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PERORÁLNÍ PODÁNÍ ACIPIMOXU BĚHEM FYZICKÉ ZÁTĚŽE ZPŮSOBUJE NEGATIVNÍ ZPĚTNOVAZEBNÝ MECHANISMUS RŮSTOVÉHO HORMONU NA SEKRECI GHRELINU U PACIENTEK S MENTÁLNÍ BULIMIÍ A ZDRAVÝCH ŽEN: ÚLOHA LIPOLÝZY

ACIPIMOX DURING SHORT-TERM EXERCISE EXERTS A NEGATIVE FEEDBACK OF GROWTH HORMONE ON GHRELIN SECRETION IN PATIENTS WITH BULIMIA NERVOSA AND IN HEALTHY WOMEN: THE ROLE OF LIPOLYSIS

Disertační práce

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ABSTRAKT

Úvod: Orexigenní peptid ghrelin je produkovaný zejména žaludkem a předpokládá se zpětnovazebný mechanismus růstového hormonu (STH) a ghrelinu mezi žaludkem a hypofýzou. Neuropeptid Y (NPY) je důležitý centrální orexigen hojně produkovaný v hypothalamu a podle nedávného zjištění je také secernovaný tukovou tkání (TT). NPY patří k systémům, které regulují stresovou odpověď a emocionalitu a také patří k hormonům vztahujícím se k mentální bulimii (MB) a mentální anorexii. Anorexigenní leptin produkovaný TT a orexigenní ghrelin vzájemně regulují sekreci NPY. Dysregulace uvolňovaných hormonů gastrointestinálním traktem, centrální nervovou soustavou a TT se podílí na patogeneze MB.

Metodika: Zkoumali jsem odpovědi plazmatického ghrelinu, STH, NPY, leptinu, inzulinu, glukózy, volných mastných kyselin, glycerolu a dialyzovaného glycerolu u pacientek s MB a zdravých žen (ZŽ) během fyzické zátěže po perorálním podání antilipolytického acipimoxu (Aci) nebo placeba. Sedm ZŽ a sedm pacientek s MB bylo zahrnuto do randomizované, placebem kontrolované, jednoduše zaslepené studie. Aci nebo placebo byly podány perorálně 60 minut před fyzickou zátěží (45 minut, 2W/kg aktivní tělesné hmoty, [ATH]). STH, ghrelin, NPY, volné mastné kyseliny, glycerol v plazmě a glycerol v extracelulární tekutině a plazmě byly stanoveny komerčními kity. Glycerol byl měřen *in vivo* v podkožní (sc) TT mikrodialyzační technikou.

Výsledky: Fyzická zátěž indukovala zvýšení STH, NPY, glycerolu a volných mastných kyselin u obou skupin a snížení ghrelinu jen u pacientek s MB a podobné snížení leptinu u obou skupin. Perorální podání Aci během fyzické zátěže vedlo ke zvýšení STH, NPY a poklesu ghrelinu u obou skupin; NPY (45 minut po cvičení) a leptin (90 minut po zotavení) se zvýšily více u MB; plazmatické hladiny volných mastných kyselin byly snížené u obou skupin a plazmatická hladina glycerolu poklesla více u pacientek s BN. U pacientek s MB fyzická zátěž indukovala signifikantně vyšší stimulaci produkce extracelulárního glycerolu v sc TT, zatímco perorální podání Aci během cvičení vedlo k většímu poklesu extracelulárního glycerolu u pacientek s MB oproti ZŽ.

Závěry: Potvrzujeme výsledky randomizované, placebem kontrolované studie, že Aci indukovaná elevace plazmatických hladin STH a NPY během fyzické zátěže je větší u pacientek s MB a že Aci indukovaná suprese ghrelinu během zátěže u obou skupin vzbuzuje inhibiční zpětnovazebný mechanismus STH na sekreci ghrelinu. Pozátěžové zvýšení extracelulárního glycerolu v sc abdominální TT je mnohem více suprimováno akutním podáním Aci u pacientek s MB než u kontrol, což ukazuje na hypersenzitivitu sympatické nervové aktivity v sc abdominální TT u pacientek s MB. Současně jsme nalezli facilitovaný obrat plazmatického glycerolu během fyzické zátěže po podání Aci u pacientek s MB. Sledované změny v plazmatických hladinách volných mastných kyselin neodpovídají změnám v hladinách ghrelinu a STH. Tato sledování nás vedou k zjištění, že Aci účinkuje na nezávislém mechanismu volných mastných kyselin. Také ukazujeme, že antilipolýza během cvičení zabránila nejenom poklesu plazmatických hladin leptinu u obou skupin, ale také zvýšila plazmatické hladiny leptinu 90 minut po zotavení u pacientek s MB. Zaznamenali jsme opačné změny v plazmatických hladinách ghrelinu a leptinu po perorálním podání Aci během cvičení a v zotavení u obou skupin. Nižší bazální lipolýza v TT u pacientek s MB může být způsobena protektivním mechanismem, který zabraňuje vyčerpání energetických zásob organismu.

Klíčová slova: Ghrelin • Růstový hormon • Neuropeptid Y • Leptin • Mentální bulimie • Acipimox • Fyzická zátěž • Tuková tkáň • Volné mastné kyseliny • Glycerol • Mikrodialýza

ABSTRACT

Background: Feeding-stimulatory peptide ghrelin is predominantly produced by the stomach and the growth hormone (GH)-ghrelin feedback loop between the stomach and the pituitary gland has recently been suggested. Neuropeptide Y (NPY) is an important central orexigenic hormone predominantly produced by the hypothalamus, and recently found to be secreted in adipose tissue (AT). NPY is one of the primary systems regulating the stress response, emocionality and hormones relevant to bulimia (BN) and anorexia nervosa. Anorexigenic leptin produced by AT and orexigenic ghrelin regulate NPY secretion. Dysregulations of the gut-brain-AT axis peptides might be involved in binge eating as is the case in BN.
Methods: We investigated responses of plasma ghrelin, GH, NPY, leptin, insulin, blood glucose, free fatty acids (FFA) and glycerol concentrations to exercise in BN patients and healthy women (C) given the anti-lipolytic drug Acipimox (Aci) or placebo. Seven BN and seven C women were recruited for this randomized, single-blind study. Aci or placebo was given 60 minutes before the exercise (45 min, 2 W/kg of lean body mass [LBM]). Ghrelin GH, NPY, leptin, FFA, glycerol plasma and AT glycerol levels were measured using commercial kits. Glycerol was measured *in vivo* in subcutaneous (sc) abdominal AT using microdialysis.
Results: The exercise induced similar increases in plasma GH, NPY, FFA, glycerol levels in both groups and a decrease in plasma ghrelin levels only in BN patients, and a similar decrease in the plasma leptin level in both groups. The exercise with Aci administration resulted in plasma GH and NPY increase and a decrease in plasma ghrelin in both groups; NPY (after 45 minute exercise) and leptin (90 minutes after post-exercise recovering phase) increased more in BN patients; plasma FFA levels were depressed in both groups, and plasma glycerol levels decreased more in BN patients. The exercise induced a higher increase of glycerol concentrations in sc abdominal AT of BN patients, while exercise with Aci administration induced a higher decrease of extracellular glycerol in BN patients compared to the C group.
Conclusions: We confirm the results of a randomized placebo-controlled study, *i.e.* that the Aci-induced elevation in plasma GH and NPY levels during the exercise is higher in BN patients and that the Aci-induced suppression in plasma ghrelin levels during exercise in both groups suggests a negative feedback of GH on ghrelin secretion. The post-exercise rise (45 minute) in AT glycerol is much more attenuated by acute Aci treatment in BN patients and that hypersensitivity of SNS in sc abdominal AT may exist in patients with BN. Simultaneously, we found facilitated turnover of plasma glycerol after short-term exercise together with Aci administration in BN. Observed changes in plasma FFA levels did not respond to changes in GH and ghrelin levels. Therefore, these observations led us to suggesting that Aci affects a FFA-independent mechanism. In addition, we demonstrate that antilipolysis during short-term exercise not only might prevent falling of plasma leptin levels in both groups but also increased plasma leptin levels in the post-exercise recovering phase (90 minute) more in BN patients. We observed opposite changes in plasma ghrelin and leptin levels during the exercise with Aci administration and in the post-exercise recovering phase in both groups. Lower basal lipolysis in AT in BN patients may be due to the protective mechanism that prevents the exhaustion of energy reserves.

Keywords: Ghrelin • Growth hormone • Neuropeptide Y • Leptin • Bulimia nervosa • Acipimox • Exercise • Adipose tissue • Free fatty acids • Glycerol • Microdialysis

CONTENTS

1. LIST OF ABBREVIATIONS

Aci	Acipimox (5-Methylpyrazine-2-carboxylic acid 4-oxide)
AM	Ante meridiem
AN	Anorexia nervosa
ATGL	Adipose triglyceride lipase (Desnutrin)
AT	Adipose tissue
BMI	Body mass index
BN	Bulimia nervosa
C	Controls
cAMP	3´, 5´- cyclic adenosine monophosphate
CART	Cocaine amphetamine transcript
DPP-IV	Dipeptidyl peptidase IV
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders
ECG	Electrocardiogram
EDTA	Ethylenediamine tetra-acetate
FFA	Free fatty acids
G_i-type	G-inhibitory type G protein-coupled receptors
GH	Growth hormone
GHRH	Growth hormone releasing hormone
GHS	Growth hormone secretagogue
GHS-R	GH secretagogue receptors
GPR 109A	G-protein-coupled nicotinic acid receptor
CRF_2-R	Corticotropin releasing factor-receptor subtype 2
G_s-type	G-stimulatory type G protein-coupled receptors
HM74A	Nicotinic acid receptor in humans
HSL	Hormone-sensitive lipase
i.v.	Intravenous
LBM	Lean body mass
n	Number of subjects
NPY	Neuropeptide Y
NS	Not significant
OB-R	Soluble leptin receptor
PM	Post meridiem
PUMA-G	Nicotinic acid receptor in mice
PYY	Peptide YY
RGR	Relative glycerol recovery
P	Probability
p.o.	Per os
S.E.M.	Standard error of the mean
SNRI	Serotonin Norepinephrine Reuptake Inhibitors
SNS	Sympathetic nervous system
SSRI	Selective Serotonin Reuptake Inhibitors
sc	Subcutaneous
VO_2 max	Maximal oxygen uptake
% BF	Percentage of body fat

2. INTRODUCTION

Bulimia nervosa (BN) is an eating disorder characterized by repeated episodes of binge eating followed by inappropriate compensatory behavior with no pathological change in body weight leading to so far poorly understood neuroendocrine dysfunction of the hypothalamic-pituitary-adrenal axis (Torsello et al. 2007).

Exercise and anti-lipolytic drugs are enhancers of growth hormone (GH) secretion (Kok et al. 2004, Vendelbo et al. 2010), but the underlying mechanisms as well as the physiological significance remain elusive. The nutritional background and neurotransmitters involved in GH secretion during exercise remain uncertain; it has been suggested that exists GH - ghrelin feedback loop between the pituitary gland and stomach, the major site of ghrelin synthesis for release into systemic circulation (Qi et al. 2003). Ghrelin is an orexigenic peptide produced mainly in the stomach. It is an endogenous ligand of GH secretagogue (GHS) receptors with high *in vivo* and *in vitro* GH-releasing activity (Kojima et al. 1999), and thus, it is anticipated that circulating levels of this peptide would be linked with an endogenous GH secretion. In addition, ghrelin plays an essential role in appetite and meal initiation, and in regulation of energy homeostasis (Gil-Campos et al. 2006). There is important link between ghrelin secretion and fat metabolism. The orexigenic effects of ghrelin are inhibited during conditions of nutrient surplus (Gormsen et al. 2007). Ghrelin contributes to lower intracellular long-chain fatty acids concentrations and by this manner probably stimulates hypothalamic neuropeptide Y (NPY) and agouti-related protein, the main hormones of appetite regulation (Janas-Kozik et al. 2006). NPY leads to a net increase of stored energy, including decreased lipolysis, as well as overall de novo lipogenesis (Valet et al. 1990). NPY receptors are highly expressed both in the paraventricular nucleus of the

hypothalamus and on human adipocytes where inhibit lipolysis and promote leptin secretion (Serradeil-Le Gal et al. 2000). Recently, studies in rodents suggested a possible mediation of ghrelin action on GH by NPY. GH may also be involved in maintaining feeding (Hozumi et al. 2006). Orexigenic hypothalamic peptide NPY, influences the release of GH from the pituitary gland (Mazumdar et al. 2006) and there is a stimulator of pathways favouring food intake and energy storage. Ghrelin-NPY interaction and stimulatory effect of ghrelin on GH release in humans remains has been provided. The role of appetite-regulating neuropeptides in mediating lipolysis in humans is not well understood (Bradley et al. 2005). NPY is a co-transmitter with norepinephrine in peripheral sympathetic nerve fibers and has powerful vasoconstrictor effects (Cleary et al. 2007).

GH induces lipolysis, insulin resistance and hyperinsulinemia (Norrelund et al. 2003). Administration of Acipimox (Aci), a potent inhibitor of lipolysis, antagonizes the insulin antagonistic effects of GH in humans (Nielsen et al. 2002). The inhibition of lipolysis via supression of the hormone-sensitive lipase (HSL) in combination with GH administration significantly changed plasma ghrelin levels (Vestergaard et al. 2005). Ghrelin exerts a variety of metabolic functions including stimulation of appetite and weight gain, and suppression of insulin secretion (Esler et al. 2007). Whereas ghrelin administration in humans elicits a pronounced increase in pituitary GH secretion, a stimulatory effect of ghrelin on GH release remains to be convincingly demonstrated in healthy subjects (Vestergaard et al. 2007a, Veldhuis et al. 2008).

The peripheral ghrelin secretion is actively regulated by cephalic mechanisms (Power and Schulkin 2008), and it is hypothesized that activation of the sympathetic nervous system (SNS), which is known to regulate the secretion of other gut hormones, is one mediator of increased ghrelin secretion before scheduled meals (Mundinger et al. 2006).

Disruption of the gut-brain axis peptides might be involved in the pathophysiology of binge eating, as is BN. In BN observed some authors increased plasma ghrelin levels in spite of normal body mass index (BMI) (Tanaka et al. 2002) and increased plasma GH levels, similarly as is in anorexia nervosa (AN) patients with low BMI (Coiro et al. 1992, our observations), on the other hand, patients with AN have decreased plasma insulin levels with increased insulin sensitivity (Dostálová et al. 2007b). There is no information about the effects of a single bout of exercise or anti-lipolytic agent Aci on plasma ghrelin and GH levels in BN patients. In healthy subjects is hypothesized that circulating ghrelin is suppressed by exercise-induced GH release (Vestergaard et al. 2007b). On the other hand, Kraemer and Castracane (2007) demonstrated that ghrelin levels are lower with higher GH concentrations during aerobic exercise.

In humans, the first demonstration of an inhibitory action of increased plasma free fatty acids (FFA) levels at hypothalamic or higher centres in the central nervous system under physical exercise was shown by Coiro et al. (2007) and that supraphysiological FFA levels inhibited lipolysis in a feedback fashion.

NPY is expressed predominantly in the neurons of the hypothalamic arcuate nucleus and coexists with catecholamines in the central and the SNS, which play an important role in the up- or down-regulation of adipose tissue (AT) lipolysis (Turtzo and Lane 2006). Morover, NPY receptors are highly expressed on human adipocytes where they inhibit lipolysis (Kos et al. 2009b). Recently, it was found that NPY is synthesized in human AT and stimulates proliferation and differentiation of new adipocytes (Kos et al. 2007, Yang et al. 2008), and that adipose-derived NPY may have implications for central feedback of adiposity signals.

BN and AN are often accompanied by a stressful events and stressors, such as starvation, excessive physical exercise, energy excess or severe emotional stress including high anxiety (Fetissov et al. 2005). Both fasting and exercise are catabolic stress states during

which increase GH secretion and activation of the GHS receptors, abundantly expressed in the arcuate nucleus, up-regulates expression of NPY (Vendelbo et al. 2010, Rask-Andersen et al. 2010). It is supported that NPY can attenuate specific behavior when organism is stressed and anti-stress effects of NPY are relevant to psychiatric conditions, such as AN and BN (Eaton et al. 2007, Gruninger et al. 2007) and that NPY has an anxiolytic and anti-depressive behavior profile (Hökfelt et al. 2008). Thus, plasma NPY may be a good biomarker of sympathetic response to chronic stress (Eaton et al. 2007). Indeed, the same patients often switch between AN and BN suggesting that anorectic and bulimic manifestations are two stages of the same AN/BN syndrome (Fairburn and Harrison 2003).

