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Increased Lipolysis of Subcutaneous Abdominal Adipose Tissue and Altered Noradrenergic Activity in Patients with Cushing's Syndrome: An In-vivo Microdialysis Study

M. KRŠEK[1], M. ROSICKÁ[1], J. NEDVÍDKOVÁ[2], H. KVASNIČKOVÁ[2], V. HÁNA[1], J. MAREK[1], M. HALUZÍK[1], E.W. LAI[3], K. PACÁK[3]

[1]Third Department of Medicine, First Faculty of Medicine, Charles University, [2]Institute of Endocrinology, Prague, Czech Republic and [3]Reproductive Biology and Medicine Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA

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Summary

Cushing's syndrome is associated with typical central redistribution of adipose tissue. The aim of the study was to assess lipolysis and catecholamines and their metabolites in subcutaneous abdominal adipose tissue using an *in-vivo* microdialysis technique. Nine patients with Cushing's syndrome and nine age-, gender- and body mass index (BMI)-matched control subjects were included in the study. Local glycerol concentrations were significantly increased in subcutaneous adipose tissue of patients with Cushing's syndrome ($p<0.001$). Plasma noradrenaline, dihydroxyphenylglycol and dihydroxyphenylalanine were decreased in patients with Cushing's syndrome ($p<0.02$, $p<0.05$, and $p<0.02$, respectively). Adrenaline, noradrenaline, dihydroxyphenylglycol and dihydroxyphenylalanine concentrations in subcutaneous abdominal adipose were non-significantly higher in patients with Cushing's syndrome. In conclusion, we showed that lipolysis in subcutaneous adipose tissue of patients with Cushing's syndrome is significantly increased as compared to healthy subjects. This finding together with non-significantly increased local catecholamine concentrations in these patients suggests a possible link between increased lipolysis and catecholaminergic activity in subcutaneous adipose tissue.

Key words
Cortisol • Catecholamines • Noradrenaline • Glycerol • Fat

Introduction

The sympathetic nervous system plays an important role in the regulation of adipose tissue metabolism. Its activation stimulates lipid mobilization from the dipose tissue. The sympathetic function in adipose tissue involves various adrenoreceptor subtypes (Lafontan and Berlan 1993, Mauriege *et al.* 1987). Generally, β-adrenoceptors (β_1, β_2, β_3) are thought to increase and α_2-adrenoceptors to decrease the rate of lipolysis in adipose tissue through the activation or inhibition of adenylate cyclase. In human fat cells, the

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

numerical predominance of α_2-adrenoceptors has been reported and therefore lower catecholamine concentrations can cause inhibition of lipolysis (Mauriege *et al.* 1987). The β-adrenoceptor induced lipolysis occurs at higher concentrations of catecholamines in the adipose tissue. The physiological importance of this dual effect of catecholamines is still not clear. The effect of sympathetic function on lipolysis has been extensively explored but no study focusing directly on the local changes of sympathetic function in the adipose tissue of patients with Cushing's syndrome (CS) has yet been performed.

Current data suggest that there is probably significant interplay between cortisol and sympathetic nerve activity at not only systemic but also at the tissue level. Obesity, particularly abdominal and the regulation of hypothalamic-pituitary-adrenal axis (HPA) are closely related (Björntorp 1996, Rosmond *et al.* 1998). On the other hand, hypercortisolism is associated with the typical changes in fat distribution characterized typically with central (visceral) obesity (Lamberts and Birkenhager 1976). The mechanism of the typical fat redistribution in hypercortisolism is not completely understood. One of the reasons is probably the site-specific regulation of enzymes of intracellular lipolysis (hormone-sensitive lipase) and intravascular lipolysis (lipoprotein lipase) (Samra *et al.* 1998). Catecholamines are hormones that also play an important role in the regulation of lipolysis (Mauriege *et al.* 1987). For this reason, we decided to study sympathetic nerve activity in subcutaneous adipose tissue with a special attention to differences between simple obesity and cortisol-induced obesity.

