



Comparison of adipose tissue-derived genes in endogenous Cushing's syndrome versus diet-induced obesity

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Abstract

Introduction: Dysregulation of adipokine secretion and action is a characteristic feature of obesity and a key clinical feature of Cushing's syndrome (CS). We have investigated whether endogenous glucocorticoid excess influences adipose tissue-derived gene expression.

Material and methods: mRNA expression of adipokines; adiponectin, resistin, tumour necrosis factor- α , interleukin-6 (IL-6), angiotensinogen (AGT), plasminogen activator inhibitor type 1, retinol binding protein 4, visfatin, and cystatin C was assessed by quantitative real-time RT-PCR in visceral adipose tissue removed during abdominal surgery of eight patients with CS, and six control patients.

Results: We did not find any significant difference in the investigated genes; however, the almost significant overexpression of AGT and underexpression of IL-6 might be noteworthy ($p = 0.06$ in both cases).

Conclusions: No significant differences were found in the expression of the investigated genes known as cardiometabolic risk factors. This indicates that there are no major differences between endogenous hypercortisolism or diet-induced obesity regarding the expression of adipokines involved in cardiometabolic disorders. However, the difference in AGT and IL-6 expression might be included in pathways affecting fat distribution in CS. (Endokrynol Pol 2019; 70 (2): 131–134)

Key words: Cushing's syndrome; obesity; adipokines; visceral adipose tissue, glucocorticoids

Introduction

The pathogenesis of obesity is an intensively studied research field because of its high prevalence and severe comorbidities. Especially visceral/abdominal obesity has a strong link to the pathophysiology of comorbidities (insulin resistance, impaired glucose tolerance or type 2 diabetes, dyslipidaemia, inflammatory disorders, hypercoagulability, hypertension, atherosclerosis, or other cardiovascular disorders) [1]. One of the typical features of Cushing's syndrome (CS) is central obesity. CS is the consequence of chronic endogenous glucocorticoid (GC) excess, and the above-mentioned associated disorders can also join the list of its clinical properties.

Glucocorticoids are involved in several physiological processes, among others in the regulation of food intake, gluco- and adipogenesis, strongly influencing energy metabolism and body weight [2]. GCs have several targets in lipid metabolism, are powerful regulators of fat deposition and distribution, and are essential in adipogenesis and adipocyte differentiation [3]. Glucocorticoids might play an important role in the development of visceral adiposity [4]. White adipose tissue secretes a large number of adipokines, which

have effects on inflammation/oxidative stress (leptin, adiponectin, resistin), energy wasting (leptin, adiponectin), insulin signalling (adiponectin, leptin, visfatin), endothelial dysfunction (visfatin), and vascular damage (adiponectin, leptin, resistin), which are relevant in obesity. Obesity disrupts the dynamic role of adipocytes, resulting in altered production of adipokines. This is involved in the well-known obesity-related complications. In central obesity altered adipokine secretion has been observed. Both *in vitro* and *in vivo* studies have shown a relationship between cortisol and adipokines [5]. Altered cytokine levels in CS lead to increased mortality [5]. We have to note that mainly circulating cytokine levels have been measured in these studies, or if gene expression analysis was performed, it was done on subcutaneous adipose tissue samples [6]. We have therefore investigated the gene expression of several adipokines, which have been shown to have a major influence on cardiovascular complications, and are produced mainly by visceral but not subcutaneous adipose tissue [adiponectin (ADIPDQ), resistin (RETN), tumour necrosis factor- α (TNF- α), interleukin-6 (IL-6), angiotensinogen (AGT), plasminogen activator inhibitor type 1 (PAI-1), retinol binding protein 4 (RBP4), visfatin,



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cystatin C (CST3)] in visceral adipose tissue of patients with CS and control subjects.

Material and methods

Patients

Altogether 14 obese patients (BMI > 30 kg/m²) were involved in this study: eight patients with adrenal Cushing's syndrome undergoing adrenalectomy and six control patients undergoing elective abdominal surgery (such as cholecystectomy, appendectomy). To avoid the gender differences of gene expression, we performed an only female study. The control patients had no symptoms and laboratory signs of hypercortisolism. The diagnosis of Cushing's syndrome was made on the basis of clinical appearance, laboratory testing (overnight and low-dose dexamethasone test, ACTH) and imaging. Table I summarises the clinical characteristics of the patients. There was no significant difference between the two groups in terms of age or body mass index. The study was approved by the local research ethics committee, and all patients involved gave written, informed consent in accordance with the guidelines in the declaration of Helsinki.

Tissue handling

Abdominal adipose tissue was taken by excision at the end of the surgical intervention. The tissue samples were immediately frozen on dry ice in the operating room for RNA extraction and transferred to the laboratory. Samples were stored at -80°C until use.