Recent experimental evidence suggests that plasma NPY is increased in low plasma leptin states, and that plasma leptin levels are negatively correlated with NPY in patients with AN (Gendall et al. 1999). Furthermore, leptin is involved in the control of glucose-lipid homeostasis, energy storage and multiple neuroendocrine functions, which are impaired in BN and AN patients, including the pathological adaptation of the organism to starvation (Nedvídková et al. 2000, Scherag et al. 2010). Leptin may decrease food intake and increase metabolic rate and reduce body weight by inactivating the expression of orexigenic peptides such as NPY. On the contrary, hypothalamic neurons release NPY in response to falling leptin levels to maintain the stability of adipose stores (Leibowitz and Wortley 2004). In addition, leptin is likely to exert an inhibitory action on GH release in humans and it is possible that the malnutrition-dependent reduction of leptin levels may play a role in the hypersomatotropism of AN (Scacchi et al. 2003). This GH secretory pattern, which may reflect an adaptive phenomenon, is favouring the preferential utilization of FFA. Indeed, we have previously shown that anorectic patients have higher rate of lipolysis relative to healthy women (Barták et al. 2004). It has been suggested that fasting and exercise-enhanced appetite and food intake are due to an increased negative energy balance, causing the change of the levels of some

hypothalamic and extra-hypothalamic peptides such as NPY. Karamouzis et al. (2002) examined the effect of intense prolonged exercise on plasma leptin and NPY levels in man. This indicates that the leptin and NPY are involved in the regulation of energy expenditure and that low level of leptin facilitates expression of NPY synthesis. The effect of exercise on decreased plasma leptin is suggested *via* increased plasma FFA levels (Duclos et al. 1999), although direct action of FFA on plasma leptin levels cannot be concluded (Stumvoll et al. 2000). However, leptin production was stimulated by the anti-lipolytic agent Acipimox (Aci) from isolated rat adipocytes (Wang-Fisher et al. 2002).

In our previous study, we showed that malnourished and underweight anorexic patients associated with low plasma leptin did not find any changes in increased plasma NPY before and one month after partial weight recovery (Nedvídková et al. 2000). During the preparation of this article, Sedlačková et al. (2010) reported that increased fasting NPY levels, unchanged after a high-carbohydrate breakfast, indicated that NPY might be an important biomarker for disturbed eating behavior in BN.

Physical exercise and anti-lipolytic drugs are well-recognized stimulators of several hormones, such as GH and NPY (Fruehwald-Schultes et al. 1996, Karamouzis et al. 2002, , Kok et al. 2004, Vendelbo et al. 2010), however, molecular mechanisms and receptor array underlying these regulations are different and remain unidentified yet (de Vries et al. 2003, Kok et al. 2004, Taylor et al. 2008, Teske et al. 2008, Jürimäe et al. 2011).

Recent studies have suggested that NPY is not merely an ″orexigen″ but also acts to stimulate behavior which precedes food intake and actually inibits the intake per se (Sederholm et al. 2002, Ammar et al. 2005). It was found that the treatment with NPY increased physical activity and decreased food intake and caused a loss of body weight in rats (Nergårdh et al. 2007). From this point of view, it is possible that AN patients are physically hyperactive and eat only little food in spite of having depleted body fat and pathologically up-

regulated hypothalamic orexigenic peptides (Nergårdh et al. 2007). The discovery of the orexigenic peptide ghrelin, which represents an endogenous ligand for GHS receptors, augmented the complexity of the GH neuroendocrine axis even further (Wagner et al. 2009). The link between ghrelin and NPY has been established by co-localization studies of NPY-containing neurons and ghrelin receptors, which do overlap, indeed (Willesen et al. 1999, Tannenbaum et al. 2003), and an intravenous (i.v.) injection of ghrelin was reported to increase plasma NPY levels in humans (Coiro et al. 2006).

In our recent studies, we reported increased response of GH and ghrelin to exercise and anti-lipolytic drug administration in BN patients, and we confirmed that GH exerted an inhibitory feedback effect on plasma ghrelin during exercise only in BN patients; however, this effect was exerted in both BN patients and healthy women during exercise with Aci administration (Smitka et al. 2008, Nedvídková et al. 2011). This evidence suggests a FFA-independent mechanism of Aci.

Aci (5-Methylpyrazine carboxylic acid 4-oxide; Olbetam) is a nicotinic acid-derived anti-lipolytic drug devoid of major side effects, and has been used in a number of human trials, but the cellular mechanism by which Aci exerts its main effect is not fully known. Aci is a potent and long-acting anti-lipolytic drug, derived from niacin. The inhibition of adipocyte lipolysis by Aci is mediated through suppression of intracellular cyclic adenosine monophosphate (cAMP) levels, which inhibit adipocyte lipases such as HSL and adipose triglyceride lipase (ATGL, *i.e.* desnutrin) *via* alternative cAMP-independent pathways (Wang-Fisher et al. 2002, Karpe and Frayn 2004, Langin 2006, Soudijn et al. 2007), thereby lowering circulating plasma FFA and glycerol levels.

Leptin-ghrelin-NPY-GH interactions and the role of appetite-regulating neuropeptides in mediating lipolysis in humans are not well understood. Dysregulations of the gut-brain-AT axis peptides might be involved in the pathophysiology of BN patients (Meguid et al. 1996,

Roemmich and Rogol 1999, Romijn et al. 2008, Smitka et al. 2008, Nedvídková et al. 2011). In this eating disorder characterized by repeated episodes of binge eating followed by inappropriate compensatory behavior, increased baseline plasma GH and NPY levels were documented, similarly as in restrictive-type AN patients (Coiro et al. 1992, Scacchi et al. 2003, Nedvídková et al. 2003a, Fassino et al. 2005, Sedláčková et al. 2010).

Catecholamines of the SNS play an important role in the regulation of AT lipolysis, which is a key step in the metabolic processes leading to the decrease of fat mass. Catecholamines influence lipolysis *via* beta-adrenergic G-protein-coupled receptors of adipocytes. In our previous studies (Nedvídková et al. 2004, Barták et al. 2004), we found increased norepinephrine concentrations and increased production of glycerol as an index of lipolysis rate in subcutaneous (sc) abdominal AT in patients with anorexia nervosa (AN), measured *in vivo* by microdialysis.

Therefore, the aim of this randomized, placebo-controlled, single-blind, microdialysis study was to examine the changes of plasma GH, ghrelin, NPY, leptin, insulin and blood glucose levels induced by physical exercise alone or in association with anti-lipolytic drug Aci administration during short-term exercise in BN patients and to find out whether ghrelin is involved in exercise-induced GH release in BN patients with higher basal plasma levels and to determine how exercise and Aci affect these peptides in energy balance. At the same time, we measured FFA and glycerol metabolites in the circulation and dialysate glycerol in sc abdominal AT using microdialysis. Healthy women were used as the control group.

3. EATING DISORDERS

BN and AN are eating disorders characterized by severe disturbances in eating behavior. AN is characterized by self induced starvation and refusal to gain and maintain a minimal normal body weight (weight criterion for the diagnosis is under 85% of normal body weight) while for BN repeated episodes of binge eating followed by inappropriate compensatory behavior, such as self-induced vomiting, laxative and diuretics misuse, fasting or excessive exercise are typical (Diagnostic and Statistical Manual of Mental Disorders [DSM-IV], American Psychiatric Association, 1994). These disorders affect 2-3% of young women and adolescents (Hsu 1996). AN is highly morbid pathologic condition with the highest mortality rate among psychiatric disorders (Vitiello and Lederhendler 2000). In addition to that the multiple negative social and psychological impacts of eating disorders are considerable.

The cause of pathogenesis of BN and AN, however, remains unknown. The phenomenon of binge eating *i.e.* consumption of large amounts of food in a short time period accompanied by a sensation of losing control over eating in BN, and/or intense fear of losing control over eating and becoming overweight in BN suggest a deficit in the normal mechanisms that turn off eating.

3.1. Diagnostic Criteria for BN (DSM-IV)

The criteria for diagnosing a patient with bulimia are:

A. Recurrent episodes of binge eating. An episode of binge eating is characterized by both of the following:

Eating within any 2-hour period of time, an amount of food that is definitely larger than most people would eat under similar circumstances.

A lack of control over eating during the episode; a feeling that one cannot stop eating or control what or how much one is eating.

B. Recurrent inappropriate compensatory behavior to prevent weight gain, such as self-induced vomiting; misuse of laxatives; diuretics, enemas, or other medications; fasting or excessive exercise.

C. The binge eating and inappropriate compensatory behavior both occur, on average, at least twice a week for 3 months.

D. Self-evaluation is unduly influenced by body shape and weight.

E. The disturbance does not occur exclusively during episodes of AN.

There are two sub-types of BN: Purging and non-purging

3.1.1. Purging and Non-Purging Sub-Types of BN

Purging bulimia is the more common of the two and involves self-induced vomiting, which may include use of emetics, such as syrup of ipecacuanha, and self-induced purging, which may include use of laxatives, diuretics and enemas to rapidly remove food from the body before it can be ingested.

Non-purging bulimia, which occurs in only approximately 6%-8% of cases, which involves excessive exercise or fasting after a binge to offset the caloric intake after eating. Purging sub-type bulimics may also exercise or fast but as a secondary form of weight control.

4. APPETITE-REGULATING PEPTIDES IN EATING DISORDERS

4.1. An Overview of Feeding-Stimulatory and Feeding-Inhibitory Signals

4.1.1. Ghrelin

Ghrelin is a 28-amino acid peptide which increases food intake and acts as an endogenous stimulator of GH (Ariyasu et al. 2001) The ghrelin gene encodes a polypeptide preproghrelin containing 117 residues which undergoes stepwise processing to form ghrelin (Zhu et al. 2006). Ghrelin is predominantly produced by the stomach but is also expressed in many other tissues and is the first identified hormone containing acylated n-octanoic acid in its residues (Kojima et al. 1999). This acylation is essential for binding to the GHS receptor type 1a (GHS-R 1a) and for the GH-releasing and appetite-stimulating activities. Asakawa et al. (2005) indicated that in contrast to acylated ghrelin, nonacylated ghrelin induces a negative energy balance by decreasing food intake and delaying gastric emptying. Nonacylated ghrelin present in plasma in far greater quantities than acylated ghrelin seems to be devoid of any endocrine action. However, nonacylated ghrelin is able to exert some nonendocrine actions including cardiovascular and anti-proliferative effects by binding different GHS-R subtypes or receptor families (Cassoni et al. 2001). It was described that acylated ghrelin (Banks et al. 2002) and nonacylated ghrelin pass the blood-brain barrier by means of transmembrane diffusion (Chen et al. 2005). Nonacylated ghrelin induces an increase in neuronal activity in the arcuate nucleus is involved in the regulation of the synthesis of anorexigenic mediators like urocortin and cocaine amphetamine regulated transcript (CART) in the hypothalamus and

interacts with the corticotropin releasing factor-receptor subtype 2 (CRF_2-R). Therefore, the neuromodulatory peptides CART and urocortin might thus play a key role in the anorexigenic effect of nonacylated ghrelin *via* CRF_2-R dependent signalling (Inhoff et al. 2009).

The role of acylated ghrelin, nonacylated ghrelin and other ghrelin gene derived peptides in the postprandial regulation of satiety was not established. Recently, we found decreased levels of plasma total, acylated and nonacylated ghrelin and obestatin levels after a high-carbohydrate breakfast in healthy women (Sedláčková et al. 2008). It is possible that obestatin may postprandially blunt the effect of ghrelin in healthy normal weight women. Furthermore, our group assumes that the preproghrelin is cleaved differently in eating disorders such as AN than under physiological conditions. Thus, intact acylated ghrelin 1-28 is rapidly degraded to nonacylated forms or smaller fragments in AN patients (unpublished data). This is in keeping with the finding of Hotta et al. (2004) who reported decreased levels of plasma acylated ghrelin levels in AN patients.

Ghrelin increases food intake through effects on NPY (Chen et al. 2004). Plasma ghrelin levels are elevated during fasting and suppressed after meal (Cummings et al. 2001). Fasting plasma ghrelin concentrations in humans are negatively correlated with BMI (Shiiya et al. 2002). In obese individuals, dieting is associated with an increase in plasma ghrelin levels (Cummings et al. 2002). In women with AN, Karczewska-Kupczewska et al. (2010) reported significant positive correlation between fasting ghrelin and insulin sensitivity and that the progressive decline in circulating insulin would favour ghrelin production in AN (Misra et al. 2005a). Both peripherally and centrally administered ghrelin produce a positive energy balance and lead to body weight gain (Nakazato et al. 2001). In humans, acylated ghrelin induces a rapid rise in blood glucose and plasma insulin levels. However, co-administration of nonacylated ghrelin counteracts this effect. Thus, i.v. administration of nonacylated ghrelin improves glucose metabolism and insulin sensitivity and inhibits lipolysis

in humans (van der Lely 2009). Based on these data, van der Lely (2009) suggests the existence of a specific receptor for nonacylated ghrelin another than the CRF_2-R and GHS-R 1a.

Current analysis has highlighted the expression of ghrelin in a number of endocrine tissues such as AT (Kojima and Kangawa 2005). Recently, Liu et al. (2009) have explored the effects of ghrelin on the proliferation and differentiation of preadipocytes *in vitro* and confirm that ghrelin induces the differentiation of 3T3-L1 preadipocytes into mature adipocytes. Rodent and human studies indicate that ghrelin elicits an anti-lipolytic effect mediated by both acylated and nonacylated ghrelin and promotes adipogenesis (Rodríguez et al. 2009). However, predominant nonacylated ghrelin does not appear to activate GHS-R1a and it remains unclear through which receptor nonacylated ghrelin mediates its action in AT, although it has been suggested that the anti-lipolytic effect of ghrelin could be mediated by an unidentified non-GHS-R 1a receptor (Broglio et al. 2004a). Interestingly, the ratio of acylated and nonacylated ghrelin production might help to regulate the balance between adipogenesis and lipolysis in response to nutritional status (Thompson et al. 2004). Recently, Tebbe *et al.* (2005) have shown that ghrelin effects in the rat central nervous system appear to be mediated through receptor Y1, which also mediates the anti-lipolytic action of NPY_{1-36} and peptide YY_{1-36} (PYY_{1-36}). Thus, ghrelin may mediate its peripheral action in AT through Y1 receptor. Kos et al. (2009a) have demonstrated anti-lipolytic action of ghrelin in human AT and showed that acylated and nonacylated ghrelin may be ligands for Y1 mediating lipogenic effect in humans. Taken together, these studies suggest the importance of the gut-brain-AT axis in determining ghrelin influence on metabolism and potential interaction between organs.

4.1.1.1. Ghrelin Levels in Eating Disorders

Fasting plasma ghrelin levels have been reported to be increased in underweight patients with AN, especially in patients with binge-purge subtype of AN as compared to patients with restrictive type of AN suggesting that binge-purging behavior has some influence on plasma ghrelin (Otto et al. 2001, Misra et al. 2005a). These findings were not confirmed by Otto et al. (2004), who did not find difference in plasma ghrelin between restrictive and binge-purge subtypes of AN, and by Troisi et al. (2005), who detected opposite results with higher plasma ghrelin levels in restrictive type of AN as compared to patients with binge-purge subtype of AN and BN individuals. Ghrelin is increased in the case of AN and this increase in plasma ghrelin levels may occur either as an adaptive response to correct the abnormal energy status or as a result of relative resistance to ghrelin (Yin et al. 2009). The enhanced plasma ghrelin levels of underweight AN patients tend to normalize after refeeding (Janas-Kozik et al. 2007). Furthermore, patients with AN do not show a decrease in plasma ghrelin following a standardized meal that is observed in healthy women (Nedvídková et al. 2003a) and anorectic patients would be refractory to the orexigenic action of ghrelin to regain a normal weight and replenish energy stores.

It is accepted that plasma levels of active acylated ghrelin represent less than 10% of circulating total ghrelin levels, which include acylated and inactive nonacylated ghrelin. We found increased plasma nonacylated ghrelin but not acylated ghrelin levels in AN patients (unpublished data). While high plasma total ghrelin in AN has been consistently observed (Nedvídková et al. 2003a, Germain et al. 2009), elevated acylated ghrelin was found in few studies (Nakai et al. 2003, Harada et al. 2008).

It was reported that fasting plasma ghrelin levels were higher in the purging type of BN in comparison to the non-purging type and in comparison to controls (Tanaka et al. 2002, 2003); this supports the idea that binge-purge cycles have an influence on fasting plasma

ghrelin. However, subsequent studies did not detect any significant difference in plasma ghrelin levels between binge-purge BN patients and controls (Troisi et al. 2005, Monteleone et al. 2005) though Kojima et al. (2005) found that BN patients exhibited elevated ghrelin levels despite higher BMI. In our recent study, we reported increased response of GH and ghrelin to short-term exercise and anti-lipolytic drug Aci in BN patients and confirmed that GH exerted an inhibitory feedback effect on plasma ghrelin during exercise only in BN patients but in both BN patients and healthy women during exercise with Aci administration (Smitka et al. 2008, Nedvídková et al. 2011). Therefore, these data established ghrelin as a potential discriminator between women with eating disorders and healthy women (Troisi et al. 2005). Furthermore, the ghrelin responses to a standardized meal have been reported to be blunted in symptomatic binge-purge BN patients as compared to healthy controls (Monteleone et al. 2003, 2005, Kojima et al. 2005). However, in our recent study we documented decreased ghrelin levels in BN patients after a high-carbohydrate breakfast (Sedláčková et al. 2010).

