

Increased Subcutaneous Abdominal Tissue Norepinephrine Levels in Patients With Anorexia Nervosa: an *In Vivo* Microdialysis Study

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Summary

The present study was designed to measure interstitial levels of norepinephrine-regulating lipolysis (NE) in subcutaneous abdominal adipose tissue of anorexia nervosa (AN) patients and control subjects under basal conditions and after the local administration of an inhibitor of NE re-uptake, maprotiline. *In vivo* microdialysis technique was used to assess subcutaneous adipose NE levels in five women with AN (body mass index 14.62 ± 0.47 kg/m²) and six age-matched controls (22.1 ± 0.52 kg/m²). NE was assayed using high performance liquid chromatography with electrochemical detection after batch alumina extraction. Measured basal adipose tissue NE levels reflecting its interstitial levels were significantly increased in AN patients compared to the controls (106.0 ± 20.9 vs. 40.0 ± 5.0 pg/ml). The local maprotiline administration resulted in a significant increase in adipose tissue NE levels (AN patients: 440.0 ± 28.6 vs. 202.0 ± 33.0 pg/ml in the controls) in both groups. Markedly increased subcutaneous abdominal adipose tissue NE levels in AN patients compared to control subjects reflect increased sympathetic nervous system activity but not altered membrane noradrenergic transporter system in anorexia nervosa patients.

Key words

Anorexia nervosa • Catecholamines • Adipose tissue • Microdialysis • Maprotiline

Introduction

Anorexia nervosa (AN) is a severe psychiatric disorder with abnormal food behavior, leading to a weight decrease associated with fat mass loss. The adipose tissue, which is strongly reduced in AN patients, is currently considered as a hormonally active system, i.e. not only as a store of excess energy but also as an active system in the control of metabolism (Trayhurn and

Beattie 2001). For example, our previous study in AN patients showed lowered plasma levels of adipocyte-derived hormone leptin that is considered to reflect the body fat content (Haluzík *et al.* 1999). The adipose tissue is richly innervated by neurons of the sympathetic nervous system (SNS) that is a major contributor to body homeostasis, energy balance and intermediary metabolism (Lawrence and Coppack 2000). Alterations of SNS signalling at the target fat cell level could have a

major impact on metabolic events, such as altered lipolysis in AN patients. Catecholamines, especially norepinephrine (NE), are the main lipolytic hormones in man (Langin *et al.* 2000, Lawrence and Coppack 2000). In some studies, lower basal SNS activity was demonstrated in AN patients compared to healthy controls (Gross *et al.* 1979, Kaye *et al.* 1985, Pirke 1996). However, all these studies were based on measurements of plasma, urine and cerebrospinal fluid NE concentrations but not local specific tissue catecholamine concentrations. Thus, it is unknown whether subcutaneous abdominal adipose tissue sympathetic activity is altered in AN patients.

The present study is focused on measurements of subcutaneous abdominal adipose tissue NE levels using a microdialysis technique *in vivo* under basal conditions and after local administration of NE re-uptake blocker, maprotiline.

Methods

Study subjects

Five women with AN (age 23.0 ± 1.3 years; BMI: 14.7 ± 0.65 kg/m²) and six healthy women (22.3 ± 1.0 years, 22.16 ± 0.32 kg/m²) were recruited for this study. All participants provided written informed consent before being enrolled in the study that was approved by the Human Ethical Review Committee, Institute of Endocrinology, Prague. All subjects included in the study were non-smokers, had no allergies and had been free of medication for at least 1 month prior to the study. Healthy subjects had no history of obesity or malnutrition, endocrine or cardiovascular diseases, eating disorders or other psychiatric afflictions, and had normal physical examination and electrocardiogram. AN patients were diagnosed according to the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 1994). All AN patients were clinically stable and in relatively good health, except for their eating disorder. They were investigated after one week of hospitalization at the Department of Psychiatry. All subjects were asked to fast and drink only water on the night prior to the study and not to use stimulant drugs, such as caffeine, nicotine or alcohol, for two days prior to the experiment.

