

**11- β HYDROXYSTEROID DEHYDROGENASE TYPE 2 EXPRESSION IN WHITE ADIPOSE
TISSUE IS STRONGLY CORRELATED WITH ADIPOSITY**

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Abstract:

Glucocorticoid action within the cells is regulated by the expression levels of the glucocorticoid receptor and two enzymes, 11 β hydroxysteroid dehydrogenase types 1 and 2. Thus, 11- β dehydrogenase type 1 activates glucocorticoid action, whereas 11- β dehydrogenase type 2 regulates the access of 11- β hydroxyglucocorticoids to the mineralocorticoid receptor by converting cortisol/corticosterone to the glucocorticoid-inactive form cortisone/ dehydrocorticosterone. Male Wistar rats developed obesity by being fed on a high-fat diet for 56 days, and were compared with control-diet fed animals. Gene expression analysis of 11- β hydroxysteroid dehydrogenase type 2, 11- β hydroxysteroid dehydrogenase type 1, and glucocorticoid receptor were performed by RT-PCR in subcutaneous and retroperitoneal adipose tissue. High-fat fed animals overexpressed 11- β hydroxysteroid dehydrogenase type 2 in subcutaneous adipose tissue, but not in retroperitoneal fat pad. Interestingly, mRNA levels strongly correlated in both tissues with different parameters related to obesity, such as body weight, adiposity or insulin resistance, suggesting that this gene is a reliable marker of adiposity in this model of overweight in rats. These results state that 11- β hydroxysteroid dehydrogenase type 2 is expressed in adipose tissue by adipocytes and stromal-vascular cells, and suggest that this enzyme could play an important role in combating fat accumulation in adipose tissue.

Keywords: Hydroxysteroid 11- β dehydrogenase type 2, obesity, high-fat diet, RT-PCR, glucocorticoid receptor, leptin, insulin resistance

Abbreviations: 11 β HSD2: 11- β hydroxysteroid dehydrogenase type 2; 11 β HSD1: 11- β hydroxysteroid dehydrogenase type 1; GR: Glucocorticoid Receptor; GC: Glucocorticoid; WAT: White adipose tissue.

Introduction

A cause of obesity and metabolic complications in humans is the exposure to excessive levels of circulating glucocorticoids (GC), being a necessary condition for the development of diet-induced obesity [1, 2]. GC action within the cells is regulated by the expression levels of the glucocorticoid receptor (GR) and the two enzymes type 1 and type 2 11 β -hydroxysteroid dehydrogenase (11 β HSD1 and 11 β HSD2) [3]. 11 β HSD1 is the enzyme that catalyses the regeneration of active GCs from inert 11-keto forms, thus amplifying local GC action. This enzyme is abundantly expressed in white adipose tissue (WAT), specifically in omental fat, where facilitates GC-induced adipocyte differentiation [4]. As reported in the literature, its expression is increased in subcutaneous adipose tissue from obese humans and rodents [3, 5], but does not change or is slightly reduced in omental fat [6]. However, it appears downregulated in rodent fat in response to high-fat diets [7]. Pharmacological inhibition of 11 β HSD1 in obese rodents is able to improve glucose tolerance, insulin sensitivity, and lipid profiles, whereas adipocyte-specific overexpression of 11 β HSD1 develops visceral obesity and metabolic syndrome in mice [8].

Meanwhile, 11 β HSD2 regulates the access of 11 β -hydroxyglucocorticoids to the mineralocorticoid receptor by converting cortisol/corticosterone to the GR-inactive form cortisone/dehydrocorticosterone [9]. This gene is selectively expressed in mineralocorticoid target tissues, such as kidney and colon [10], where it confers aldosterone selectivity for its own receptor [3]. However, the expression of 11 β HSD2 in WAT has not been well-established and is controversial [11], with some authors reporting extremely low levels in mice [9] and humans [5].

The aim of the present study was to establish whether 11 β HSD2 is really expressed in rat adipocytes, and whether its expression in WAT is influenced by high-fat feeding.

Materials and Methods

Animals. Sixteen male Wistar rats, supplied by the Applied Pharmacobiology Center (CIFA, Pamplona, Spain), were assigned into two different dietary groups. One group (n=8, Control group) was fed on a standard pelleted diet (Harlan Iberica, Barcelona, Spain), and a second group (n=8, Cafeteria) was fed on a high-fat diet in order to produce an obesity model as previously reported [1, 2]. At the end of the experimental period (56 days), rats were anaesthetized in the fasted state with Ketamine (50 mg/kg *ip*, Parke-Davis, Madrid, Spain) and Medetomidine (0.025 mg/kg *ip*, Pfizer S.A., Madrid, Spain). Before the animals were killed by decapitation, body composition was measured with EM-SCAN (Springfield, IL,

USA) model SA2 [1, 2]. Blood and WAT depots (subcutaneous and retroperitoneal) were immediately collected, weighed and frozen. Adipocytes were isolated after collagenase digestion [2], and stromal-vascular cells were obtained by centrifugation at 500g for 7 minutes. All the procedures were performed according to national and institutional guidelines of the Animal Care and Use Committee at the University of Navarra.