RNA isolation

Total RNA extraction from fresh-frozen, 500 µl human adipose tissues was performed after tissue homogenisation with a Qiagen miRNeasy Mini Kit (Qiagen GmbH, Hilden, Germany) according to the instructions of the manufacturer. On-column DNase digestion was carried out with the RNase-Free DNase set (Qiagen GmbH, Hilden, Germany). RNA quantity was determined by NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA), and then RNA integrity was examined by an Agilent 2100 Bioanalyzer System (Agilent Technologies Inc., Santa Clara, CA, USA). Samples with an RNA integrity number (RIN) above 6.0 were used for further analysis. RNA was stored at -80 °C until use.

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA, USA) were used as follows: ADIPOQ (00605917), RETN (00220767), TNF-α (01113624), IL6 (00985639), AGT (01586213), SERPINE 1 (01126606), RBP4 (00198830), NAMPT (00237184), CST3 (00264679).

Hypoxanthine phosphoribosyltransferase 1 (HPRT1) (02800695) was used as a reference gene (Hurtado 2010).

Table I. Clinical characteristics of the patients

	Patients with CS	Control patients
Number of patients/ /gender	8 females	6 females
BMI	35.4 ± 0.6	34.6 ± 0.4
Age at surgery	48 ± 8 years	47 ± 7 years
Size of the adrenal gland (CT)	23 ± 8 mm	NA
Cortisol (after 1 mg dexamethasone)	227 ± 37 nmol/l	28 ± 7 nmol/l
ACTH (10–60 pg/ml)	6 ± 3 pg/ml	NA

CS — Cushing's syndrome; BMI — body mass index; CT — computed tomography; ACTH — adrenocorticotrophic hormone; NA — not available

Total RNA (10 ng) was reverse transcribed to cDNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). qRT-PCR was performed by TaqMan Fast Universal PCR Master Mix (2x) (Applied Biosystems) on a 7500 Fast Real-Time PCR System (Applied Biosystems) according to the manufacturer's instruction. All samples were run in triplicate. Normalised signal levels for each gene were calculated using the comparative cycle threshold (ddCT) method relative to HPRT1 following the instructions of the manufacturer (SDS Program, Applied Biosystems).

Statistical analysis

For the identification of differentially expressed genes between Cushing's adipose tissue groups and control adipose tissue groups, F-test was used followed by t-test. p values < 0.05 were considered statistically significant.

Results

Nine genes (*ADIPOQ*, *RETN*, *TNF-α*, *IL6*, *AGT*, *SERPINE-1*, *RBP4*, *NAMPT*, *cystatin C*) were selected based on literature data, and their expression was studied by qRT-PCR. None of these genes turned out to be significantly differentially expressed between CS and normal samples; however, the expression of two genes was almost significant. *AGT* was overexpressed in Cushing's adipose samples versus control samples (p = 0.06). Underexpression of *IL-6* was validated in Cushing's adipose samples versus control samples (p = 0.06) (Fig. 1).

RETN could not be measured in tissue samples, and the expression of *TNF-α* only exceeded the detection limit in some samples.

Discussion

Adipose tissue plays not only a role in energy storage, but it is also an endocrine organ that secretes numer-

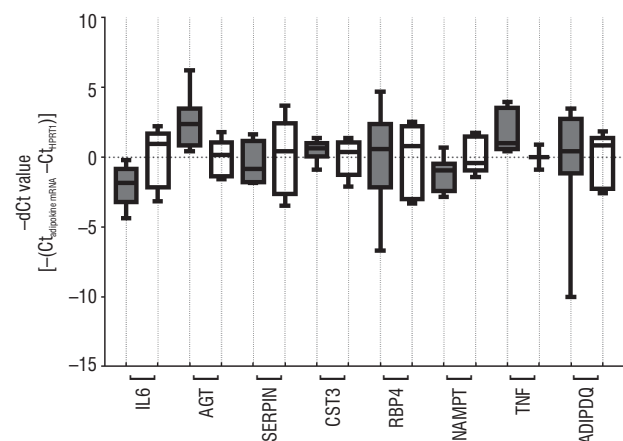


Figure 1. Relative expression of different adipokine mRNAs in Cushing's syndrome (n = 8) compared to controls (n = 6) by qRT-PCR. Dark bars represent Cushing's syndrome patients, whereas white bars are controls. Mean ± SD of -dCt values of mRNAs relative to HPRT1 are shown

ous hormones and bioactive molecules, the so-called “adipokines”.

Several data suggest meaningful influence of GCs on the expression of different adipokines. In the current study we have investigated the expression level of several adipokines in the visceral adipose tissue of patients with CS and control obese patients, to investigate the potential relevance of adipokines in the abdominal obesity characteristic for CS. Genes have been selected based on literature data [5]. Below, we briefly summarise the most important characteristics of the studied adipokines. Two adipokines showed almost significant alterations in expression in our small cohort.