Microdialysate and blood sampling

All subjects were studied while resting supine on a comfortable bed covered with blanket to achieve their thermoneutrality in a room kept at 23-25 °C. At 08:00 h,

after overnight fasting, a venous catheter was inserted into the antecubital vein and a CMA-60 catheter with cut-off of 20 kDa (CMA Microdialysis, Stockholm, Sweden) was inserted subcutaneously under sterile conditions (8-10 cm left of the umbilicus, at least 60 min prior to blood and microdialysate sampling). Sterile Ringer buffer was used as the perfusate. After insertion of the CMA-60 catheter, perfusion with Ringer solution supplemented with 50 mM of ethanol was started at a flow rate of 2 µl/min using CMA 107 microdialysis pump (CMA Microdialysis, Stockholm, Sweden). Ethanol was added to the perfusate to estimate changes in the adipose tissue blood flow, as previously described (Štich *et al.* 1999, 2000).

Blood samples were collected into heparinized tubes, placed immediately on ice, centrifuged and stored at -80 °C until analyzed for NE. Microdialysate samples for catecholamine determination were collected into microvials containing conservation agents (acetic and phosphoric acid), placed on ice, and stored at -80 °C until analyzed for NE. Microdialysate samples were collected at 30 min intervals. After 60 min maprotiline was added to Ringer solution in concentration 3.0 mM and the local perfusion continued for 120 min. The microdialysate samples were collected 120 min after the end of maprotiline administration.

Analytical procedures

NE levels in the dialysate and plasma was assayed by high-performance liquid chromatography, using electrochemical detection after purification on alumina (Eisenhofer *et al.* 1986, Holmes *et al.* 1994).

Blood flow measurements

Changes in subcutaneous abdominal adipose tissue blood flow were determined using the qualitative ethanol dilution technique, based on the Fick principle (Bernst and Gutmann 1974). According to this method, differences between ethanol concentration in the perfusate (inflow) and in the dialysate (outflow) reflect changes in blood flow in the same subject. Ethanol was measured using a standard enzymatic assay (Sigma Diagnostics, USA). For simplicity, the microdialysate ethanol concentration/perfusate ethanol concentration ratio is referred to as „ethanol ratio“.

Data analysis

Results are presented as mean values \pm S.E.M. Differences between basal and maprotiline-stimulated NE concentrations were analyzed by the paired t-test.

Differences between groups of subjects were analyzed by two-way ANOVA, followed by Student-Keuls *post-hoc* test. The level of statistical significance was set at $P < 0.05$.

Results

Baseline characteristics of subjects are summarized in Table 1. AN patients had significantly lower BMI, blood pressure and heart rate in comparison with the controls.

Table 1. Baseline characteristics and circulatory response of the study subjects.

	Controls (n=6)	Anorexia nervosa (n=5)
Age (years)	22.7±1.7	23.9±1.6
BMI (kg/m ²)	22.1±0.5	14.6±0.5*
Heart rate (bpm)	64.5±2.3	62.1±3.7
Systolic BP (mm Hg)	104.6±2.5	93.8±3.1*
Diastolic BP	69.3±2.7	56.9±1.7*

Values are means ± S.E.M. C = controls; AN = anorexia nervosa; n = number of subjects, BMI = body mass index. *significant difference ($P < 0.05$) between the controls and AN patients.

Basal plasma NE levels in AN patients (150.2±28.0 pg/ml) did not differ from control subjects (242.3±75.8 pg/ml). Basal subcutaneous adipose tissue NE and glycerol levels were increased in AN patients compared to control subjects (Table 2). The local maprotiline administration increased significantly adipose tissue NE levels in both groups (Table 2), but the relative increases did not differ between AN and control subjects (Table 3). Two hours after the end of maprotiline perfusion, adipose tissue NE levels almost returned to basal levels.

The measurement of ethanol in the dialysate did not show any changes in adipose tissue blood flow between AN patients and control subjects (47.6±3.8 vs. 48.0±4.2 % outflow/inflow).

Discussion

The important finding of the present study is that AN patients had markedly increased basal NE microdialysate levels reflecting its interstitial levels in the

subcutaneous abdominal adipose tissue in comparison with control subjects. Maprotiline-induced increase in subcutaneous abdominal adipose tissue NE levels did not significantly differ between both experimental groups suggesting no altered membrane noradrenergic transporter system in AN patients.

