Original Article

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The Effect of Chronic Glucocorticoid Exposure on Brown Adipose Tissue in Cushing's Disease

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Abstract

Aim: In this study, we aimed to evaluate the levels of brown adipose tissue markers uncoupling protein-1 (UCP-1), irisin, bone morphogenetic protein-7 (BMP-7) and PR-Domain Zinc Finger Protein-16 (PRDM-16) in Cushing's disease (CH) with hypercortisolism.

Methods: The study was conducted prospectively with 48 CH patients and 40 healthy volunteers between 2018 and 2019. Two cc of peripheral blood taken from the participants was centrifuged and stored at -80 °C degrees. Cushing's syndrome was excluded by performing 1 mg dexamethasone suppression test in the control group. Blood samples were analyzed by Enzyme-linked Immunosorbent Assay method.

Results: The patient group included 11 males (22.9%), 37 female (77.1%); 9 male (22.5%) and 31 females (77.5%) in the control group. Body mass index was 31.29±5.76 kg/m^2 in the patient group and 33.42±3.11 kg/m^2 in the control group. PRDM-16, Irisin, BMP-7, UCP-1 levels were not significantly different between the two groups. While there was a positive correlation between serum cortisol and irisin, a negative correlation was observed between urinary free cortisol and UCP-1.

Conclusion: These data suggest that long-term exposure to high doses of glucocorticoids in CH patients causes loss of adipose tissue functionality and development of resistance *in vivo*.

Keywords: Cushing's disease, glucocorticoid, brown fat tissue, UCP-1, irisin, BMP-7, PRDM-16

Introduction

Cushing syndrome (CS) results from long-lasting and inappropriate exposure to excessive concentrations of free glucocorticoids in the bloodstream. It leads to many comorbid conditions including chronic hypercortisolism, obesity, hypertension, diabetes, dyslipidemia and cerebrovascular diseases (1). Adipose tissue plays a central role in the interaction between nutrition, energy balance and human health. White adipose tissue (WAT) stores the energy, whereas brown adipose tissue (BAT) distributes the energy. With the discovery of BAT that has a high metabolic activity by functional imaging methods, research on importance of BAT has been increasing (2). Uncoupling protein-1 (UCP-1) is localized in the inner membrane of mitochondria of BAT cells and leads to a very high amount of fatty acid oxidation that directly produces heat through annihilation of the negative feedback inhibition on the mitochondrial Krebs cycle executed by high adenosine triphosphate and/or low adenosine diphosphate levels (3). In studies, several mediators which regulates UCP-1 expression and are involved in differentiation of BAT, including irisin, bone morphogenetic protein-7 (BMP-7) and PR-Domain Zinc Finger Protein-16 (PRDM-16), have been described (4-6).

Knowledge on effect of glucocorticoids on functions of BAT in humans is insufficient. Glucocorticoids have

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been shown to inhibit the reaction of adipocytes in human BAT cultures to adrenergic stimulation *in vitro* (7). While glucocorticoids alter the brown preadipocyte tissue, it suppresses UCP-1 expression and activity in brown adipocytes. In animal trials, lipid deposition in BAT increases with the administration of glucocorticoids, whereas thermogenic activity and production of UCP-1 are decreased (8).

In our study, it was aimed to evaluated levels of UCP-1, irisin, BMP-7 and PRDM-16, which are considered as markers for brown adipose tissue, in Cushing's disease (CH) cases with hypercortisolemia.

Methods

This study was approved by Kocaeli University Ethics Committee on Noninvasive Clinical Research with the project number KU GOKAEK 2018/274 on 27.10.2018. For the clinical prospective study in which the levels of UCP-1, irisin, BMP-7 and PRDM-16, which are considered as markers for brown adipose tissue, were evaluated in CH cases, 48 patients diagnosed with CH who were being followed-up between 15.11.2018 and 01.04.2019 in Kocaeli University Department of Endocrinology and Metabolism. As a control group, 40 volunteers at 30-60 years of age who were overweight or obese, screened for CH and found to be negative, had normal glucose tolerance and had not used any hormone therapy for last 6 months were recruited. Data of those with CH and volunteers were obtained from the hospitals' automation system and patients' files.