Alterations in regional sympathetic nervous system outflows alter the release of from noradrenaline (NA) nerve terminals into the interstitial fluid. Most of released noradrenaline undergoes inactivation by reuptake, via a specific membrane-bound transporter (Uptake-1). Only a small portion of released NA enters the circulation (Kopin 1985). Clinical assessment of sympathetic function has generally relied on concentrations of NA in the plasma compartment. Factors such as protein binding, capillary permeability and kinetic differences in the distribution volumes and mechanisms of uptake and release among different tissues limit the validity of plasma NA concentrations in reflecting the release into interstitial fluid in particular organs (Esler *et al.* 1990).

Microdialysis allows direct sampling of interstitial fluid and enables measurements of concentrations of neurochemicals and other analytes in interstitial fluid *in vivo*. Microdialysis has been used extensively in animals (Pacak *et al.* 1995a) and is being used increasingly in humans. Since 1987, over 200 clinical microdialysis studies have been published, mainly based on findings in muscle and adipose tissue (Lonnroth *et al.* 1987). Very few studies, however, have examined microdialysate levels of catecholamines together with the measurement of their metabolites. In the present study, *in vivo* microdialysis was combined with assays of A, NA and metabolites related to NA turnover and synthesis such as dihydroxyphenylalanine (DOPA) and dihydroxyphenylglycol (DHPG). Levels of DHPG, the deaminated neuronal metabolite of NA, are known to reflect the intraneuronal metabolism of NA and therefore to be related to NA turnover. DOPA is a NA precursor and may reflect its synthesis.

Microdialysis is a novel, minimally traumatic technique that allows continuous, direct measurements of concentrations of substances of interest in the interstitial space. The measurements have a unique possibility to provide insights about local metabolic processes and pharmacological effects at the cellular level. The principle of microdialysis is quite simple. A tubular dialysis membrane is introduced into the tissue, and a liquid is perfused that allows bi-directional exchange with the interstitial fluid outside the tube. Endogenous compounds in the interstitial fluid that enter the microdialysate can be assayed, so that concentrations in the microdialysate reflect concentrations in the interstitial fluid (Pacák *et al.* 1995ab). In our previously published study examining the effect of hyper- and hypothyroidism on noradrenergic activity and glycerol concentrations in subcutaneous adipose tissue we have proved the feasibility of measuring catecholamine levels in samples from subcutaneous adipose tissue (Haluzík *et al.* 2003, Nedvídková *et al.* 2004).

This study was designed to compare local sympathetic activity in the subcutaneous abdominal adipose tissue of patients with CS with control subjects matched for the age, gender and body mass index (BMI) in order to clarify whether and to what extent these changes participate in the regulation of lipolysis and fat tissue distribution seen in patients with hypercortisolism.

Methods

Study subjects

Nine patients (8 women and 1 man) with overt

CS and nine control subjects (8 women and 1 man) were included in the study. Patients with CS were characterized by typical clinical appearance of CS, elevated urinary free cortisol excretion (UFC), blunted circadian variability of plasma cortisol levels with elevated midnight cortisol levels and lack of appropriate suppression in the low dose dexamethasone suppression test (LDDST – overnight variant with 1 mg of dexamethasone given at 23:00 h.). Control subjects were matched to the CS patients according to their age, BMI and gender distribution. Therefore, they were only slightly overweight, otherwise completely healthy subjects. The principal characteristics of both groups of subjects are given in Table 1. All participants were non-smokers, without medication with known interference with the sympathetic nervous system. Upon enrolment, all patients were placed on a low monoamine diet. The body fat content was calculated from the skinfold thickness measured at four sites using Best's calipper. The study was conducted according to the declaration of Helsinki and was approved by the Ethics Committee of the First Faculty of Medicine of Charles University in Prague. All study subjects signed a written Informed consent.

Blood and microdialysate sampling

All subjects were studied after an overnight fast while resting in the supine position on a comfortable bed at room temperature 23-25 ^{o}C. At 08:00 h, a microdialysis catheter CMA-60 with cut off of 20 kDa (CMA Microdialysis, Stockholm, Sweden) was inserted s.c. under sterile conditions (8-10 cm left of the umbilicus, at least 45 min before blood and microdialysate sampling). A sterile Ringer buffer was used as the perfusate. After insertion of a CMA-60 catheter, perfusion with Ringer solution supplemented with 50 mmol/l of ethanol was started at a flow rate of 2 μl/min using CMA 107 microdialysis pump (CMA Microdialysis, Stockholm, Sweden). Microdialysate samples for catecholamine determination were collected into microvials containing the preserving buffer (0.2 N acetic acid and 0.04 M H_3PO_4). Samples were placed on ice and immediately after collection stored at –80 ^{o}C until analysis.