4.1.2. NPY

NPY is a 36-amino acid peptide that has potent orexigenic properties (Neary et al. 2003). Experimental evidence indicates that NPY is the strongest orexigenic factor in the hypothalamic control of feeding behavior (Sahu and Kalra 1993). NPY coexists with catecholamines in the central and SNS and in the adrenal medulla (Turtzo and Lane 2006). Furthermore, NPY containing nerves are present in the gut of many species. The role of NPY can be considered as helping to coordinate protective antistarvation activity and preventing further depletion of existing energy stores. Recent studies have suggested that NPY is not merely an ″orexigen″, but acts to stimulate behavior which precedes the food intake and

actually inibits intake per se (Sederholm et al. 2002, Ammar et al. 2005). The treatment with NPY increased physical activity and decreased food intake and caused a loss of body weight in rats (Nergårdh et al. 2007). These findings can be in line with clinical observation in AN patients who are physically hyperactive and eat only a little food in spite of having depleted body fat and pathologically up-regulated hypothalamic orexigenic peptides (Nergårdh et al. 2007). NPY´s activity in cellular metabolism is mediated through binding to G-protein coupled receptors, of which at least four subtypes exist in humans (Y1, 2, 4 and 5) and which are present in most peripheral tissues. The hypothalamic Y1, Y2, Y4 and Y5 receptors have all been hypothesized to mediate the orexigenic effects of NPY (Wynne et al. 2005).

Intracerebroventricular injection of NPY appears to mediate upregulation of the key enzyme of lipogenesis: lipoprotein lipase expression and activity in AT (Billington et al. 1991). It was recently found that NPY is synthesized in human AT and stimulates the proliferation and differentiation of new adipocytes (Kos et al. 2007, Yang et al. 2008). Although to date the role of most of these receptors in human AT is poorly understood, binding studies (Bradley et al. 2005) have suggested that Y1 receptor may mediate the anti-lipolytic effect of NPY in AT. NPY_{1-36} is cleaved by dipeptidyl peptidase IV (DPP-IV) to generate the truncated NPY_{3-36} with which DPP-IV diverts affinity of NPY from Y1 to other receptors such as receptor Y5 whose function remains elusive (Kos et al. 2009b). DPP-IV inhibitors are therefore likely to enhance the anti-lipolytic action of NPY_{1-36} as well as PYY_{1-36} (Kos et al. 2009b). Furthermore, in order to better understand the interactions between sympathetic neurotransmitters and glucocorticoids in AT, Kuo et al. (2008) treated sympathetic neural cells with dexamethasone upon which the expression of NPY and its Y2 receptor was more than doubled. Therefore, cortisol and the adrenergic activity seem to converge on the NPY-Y2 adipogenic system. Thus, adipose-derived NPY may have implications for central feedback of adiposity signals.

4.1.2.1. NPY Levels in Eating Disorders

In reports with AN, basal plasma NPY levels in AN patients did not differ from the levels in the controls (Baranowska et al. 1997, Nedvídková et al. 2000). Discordant data have been published concerning NPY levels in AN patients. Plasma levels of NPY were significantly lower in anorectic women than in the control group (Baranowska et al. 2001) and plasma NPY were decreased during treatment of anorectic girls. These changes do not correspond with increasing body weight suggesting dysregulation of appetite and body weight control mechanisms in AN (Oswiecimska et al. 2005). The study by Sedláčková et al. (2010) published during the preparation of this paper assumes that increased fasting NPY levels unchanged after a high-carbohydrate and high-protein breakfast indicate that NPY may be an important biomarker for disturbed eating behavior in AN and BN patients.

Plasma levels of NPY during symptomatic and remission phases of BN are unchanged compared with age and weight matched controls (Takimoto et al. 2003). However, plasma concentrations of NPY in patients with BN were significantly elevated in comparison to controls (Baranowska et al. 2001, Nedvídková et al. 2011) indicating the effects of variations in nutrition and body weight on plasma levels of NPY.

4.1.3. Leptin

Leptin is a 167-amino acid protein known to suppress appetite and regulate energy expenditure. Leptin is secreted exclusively by adipocytes (Zhang et al. 1994) and leptin has also been found in the stomach (Bado et al. 1998) and the pituitary gland (Jin et al. 1999). Nevertheless, AT remains its main source responsible for 95% of leptin production (Frayn et al. 2003). Leptin acts through the leptin receptor (OB-R), which is expressed in the hypothalamus and peripheral tissues such as the gut and AT. This ubiquitious distribution of

OB-R underlies the pleiotropic roles of leptin (Anubhuti and Arora 2008). Soluble OB-R represents the major leptin binding activity in human plasma (Lammert et al. 2001).

Plasma leptin levels reflect both energy stores and acute energy balance. Circulating leptin levels are positively correlated with BMI and AT mass, food restriction results in suppression of plasma leptin levels, which can be reversed by refeeding (Stanley et al. 2005). Peripheral leptin administration reduces food intake resulting in loss of fat mass (Halaas et al. 1995). It was suggested that leptin-induced increases in energy expenditure may reflect an activation of the SNS (Haynes et al. 1997). A report by Tang-Christensen et al. (1999) supporting that central leptin administration activates the SNS and that increases plasma norepinephrine levels in primates.

As shown by recent studies, leptin dose-dependently inhibits ghrelin transcription *in vitro* (Zhao et al. 2008) and decreases ghrelin release from isolated rat stomach (Kamegai et al. 2004). These findings raised the possiblity that hyperleptinemia may suppress ghrelin secretion in obese patients (Tschöp et al. 2001). There is also an opposing relation that ghrelin hypersecretion is in conjunction with hypoleptinemia in AN (Scacchi et al. 2003). The importance of leptin as adiposity signal to the brain is revealed by evidence that leptin inhibits the activity of orexigenic ghrelin-NPY network, whereas low plasma leptin levels up-regulate the expression of NPY neurons which co-express ghrelin receptors (Kalra et al. 2005).

Furthermore, the production of leptin is influenced by several regulators, being stimulated by anti-lipolytic insulin and blood glucose but inhibited by sympathetic activity, lipolytic catecholamines and FFA (Frühbeck et al. 1998). As reported by Frühbeck et al. (1998), leptin appears to be involved in the regulation of AT metabolism by both inhibiting lipogenesis (Bai et al. 1996) and stimulating lipolysis (Frühbeck et al. 1997).

It was shown that leptin is the major hormone to trigger the adaptation of an organism to food restriction (Ahima et al. 1996). These findings indicate that the drop in

leptin secretion associated with weight loss induced *via* a reduced energy intake is a major trigger underlying adaptation to starvation in AN (Holtkamp et al. 2003).

4.1.3.1. Plasma, Soluble OB-R and AT Leptin Levels in Eating Disorders

In malnourished and underweight AN patients, plasma leptin levels are consistently found to be markedly lower than in normal weight controls (Nedvídková et al. 2000, Holtcamp et al. 2003, Dostálová et al. 2005, 2007a, b, Monteleone et al. 2008) and weight recovery in AN is associated with a trend towards increases in plasma leptin levels (Misra et al. 2005b). In contrast, plasma soluble OB-R level was reported to be increased (Dostálová et al. 2005, Housová et al. 2005). This increase may reflect a protective mechanism that decreases free leptin bioavailability and thus further facilitates energy conservation in AN patients (Housová et al. 2005). Interestingly, in our study, Dostálová et al. (2005) reported significantly reduced plasma leptin levels but normal dialysate leptin concentrations in sc abdominal AT in AN patients. This finding could be explained by the increased number of smaller adipocytes in sc abdominal AT leading to a higher number of adipocytes per volume in AN patients when compared with the controls. Another explanation of this may be due to a reduced efficiency of the SNS inhibiting adipocyte leptin production (Monteleone et al. 2008). On the other hand, because of reduced volume of sc abdominal AT less leptin is secreted into plasma.

Very recently, Fazeli et al. (2010) have shown that administration of supraphysiological recombinant GH in patients with AN leads to significantly decrease in plasma leptin levels when compared with the placebo AN group.

In normal weight subjects with BN, plasma leptin levels have been reported to be either decreased (Monteleone et al. 2000, Brewerton et al. 2000, our observations) or normal

(Zipfel et al. 1998, Housová et al. 2005). It has been reported that BN patients with a significantly higher number of daily binge/vomiting episodes hyposecrete leptin in spite of no changes in their BMI (Jimerson et al. 2000, Monteleone et al. 2002). Plasma soluble OB-R was unaffected in BN patients when compared with the controls (Housová et al. 2005).

5. THE METABOLIC ROLE OF GH AND GH SECRETION IN EATING DISORDERS

5.1. The Metabolic Role of GH under Resting Conditions and during Physical Exercise

Human GH is a 191-amino acid, 22-kDa polypeptide, that is secreted from the pituitary gland (Møller and Jørgensen 2009). It has been reported that free monomeric 22-kDa GH represents only 20% of total immunoreactivity in plasma (Baumann 1991). Furthermore, a novel assay of free GH shows that free GH depends much on total GH and GH binding protein concentrations (Frystyk et al. 2009) and that the pituitary gland secretes a spectrum of homo- and heterodimers and -multimers of a variable spectrum of GH isoforms (Bidlingmaier and Strasburger 2010).

GH release is stimulated by hypothalamic GH relasing hormone (GHRH) and inhibited by somatostatin (Anderson et al. 2004). Recently, ghrelin was identified as the specific endogenous ligand for the GHS receptor and was isolated from rat stomach (Kojima et al. 1999). The effect of ghrelin on GH release is two to three times greater than that of GHRH in humans (Arvat et al. 2000). Morover, peripherally administered ghrelin signals *via* the vagus nerve to the brain where it triggers the release of GHRH and contributes to the activation of the food intake signaling cascade by NPY neurons in the arcuate nucleus of the hypothalamus (Date et al. 2002), when the vagus nerve is cut, the induction of GH release after ghrelin injection is dramatically decreased. It was documented that GH inhibits stomach ghrelin secretion. These findings indicate that the vagal circuit between the brain and the stomach has a crucial role in regulating plasma ghrelin levels (Nonogaki 2008).

GH secretion is also regulated by peripheral and central peptides participating in the control of food intake and energy expenditure, such as above mentioned ghrelin, leptin, insulin and NPY. The i.v. administration of NPY has no effect on GH secretion in healthy humans (Antonijevic et al. 2000), however, stimulatory effects of NPY on GH secretion has been reported in prolactinoma and acromegalic patients (Watanobe and Tamura 1996, 1997). In addition, some of these authors also described inhibition of GH secretion by NPY (Watanobe and Tamura 1997). Adipocytes possess large numbers of GH receptors, and it was shown that GH directly regulates leptin gene production (Lisset et al. 2001) and that hyperleptinemia may suppress ghrelin secretion (Kalra et al. 2005). Furthermore, leptin has been shown to play a stimulatory role in GH secretion in rats (Carro et al. 1998), however, leptin is likely to exert an inhibitory action on GH secretion *via* a stimulatory effect on hypothalamic somatostatin activity in humans (Støving et al. 2002) and/or GH hyposecretion might be explained by a resistance to leptin action because hyperleptinemia might contribute to the GH hyposecretion of obese patients (Dieguez et al 2002) and it is suggested that the malnutrition-dependent reduction of leptin levels may play a role in the hypersomatotropism of AN (Sccachi et al. 2003, Misra et al. 2005b). Considering that anti-lipolytic drug Aci is known to strongly decrease plasma insulin levels in obese patients (Worm et al. 1994) and that hyperinsulinemia might be involved in the GH hyposecretion of obese subjects (Sccachi et al. 2010). Similarly, a possible involvement of malnourishment-induced hypoinsulinemia in the GH hypersecretion of AN has also been hypothesized (Sccachi et al. 2003) because insulin appears to be involved in the control of GH secretion and exerts an inhibitory effects on GH synthesis and release in anterior pituitary cells (Yamashita and Melmed 1986).

GH secretion is amplified during fasting, physical exercise, stress and catabolic conditions, whereas excess of fuels such as blood glucose and lipids inhibits GH release in humans (Møller and Jørgensen 2009).Thus, a classic feedback relationship between GH and

FFA has been reported, FFA exerts an inhibitory effect on GH secretion (Alvarez et al. 1991), while the reduction of plasma FFA following the administration of Aci stimulates GH release (Peino et al. 1996). A key metabolic effect of GH is stimulation of lipolysis in AT and release of FFA and glycerol (Gravholt et al. 1999) and increased lipid availability has been suggested to be responsible for the impact on insulin resistance (Nørrelund 2005). Thus, GH stimulates lipolysis and causes insulin resistance, hyperglycaemia and hyperinsulinemia (Nørrelund 2005). It has also been observed that pulsatile GH exposure increases FFA and glycerol turnover in humans (Cersosimo et al. 1996, Gravholt et al. 1999). Thus, administration of GH to normal subjects under resting conditions increases lipolysis, insulin secretion, FFA availability and fat oxidation but there was no effect of GH replacement on FFA turnover during exercise in healthy humans (Irving et al. 2004, Hansen et al. 2005). However, a greater increment in FFA turnover following GH administration during exercise was observed in GH-deficient subjects (Kanaley et al. 2004).

The lipolytic effects of GH are partly mediated *via* the HSL (Yip and Goodman 1999). Furthermore, GH suppresses the lipoprotein lipase activity in human AT (Ottosson et al. 1995) and antagonizes the anti-lipolytic effect of insulin (Nørrelund 2005). GH also increases lipolysis indirectly by altering the effect of adipocytes to respond to lipolytic factors such as catecholamines, consequently, the GH-related relief of G_i alpha$_2$-adrenergic dependent inhibition of cAMP production increases lipolysis (Marcus et al. 1994, Lafontan and Langin 2009). This is in accordance with the administration of Aci, a nicotinic derivative that blocks the actions of HSL has been shown to suppress the lipolytic effects of GH in humans (Nielsen et al. 2001) and reverse the inhibitory effects of GH on insulin-stimulated glucose uptake (Nørrelund 2005). It was suggested that FFA play a causal role in the development of insulin resistance associated with GH replacement by demonstrating that co-administration of Aci is able to improve insulin sensitivity (Nielsen et al. 2001).

5.1.1. The Neuroendocrine Control of GH Secretion in Eating Disorders

In AN and BN, plasma GH levels are increased under basal conditions (Sccachi et al. 2003, Fassino et al. 2005) as a consequence of prolonged starvation, inadequate caloric intake, persisting binge/vomiting behavior and stressful events.

AN is asssociated with a nutritionally acquired resistance to GH with elevated GH levels and low levels of the GH-binding protein indicate decreased expression of the GH receptor, which accounts for the state of GH resistance in the starved state (Misra and Klibanski 2011). This is consistent with results reported by Fazeli et al. (2010) that administration of supraphysiological recombinant human GH in patients with AN does not overcome the state of GH resistance. The administration of recombinant human GH was not associated with a significant change in plasma levels of blood glucose, insulin or FFA in AN. Importantly, these findings suggest that patients with AN would have a relatively high resistance to the effects of GH (Fazeli et al. 2010).

GH levels are higher in patients with AN than in controls and these higher levels are consequent to higher levels of ghrelin, a GH secretagogue. Thus, the hypersecretion of ghrelin might contribute to the hypersomatotropism of AN (Sccachi et al. 2003). Furthermore, Støving et al. (2002) suggest that GH hypersecretion in AN is due to decreased hypothalamic somatostatinergic tone restored by weight gain in these patients. Although patients with AN showed a hyperresponsiveness to GHRH administration (Gianotti et al. 2000), their GH response to ghrelin administration is surprisingly blunted. This finding is consistent with desensitization of the GHS receptor induced by the chronic elevation of ghrelin levels in AN or impaired metabolic status in AN because ghrelin administration was not followed by increase in blood glucose levels in these patients (Broglio et al. 2004b). However, the normal GH response to ghrelin administration was observed in BN patients and ghrelin

administration was followed by increase in blood glucose in BN (Fassino et al. 2005). These authors hypothesize that ghrelin hypersecretion may have a role in eating behavior but normal GH and blood glucose response to ghrelin administration may reflect less impaired nutritional status in BN patients (Fassino et al. 2005).

The physiological inhibitory role of FFA on GH secretion seems to be preserved in patients with AN. In fact, the infusion of FFA inhibited the elevated basal GH levels and abolished the exaggerated GH response to the GHRH, whereas the administration of Aci led to the decrease in plasma FFA and markedly enhanced the GHRH-induced GH rise in patients with AN but not in healthy women (Giannoti et al. 2000).

6. THE ROLE OF SNS ON AT METABOLISM

The sympathoadrenal system is considered to be as a major system that regulates AT metabolism.

6.1. Catecholamines; Beta- and Alpha$_2$-Adrenoceptors in Sc AT

Key hormones controlling adipocyte function are catecholamines, such as norepinephrine and epinephrine (Lawrence and Coppack 2000). Catecholamines stimulate or inhibit lipolysis depending on the presence of tissue adrenoceptors involved in their effect. Norepinephrine stimulates lipolysis through the activation of beta adrenergic G_s-type G protein-coupled receptors and epinephrine exhibits a higher affinity and inhibits lipolysis through activation of the alpha$_2$-adrenergic G_i-type G protein-coupled receptors as has been described *in vitro* (Wellman 2000). In humans, sc fat cells alpha$_2$-adrenoceptors numerically predominate over beta-adrenoceptors and therefore lower catecholamine concentrations can cause inhibition of lipolysis (Mauriege et al. 1987, Lawrence and Coppack 2000). The physiological importance of this dual effect of catecholamines is still not clear. Beta-adrenoceptors stimulates lipolysis at higher concentrations of catecholamines in AT *via* adenylate cyclase system resulting in HSL activation. Ending products are glycerol and free fatty acids. Alpha$_2$-adrenoceptor agonists inhibit lipolysis *via* the inhibition of adenylate cyclase system. Adrenoceptors often display opposing on adipocytes in response to ligand binding and their relative membrane densities show not only marked regional heterogenity (Arner 1999a) but are also subject to rapid changes in response to different metabolic and endocrine influences. Differences in the lipolytic response to catecholamines are associated with variation in the functional balance between alpha and beta-adrenoceptors in AT (Langin

et al. 2000). Multiple alterations in catecholamine signal transduction pathways could explain the lipolytic defects in obese and lean subjects.