Those who were on an insulin or incretin-based therapy had a chronic inflammatory disease, had an active malignancy, used steroids for a long period of time before 6 months, were using a medication that may influence energy homeostasis and had renal or liver dysfunction were excluded from the study.

Of the volunteers and the patients diagnosed with CH included in the study; 2 cc of blood were taken in a dry tube after obtaining informed consent. Bloods taken were centrifuged at a speed of 3,000 rpm for 15 minutes and the plasmas were stored at -80 degrees on the same day. In addition, patients' anthropometric and demographic characteristics, as well as history of drug use and smoking were also recorded. The volunteers included in the control group underwent 1 mg DST screening test and CS was excluded.

UCP-1, irisin, PRDM-16 and BMP-7 were analyzed by using the brand elabscience commercial kits in the Radim Diagnostics Rome (Italy) device with Enzyme-linked Immunosorbent Assay method in Kocaeli University Faculty of Medicine Biochemistry Research Laboratory. The unit was determined to be ng/mL.

Statistical Analysis

Statistical evaluation was performed by using the IBM SPSS 20.0 (IBM Corp., Armonk, NY, USA) package program. Conformity to normal distribution was evaluated with Kolmogorov-Smirnov test. Numerical variables exhibiting normal distribution were given as mean ± standard deviation and those not exhibiting normal distribution as median (25^{th}-75^{th} percentiles), whereas the categorical variables, however, were given as frequency (percentage). Intergroup difference was determined by using student t-test for numerical variables exhibiting normal distribution and using Mann-Whitney U test for numerical variables not exhibiting normal distribution. Correlations between categorical variables were evaluated by using chi-squared analysis. For analysis of correlations between numerical variables, Spearman correlation analyses were used, as assumption of normal distribution could not be made. Considering the factors with an impact on the study, moderator analysis was used and the corrected values of the markers were interpreted again. For testing bidirectional hypotheses, a $p<0.05$ was considered sufficient for statistical significance.

Results

For the study, 48 patients diagnosed with CH (37 female, 11 male) and 40 volunteers (31 female, 9 male) as a control group were recruited. The mean age of the groups was similar. General characteristics and laboratory values of the patient and control groups are represented in Table 1.

When the markers for BAT were compared between the patient and control groups, the markers PRDM-16, irisin, BMP-7 and UCP-1 were determined to be similar between both groups (Table 2).

By using the moderator analysis in our analysis as weight and body mass index (BMI) were included as the factors influencing BAT, corrected values of markers of both groups were re-calculated in accordance with weight and BMI and then compared again, and the results were found to be similar between both groups ($p>0.05$).

When the group with CH was evaluated in itself, it was determined that 19 (40.4%) had a macroadenoma and 28 (59.6%) a microadenoma. While 82% of patients underwent early remission, the disease persisted in 18% of patients. Furthermore, during their 6-year follow-up, 23% of the diagnosed patients were found to have a recurrence and 77% continued to be in remission.

Data on effects of the size adenomas, time to postoperative remission and rate of recurrence on markers for BAT are represented in Table 3.

A positive correlation was observed between preoperative basal cortisol and night-time cortisol

and irisin, among the markers for BAT, and a negative correlation was observed between urinary free cortisol (UFC) and UCP-1 (Table 4).

By dividing the patient group as those with a BMI under 30 and over 30, irisin, PRDM-16, UCP-1 and BMP-7 were compared. BMP-7 was determined to be higher in the group with a BMI >30 (p<0.05). No significant difference was detected for other markers for BAT (p>0.05). In the comparison by triglyceride (TG) levels, PRDM-16 and BMP-7 were determined to be higher in patients with a TG <150 (p<0.05). No significant difference was determined between HbA1c and markers for BAT (p>0.05).