Serum measurements. Glucose was measured with the HK-CP kit (ABX diagnostic, Montpellier, France) adapted for a COBAS MIRA equipment (Roche, Basel, Switzerland). Serum leptin and insulin were assayed by radioimmunoassay, as described by the supplier (Linco Research, Missouri, USA). The homeostatic model assessment (HOMA) as an insulin resistance index was calculated as fasting plasma glucose times fasting serum insulin divided by 22.5.

Real Time PCR. Total RNA was isolated from frozen retroperitoneal and subcutaneous adipose tissue using *Trizol* (Invitrogen, CA, USA) according to the manufacturer's instructions. DNase treatment was performed with *DNA-free kit* (Ambion, TX, USA). The purified total RNA was used as a template to generate first-strand cDNA synthesis using M-MLV reverse transcriptase (Invitrogen) as described by the manufacturer. Quantitative real-time PCR was performed as described by the provider (Applied Biosystems, CA, USA) using an ABI PRISM 7000 HT Sequence Detection System [1]. Taqman probes for rat GR, 11 β HSD1, and 11 β HSD2 mRNA, glyceraldehyde 3-phosphate dehydrogenase and 18s rRNA were also supplied by Applied Biosystems (CA, USA). Gene expression levels were normalized using glyceraldehyde 3-phosphate dehydrogenase and 18s as internal controls. Fold change between cafeteria and control rats was calculated using the $2^{-\Delta\Delta C_t}$ method by applying GeNorm software [12].

Statistical analysis. Results were expressed as mean \pm standard error of the mean (SEM). The differences between the groups were evaluated by student's t test, and the correlation analysis was performed using Pearson Correlation coefficient. Statistical analyses were calculated using SPSS 13.0 packages (Chicago, IL, USA).

Results

Animals fed on the high-fat diet during 8 weeks gained more body weight than those animals fed on the standard-fat diet (23%, $p < 0.01$). Moreover, the total body fat and the weights of subcutaneous and retroperitoneal depots from overweight rats were also greater than in lean animals (94%, $p < 0.001$; 225%, $p < 0.05$; and 111%, $p < 0.01$, respectively). Also serum measurements were significantly increased by the

high-fat diet as follows: leptin (324%, $p < 0.01$), insulin (203%, $p < 0.05$), glucose (35%, $p < 0.05$), and HOMA insulin resistance index (291%, $p < 0.05$).

On the other hand, gene expression analysis of three key genes involved in GC action (11 β HSD1, 11 β HSD2 and GR) showed detectable levels in subcutaneous and retroperitoneal WAT fragments, being the magnitude of expression: GR > 11 β HSD1 > 11 β HSD2 mRNA (data not shown). Being the presence of 11 β HSD2 mRNA a matter of debate, we analyzed also 11 β HSD2 gene expression separately in stromal vascular cells and adipocytes from both WAT depots, presenting similar levels of mRNA in both cellular types (data not shown). Regarding gene expression, high-fat feeding induced an increase in subcutaneous fat's 11 β HSD2 and GR, but no changes in 11 β HSD1 (Figure 1B). As a result, the ratio of 11 β HSD1 to 11 β HSD2 expression in this depot was 11.8 ± 1.5 in control rats, but only 4.0 ± 0.8 in high-fat fed rats ($p < 0.001$). No differences were detected in retroperitoneal fat pad in any of the assessed genes in comparison to the controls.

Finally, correlation analysis between gene expression levels of 11 β HSD1, 11 β HSD2, and GR from subcutaneous and retroperitoneal fat pads and rat metabolic parameters (Table 1), showed a highly significant correlation between 11 β HSD2 gene expression in both depots and all the diet-induced parameters, with the HOMA insulin resistance index presenting the strongest correlation ($r = 0.865$, $p < 0.001$, and $r = 0.891$, $p < 0.001$ for subcutaneous and retroperitoneal pads, respectively). Interestingly, 11 β HSD1 mRNA levels did not show any significant correlation with the analyzed parameters, meanwhile GR expression in subcutaneous WAT correlated with some metabolic measurements (Table 1).

Discussion

Our data report for the first time that 11 β HSD2 is expressed in rat WAT in both adipocytes and stromal-vascular cells, being mRNA levels higher (2 fold) in the retroperitoneal location than in subcutaneous adipose tissue. Although 11 β HSD2 is expressed in different tissues, some authors had not detected its expression in adipocytes [13]. However, Engeli et al [5] found an under-regulation by half of this gene in adipose tissue of obese women, which has been considered by other authors as a vascular contamination of biopsies [11]. Responding to this, our data state that 11 β HSD2 is expressed at a similar level in adipocytes and stroma-vascular cells, in both subcutaneous and retroperitoneal locations.

The three genes analyzed are differentially regulated in our obesity model concerning the studied fat pads, being GR and 11 β HSD2 overexpressed in subcutaneous WAT but unchanged in the retroperitoneal depot. At this respect, although some differences have been observed by other authors when comparing the expression pattern of genes related with GC metabolism [5, 7, 9, 11, 14], they could be explained by differences in the specie, fat depot, and obesity model.