6.1.1. Catecholamines during Physical Exercise in Anorexic and Obese Patients

The increase in lipolysis in AT during physical exercise results from the interplay of stimulatory and inhibitory actions of catecholamines mediated by beta-adrenoceptors and alpha$_2$-adrenoceptors (Stich et al. 2000). The relative contribution of the specific signaling pathway and the overall lipolytic response to physical exercise are dependent on nutritional status, presence of obesity and/or AN or other pathophysiological states. It can be proposed that under hypocaloric diet or high-fat meal, the balanced interplay between activation of beta-adrenoceptors and alpha$_2$-adrenoceptors pathways is modified during exercise in sc abdominal AT in obese women and obese male subjects (Stich et. al. 2002, Polak et al. 2007) and that reduced exercise-induced alpha$_2$-adrenergic anti-lipolytic effect was observed in above mentioned both cases. However, dynamic strength training increases sensitivity and responsiveness to the adrenergic beta receptor stimulation of lipolysis and to the anti-lipolytic action of catecholamines mediated by alpha$_2$-adrenergic receptors in obese men (Polak et al. 2005). Our group has previously found that malnourished and underweight anorexic patients are associated with *in vivo* increased basal SNS and AT lipolysis (Nedvídková et al. 2004) as well as exercise-stimulated SNS activity (Barták et al. 2004), especially norepinephrine levels, and unchanged basal but increased significantly exercise-stimulated lipolysis in sc abdominal AT (Barták et al. 2004). Our observations suggest that the increased lipolysis induced by norepinephrine is mediated *via* second messenger cAMP leading to the increase in phosphorylation of HSL and release of glycerol from adipocytes.

6.2. Mechanism of Anti-Lipolytic Action of Aci in AT

6.2.1. Aci and cAMP-Dependent Signal Transduction Pathway

Aci is an anti-hyperlipidaemic analogue of niacin (5-Methylpyrazine-2-carboxylic acid 4-oxide, molecular weight: 154.1) and has been used in a number of human trials (Ball et al. 1986, Fulcher et al. 1992) but the cellular mechanism by which Aci exerts its main effect (*i.e.* suppression of lipolysis from AT) is not fully known. It is supposed that the anti-lipolytic action of Aci is mediated through suppression of intracellular cAMP levels. Aci initiates this inhibitory process *via* binding to a G_i-type G protein-coupled receptors termed GPR 109A (HM74A in humans and PUMA-G in mice) on the membrane of adipocytes, subsequent suppression of cAMP through adenylate cyclase system with the decrease in protein kinase A activity leading to the key´s decrease in phosphorylation of HSL and reduce the association of HSL with its triacylglycerol substrate in the lipid droplet of adipocytes (Christie et al. 1996, Lorenzen et al. 2001, Wise et al. 2003, Tunaru et al. 2005). Importantly, on the basis of above mentioned mechanism in action of catecholamines, anti-lipolytic influences include $alpha_2$-adrenergic G_i- type G protein-coupled receptors activation and activation of nicotinic acid receptor HM74A (Karpe and Frayn 2004). Interestingly, plasma epinephrine levels increased after Aci administration in obese and lean males (Allick et al 2004) and that there was a trend towards higher plasma norepinephrine values during and after exercise with Aci administration in patients with non-insulin-dependent diabetes mellitus (Akanji et al. 1993).

6.2.2. Aci and Non cAMP-Dependent Signal Transduction Pathway

Moreover, anti-lipolytic effect of Aci is the result of the suppression of adipocyte lipases, such as HSL and ATGL (desnutrin), although a direct link between Aci and either HSL or ATGL has not been demonstrated yet (Soudijn et al. 2007). Interestingly, *in vitro* ATGL gene expression was not regulated by cAMP suggesting that activation and/or inhibition of ATGL could mediate alternative non cAMP-dependent signal pathway (Villena et al. 2004). Indeed, Aci may bypass the membrane receptor control to reach the target regulatory machinery *via* alternative non cAMP-dependent signal transduction pathways. Nevertheless, Wang-Fisher et al. (2002) showed that Aci stimulation was efficiently suppressed by norepinephrine, consistent with our hypothesis that Aci acts mainly *via* its inhibition on cAMP production.

6.3. Principles of Microdialysis

Microdialysis has been used to determine the extracellular concentrations and local changes of tissue metabolism (glycerol, catecholamines, hormones, etc.) *in vivo* (Nedvídková et al. 2003b, 2004, Dostálová et al. 2003, 2009). A microdialysis catheter is an artificial blood vessel system which can be placed in the extracellular space of various tissues such as AT *in situ*. (Arner 1999b). Conceptually, microdialysis is 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 of 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 (Pacak et al. 1995 a, b). The composition of the perfusate should resemble that of human extracellular fluid.

Microdialysis probe is inserted after local anaesthesia of the overlying skin with lidocaine. An approximately 18-gauge guide-cannula (introducer) containing the probe is

inserted. The introducer is then removed, with the microdialysis probe remaining in place. The microdialysis catheter is held in place by adhesive tape. There is no threat of infection from perfusion fluid since there is no exchange of high molecular weight substances, bacteria or endotoxin. After the experiment, the probe is removed, each probe is inspected for fragmentation or irregularities, and the sites of insertion are inspected and then bandaged if necessary. The subject is given haematoma precautions.

Analyte concentrations in the microdialysate depend on several factors, including the following:

6.3.1. The Microdialysis Probe

In this region, substances diffuse across a semipermeable membrane from the extracellular fluid to the microdialysate. The diameter, length, and molecular permeability of the membrane determine the function of the probe. The microdialysis probe CMA/60 (CMA Stockholm, Sweden) with a membrane length of 30 mm and a molecular cut-off of 20 kDa allows free diffusion of glycerol and catecholamines. The catheter CMA/65 (CMA, Stockholm, Sweden) with a membrane length of 30 mm and a molecular cut-off of 100 kDa is used for determination of higher molecules.

6.3.2. The Perfusate

The composition of the perfusate should resemble that of human extracellular fluid. The concentration of substances in the perfusate and extracellular fluid determine the net exchange across the membrane. The perfusates most commonly used are Ringer´s solution (Jansson et al. 1992) and Krebs-Henseleit buffer with dextran to prevent perfusion fluid loss (Rosdahl et al. 1997).

6.3.3. The Perfusion Rate

The perfusion rate (0.1, 0.3, 0.5, 1.0, 2.0, 5.0 µl/min) should be as low as possible to maximize equilibration with the interstitial fluid. The duration of fraction collection should be long enough to obtain sufficient volumes of microdialysate. The longer time for equilibration with the interstitial fluid, the higher the microdialysate concentration. Theoretically, optimal recovery occurs at zero flow rate (less than 0.3 µl/min) or infinite exchange across the surface length.

6.3.4. The Duration of Collection

The lower flow rate the longer time required for sample collection, which could last up to 6 hours with the same catheter.

6.3.5. The Recovery of Glycerol In Vitro and In Vivo

The relative glycerol recovery (RGR) *in vitro* is defined as the ratio between its concentration in the diylasate and the concentration in the fluid surrounding the probe (expressed in % values) (Lafontan and Arner 1996). *In vitro* RGR has been reported 76 ± 6% (Maggs et al. 1995). The *in vivo* relative recovery was determined by zero flow method (Jacobson et al. 1985). The zero flow method is based on the principle that recovery of a substance across the dialysis membrane depends on dialysis flow rate such that at zero flow rate (less than 0.3 µl/min) the interstitial space and dialysate fluid are in complete equilibrium. This zero flow method involved the insertion of two microdialysis probes having measured dialysate concentration and estimated interstitial concentration of glycerol in the two tissue beds. Dialysate concentrations were plotted (after log-transformation) against the perfusion rates. Regression analysis was used to calculate the glycerol concentration at zero flow, corresponding to the interstitial glycerol concentration. The ratio (dialysate glycerol

concentration)/(interstitial glycerol concentration) x 100 expressed the % recovery rate. *In vivo* recovery of glycerol has been reported 35 ± 3% at a perfusion rate 2.5 µl/min (Barbe et al. 1996).

6.3.6. Local Blood Flow

Changes in abdominal AT blood flow were determined using the ethanol dilution technique based on the Fick principle (Arner and Bülow 1993). It was shown that local blood flow modifies glycerol concentration in AT and that vasoconsctriction increases and vasodilatation decreases extracellular glycerol concentration in AT (Enoksson et al. 1995). Differences between the ethanol concentration in the perfusate and that in the dialysate reflect changes in AT blood flow (dialysate to perfusate ratio). This method was found to be comparable to use of ^{133}Xe clearance (Karpe et al. 2002).

7. THE AIMS

1. To find whether GH may play an inhibitory role on ghrelin secretion during exercise alone or during exercise after systemic administration of Aci.

2. To find the gut-brain-AT function in BN patients compared to the controls with the attention to exercise alone or together with Aci administration responses of GH, ghrelin, NPY, leptin, insulin, blood glucose, FFA and glycerol in circulation.

3. To determine the abdominal AT function and its relationship to gut-brain hormones and lipid metabolites on tissue level using *in vivo* microdialysis technique in patients with BN and compared to healthy women.

4. To determine glycerol turnover, as the index of local abdominal AT and systemic lipolysis by the measurements of extracellular and plasma levels of glycerol under basal conditions and during exercise alone or together with Aci administration in bulimic patients and compared to healthy women.

5. To find if Aci acts through a FFA-dependent mechanism.

8. THE WORKING HYPOTHESES

In BN, AN and chronic undernutrition, ghrelin and GH are increased (Tanaka et al. 2002, 2004). Exogenous ghrelin administration stimulates GH in a dose dependent fashion in humans (Takaya et al. 2000), and it is therefore possible that increased endogenous ghrelin may contribute to increased GH in conditions of negative energy balance, *i.e.* exercise. Exercise and anti-lipolytic drugs are known to be a potent stimulus of GH secretion, and as mentioned above, ghrelin is a potent GH secretagogue and ghrelin may contribute to the regulation of GH secretion in response to exercise, thus, we hypothesize that ghrelin can affect GH responses to exercise alone or together with Aci administration *via* a positive feedback action or GH can alter ghrelin levels *via* a negative feedback action when GH levels are high (Kraemer and Castracane 2007, Vestergaard et al. 2007b).

We suppose that stressor such as acute exercise alone or together with anti-lipolytic drug Aci may lead to activation of SNS and to release of NPY from sympathetic nerves and AT, which in turn may up-regulate NPY and its receptors in sc abdominal AT.

The sympathoadrenal system is considered to be an important system that regulates AT metabolism. Thus, function alterations of this system may contribute to dysregulation of adipocyte metabolism. In the present study, we will use *in vivo* microdialysis technique to measure interstitial AT glycerol levels to assess local lipolysis in patients with BN. On the basis of our previous studies, we hypothesize, that higher sensitivity of SNS to anti-lipolytic drug Aci during exercise in sc abdominal AT may exist in BN patients, and that Aci influences the same signal transduction patway as norepinephrine (Wang-Fisher et al. 2002), the major representative of SNS.

9. STUDY DESIGN AND METHODS

The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Institute of Endocrinology in Prague. Each participant signed an informed consent form before entering the study.

9.1. Study Timeline

2004:

Normal volunteers: 5 women, age 18-30

Patients with BN: 8 women, age 18-30

Assay of systemic administration (per os, p.o.) of anti-lipolytic drug Aci or placebo

2005:

Normal volunteers: 5 women, age 18-30

Patients with BN: 6 women, age 18-30

Assay of systemic administration (p.o.) of anti-lipolytic drug Aci or placebo

9.2. Bulimic Patients and Healthy Women

The recruitment phase was from January 2004 to June 2005. Main inclusion criteria were: age between 18 and 30 years, BMI between 18 and 23 kg/m^2 , patients with a diagnosis of BN (DSM-IV). Women with diabetes type 1 or 2, hypo- or hyperthyroidism,

cardiovascular disease, pregnant or lacting women, patients with any severe active infection or cancer, patients with impaired mental capacity or other psychiatric diseases were not eligible for participation in the study. Other exclusion criteria were: hypertension, abnormal blood tests with significant hyperlipidaemia, history or presence of hepatic or renal disorders. For two weeks before the study they had to refrain taking anti-depressant and anti-conceptive drugs. For five days before study they had to refrain taking aspirin, anti-histamine drugs and Tylenol (acetaminophen). For two days before study they had to refrain from tea, coffee (even decaffeinated), cigarettes, alcohol, chocolate, cocoa, nuts and bananas. They had to fast from the morning, except for water or no caloric fluids. From all bulimic patients (14) and healthy women (10), 7 BN patients and 7 healthy women were acceptable during the inclusion procedure (the recruitment phase) for to be randomized, *i.e.* 3 BN patients were excluded, 2 BN patients were not meeting inclusion criteria, 2 BN patients declined to participate in the study, 2 healthy women were not meeting criteria and 1 healthy women declined to participate in this study.

Seven women with BN (mean ± S.E.M.; age: 24.33 ± 1.38 years; BMI: 20.63 ± 0.80 kg/m^2; percentage of body fat [% BF]: 24.83 ± 1.92) and seven healthy women (age: 25.83 ± 1.69 years; BMI: 19.98 ± 0.44 kg/m^2; % BF: 24.5 ± 0.47) were recruited for this study. All subjects included in the study were nonsmokers, had no allergies and had been free of medications for at least two weeks prior to the study. Healthy volunteers had no history of cardiovascular disease, eating disorders or other psychiatric diseases. All healthy women were in the first two weeks of the follicular phase of their menstrual cycle. Patients with BN were diagnosed according to the 4^{th} edition of the DSM-IV, American Psychiatric Association, 1994. All BN patients were clinically stable and in relatively good health, except for their eating disorder and amenorrhoea. In BN patients, the average frequency of binge-purging episodes was 2.5 times per day and the average duration of their eating disorder was 6 years

and 8 months. They were investigated after 1 week of hospitalization at the Department of Psychiatry of the Charles University, Prague.

9.3. Patient Informed Consent

Dear Madam/Miss,

We invite you to take part in a research study at the Institute of Endocrinology in Prague.

You are being asked to provide your consent with your cooperation in carrying out a grant project No. 303/03/0376 of the Grant Agency Czech Republic.

First, we want you to know that taking part in this research is entirely voluntary. You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with our research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at our institution, or with family, friends or your personal physician or other health professional.

The disorder you are suffering is called bulimia nervosa. This disorder is very often associated with hormonal and other abnormalities. This research study is design to determine whether some compounds or gut-brain-adipose tissue hormones such as ghrelin, growth

hormone, neuropeptide Y, norepinephrine, insulin and leptin or metabolites glycerol and free fatty acids may abnormally affect your adipose tissue and contribute to your weight loss or gain.

You will be admitted to the Institute of Endocrinology for standard medical as well as research tests. Standard tests include taking blood through an intravenous (i.v.) line and anthropometrical examination. Research experiment includes taking blood through i.v. line for hormones estimation and a method called microdialysis. Microdialysis is a technique that is based on the exchange or diffusion of compounds across a special type of membrane, originally and still used in kidney dialysis machines. The microdialysis probe is a hollow, thin, straw-like tube (about the width of i.v. catheter), with a part of the tube's wall consisting of a membrane across which norepinephrine, glycerol or other compounds can diffuse. Through the tube, a solution resembling normal body fluid is passed at a very slow rate. Molecules in the tissue fluid pass through the membrane, and enter the perfusion solution. In kidney machines, this principle is used to remove molecules from the body that normally are removed by healthy kidneys.

The microdialysis catheter has an inlet as well as an outlet. Compounds in the tissue diffuse into the microdialysis catheter, and the microdialysis fluid is collected and subsequently analyzed. In this study microdialysis will be also preformed before and after exercise or after systemic or local (*via* the microdialysis catheter) administration of drugs that may affect metabolism of adipose tissue.

9.3.1. Description of The Procedures

For five days before the study you must refrain from taking aspirin, anti-histamine drugs, Tylenol (acetaminophen), and any other drugs unless approved by the investigator.
On the morning of the study, you report to a special patient observation room. You need to fast from the morning, except for water or no calorie fluids, and refrain from tea, coffee (even decaffeinated) for two days.

For microdialysis experiment, we will use a small catheter that will be inserted under aseptic conditions into your abdominal fat tissue. The microdialysis catheter is inserted with the use of a u-shaped introducer that is removed after insertion. The catheter is specifically designed for human use and is left under the abdominal wall (adipose tissue), up to 6 hours. The catheter is so designed, that it takes only one puncture to insert the probe. The microdialysis catheter is attached to the skin by adhesive tape. Up to now, at least 2000 procedures have been performed worldwide, and no adverse effects have been reported.

The inlet tubing will be connected to a sterile syringe containing the perfusion solution alone or with pharmaceuticals influencing the adipose tissue metabolism. The outlet tubing will be connected to a small collecting tube. At predetermined intervals, samples of solution coming out of the tube will be collected.