Discussion

CS indicates pathological hypercortisolism as a result of excessive adrenocorticotropic hormone (ACTH)

Table 1. Characteristics of the patient and control groups

	Patient group	Control group	p
Female (n) (%)	37 (77.1%)	31 (77.5%)	>0.05
Male (n) (%)	11 (22.9%)	9 (22.5%)	>0.05
Age (years) (mean ± SD)	44.04±13.79	45.30±9.31	>0.05
Weight (kg) (mean ± SD)	82.47±13.13	89.51±11.26	<0.05
BMI (kg/m²) (mean ± SD)	31.29±5.76	32.4±3.11	<0.05
HbA1c (%)	6.3 (5.7-6.8)	5.6 (5.3-5.9)	<0.05
CRP (mg/L)	0.73 (0.4-1.2)	0.53 (0.3-0.9)	>0.05
Triglyceride (mg/dL) (mean ± SD)	190.5±80	83.5±49.2	<0.05
HDL (mg/dL) (mean ± SD)	48.5±15.5	51.3±10.7	>0.05
LDL (mg/dL) (mean ± SD)	125.1±27.5	134.6±27.9	>0.05
25-OH (mg/mL) (mean ± SD)	15.3±7.83	17.9±8.31	>0.05

Independent samples t-test
BMI: Body mass index, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, CRP: C-reactive protein, 25-OH: Vitamin D, SD: Standard deviation

Table 2. Comparison of the patient and control groups for markers for BAT

	Patient group	Control group	p
UCP-1 (ng/mL)	1.4±1.09	1.3±0.74	>0.05
İrisin (ng/mL)	3.2±0.8	2.7±1.16	>0.05
BMP-7 (ng/mL)	148.1±97.9	158.8±84.78	>0.05
PRDM-16 (ng/mL)	207.6±136.3	306.9±308.6	>0.05

UCP-1: Uncoupling protein-1, BMP-7: Bone morphogenetic protein-7, PRDM-16: PR-Domain Zinc Finger Protein-16, BAT: Brown adipose tissue

Table 3. Correlation of size of adenoma, time of remission and rate of recurrence with markers for BAT in the patient group

	Recurred	Not recurred	Those with early remission	Those without early remission	Microadenoma	Macroadenoma
İrisin (25%-75%)	3.6 (3.1-3.7)	3.7 (2.2-3.7)	3.7 (2.6-3.7)	3.7 (3.7-3.7)	3.7 (2.29-3.7)	3.7 (3.5-3.7)
p	>0.05		>0.05		>0.05	
UCP-1(25%-75%)	0.79 (0.43-1.12)	0.91 (0.56-2.43)	1.12 (0.55-2.36)	2.5 (0.5-2.7)	1.68 (0.61-2.6)	1.01 (0.49-1.27)
p	>0.05		>0.05		<0.05	
PRDM-16 (25%-75%)	180 (146-263)	153 (138-217)	157 (142-281)	164 (132-195)	160 (140-240)	152 (138-197)
p	>0.05		>0.05		>0.05	
BMP-7 (25%-75%)	98.9 (60-135)	104 (67-240)	127 (72-210)	217 (77-350)	157 (67-242)	86 (65-177)
p	>0.05		>0.05		>0.05	

Spearman correlation analyses
UCP-1: Uncoupling protein-1, BMP-7: Bone morphogenetic protein-7, PRDM-16: PR-Domain Zinc Finger Protein-16, BAT: Brown adipose tissue

Table 4. Correlation analysis of markers for BAT and hormones in the patient group								
	Basal ACTH		Basal cortisol		Night-time cortisol		Urinary free cortisol	
	r	p	r	p	r	p	r	p
irisin	0.041	0.783	0.328	0.024	0.375	0.019	0.143	0.411
UCP-1	-0.09	0.546	0.145	0.331	-0.079	0.633	-0.391	0.020
PRDM-16	-0.124	0.405	-0.097	0.518	0.155	0.346	-0.004	0.984
BMP-7	-0.164	0.272	0.094	0.528	-0.082	0.621	-0.239	0.167

Spearman correlation analyses
UCP-1: Uncoupling protein-1, BMP-7: Bone morphogenetic protein-7, PRDM-16: PR-Domain Zinc Finger Protein-16, ACTH: Adrenocorticotropic Hormone

production or autonomous adrenal cortisol production. Adrenocorticotropic hormone -dependent cortisol excess due to a pituitary adenoma is called CH and it accounts for 80% of endogenous CS. CS is associated with hypertension, diabetes, coagulopathy, cardiovascular diseases, infections and fractures, all of which may lead to significant morbidity and mortality (9).