Hormonal and biochemical assays

Concentrations of catecholamines and their metabolites (NA, A, DOPA, and DHPG) were measured using the HPLC technique with electrochemical detection after batch alumina extraction as previously described (Pacak *et al.* 1998). Plasma cortisol levels were determined with a RIA kit (Immunotech, France) with intra-assay variability 5.1 % and inter-assay variability 9.2 %. Urinary free cortisol was determined using the same RIA kit (Immunotech, France). Plasma ACTH levels were measured using IRMA kits Dyno test (Brahms Diagnostica GmbH, Germany) with intra-assay variability 7 % and inter-assay variability 10 %. Insulin serum levels were measured using RIA kits (Pharmacia-Upjohn Diagnostics, Sweden). Insulin resistance was assessed using the HOMA-R formula. Glycerol was measured colorometrically with a commercial kit (Randox). Changes in subcutaneous abdominal adipose tissue blood flow were determined using the ethanol dilution technique based on Fick's principle (Rosdahl *et al.* 1998). According to this method, differences between ethanol concentration in the perfusate (inflow) and in the dialysate (outflow) reflect changes in blood flow. Ethanol was measured using a standard enzymatic assay (Sigma Diagnostics, St. Louis, MO, USA). For simplicity, the microdialysate ethanol concentration/perfusate ethanol concentration ratio is referred to as the "ethanol ratio".

Statistical analysis

Statistical analysis of the differences between both groups was performed using Student's t-test for unpaired data or the Mann-Whitney rank sum test for non-normally distributed data. Data are given as the mean ± S.E.M. $P<0.05$ values were considered as statistically significant.

Results

Patients with CS were comparable to the control group in terms of age, gender distribution and BMI. As expected, patients with CS differed significantly from the control group in parameters of cortisol secretion and were typically characterized by significantly elevated UFC ($P<0.001$), plasma midnight cortisol levels ($P<0.001$) and abnormal plasma cortisol response after 1.0 mg dexamethasone ($P<0.001$). Both groups also had comparable glycemia, insulin serum levels and HbA1C levels. Patients with CS were slightly more insulin-resistant as assessed using HOMA-R formula (Table 1).

Ethanol ratios were comparable in both studied groups (Table 2). Local glycerol levels as a parameter of lipolysis in subcutaneous abdominal adipose tissue were significantly increased in patients with CS when

Table 1. Comparison of both study groups

	Cushing's	Controls	P Value
N (men, women)	9 (1, 8)	9 (1, 8)	N. S.
Age (years)	41.0 ± 11.6	41.2 ± 11.7	N. S.
BMI (kg/m^2)	33.9 ± 8.1	36.0 ± 9.7	N. S.
Body fat (%)	32.6 ± 10.7	33.8 ± 6.3	N. S.
UFC (nmol/24 h)	3282.6 ± 1157.1	186.0 ± 79.9	P < 0.0001
P-cortisol in LDDST (nmol/l)	1925.9 ± 856.7	53.9 ± 12.5	P < 0.0001
Insulin (mU/l)	19.8 ± 3.3	14.8 ± 1.7	N.S.
Glycemia (mmol/l)	5.8 ± 0.5	5.1 ± 0.1	N.S.
HbA_{1C} (%)	6.4 ± 0.4	5.8 ± 0.1	N.S.
HOMA-R	4.9 ± 0.7	3.3 ± 0.4	P < 0.05

BMI – body mass index; UFC – urinary free cortisol; LDDST – low dexamethasone suppression test; HbA_{1C} – glycated hemoglobin; HOMA-R – homeostasis model assessment ratio formula; N.S. – non- significant.

Table 2. Ethanol ratio (dialysate ethanol concentration/perfusate ethanol concentration) in healthy control and patients with Cushing's syndrome

	Cushing's	Controls	P Value
Ethanol ratio (%)	41.0±3.6	40.8±3.0	N.S.