It has been reported that visceral fat is the physiological GC target in the high-fat diet-induced weight gain [8]. Consistent with this, the levels of GR and 11 β HSD1 mRNAs were higher (4 fold) in retroperitoneal compared with subcutaneous adipose tissue. Nevertheless, the ratio between 11 β HSD2 and 11 β HSD1 expression does not change significantly in retroperitoneal location, but drops from twelve- to four-fold in subcutaneous fat. As 11 β HSD2 shows a several-fold higher affinity for cortisol than does 11 β HSD1 for cortisone [11], it is suggested that 11 β HSD2 could be important in determining GC metabolism in this location.

Hence, the upregulation in subcutaneous WAT of 11 β HSD2 mRNA in the cafeteria-fed model could represent an additional defense against excess fat storage and GC responsiveness. The significance of this enzyme as a potential marker of adiposity is firmly reinforced from the findings of Kershaw et al. [9] reporting resistance to gaining weight in transgenic mice overexpressing this protein, but also by the strong correlation between 11 β HSD2 mRNA levels in both analyzed fat depots and body weight, adiposity or insulin resistance.

Summarizing, GC metabolism, including 11 β HSD1 and 11 β HSD2, plays a critical role in the development of obesity, affecting glucose homeostasis, insulin sensitivity, lipid storage, and adipogenesis. The adaptive upregulation of adipose 11 β HSD2 expression in response to chronic high-

fat diet represents a novel mechanism that may counteract the GC action occurring in obesity, opening the door to study this enzyme as a target regulating excess fat deposition.

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Figure 1. mRNA expression of 11 β HSD1, 11 β HSD2, and GR in subcutaneous and retroperitoneal WAT samples of high-fat fed rats when compared with control diet-fed animals. Statistical differences by Student's t test: * p<0.05.

FIGURE 1

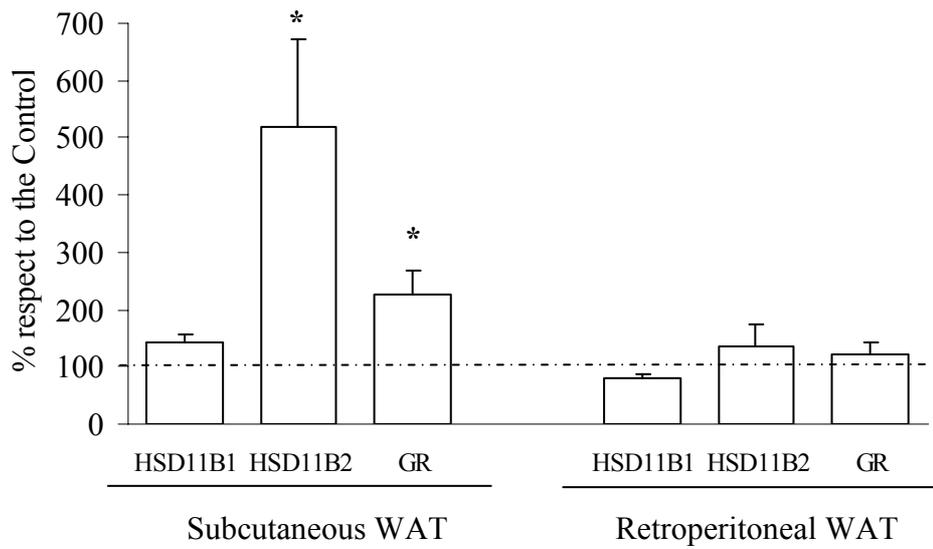


Table 1. Correlation analysis between gene expression levels of 11 β HSD1, 11 β HSD2 and GR from subcutaneous and retroperitoneal adipose tissue and body weight, total fat weight, leptin and HOMA insulin resistance index. Gene expression levels were normalized with those analyzed by GENORM and calculated from $2^{-\Delta\Delta C_t}$ as described in Materials and Method (r = Pearson Correlation coefficient; Control vs. Cafeteria: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

	r	<i>Subcutaneous WAT</i>						<i>Retroperitoneal WAT</i>					
		HSD11B1		HSD11B2		GR		HSD11B1		HSD11B2		GR	
<i>Body weight (g)</i>	r	0.405	n.s.	0.828	***	0.782	***	-0.403	n.s.	0.685	**	0.308	n.s.
<i>Subcutaneous WAT (g)</i>	r	0.366	n.s.	0.790	***	0.446	n.s.	-0.455	n.s.	0.747	**	-0.128	n.s.
<i>Retroperitoneal WAT (g)</i>	r	0.350	n.s.	0.789	***	0.583	*	-0.329	n.s.	0.723	**	0.167	n.s.
<i>Body fat (g)</i>	r	0.410	n.s.	0.854	***	0.604	*	-0.420	n.s.	0.732	**	0.058	n.s.
<i>Leptin (ng/mL)</i>	r	0.432	n.s.	0.778	***	0.680	**	-0.369	n.s.	0.671	**	0.223	n.s.
<i>HOMA</i>	r	0.259	n.s.	0.865	***	0.547	*	-0.384	n.s.	0.891	***	-0.016	n.s.