Glucocorticoids are an important part of human and animal physiology as a modulator of inflammation and glucose homeostasis, mainly during the stress response. Furthermore, glucocorticoids have been found to play a significant role in energy homeostasis and physiology of adipose tissue over the years (10). Abnormal levels of circulating glucocorticoids directly affect the physiology of WAT at both cellular and molecular level, stimulating adipogenesis and leading to enlargement of adipose tissue (11). Observed importance of the need for glucocorticoids for WAT function has led to foresight that glucocorticoids may also modulating BAT function. In fact, BAT has also been shown to be a target organ for glucocorticoids in early studies (12). Similar to effect on WAT, glucocorticoids may induce lipid deposition in BAT and this may reflect an impaired UCP-1-related thermogenic capacity. If excessive exposure to glucocorticoids suppresses BAT thermogenesis, this may reduce dietary thermogenic energy expenditure and it has been suggested that this may contribute to the development of glucocorticoids-induced obesity in mice and humans (13).

In this study, it was aimed to demonstrate alterations in UCP-1, irisin, PRDM-16 and BMP-7, which are considered to be markers for BAT, in CH that is a hypercortisolemic condition compared to healthy volunteers, under the light of the data obtained from *in vivo* and *in vitro* studies.

Activity of the hypothalamic-pituitary-adrenal axis has an impact on production and secretion of glucocorticoids. Glucocorticoids may modulate UCP-1-induced thermogenesis in BAT. High levels of ACTH appear to up-regulate UCP-1 transcription in vitro. Accordingly, an increase in UCP-1 mRNA and UCP-1 protein levels after treatment with ACTH was determined in studies on cultured adipocytes (14,15). ACTH-induced up-regulation of UCP-1 levels most likely occurs via activation of Gs-cAMP-protein kinase A (PKA) signaling following binding of ACTH to the cognate melanocortin 2 receptor (16). When exposed to a stress factor, while circulating glucocorticoids levels increase after 20 minutes, circulating ACTH levels increase much more rapidly. Therefore, ACTH may initially promote the activity of BAT, and this effect may then be suppressed with an increase in glucocorticoids. However, ACTH concentrations used in animal studies are at least 5-fold higher than the maximal physiological ACTH concentration in case of stress (15,17,18). Because effects of ACTH on BAT are dose-dependent and they occur at these supraphysiological doses, it is unlikely for ACTH-induced activation of UCP-1-induced thermogenesis to be related under physiological conditions (19). In our research, serum basal ACTH levels were found to have no effect on BAT. This, in turn, supports the idea in the literature that exposure to ACTH at physiological doses has no significant effect on BAT.

First clues for a potential association between glucocorticoids and BAT function were obtained from studies on adrenalectomized rats. The researchers observed that there was a significant reduction in lipid stores in BAT in adrenalectomized rats and mice as earlier as 1949 (20). In such adrenalectomized rodents, regaining of lipid stores after glucocorticoid injection indicates that the effect of adrenalectomy on BAT actually results from absence of glucocorticoids (21). Based upon this, in earlier studies, the hypothesis that adrenalectomy inhibits glucocorticoids-induced suppression of BAT thermogenesis and, thusly, increases energy expenditure and reduces the incidence of obesity was proposed (22). Therefore, consistent with this, BAT function may be clearly suppressed in presence of glucocorticoids. In animal studies, chronic glucocorticoid treatment has been reported to cause deep lipid deposition in BAT and a reduction in UCP-1 mRNA and UCP-1 protein levels (8,14). However, there also are studies which did not found any alteration in BAT UCP-1 protein levels or UCP-1-induced thermogenic capacity (23). Direct human studies on effects of glucocorticoids on BAT are very limited due to challenges in sample tissue collection. Nevertheless, there are indicators demonstrating a suppressive effect of glucocorticoids on BAT in humans. In retroperitoneal

adipose tissue analysis of 57 patients, the intensity of BAT in patient groups with cortisol-secreting adenomas and secondary hypercortisolism was found to be lower compared to those with aldosterone-secreting adenomas, pheochromocytomas and nonfunctional adenomas (24). Again in this study, a negative correlation was also determined between UFC level and retroperitoneal UCP-1 expression. Similarly, a negative correlation was also determined between the group with UFC and UCP-1 in the group with CH in our study.