N.S. – non-significant

Table 3. Local levels of glycerol in microdialysate from abdominal subcutaneous adipose tissue in patients with Cushing's syndrome and controls

Glycerol in Microdialysate	Cushing's	Controls	P Value
Glycerol (mmol/l)	47.4±8.1	34.2±2.5	P<0.001

Table 4. Plasma levels of catecholamines in patients with Cushing's syndrome and controls.

Plasma Catecholamines	Cushing's	Controls	P Value
Noradrenaline (pg/ml)	56.4±17.4	539.6±300.4	P<0.02
Adrenaline (pg/ml)	46.6±17.3	17.4±3.0	N.S.
DHPG (pg/ml)	223.0±81.2	660.7±148.8	P<0.05
DOPA (pg/ml)	241.3±82.4	1042.7±243.1	P<0.02

DHPG – dihydroxyphenylglycol; DOPA – dihydroxyphenylalanine; N.S. – non-significant

compared to the controls (P<0.001) (Table 3). When evaluating the plasma levels of catecholamines and their metabolites, we found significantly decreased levels of NA, DHPG and DOPA (P<0.02, P<0.05, and P<0.05, respectively) in patients with CS when compared to the controls (Table 4). Furthermore, we investigated local concentrations of catecholamines (A, NA) and their metabolites (DHPG, DOPA) in subcutaneous adipose tissue. We observed a tendency towards an increase in local catecholamine concentrations from subcutaneous abdominal adipose tissue of patients with CS, but this difference did not achieve statistical significance (Table 5).

Discussion

One of the characteristic features of Cushing syndrome is typical central redistribution of fat tissue giving the typical appearance of these patients. The mechanism of these changes has not been elucidated so far. We therefore decided to examine the local lipolysis rate and catecholamine levels in subcutaneous adipose tissue of patients with CS and in BMI-, age- and gender-matched control group using the *in-vivo* microdialysis technique. We showed significantly increased lipolysis in subcutaneous adipose tissue of patients with CS. Furthermore, we found increased local concentrations of

Table 5. Local levels of catecholamines in microdialysate from abdominal subcutaneous adipose tissue in patients with Cushing's syndrome and controls.

Local Catecholamines	Cushing's	Controls	P Value
Noradrenaline (pg/ml)	32.4±9.4	20.0±9.4	N.S.
Adrenaline (pg/ml)	86.8±19.7	46.0±6.0	N.S.
DHPG (pg/ml)	60.5±11.7	42.0±13.4	N.S.
DOPA (pg/ml)	268.8±50.1	199.5±41.4	N.S.

DHPG – dihydroxyphenylglycol; DOPA – dihydroxyphenylalanine; N.S. – non-significant

catecholamines and their metabolites in subcutaneous adipose tissue of patients with CS. In spite of the fact that these changes did not attain statistical significance we can speculate that the changes in local concentrations of catecholamines and their metabolites might be linked to the changes in lipolysis in subcutaneous abdominal adipose tissue.

The relationship between activity of the HPA axis and catecholamines has been investigated in a number of different studies focusing on either systemic catecholamine levels or local sympathetic activity in the central nervous system (Pohorecky and Wurtman 1971, Mobley *et al.* 1983, Axelrod and Reisine 1984, Brown and Fischer 1986, Udelsman *et al.* 1987). In previous studies on patients with CS an increase in urinary levels of dopamine (but not A and NA) was described (Wocial *et al.* 1978). More recently, Cameron *et al.* (1995) found decreased NA plasma levels in patients with CS and their inverse correlation with urinary free cortisol levels. Our data on systemic catecholamine levels are in agreement with the above mentioned reports although large variations of measured values have been observed. The mechanism of decreased sympathetic activity in patients with hypercortisolism is complex and not entirely understood. It may involve decreased production of catecholamines and their release into the systemic circulation and/or their increased clearance. Increased clearance could involve processes of removal from the blood by excretion and/or increased reuptake into cells and also changes in their metabolism (Ziegler *et al.* 1993, Cameron *et al.* 1995). The documented decrease of NA concentrations together with the decrease of DHPG and DOPA concentrations allow us to conclude that not only NA release but also its synthesis is decreased in Cushing syndrome.