During the experimental procedure, you remain supine in a hospital bed and we will 45 minute train on bicycle ergometer also. Research experiment includes giving Acipimox or placebo by mouth in a usual dose of 500 mg (two 250 mg capsules of Acipimox or placebo) one hour before single exercise bout for two consecutive weeks. Acipimox (Olbetam capsules, Farmitalia Carlo Erba, Milan, Italy) is a lipid regulating drug related to nicotinic acid (*i.e.* vitamin B_3, niacin). It is used to reduce cholesterol and triglycerides in the management of hyperlipidaemias. Acipimox may cause peripheral vasodilatation resulting in

flushing, ichting, and a sensation of heat. In some cases, gastrointestinal disturbances including heart-burn, epigastric pain, nausea, as well as headache, and dry eye. Urticaria, angioedema, and bronchospasm may occur rarely. Acipimox is contra-indicated in patients with peptic ulcer disease and renal impairment. Placebo is a medicine that is ineffective and it has not any effect on your body instead of Acipimox. Placebos are used in our experiments in which patients and healthy volunteers take real medicine (Acipimox) and other weeks take a placebo, so that researchers can compare the reults to see if Acipimox works properly. During experiment you may watch television or listen to the radio. During the study you will be connected over the phone or personally with your nurses and attending physicians. They will be pleased to answer any questions you may have during the procedure.

At the end of the experiment, the catheter is pulled out and the site is covered with a bandage. You will be instructed to avoid strenuous exercise during the subsequent day.

As part of the experimental procedure, an i.v. catheter will be inserted to allow blood sampling during the tests. These can be used to compare glycerol and other compounds or hormones in the blood with that in the microdialysis fluid.

As a result of participation in this study, you will also receive a standard evaluation of bulimia nervosa. You are free to withdraw from the study at any time. Should you do so, we will not continue further research tests with you.

9.3.2. Hazards and Risks

Microdialysis catheter insertion can cause pain, bleeding or infection. No complications at all have been observed in over 2000 clinical procedures worldwide. You may develop a temporary black-blue mark or swelling at the site of the catheter, but the risk

should be minimized by not performing vigorous exercise for 24 hours after catheter removal. Infection will be prevented by use of a sterile technique for insertion of the microdialysis catheter. The area of insertion of the probe will be inspected carefully for local tissue reactions.

Having read this text, I have had an opportunity to ask about other details, not connected in the questionnaire, regarding my potential participation in carrying out the grant project of the Grant Agency Czech Republic.

In Prague…….. Signature…….

9.4. Experimental Protocol; Blood Sampling

Blood tests conducted before initiation of the study confirmed normal values for blood count, fasting blood glucose, and liver and renal function. All subjects consumed a standardized dinner at 6:00 PM and were then asked to fast overnight. Reported duration of sleep in the night preceding blood sampling was comparable in all studied subjects. Subjects were admitted to the Institute of Endocrinology at 7:00 AM. After a short medical examination (blood pressure, heart, and respiratory rate measurement, electrocardiogram [ECG]), % BF was estimated by anthropometric measurement and bioimpedancy (TANITA, Tokyo, Japan). Before starting the test, all individuals rested on the bed for 45 minutes. All subjects were randomized to receive either placebo or Aci capsules each week (two 250 mg capsules of Aci or placebo; 500 mg total – 5-Methylpyrazine-2-carboxylic acid 4-oxide, molecular weight: 154.1, Olbetam capsules, Farmitalia Carlo Erba, Milan, Italy) 1 hour before a single exercise bout. A low- to moderate-intensity exercise bout on an electromagnetically

braked bicycle ergometer (Cateye EC 1600, Japan) was performed for 45 min at power output 2W/kg of lean body mass (LBM), intended to be below the aerobic-anaerobic threshold. ECG, heart rate and blood pressure were monitored using an Eagle 3000 cardiomonitor (Marquette, Milwaukee, WI, U.S.A.) and haemodynamic parametres were measured every 5 minutes during the 45-minute exercise bout. At 8:00 AM, after overnight fasting, a venous catheter was inserted into the antecubital vein. A blood sample was collected at the beginning and in the course (45 minutes after exercise, 90 minutes after post-exercise recovering phase) of the experiment to estimate plasma ghrelin, GH, NPY, leptin, insulin, blood glucose, FFA and glycerol concentrations. Blood samples were collected into chilled tubes containing Na_2EDTA and antilysin. Plasma was separated immediately by centrifugation at 4 $^{\circ}$C and stored at -80 $^{\circ}$C until being assayed. Subjects started their 45 min exercise alone or exercise after Aci administration, assigned randomly 60 minutes before exercise for two consecutive weeks. After the 45-min exercise, all subjects assumed a resting supine position on a comfortable bed for additional 90 minutes.

9.5. Experimental Protocol; Microdialysate Sampling

The *in situ* and *in vivo* microdialysis technique was used to examine the exercise stimulated lipolysis (by measurement of dialysate glycerol) alone or with Aci randomly received (500 mg p.o., Olbetam capsules, Farmitalia Carlo Erba, Milan, Italy) for two consecutive weeks. A CMA/60 microdialysis probe (CMA Microdialysis, Stockholm, Sweden) with membrane length 3 cm and molecular weight cut-off of 20 kDa was inserted sc under sterile conditions (8-10 cm left of the umbilicus at least 60 min before microdialysate sampling). After insertion of the CMA/60 catheter, perfusion with sterile Ringer solution was started at a flow rate of 2 μl/min using a CMA 107 microdialysis pump (CMA Microdialysis,

Stockholm, Sweden). Microdialysate samples were collected every 15-30 min over a 6-hour period, 120 min before the exercise (baseline values), 45 min during the exercise and 90 min after the post-exercise recovering phase. Microvials were placed on ice immediatelly after the collection, and stored at -80 °C until analysis. Before starting microdialysis perfusion, the RGR was calculated *in vitro* at a temperature of 37 °C and maintained by a digital block heater to simulate body temperature. Catheters were immersed in test tubes containing standard glycerol concentration (100 μmol/l), which was determined from the average glycerol concentrations in human plasma. Different perfusion rate (0.1, 0.3, 0.5, 1.0, 2.0, 5.0 μl/min) were tested to investigate possible relative recovery *vs.* perfusion rate dependency. At each rate, RGR was calculated from 15 samples collected in perfusion rate-dependent intervals according to formula: RGR (%) = (glycerol concentration in dialysate/glycerol concentration in standard solution) x 100. A perfusion rate of 2μl/min was selected for *in vivo* microdialysis, based on the calculated *in vitro* RGR corrected for experiment duration. The procedure is described in detail in our previous report (Dostálová et al. 2003).

9.6. Hormonal and Biochemical Assays

GH in plasma [as a 22 kDa monomeric GH form and non-22 kDa isoforms (dimers and GH bound to plasma proteins)] was measured by a commercial RIA kit (Immunotech, Prague, Czech Republic). Intra- and inter-assay variability was 1.5% and 14%, respectively, sensitivity was 0.1 μIU/ml. Total plasma ghrelin and NPY were determined by commercially available RIA kits (Linco Research, Inc., St. Charles, Missouri, U.S.A.). The intra- and interassay variability for total ghrelin was 6.4 % and 16.3 %, respectively, and the sensitivity was 93.0 pg/ml. For NPY the intra- and interassay variability was 5.0 % and 8.4 % respectively, the sensitivity being 3.0 pmol/l. Leptin in plasma was measured by a commercial

RIA kit (Linco Research, St. Charles, Missouri, U.S.A.). Sensitivity, inter-assay and intra-assay variability were 0.05 ng/ml, 8.6% and 5.9%, respectively. Plasma blood glucose levels and plasma insulin levels were measured in a Cobas Integra 400 plus system (Roche Diagnostics, GmbH, Mannheim, Germany). Glycerol in plasma and in the dialysate was analyzed with a radiometric kit (Randox Laboratories, GY 105, Montpellier, France). Plasma FFA were estimated colorimetrically with a commercial kit (Randox Laboratories, FA 115, Montpellier, France). All assays were run twice in duplicate.

9.7. Statistical Analysis

All values are presented as the means ± S.E.M. All statistical comparisons were performed using a statistical program: General Linear Repeated Measures with Status as the between Factor and Aci and Time as the within Factors. Repeated measure linear model consisting of patient Status (the controls, bulimic patients), treatment effect (placebo, Aci) as the between-factors, subject Status (individual subjects), Time as the within-factor and all possible 2^{nd} order inter-factor interactions was used for evaluation of the results. Respecting the skewed data distribution and a non-constant variance in most of the variables, power transformation was used to approximate Gaussian data distribution and the constant variance. The linear model evaluation was followed by a least significant difference multiple comparisons. Correlations between parameters were examined using Spearman´s rank correlation coefficient. The difference between medians (Mann-Whitney and Wilcoxon Rank-Sum tests) was applied to compare baseline values with those during exercise.. A P value < 0.05 denoted statistical significance.

10. RESULTS

10.1. Tables and Figures

Baseline characteristics of the study subjects, including anthropometric, hormonal and biochemical measurements, are summarized in Table 1 and of the circulatory response to exrcise alone or together with Aci administration in Table 5. The exercise-induced changes in plasma of the study subjects during Aci and placebo treatment in plasma and in AT are shown in Table 2, Table 3, Table 4, respectively, and Fig. 1., Fig. 2., Fig. 3., Fig. 4., Fig. 5., Fig. 6., Fig. 7., Fig. 8., respectively.

10.1.1. Baseline and Exercise-Induced Plasma GH Concentrations Alone or Together with Aci Administration

Mean baseline fasting plasma GH concentrations were significantly increased in BN patients compared to the controls (11.2 ± 0.9 *vs.* 7.1 ± 0.5 mIU/l in the controls; $P < 0.05$) (Table 1). Plasma GH concentrations increased significantly after 45 minute exercise with placebo in both groups (13.1 ± 4.3 *vs.* 11.3 ± 2.2 mIU/l in the controls; $P < 0.001$). Randomly consecutive weeks of the administration of Aci 60 minute before the 45-minute exercise further increased plasma GH in both groups (73.7 ± 23.1 *vs.* 40.9 ± 8.7 mIU/l in the controls; $P < 0.0001$). Plasma GH levels decreased significantly more in the controls at 90 minutes after post-exercise recovering phase with placebo than in BN patients (2.0 ± 0.5 *vs.* 0.7 ± 0.2 mIU/l in the controls; $P < 0.0001$). On the contrary, plasma GH levels were significantly

elevated at 90 minutes after post-exercise recovering phase with Aci administration in both groups (28.9 ± 7.5 *vs.* 21.4 ± 8.1 mIU/l in the controls; $P < 0.0001$) (Table 2, Fig. 1).

10.1.2. Baseline and Exercise-Induced Plasma Ghrelin Concentrations Alone or Together with Aci Administration

Mean baseline fasting plasma ghrelin concentrations were similar in BN patients and in the controls (1099 ± 218 *vs.* 1112 ± 273 pg/ml in the controls) (Table 1). In BN patients plasma ghrelin levels significantly decreased after the 45-min exercise compared to the controls (812 ± 104.4 *vs.* 1189.7 ± 254.8 pg/ml in the controls, $P < 0.05$). In both groups plasma ghrelin levels decreased after the exercise plus Aci (690.5 ± 92.7 *vs.* 932 ± 115.2 pg/ml in the controls, $P < 0.01$). In BN patients plasma ghrelin levels remained significantly decreased 90 min after the exercise compared to the controls (952.3 ± 77.7 *vs.* 1322.2 ± 240.5 pg/ml in the controls, $P < 0.05$). In both groups plasma ghrelin levels were still significantly decreased 90 min after the exercise plus Aci (768.7 ± 73.1 *vs.* 836.7 ± 137.7 pg/ml in the controls, $P < 0.01$). (Table 2, Fig. 2.).

10.1.3. Baseline and Exercise-Induced Plasma NPY Concentrations Alone or Together with Aci Administration

Mean baseline fasting plasma NPY concentrations were significantly increased in BN patients compared to the controls (53.0 ± 3.0 *vs.* 45.0 ± 2.3 pmol/l in the controls; $P < 0.05$) (Table 1). Plasma NPY levels increased significantly after 45 minute exercise with placebo in both groups (78.3 ± 15.7 in BN patients *vs.* 69.7 ± 19.2 pmol/l in the controls; $P <$

0.01), but randomly consecutive exercise weeks with Aci administration led to a further increase of plasma NPY levels only in BN patients (111.4 ± 20.6 *vs.* 69.0 ± 9.2 pmol/l in the controls; $P < 0.001$). Plasma NPY levels decreased to the baseline values 90 minutes after post-exercise recovering phase with placebo in both groups (51.2 ± 8.6 *vs.* 45.6 ± 6.7 pmol/l in the controls), but less in BN patients if associated with Aci administration compared to the controls (65.0 ± 10.6 *vs.* 51.5 ± 12 pmol/l in the controls; $P < 0.05$) (Table 2, Fig. 3.).

10.1.4. Baseline and Exercise-Induced Plasma Leptin Concentrations Alone or Together with Aci Administration

Mean baseline fasting plasma leptin levels were significantly decreased in BN patients compared to the controls (6.96 ± 1.1 *vs.* 7.83 ± 1.3 ng/ml in the controls; $P < 0.05$) (Table 1). Plasma leptin levels decreased significantly after 45 minute exercise with placebo in both groups (6.31 ± 0.83 *vs.* 6.52 ± 0.68 ng/ml in the controls; $P < 0.05$). Plasma leptin concentrations approached the baseline values after 45 minute exercise with Aci administration in both groups (6.8 ± 1.38 *vs.* 7.73 ± 1.13 ng/ml in the controls). Plasma leptin concentrations decreased significantly at 90 minutes after post-exercise recovering phase with placebo in both groups (5.93 ± 0.69 *vs.* 6.21 ± 0.75 ng/ml in the controls; $P < 0.05$). Plasma leptin levels increased significantly at 90 minutes after post-exercise recovering phase with Aci administration in both groups when compared to the post-exercise recovering phase associated with placebo (7.98 ± 0.99 *vs.* 7.84 ± 0.94 ng/ml in the controls; $P < 0.05$) (Table 2, Fig. 4.).

10.1.5. Baseline and Exercise-Induced Plasma Insulin Concentrations Alone or Together with Aci Administration

Mean baseline fasting plasma insulin concentrations were significantly decreased in BN patients compared to the controls (2.5 ± 0.59 *vs.* 4.32 ± 0.98 mIU/l in the controls; $P < 0.05$) (Table 1). Plasma insulin concentrations approached the baseline values after 45 minute exercise with placebo in both groups (2.32 ± 0.71 *vs.* 4.05 ± 0.37 mIU/l in the controls). Randomly consecutive weeks of the administration of Aci 60 minute before the 45-minute exercise decreased significantly plasma insulin in both groups (2.00 ± 0.33 *vs.* 2.66 ± 0.98 mIU/l in the controls; $P < 0.05$). Plasma insulin levels decreased significantly at 90 minutes after post-exercise recovering phase with placebo in both groups (2.01 ± 0.5 *vs.* 2.33 ± 0.34 mIU/l in the controls; $P < 0.05$). Plasma insulin levels decreased significantly at 90 minutes after post-exercise recovering phase with Aci administration more in BN patients (1.03 ± 0.44 *vs.* 1.9 ± 0.8 mIU/l in the controls; $P < 0.01$) (Table 2, Fig. 5.).

10.1.6. Baseline and Exercise-Induced Plasma Blood Glucose Concentrations Alone or Together with Aci Administration

Mean baseline fasting plasma blood glucose concentrations were similar in BN patients and the controls (4.33 ± 0.13 *vs.* 4.72 ± 0.11 mmol/l in the controls) (Table 1). Plasma blood glucose levels were over the baseline values after 45 minute exercise with placebo in both groups (4.46 ± 0.38 *vs.* 4.8 ± 0.26 mmol/l in the controls). Plasma blood glucose levels increased significantly after 45 minute exercise with Aci administration in BN patients (5.03 ± 0.34 *vs.* 4.63 ± 0.19 mmol/l in the controls; $P < 0.05$). Plasma blood glucose levels were

under the baseline values 90 minutes after post-exercise recovering phase with placebo in both groups (4.07 ± 0.3 *vs.* 4.59 ± 0.15 mmol/l in the controls). Plasma blood glucose levels approached the baseline values 90 minutes after post-exercise recovering phase with Aci administration in both groups (4.43 ± 0.26 *vs.* 4.33 ± 0.23 mmol/l in the controls) (Table 3).

10.1.7. Baseline and Exercise-Induced Plasma FFA Concentrations Alone or Together with Aci administration

Mean baseline fasting plasma FFA concentrations were similar in BN patients and the controls (0.79 ± 0.3 *vs.* 0.86 ± 0.3 mmol/l in the controls) (Table 1). Plasma FFA concentrations increased significantly after 45 minute exercise with placebo in both groups (1.60 ± 0.28 *vs.* 1.54 ± 0.13 mmol/l in the controls; $P < 0.0001$). Plasma FFA concentrations decreased significantly to the baseline levels after 45 minute exercise with Aci administration in both groups when compared to the exercise associated with placebo (0.79 ± 0.14 *vs.* 0.82 ± 0.05 mmol/l in the controls; $P < 0.05$). Plasma FFA concentrations approached the baseline values 90 minutes after post-exercise recovering phase with placebo in both groups (0.83 ± 0.1 *vs.* 0.79 ± 0.05 mmol/l in the controls). Plasma FFA concentrations decreased significantly under the baseline values 90 minutes after post-exercise recovering phase with Aci administration in both groups (0.26 ± 0.02 *vs.* 0.25 ± 0.02 mmol/l in the controls; $P < 0.0001$) (Table 2, Fig. 6.).