The local adipose tissue maprotiline administration elicited a very rapid response of adipose tissue NE levels in both groups with maximal NE value observed in the following 30 min after maprotiline administration. Because the exposure of extracellular space of the adipose tissue to maprotiline blocked NE re-uptake in both groups similarly, we suggest that patients have increased SNS activity rather than altered NE transporter. The aim of antidepressant therapy, especially of the NE re-uptake blocker, maprotiline, was to enhance and prolong NE action in the synaptic cleft. Very high basal adipose tissue NE levels in AN patients in comparison with control subjects found in our study might be in accordance with the fact, that these patients poorly respond to NE re-uptake inhibitor's therapy. The increase of basal adipose tissue NE levels was in accordance with our observations of elevated glycerol production and also to relatively increased physical activity (requiring elevated energy support, namely lipolysis of adipose fat stores) often observed in AN patients.

It is necessary to note that NE values measured by microdialysis reflect the real situation in the adipose tissue because transporters of NE re-uptake are not found in the synapse but rather around or in perisynaptic parts of the axonal and nerve terminal membrane where the microdialysis probe is inserted (Kuhar 1998, Sesack *et al.* 1998).

The observation of altered adipose NE levels in AN patients could reflect not only the maprotiline effect on adipocytes, but could be influenced by changes in local blood flow occurring in abdominal adipose tissue of AN patients. The higher blood flow contributes to the lowering of extracellular molecule levels (Enocksson *et al.* 1995). In this study using ethanol washout, no significant variations in the local adipose tissue blood flow were demonstrated during maprotiline administration in AN patients in comparison with control subjects. Furthermore, the local blood flow, evaluated with the ethanol dilution method in the present study, was similar to that assessed by the ¹³³Xe-clearance method (Summers *et al.* 1996).

Table 2. Adipose tissue norepinephrine (NE) and glycerol levels in the controls (C) and AN patients.

	Subjects	Basal 0 min	Maprotiline 30 min	Maprotiline 60 min	Maprotiline 90 min	Maprotiline 240 min
NE (pg/ml)	C	40±14.7	202±96 ⁺	128±58	119±54	28.8±9.5
	AN	106±59*	440±81* ⁺	243±78	214±57	107±47.2*
Glycerol (μmol/l)	C	96.3±3.9				
	AN	141.9±8.9*				

Values are mean ± S.E.M. The numbers of subjects are in brackets. Significantly different (P<0.05) from: * controls, ⁺ basal values.

Table 3. The ratio of basal and maprotiline-stimulated adipose NE levels in the controls (C) and AN patients.

	Subjects	Basal 0 min	Maprotiline 30 min	Maprotiline 60 min	Maprotiline 90 min	Maprotiline 240 min
NE ratio	C	1.0	5.1±0.5	3.2±0.5	3.0±0.5	0.7±0.3
	AN	1.0	4.1±0.2	2.3±0.4	2.0±0.3	1.0±0.4

Values are mean ± S.E.M.

In contrast to the demonstrated increase of adipose tissue NE levels measured by the microdialysis technique in AN patients, we did not observe significant changes of plasma NE levels in these patients in comparison with control subjects. Thus, overall sympathetic activity was not changed despite altered local tissue SNS activity. Some authors observed lower plasma NE levels in AN patients which could rather be due to reduced overall sympathetic nervous activity in those patients (Gross *et al.* 1979, Lonati-Galligani and Pirke 1986, Pirke 1996). Pirke (1996) proposed that reduced activity of SNS is involved in starvation of AN patients. A large variance in published plasma NE levels may be explained, in part, by an individually different state of anorexia pathophysiology. These observations supported the importance of measuring catecholamines directly *in vivo* in tissues of interest. Moreover, this confirms the existence of different SNS activity at whole body level and at adipose tissue level. Plasma NE levels represent the result of catecholamine release, reuptake, receptor binding, degradation and blood intake from

various tissues.

In summary, we have shown that the microdialysis method is valuable for detection of the local catecholamine concentrations that in some cases do not correspond to plasma catecholamine values. Using the *in vivo* microdialysis technique we documented increased SNS activity, but no significant alterations of noradrenergic transporter in the abdominal adipose tissue of anorexia nervosa patients. In addition, our results confirm the existence of different SNS activity at whole body level and at adipose tissue level. We believe that the results from this study could lead to a better understanding of SNS-induced regulations of adipose tissue metabolism in anorexia nervosa and introduce a more specific and effective pharmacological intervention.

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