The intensity of BAT is reduced in individuals on chronic glucocorticoids compared to matched controls (25). In a study by which effects of glucocorticoids on human BAT in vitro, UCP-1 levels were found to be up-regulated in presence of 10 μM dexamethasone in supraclavicular human brown adipocyte cultures that differentiated for 9 days (7). However, the duration of exposure to glucocorticoids influences *in vitro* experimental results. For instance, while a 24-hour treatment with 100 nM cortisol increases basal UCP-1 expression in human adipocytes, a 48-hour treatment with the same dose of cortisol does not do so. Furthermore, while a 24-hour treatment with 1 μM cortisol does not influence UCP-1 gene expression, a 48-hour treatment with 1 μM reduces basal UCP-1 mRNA levels (25). In our study, no difference in markers for BAT was determined between those with CH and healthy volunteers. UCP-1 and BMP-7 were increased in correlation with cortisol in CH patients. The reason for this may be interpreted as that long-lasting exposure to high-dose glucocorticoids leads to loss of functioning of adipose tissue in CH patients and to high levels of markers by causing an *in vivo* resistance.

İrisin, a thermogenic adipomyokine cleaved from Fibronectin type III domain-containing protein 5, plays a role in browning of the adipose tissue. İrisin facilitates glucose uptake by skeletal muscles, improves hepatic glucose and lipid metabolisms and has a positive effect on hyperlipidemia and hyperglycemia caused by obesity and metabolic syndrome, thereby acting as an insulin-sensitizing hormone (26). In a study where irisin levels were examined in 40 BAT-positive and 40 BAT-negative women determined by using 18F-florodeoxyglucose positron emission tomography, no difference was observed between the groups (27). In a study on those with CH, circulating irisin levels were determined to be lower compared to controlled CH group (in postoperative 1st year) and the control group (28). In our study, no significant difference in irisin levels was found between the patient and control groups ($p>0.05$).

Targeting brown fat in order to increase energy expenditure and promote negative energy balance has been a strategy being sought for a long period of time for the prevention and treatment of obesity (29). In previous studies, UCP-1 deficient mice exhibited increased an increasing susceptibility to diet-induced obesity (30). In a study conducted with obese individuals, *PRDM-16* gene polymorphism was observed to be a risk factor for obesity (31). BMP-7, a member of transforming growth factor-β superfamily is known for with its osteogenic properties and plays a role induction, development and regulation of adipocytes, especially BAT (32). In our study, the patient and control groups were similar in terms of weight and BMI. In the patient group, BMP-7 was found to be higher in patients with a BMI <30 kg/m^2 compared to those with a BMI >30 kg/m^2 ($p<0.05$). This is thought to be associated with mechanisms of resistance due to chronic glucocorticoid exposure.

Study Limitations

One of them is conduction of the study with a relatively small sample size. In addition, CH is usually late-diagnosed unless it has an aggressive course and how long the patients are exposed to increased endogenous glucocorticoid levels cannot be clearly estimated.

Conclusion

Studies on the association between glucocorticoids and BAT function have been carried on for years. Data obtained from animal studies have developed an interest in the physiology of BAT in humans. However, due to technical challenges, data from human studies are limited. In this study, we aimed to evaluate the association between CH and BAT via irisin, UCP-1, PRDM-16 and BMP-7. In CH, results similar to those in healthy volunteers were obtained. A positive correlation was observed between serum cortisol and irisin, whereas a negative correlation was observed between urinary cortisol and UCP-1. Studies with larger groups will provide obtaining clear data on BAT function in CH.

Authorship Contributions

Concept: A.S., I.T., B.C., Design: A.S., I.T., B.C., Data Collection or Processing: B.F.C., D.K., E.G., Analysis or Interpretation: A.S., M.S., Z.C., Literature Search: B.F.C., M.S., Writing: A.S., M.S., B.F.C.

Conflict of Interest: No conflict of interest was declared by the authors.

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