10.1.8. Baseline and Exercise-Induced Plasma Glycerol Concentrations Alone or Together with Aci Administration

Mean baseline fasting plasma glycerol levels were significantly decreased in BN patients compared to the controls (82.2 ± 26.0 *vs.* 117 ± 33.0 μmol/l in the controls; $P < 0.05$) (Table 2). Plasma glycerol levels increased significantly after 45 minute exercise with placebo in both groups (256.0 ± 56.0 *vs.* 318.0 ± 34.0 μmol/l in the controls; $P < 0.0001$). Plasma glycerol concentrations approached the baseline values 90 minutes after post-exercise recovering phase with placebo in both groups (74.0 ± 4.2 *vs.* 85.0 ± 7.3 μmol/l in the controls). Plasma glycerol levels decreased significantly after 45 minute exercise with Aci administration more in BN patients when compared to the exercise associated with placebo (114.0 ± 13 *vs.* 157.8 ± 18.4 μmol/l in the controls; $P < 0.001$). Plasma glycerol concentrations significantly decreased under the baseline values 90 minutes after post-exercise recovering phase with Aci administration in both groups (57.0 ± 6.2 *vs.* 44.0 ± 4.7 μmol/l in the controls; $P < 0.01$) (Table 3, Fig. 7.).

10.1.9. Baseline and Exercise-Induced AT Glycerol Concentrations Alone or Together with Aci Administration

Mean baseline AT glycerol levels were significantly decreased in BN patients compared to the controls (36.39 ± 4.15 *vs.* 41.21 ± 4.43 μmol/l in the controls; $P < 0.05$) (Table 2). AT glycerol concentrations increased significantly after 45 minute exercise with placebo more in BN patients (148.6 ± 23.2 *vs.* 82.2 ± 11.82 μmol/l in the controls; $P < 0.0001$). AT glycerol levels decreased significantly to the baseline values after 45 minute exercise with Aci administration in both groups when compared to the exercise associated with placebo (38.3 ± 5.39 *vs.* 41.6 ± 4.21 μmol/l in the controls; $P < 0.0001$ for BN patients, $P < 0.01$ for the controls, respectively). AT glycerol concentrations approached the baseline values 90 minutes after post-exercise recovering phase with placebo in the controls, while AT

glycerol concentrations decreased under the baseline values in BN patients (29.0 ± 2.9 *vs.* 40.5 ± 3.81 μmol/l in the controls; $P < 0.05$). AT glycerol levels decreased significantly under the baseline levels 90 minutes after post-exercise recovering phase with Aci administration in both groups (20.4 ± 3.01 *vs.* 33.9 ± 3.16 μmol/l in the controls; $P < 0.01$) (Table 4, Fig. 8.).

10.2. The Circulatory Response under Rest and after The Exercise with Aci Administration (45 minute) in Patients with BN and in Healthy Control Women

The resting heart rate was lower in BN patients compared to the controls (57 ± 3.5 *vs.* 68 ± 1.9 beats/min in the controls; $P < 0.05$). The exercise heart rate with Aci received tended to be higher in BN patients compared to the controls (152 ± 6.2 *vs.* 146 ± 8.9 beats/min in the controls; $P < 0.001$). The systolic and diastolic blood pressure was decreased in BN patients compared to the controls under rest (systolic blood pressure: 93 ± 4 *vs.* 116 ± 3.5 mmHg in the controls; $P < 0.05$, diastolic blood pressure: 56 ± 2.2 *vs.* 69 ± 1.7 mmHg in the controls; $P < 0.05$, respectively). During the exercise after Aci administration the systolic and diastolic blood pressure tended to be higher in BN patients compared to the controls (systolic blood pressure: 138 ± 5.2 *vs.* 133 ± 5 mmHg in the controls; $P < 0.05$, diastolic blood pressure: 79 ± 2.2 *vs.* 77 ± 3 mmHg in the controls; $P < 0.05$, respectively*)* (Table 5).

10.3. Correlations Between Parameters

10.3.1. The Relationship of Hormonal, Biochemical and Anthropometric Parameters during Basal Conditions in Patients with BN and in Healthy Control Women

Fasting plasma leptin levels correlated positively with BMI in the controls (r = 0.62, P = 0.03). Fasting plasma blood glucose concentrations correlated negatively with plasma FFA concentrations in BN (r = - 0.66, P = 0.02). Fasting plasma glycerol concentrations correlated positively with plasma FFA concentrations in BN patients (r = 0.69, P = 0.0001) and the controls (r = 0.87, P = 0.0001).

10.3.2. The Relationship of Hormonal and Biochemical Parameters After The Exercise with Aci Administration (45 minute) in Patients with BN and in Healthy Control Women

Plasma GH concentrations correlated positively with plasma NPY concentrations after 45 minute exercise with Aci administration only in BN patients (r = 0.56, P = 0.01). Plasma glycerol concentrations correlated positively with plasma FFA concentrations after 45 minute exercise with Aci administration in BN patients (r = 0.91, P = 0.004) and the controls (r = 0.93, P = 0.002). Plasma insulin levels correlated positively with plasma leptin levels after 45 minute exercise with Aci administration in the controls (r = 0.64, P = 0.04). Plasma leptin levels correlated positively with blood glucose concentrations after 45 minute exercise with Aci administration in the controls (r = 0.62, P = 0.03). Plasma FFA levels correlated positively with plasma insulin levels after 45 minute exercise with Aci administration in the controls (r = 0.68, P = 0.02).

10.3.3. The Relationship of Haemodynamic Parameters after Exercise with Aci Administration (45 minute) in Patients with BN

Maximal values of heart rate attained during the exercise with Aci administration correlated positively with the systolic blood pressure in BN (r = 0.73, P = 0.007). Maximal values of heart rate attained during the exercise with Aci administration correlated positively with the diastolic blood pressure in BN (r = 0.74, P = 0.006).

Tables

Table 1. Anthropometric and major laboratory characteristics of the study subjects (means ± S.E.M.). C = controls; BN = bulimia nervosa; BMI = body mass index; % BF = percentage of body fat; neuropeptide Y (NPY); growth hormone (GH); free fatty acids (FFA); NS = not significant; $^{\$}P < 0.05$ BN *vs.* control subjects (C); *n* = the number of subjects.

	C (*n* = 7)	**BN (*n* = 7)**	***P* value**
Age (years)	25.83 ± 1.69	24.33 ± 1.38	NS
BMI (kg/m^2)	19.98 ± 0.44	20.63 ± 0.80	NS
% BF	24.50 ± 0.47	24.83 ± 1.92	NS
GH (mIU/l)	7.1 ± 0.5	11.2 ± 0.9$^{\$}$	< 0.05
Ghrelin (pg/ml)	1112±273	1099±218	NS
NPY (pmol/l)	45±2.3	53±3.0$^{\$}$	<0.05
Leptin (ng/ml)	7.83 ± 1.3	6.96 ± 1.1$^{\$}$	< 0.05
Insulin (mIU/l)	4.32 ± 0.98	2.5 ± 0.59$^{\$}$	< 0.05
Glucose (mmol/l)	4.72 ± 0.11	4.33 ± 0.13	NS
FFA (mmol/l)	0.86 ± 0.3	0.79 ± 0.3	NS
Plasma Glycerol (µmol/l)	117 ± 33	82.2 ± 26$^{\$}$	< 0.05
Dialysate glycerol (µmol/l)	41.21 ± 4.43	36.39 ± 4.15$^{\$}$	< 0.05

Table 2. Effect of exercise (45 min, 2 W/kg of lean body mass [LBM]) alone or together with Acipimox (Aci) administration on plasma gut-brain-adipose tissue (AT) peptides in the controls (C) (n = 7) and bulimia nervosa (BN) patients (n = 7).

	0 min		45 min	45 min	90 min	90 min
	Basal		Exercise	Exercise	Post-exercise	Post-exercise
			+ placebo	+ Aci	+ placebo	+ Aci
GH (mIU/l)						
C group	7.1±0,5		11.3±2.2***	40.9±8.7****	0.7±0.2****	21.4±8.1****
BN group	11.2±0.9$^{\$}$		13.1±4.3***	73.7±23.1****$^{\$}$	2.01±0.5****$^{\$}$	28.9±7.5****$^{\$}$
Ghrelin (pg/ml)						
C group	1112±273		1189.7±254.8	932.0±115.2**	1322.2±240.5	836.7±137.7**
BN group	1099±218		812.0±104.4*$^{\$}$	690.5±92.7**$^{\$}$	952.3±77.7*$^{\$}$	768.7±73.1**
NPY(pmol/l)						
C group	45±2.3		69.7±19.2**	69±9.2**	45.6±6.7	51.5±12
BN group	53±3.0$^{\$}$		78.3±15.7**$^{\$}$	111.4±20.6***$^{\$+}$	51.2±8.6	65±10.6$^{\$}$
Leptin (ng/ml)						
C group	7.83±1.3		6.52±0.68*	7.73±1.13^{+}	6.21±0.75*	7.84±0.94$^{\#}$
BN group	6.96±1.1$^{\$}$		6.31±0.83*	6.8±1.38$^{\$+}$	5.93±0.69*	7.98±0.99*$^{\#}$
Insulin (mIU/l)						
C group	4.32±0.98		4.05±0.37	2.66±0.98*$^{+}$	2.33±0.34*	1.9±0.8**
BN group	2.5±0.59$^{\$}$		2.32±0.71$^{\$}$	2.0±0.33*	2.01±0.5*	1.03±0.44**$^{\$\#}$

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$ *vs.* resting (baseline) values

$ = $P < 0.05$ BN *vs.* control subjects (C)

+ = $P < 0.05$ exercise together with Aci administration *vs.* exercise alone, 45 minute

++ = $P < 0.01$ exercise together with Aci administration *vs.* exercise alone, 45 minute

+++ = $P < 0.001$ exercise together with Aci administration *vs.* exercise alone, 45 minute

= $P < 0.05$ post-exercise together with Aci administration *vs.* exercise alone, 90 minute

Table 3. Effect of exercise (45 min, 2 W/kg of lean body mass [LBM]) alone (placebo) or together with Acipimox (Aci) administration on plasma glycerol, free fatty acids (FFA) and blood glucose levels in the controls (C) (*n* = 7) and bulimia nervosa (BN) patients (*n* = 7). Values are means ± S.E.M.; *n* = the number of subjects.

	0 min	45 min	45 min	90 min	90 min
	Baseline	Exercise	Exercise	Post-exercise	Post-exercise
		+ placebo	+ Aci	+ placebo	+ Aci
Plasma Glycerol (μmol/l)					
C group	117.0 ± 33	318.0 ± 34****	157.8 ± 18.4^{++}	85.0 ± 7.3	44.0 ± 4.7**$^{\#}$
BN group	82.2 ± 26$^{\$}$	256.0 ± 56****$^{\$}$	114.0 ± 13.0$^{\$+++}$	74.0 ± 4.2	57.0 ± 6.2**$^{\$\#}$
FFA (mmol/l)					
C group	0.86±0.3	1.54±0.13****	0.82±0.05^{+}	0.79±0.05	0.25±0.02****$^{\#}$
BN group	0.79±0.3	1.6±0.28****	0.79±0.14^{+}	0.83±0.1	0.26±0.02****$^{\#}$
Glucose (mmol/l)					
C group	4.72±0.11	4.8±0.26	4.63±0.19	4.59±0.15	4.33±0.23
BN group	4.33±0.13	4.46±0.38	5.03±0.34*$^{+}$	4.07±0.3	4.43±0.26

** = $P < 0.01$, **** = $P < 0.0001$ *vs.* resting (baseline) values

$^{\$}$ = $P < 0.05$ BN *vs.* control subjects (C)

$^{++}$ = $P < 0.01$ exercise together with Aci administration *vs.* exercise alone, 45 minute

$^{+++}$ = $P < 0.001$ exercise together with Aci administration *vs.* exercise alone, 45 minute

$^{\#}$ = $P < 0.05$ post-exercise recovering phase together with Aci administration *vs.* post-exercise recovering phase alone, 90 minute

Table 4. Dialysate glycerol concentration in subcutaneous (sc) abdominal adipose tissue (AT) during basal conditions and during exercise (45 min, 2W/ kg of lean body mass [LBM] alone or together with Acipimox (Aci) administration in the controls (C) (*n* = 7) and bulimia nervosa patients (BN) (*n* = 7). Values are means ± S.E.M.; *n* = the number of subjects.

	0 min	45 min	45 min	90 min	90 min
	Baseline	Exercise	Exercise	Post-exercise	Post-exercise
		+ placebo	+ Aci	+ placebo	+ Aci
Dialysate Glycerol (µmol/l)					
C group	41.21 ± 4.43	82.2 ± 11.82****	41.6 ± 4.21^{++}	40.5 ± 3.81	33.9 ± 3.16**$^{\#}$
BN group	36.39 ± 4.15$^{\$}$	148.6 ± 23.2****$^{\$\$}$	38.3 ± 5.39^{++++}	29.0 ± 2.9$^{\$}$	20.4 ± 3.01**$^{\$\#}$

** = $P < 0.01$, **** = $P < 0.0001$ *vs.* resting (baseline) values

$^{\$}$ = $P < 0.05$ BN *vs.* control subjects (C)

$^{\$\$}$ = $P < 0.01$ BN *vs.* control subjects (C)

$^{++}$ = $P < 0.01$ exercise together with Aci administration *vs.* exercise alone, 45 minute

$^{++++}$ = $P < 0.0001$ exercise together with Aci administration *vs.* exercise alone, 45 minute

$^{\#}$ = $P < 0.05$ post-exercise recovering phase together with Aci administration *vs.* post-exercise recovering phase alone, 90 minute

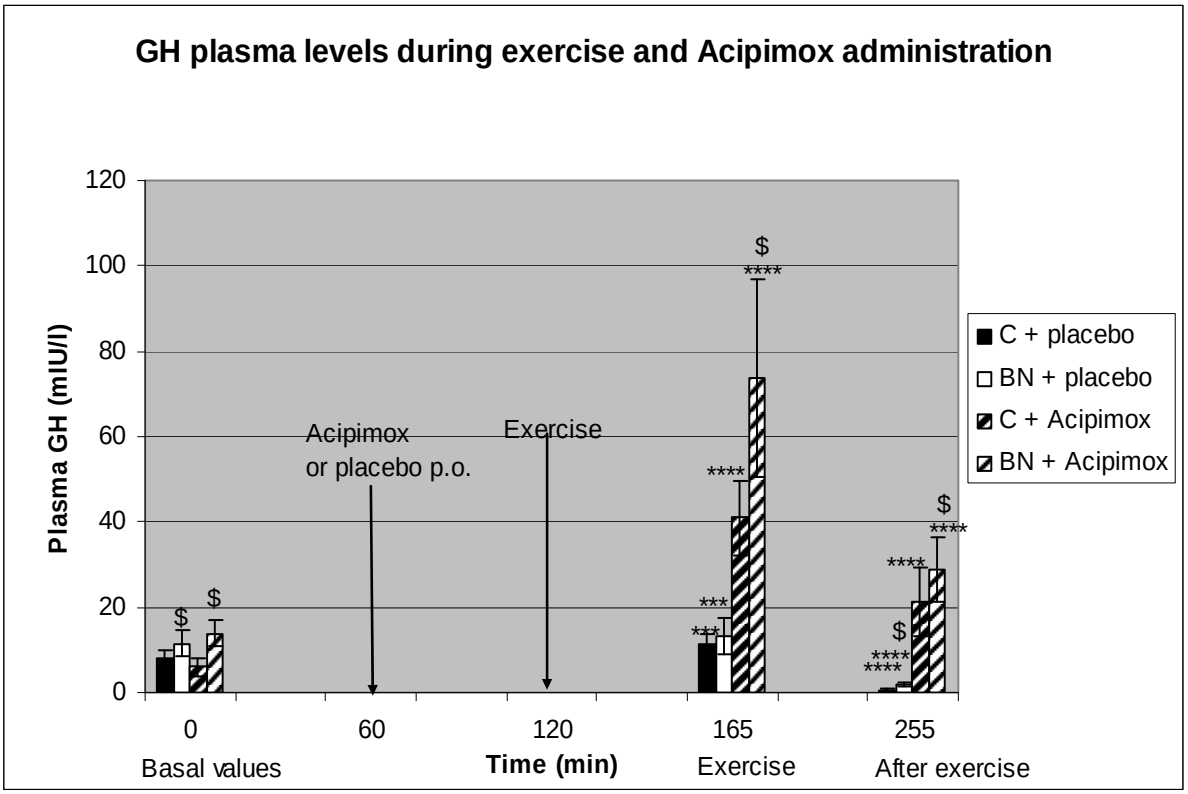
Table 5. Circulatory response of the study subjects to the exercise during Acipimox (Aci) and placebo treatment; the controls (C) (n = 7) and bulimia nervosa (BN) patients (n = 7).

	Rest	**Exercise**	**Exercise**
	Baseline	**+ placebo**	**+ Aci**
		500 mg p.o.	500 mg p.o.
Heart rate			
(beats/min)			
C group	68±1.9	138±6.3***	146±8.9***$^{+}$
BN group	57±3.5$^{\$}$	142±9.4***	152±6.2***$^{+}$
Systolic blood pressure			
(mmHg)			
C group	116±3.5	143±2.8*	133±5*$^{+}$
BN group	93±4$^{\$}$	130±3.1*$^{\$}$	138±5.2*$^{+}$
Diastolic blood pressure			
(mmHg)			
C group	69±1.7	82±2.8*	77±3*
BN group	56±2.2$^{\$}$	79±2.7*	79±2.2*

Exercise results are maximal values attained during the investigation (45 min, 2 W/kg of lean body mass [LBM]). Values are means ± S.E.M., C = controls, BN = bulimia nervosa, n = number of subjects are in brackets, p.o., per os.