Adipocytes are the most important source of *angiogenesis*. Transient or chronic overexpression of AGT in adipose tissue favours lipogenesis in adipocytes and leads to a vicious circle whereby adipose tissue development is further increased. AGT level is higher in obese than lean subjects and the AGT-mRNA expression is upregulated, especially in omental adipose tissue in obese patients [7, 8]. We showed an almost significant overexpression of AGT in visceral adipose tissue of CS patients compared to obese controls. This finding, taking into consideration that in obese patients the AGT-mRNA expression is already upregulated, might suggest that GCs might play a role in stimulation of AGT production. The adipose tissue expansion needs appropriate blood supply providing O₂, nutrition, growth factors, hormones, and stem cells [9]. Angiogenesis is an important step in adipose tissue remodelling [10]. AGT is known to be involved also in angiogenesis [11, 12]. The almost significant overexpression of AGT might be linked to angiogenesis and thereby implicated in visceral adipose tissue expansion.

mRNA expression studies show that adipocytes also can synthesise both tumour necrosis factor alpha and several interleukins, among them IL-6. TNF- α and IL-6 reduce lipid accumulation in adipose tissue, have some trophic effects, regulate insulin signalling [13], and are prominent contributors in the pathogenesis of atherosclerosis [5]. In our study, IL-6 expression was almost significantly down-regulated in patients with CS, compared to the control group. There are conflicting data on IL-6 levels in CS in the literature. The plasma levels of IL-6 have been found to be significantly higher with active CS women than in controls [14]. In contrast, in another study no significant difference was found in patients with CS and controls, but after surgery for Cushing’s syndrome, IL-6 levels rose, which could be associated with the well-known suppressive action of glucocorticoids on IL-6 secretion [15]. The anti-inflammatory effect of GCs might be related to the suppression of tissue IL-6 expression in our study. Moreover, the expression of tissue cytokines does not

always parallel the serum concentrations [16]. It has already been shown that GCs decrease inflammatory cytokines, such as IL-6 and TNF- α , in adipose tissue samples [17, 18].

Regarding the following genes, we found no difference in their expression in the CS group *vs.* the control group.

Plasminogen activator inhibitor type 1 is involved in vascular homeostasis [5], and it might play a role in the hypercoagulable and hypofibrinolytic state observed in CS. In different studies, elevated PAI-I level has been found in CS subjects compared to healthy controls [19–21]. We found no difference in the PAI-I gene expression level in CS patients compared to obese controls, but we have to note that in our study the control subjects were BMI-matched obese patients and PAI-1 is known to be elevated in obesity, while in the other studies they were healthy controls.

Retinol binding protein 4 (RBP4) elevation was shown to cause adipose tissue inflammation by activating innate immunity [22]. Insulin resistance, which is a common complication of CS, is associated with adipose tissue inflammation. Although dexamethasone was shown to induce genes related to acute-phase response or innate immunity, such as RBP4 [23], we did not find any difference in RBP4 expression in our cohort.

Regarding the level of other adipokines (adiponectin, resistin, visfatin, and cystatin C), of which gene expression level we did not find any difference, there are contradictory results based on the few studies performed on humans. Cortisol excess has either no effect or it can influence the level of these adipokines in some settings [14, 24–27].

Fat accumulation in visceral depots is associated with an increased risk of metabolic disease. Hypercortisolism is associated with visceral obesity. Glucocorticoids have an effect on adipocyte metabolic, endocrine, and immune functions. There are some known molecular mechanisms that mediate depot differences in CS (11 β -HSD1, AMPK, leptin, and lipoprotein lipase demonstrated differences in sensitivity and responsiveness to GC effect) [28–31], but the molecular mechanism by which GCs exert their effect on adipose tissue is not fully understood. We performed our study directly on visceral adipose tissue of patients with hypercortisolism and on control subjects, and we did not find any difference between the two groups in the investigated genes.

Conclusions

Dysregulated adipokine secretion and action in obesity play an important role in the development of

cardiometabolic disorders. Cushing's syndrome with endogenous glucocorticoid excess is associated with central obesity. Glucocorticoids regulate differentiation, distribution, endocrine function, and metabolism of adipose tissue. Previous studies have found an imbalance in adipokine production in conditions with central obesity, such as Cushing's syndrome. In these studies, mainly circulating cytokine levels were measured, while we investigated the gene expression of several adipokines produced mainly by visceral adipose tissue. We did not show any difference in the genes that are marked as cardiometabolic risk factors. This indicates that there are no major differences between endogenous hypercortisolism or diet-induced obesity regarding the pathogenesis of complications.

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