Only a few reports concerning local changes in sympathetic activity in humans or experimental animals with hypercortisolism are available. Chronic hypercortisolemia has been shown to suppress NA-stimulated cAMP formation in the hippocampus (Robertis *et al.* 1984) and synthesis and release of catecholamines in the paraventricular nucleus (Pacák *et al.* 1995b). On the other hand, adrenalectomy increases noradrenergic activity in the paraventricular nucleus (Jhanwar-Uniyal 1989, Shen and Gannong 1976). It has also been shown that glucocorticoids decrease sympathetic nerve activity in humans (Lenders *et al.* 1995, Golczynska *et al.* 1995). The effects of glucocorticoids on sympathetic activity are not completely understood. They are thought to involve glucocorticoid-induced inhibition of catecholamine synthesis and inhibition of sympathoneural outflows. Glucocorticoids are able to attenuate extraneural uptake of catecholamines and to inhibit sympathetic activity at the level of central nervous system and sympathetic ganglia. Decreased levels of DHPG reflect decreased NA turnover in sympathetic nerves (Goldstein *et al.* 1988).

In contrast to some information about the influence of chronic hypercortisolemia on plasma and brain sympathetic activity, no study has evaluated local levels of catecholamines and their metabolites in subcutaneous abdominal adipose tissue of patients with CS. In the present study we could confirm the results of several previous studies showing decreased sympathetic activity in patients with chronic hypercortisolemia (Lenders *et al.* 1995, Golczynska *et al.* 1995). Here, for the first time, we demonstrate the changes in local sympathetic activity in subcutaneous abdominal adipose tissue of patients with CS. We observed a tendency towards an increase in concentrations of catecholamines and their metabolites in subcutaneous abdominal adipose tissue of patients with CS. However, these differences did not attain statistical significance. Since the concentrations of catecholamines from abdominal adipose tissue of patients with CS tend to be slightly increased, although without achieving statistical significance, there is still a possibility that their elevations can contribute at least in part to increased lipolysis as evident from elevated local glycerol levels. Therefore, our future studies will be directed not only to find other possible mechanisms involved in the regulation of local lipolysis in

subcutaneous abdominal adipose tissue of patients with CS, but also to conduct a larger study to increase the statistical power of our results.

There are also other possible mechanisms that could contribute to the altered lipolysis in patients with CS. One possible explanation is that increased lipolysis in subcutaneous adipose tissue of patients with CS is the direct consequence of hypercortisolemia itself. Ottosson *et al.* (2000) described that cortisol increased lipolysis rate in cell cultures of human adipocytes. This observation was later confirmed by several *in vivo* studies (Gravholt *et al.* 2002), including studies using microdialysis (Djurhuus *et al.* 2002, 2004). Another possibility is that hypercortisolism induced changes in the number of adrenoreceptor subtypes and/or their sensitivity or affinity for particular catecholamines. In such a case, lipolysis could be increased without any alterations of local sympathetic activity. Finally, the influence of cortisol on the endocrine function of adipose tissue must be taken into account. It has been shown by numerous studies that cortisol can affect the release of adipose tissue-derived hormones such as leptin, adiponectin, tumor necrosis factor-α and others. These hormones can in turn directly affect numerous metabolic processes in the adipose tissue including lipolysis Haluzík *et al.* 2004).

In summary, we demonstrated in the present study that patients with CS have markedly increased lipolysis in the abdominal subcutaneous adipose tissue. We also confirmed the results of previous studies showing decreased sympathetic activity in patients with CS. Furthermore, we showed opposite changes in local concentrations of catecholamines and their metabolites in subcutaneous abdominal adipose tissue of patients with CS, where they tended to be increased. These results allow us to speculate that there could be a link between local sympathetic activity and lipolysis in subcutaneous abdominal adipose tissue. It is also necessary to point out that other mechanisms such as a direct effect of cortisol on lipolysis, changes in the local adrenoreceptor number and/or affinity, decreased synthesis and bioavailability of nitric oxide in Cushing's syndrome as well as possible alterations in the endocrine function of adipose tissue may also be involved in the regulation of local lipolysis in patients with CS. This will be under the scope of our future studies.

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Reprint requests

M. Kršek, Third Department of Medicine, First Faculty of Medicine, Charles University, U nemocnice 1, 128 08 Prague 2, Czech Republic. E-mail: mkrse@lf1.cuni.cz