*$P < 0.05$, ***$P < 0.001$ *vs.* resting (baseline) values, $^{\$}P < 0.05$ *vs.* control subjects (C), $^{+}P <$ 0.05 exercise together with Aci administration *vs.* exercise alone, 45 minute

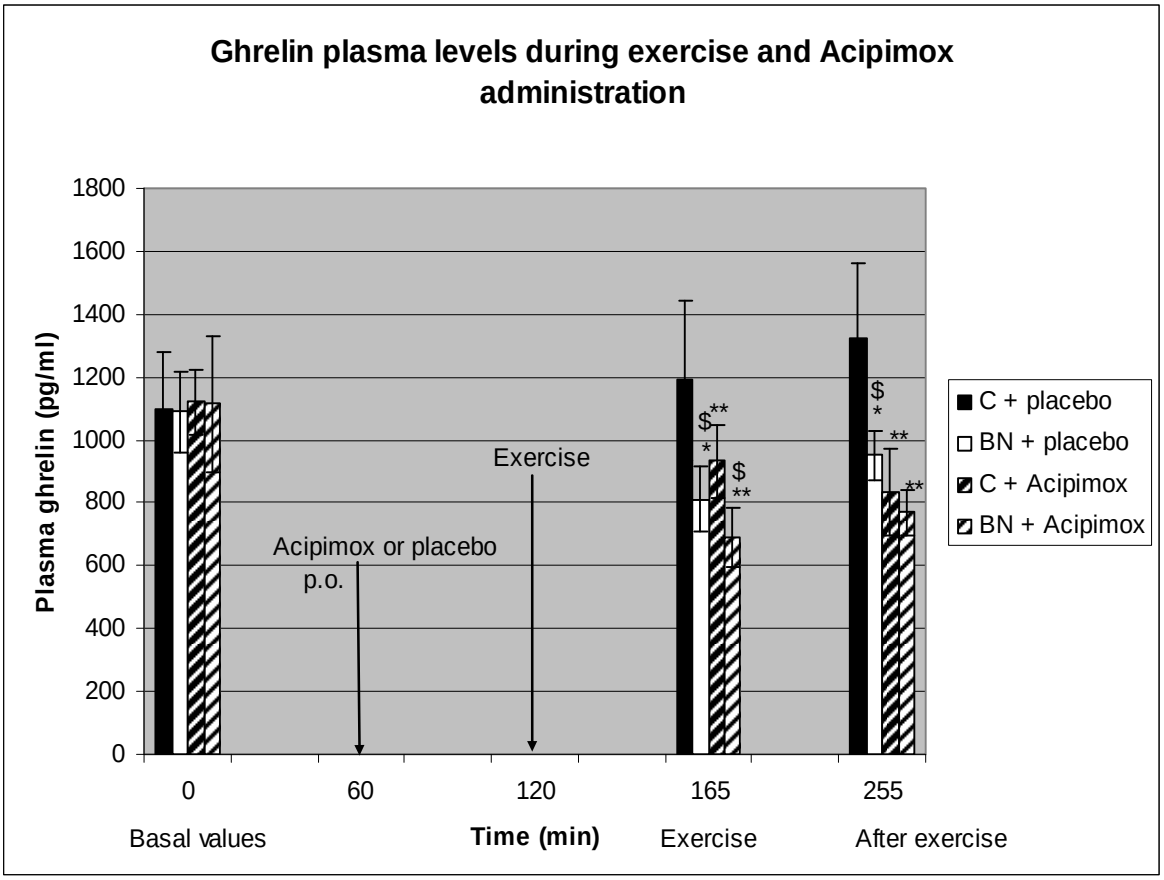
Fig. 1. Effect of exercise (45 min, 2 W/kg of lean body mass, LBM) alone or together with Acipimox (Aci) administration on plasma growth hormone (GH) levels (means ± S.E.M.) in the controls (C) (n=7) and bulimia nervosa (BN) patients (n=7).



$ = $P < 0.05$ *vs.* control subjects (C)

*** = $P < 0.001$, **** = $P < 0.0001$ *vs.* resting (basal) values

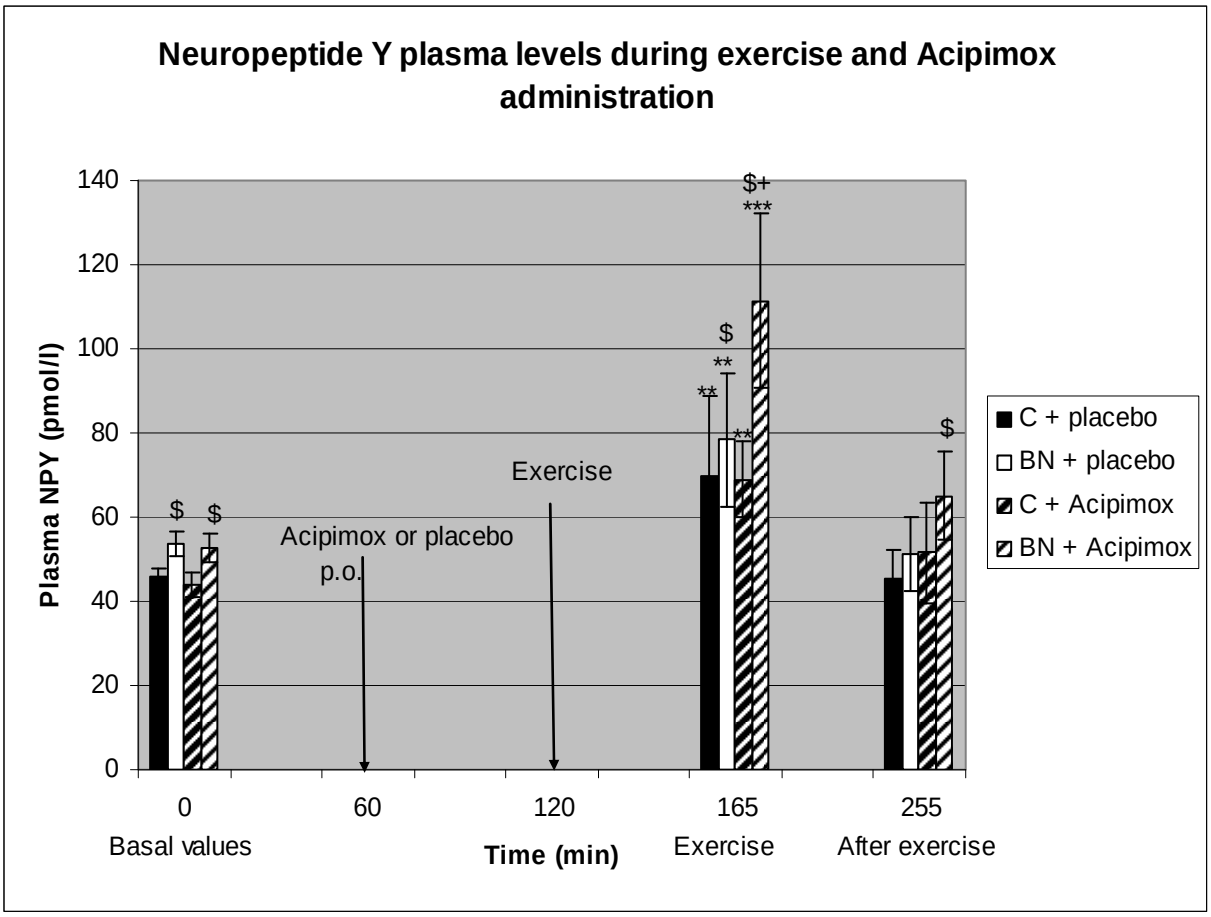
Fig. 2. Effect of exercise (45 min, 2 W/kg of lean body mass, LBM) alone or together with Acipimox (Aci) administration on plasma ghrelin levels (means ± S.E.M.) in the controls (C) (n=7) and bulimia nervosa (BN) patients (n=7).



$ = $P < 0.05$ BN *vs.* control subjects (C)

* = $P < 0.05$, ** = $P < 0.01$ *vs.* resting (basal) values

Fig. 3. Effect of exercise (45 min, 2 W/kg of lean body mass, LBM) alone or together with Acipimox (Aci) administration on plasma neuropeptide Y (NPY) levels (means ± S.E.M) in the controls (C) (n=7) and bulimia nervosa (BN) patients (n=7).

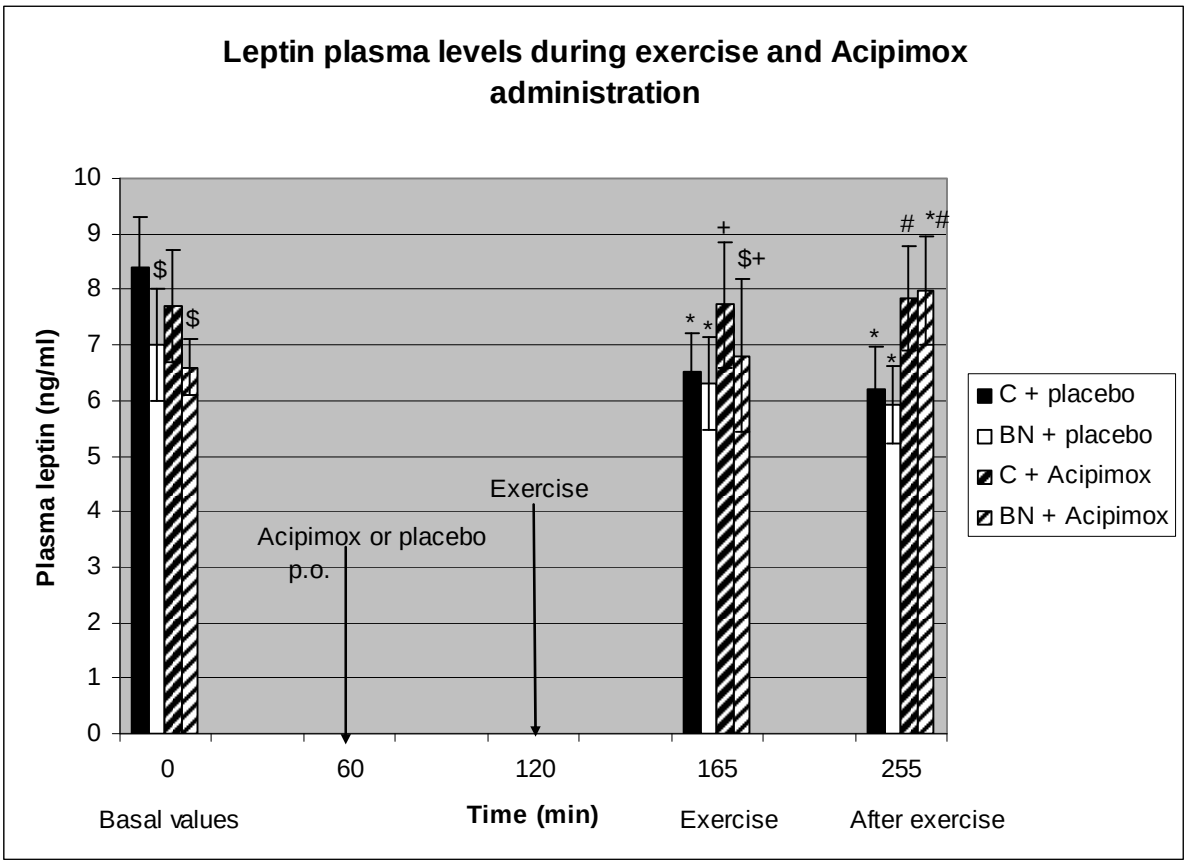


$ = $P < 0.05$ BN *vs.* control subjects (C), p.o., per os

** = $P < 0.01$, *** = $P < 0.001$ *vs.* resting (baseline) values

+ = $P < 0.05$ exercise together with Aci administration *vs.* exercise alone, 45 minute

Fig. 4. Effect of the exercise (45 min, 2 W/kg of lean body mass [LBM]) alone or together with Acipimox (Aci) administration on plasma leptin levels (means ± S.E.M.) in the controls (C) (n = 7) and bulimia nervosa (BN) patients (n = 7).



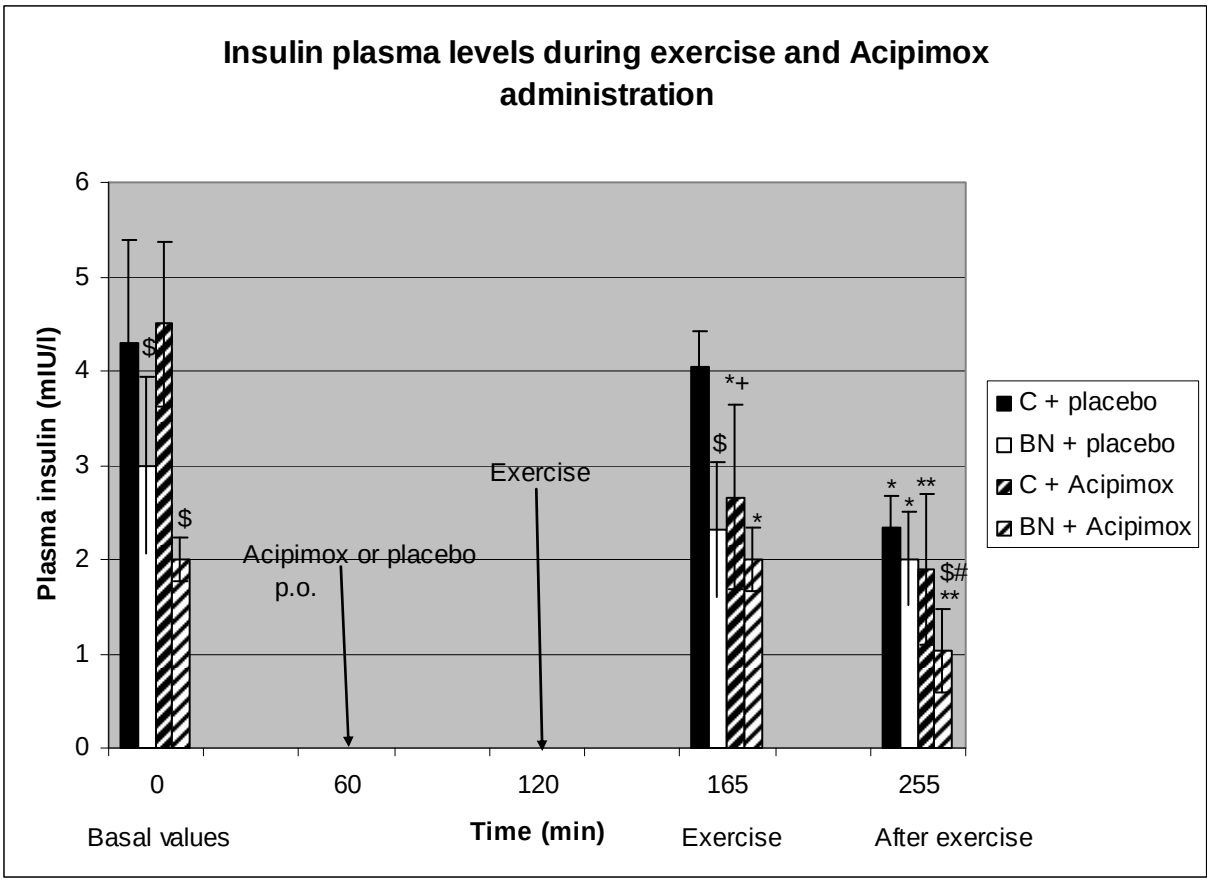
$ = $P < 0.05$ *vs.* control subjects (C), p.o., per os

* = $P < 0.05$ *vs.* resting (baseline) values

+ = $P < 0.05$ exercise together with Aci administration *vs.* exercise alone, 45 minute

= $P < 0.05$ post-exercise recovering phase together with Aci administration *vs.* post-exercise recovering phase alone, 90 minute

Fig. 5. Effect of the exercise (45 min, 2 W/kg of lean body mass [LBM]) alone or together with Acipimox (Aci) administration on plasma insulin levels (means ± S.E.M.) in the controls (C) (n = 7) and bulimia nervosa (BN) patients (n = 7).



