings suggest that the level of IR negatively modulates the amplitude of *GRα* downregulation. IR in visceral fat is less severely affected, allowing a more intense reduction of *GRα* expression and consequently greater reduction in GC sensitivity. Conversely, in IR tissues such as muscle, *GRα* expression is not downregulated, and therefore high GC sensitivity is maintained, creating a vicious cycle that aggravates insulin resistance.

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