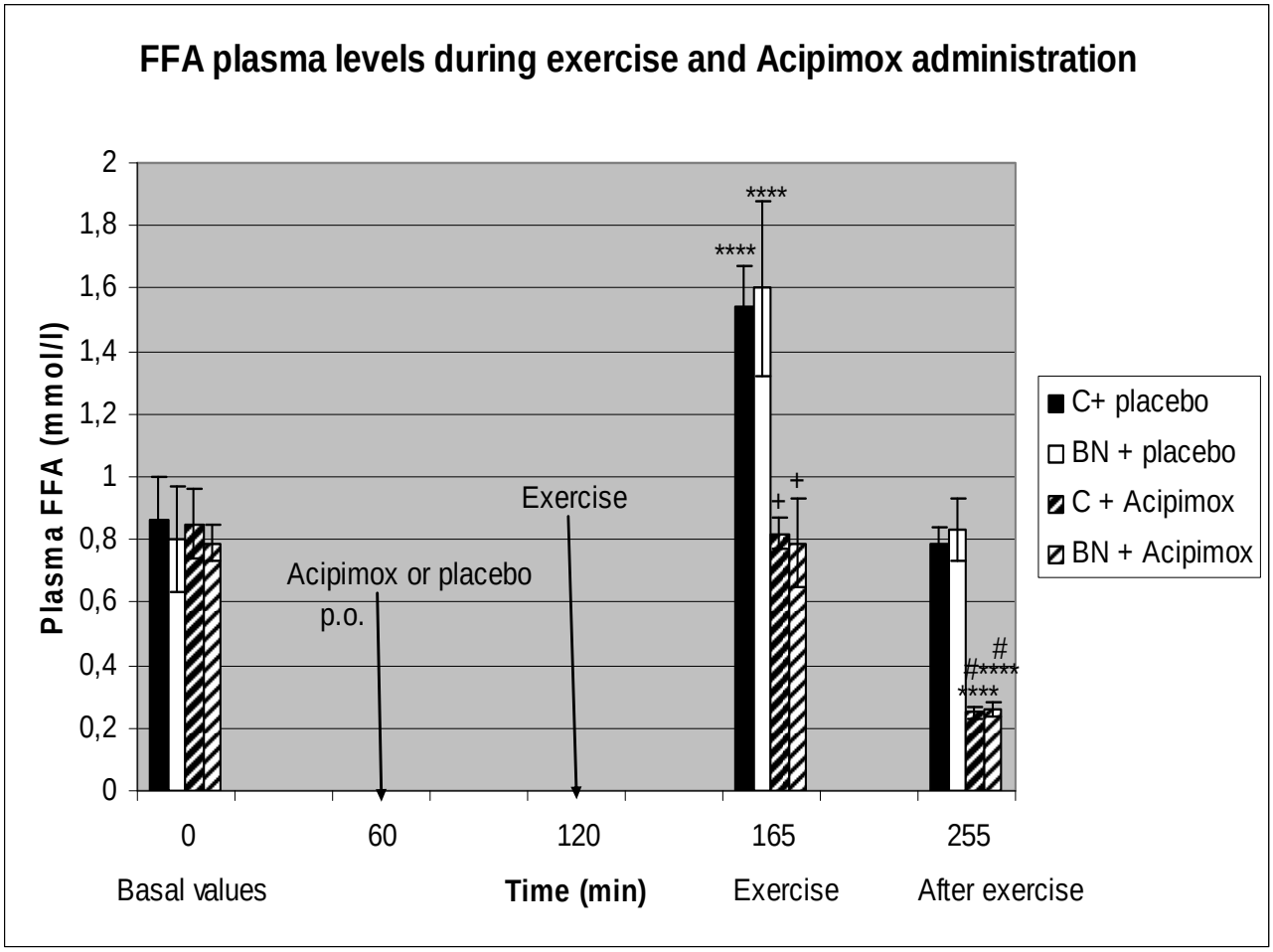
$ = $P < 0.05$ BN *vs.* control subjects (C), p.o., per os

* = $P < 0.05$, ** = $P < 0.01$ *vs.* resting (baseline) values

+ = $P < 0.05$ exercise together with Aci administration *vs.* exercise alone, 45 minute

= $P < 0.05$ post-exercise recovering phase together with Aci administration *vs.* post-exercise recovering phase alone, 90 minute

Fig. 6. Effect of the exercise (45 min, 2 W/kg of lean body mass [LBM]) alone or together with Acipimox (Aci) administration on plasma free fatty acids (FFA) levels (means ± S.E.M.) in the controls (C) (n = 7) and bulimia nervosa (BN) patients (n = 7).

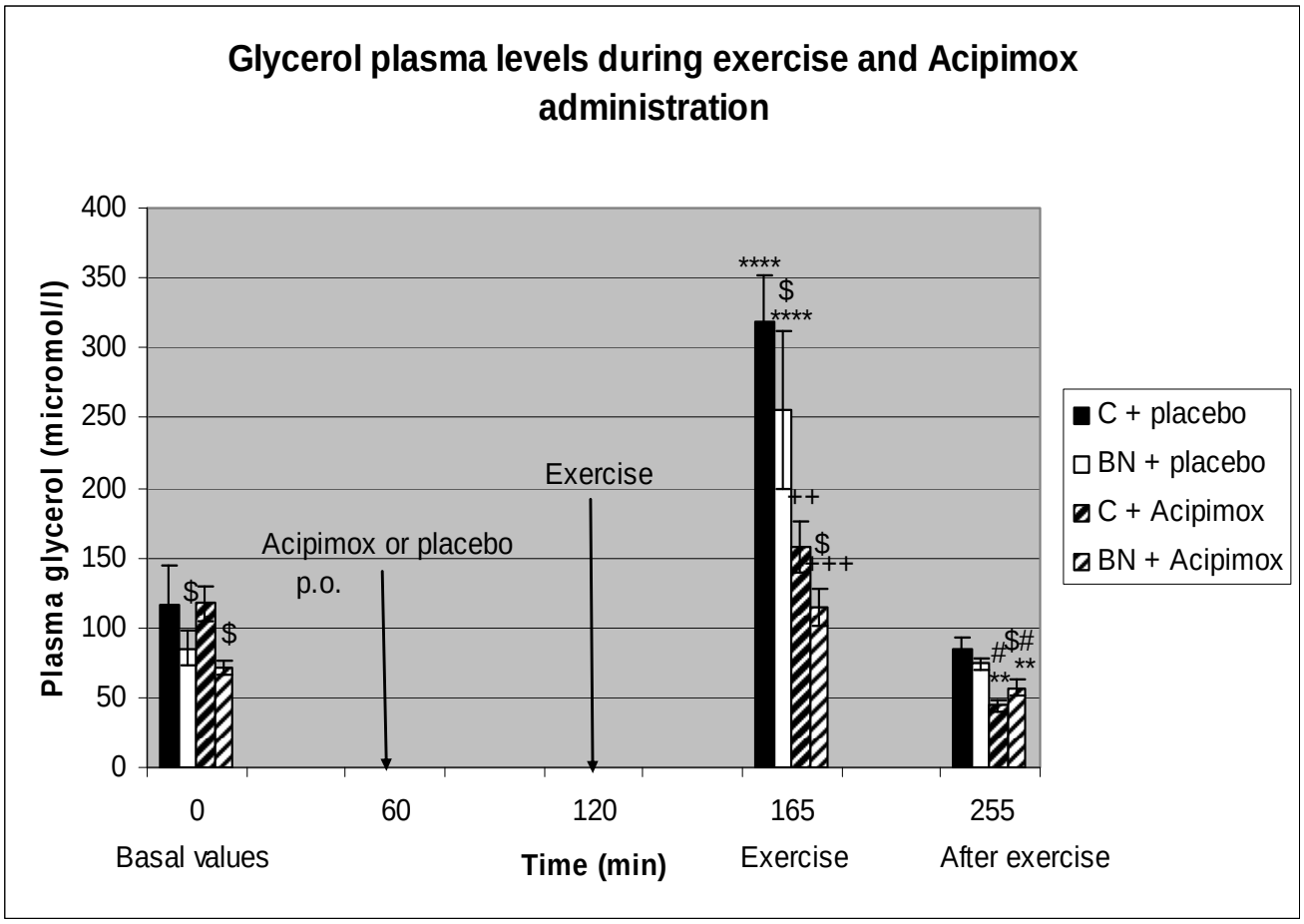


**** = $P < 0.0001$ *vs.* resting (baseline) values, p.o., per os

$^{+}$ = $P < 0.05$ exercise together with Aci administration *vs.* exercise alone, 45 minute

$^{\#}$ = $P < 0.05$ post-exercise recovering phase together with Aci administration *vs.* post-exercise recovering phase alone, 90 minute

Fig. 7. Effect of the exercise (45 min, 2 W/kg of lean body mass [LBM]) alone or together with Acipimox (Aci) administration on plasma glycerol levels (means ± S.E.M.) in the controls (C) (n = 7) and bulimia nervosa (BN) patients (n = 7).



$^{\$}$ = $P < 0.05$ BN *vs.* control subjects (C), p.o., per os

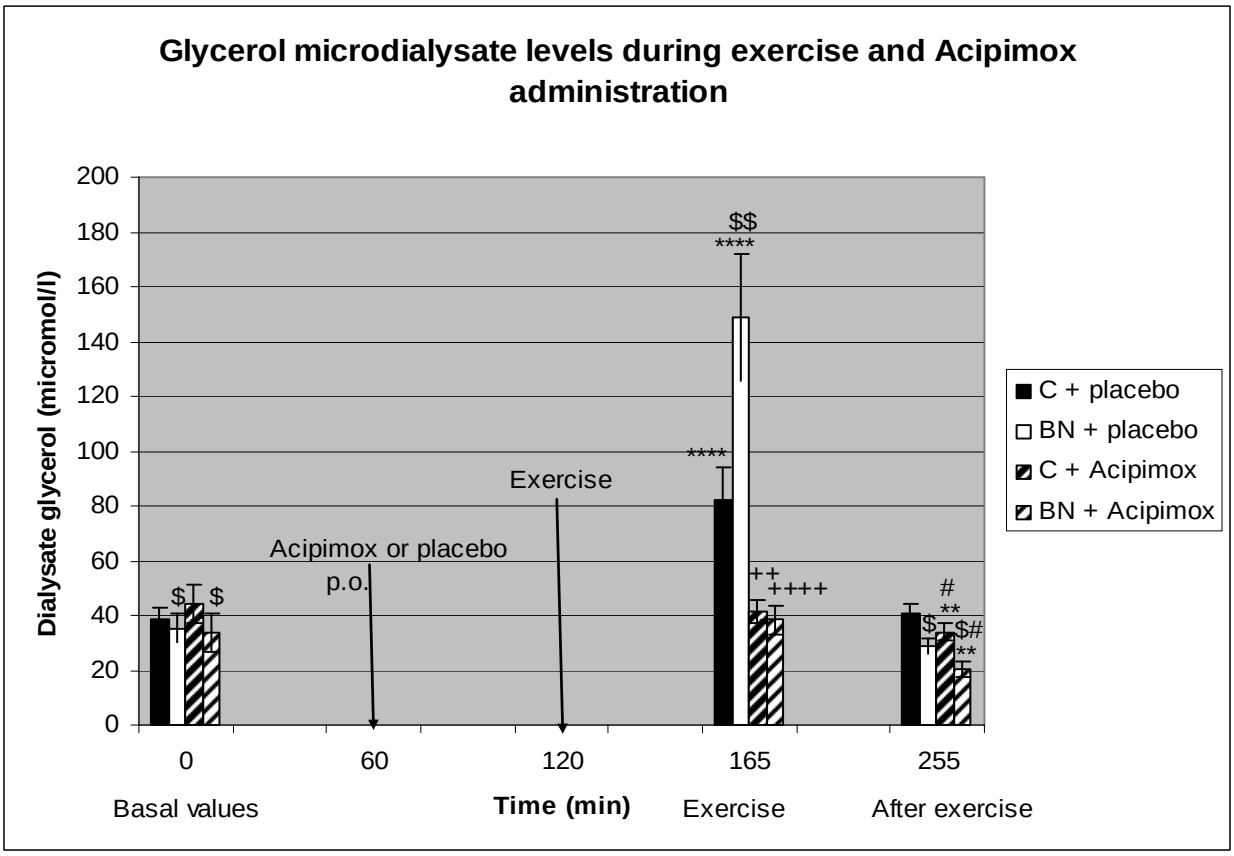
** = $P < 0.01$, **** = $P < 0.0001$ *vs.* resting (baseline) values

$^{++}$ = $P < 0.01$ exercise together with Aci administration *vs.* exercise alone, 45 minute

$^{+++}$ = $P < 0.001$ exercise together with Aci administration *vs.* exercise alone, 45 minute

$^{\#}$ = $P < 0.05$ post-exercise recovering phase together with Aci administration *vs.* post-exercise recovering phase alone, 90 minute

Fig. 8. Effect of the exercise (45 min, 2 W/kg of lean body mass [LBM]) alone or together with Acipimox (Aci) administration on microdialysate glycerol levels (means ± S.E.M.) in the controls (C) (n = 7) and bulimia nervosa (BN) patients (n = 7).



$ = $P < 0.05$ BN *vs.* control subjects (C), p.o., per os

$$ = $P < 0.01$ BN *vs.* control subjects (C)

** = $P < 0.01$, **** = $P < 0.0001$ *vs.* resting (baseline) values

++ = $P < 0.01$ exercise together with Aci administration *vs.* exercise alone, 45 minute

++++ = $P < 0.0001$ exercise together with Aci administration *vs.* exercise alone, 45 minute

= $P < 0.05$ post-exercise recovering phase together with Aci administration *vs.* post-exercise recovering phase alone, 90 minute

11. DISCUSSION

Eating disturbances, such as BN and AN, are severe clinical conditions associated with increased morbidity and mortality. These patients often deny their illness, that makes most of the current treatments unsuccessful. New hopes have arisen through by the recent progress in understanding the neuroendocrine regulation of energy metabolism and feeding behavior.

Our data have demonstrated for the first time that fasting plasma GH, but not ghrelin levels, were elevated in BN patients compared with the healthy women. Exercise alone induced minor increase in plasma GH levels, induced increase of plasma FFA and glycerol levels in both groups, however, plasma ghrelin levels decreased after acute exercise with placebo only in BN patients. In fact, Aci administered 60 minute before short-term exercise induced important increase of plasma GH levels and a decrease of plasma ghrelin levels in both groups (Smitka et al. 2008, Nedvídková et al. 2011).

The lipolytic effect of GH is one of the most important metabolic actions of GH favouring the use of FFA as an energy source during fasting and stress (Nørrelund 2005). An exercise is a well-known physiological stimulus for GH secretion (Vendelbo et al. 2010) and it is hypothesized that GH feedback inhibits ghrelin secretion (Vestergaard et al. 2007b). The present data, however, do not support this hypothesis after exercise alone in healthy women. Also Kraemer et al. (2003) and others (Dall et al. 2002, Schmidt et al. 2004) did not observe after exercise the changes of plasma ghrelin levels in healthy subjects.

As ghrelin is an endogenous ligand of the GH secretagogue receptor, it may be supposed that circulating ghrelin levels would be linked to the endogenous GH secretion. However, we did not confirm a positive feedback loop between ghrelin and GH during exercise and that ghrelin does not contribute to exercise-induced increase in GH. However, different studies have reported exercise-induced alterations seem to be depended on several factors, including exercise mode, intensity and postprandial conditions as well as blood

sampling protocol used in the study (Kraemer and Castracane 2007). Short- and long-term exercise studies have been used to investigate how ghrelin is affected. Interestingly, ghrelin levels were increased during duration of 30, 60 or 120 min of low-intensity exercise (50W) below the aerob-anaerobic threshold, on the other hand ineffective effect on ghrelin was the short-term increase of the intensity to 100 W. These data suggest that low rather than high intensity exercise stimulates ghrelin levels independent of exercise duration (Erdmann et al. 2007). In addition, Jürimäe et al. (2007) described the opposite changes in ghrelin and leptin concentrations in elite male rowers after maximal short-term exercise with significant increasing of ghrelin levels. Interestingly, this study investigated ghrelin responses to maximal short-term exercise in postprandial conditions (pre-exercise snack) in rowers, thus, it is possible that a nutrition-induced decrease in ghrelin concentration influenced a possible exercise-induced effect of ghrelin secretion. Furthermore, GH responses to prolonged exercise bouts (60-90 min) at 80% of VO_2 max are associated with decrease ghrelin levels in male athletes (Sartorio et al. 2008). The effects of resistance exercise, as a concentric muscle contractions produced greater increase in GH may lead to a suppression of ghrelin *via* a negative feedback (Ghanbari-Niaki 2006). Dall et al. (2002) observed the decrease ghrelin levels during physical exercise and i.v. GH infusion (*i.e.* supraphysiological GH concentration), but not during physical exercise alone. The basal and post-exercise decrease of ghrelin levels during exercise was supported by a further suppression of basal as well as post-exercise ghrelin levels after administration of high-dose GH (Vestergaard et al. 2007b). Our group determined responses to 45 min of cycling reaching 2W/kg, which is a low to moderate exercise intensity below individual aerobic-anaerobic threshold (30-40% of VO_2 max). Further the increase of plasma GH levels and unchanged plasma ghrelin levels after exercise in the controls lead us to supposition that a moderate intensity exercise induced small increase in GH levels without feedback inhibition of ghrelin secretion in the controls but not

in BN patients. Indeed, higher basal plasma GH levels and the increase of plasma GH levels induced by exercise induced the reduction of plasma ghrelin levels in BN patients, thus, plasma ghrelin levels better reflect nutritional status (Troisi et al. 2005) and our data established ghrelin as a potential discriminator between patients with endocrine aberrations and disturbances, such as eating disorders.

However, changes in ghrelin were associated with supraphysiological levels of GH induced by Aci administration and exercise in both groups. It is possible that a high absolute GH levels induced by exercise together with Aci administration resulted to significant decrease of plasma ghrelin levels also in the controls. Another possibility is that plasma ghrelin decreased in dependence on changes in plasma FFA and glycerol. It has been reported that administration of FFA synthase inhibitor suppresses ghrelin secretion from the stomach in mice (Hu et al. 2005) and similar effect has been observed with i.v. infusion of FFA (Coiro et al. 2007). Ghrelin is meal initiating signal and in line with this view that suppression lipolysis *via* Aci in AT would suppress ghrelin secretion since mobilization of FFA is increased with fasting and blocked by food intake. Thus, the role of FFA induced suppression on ghrelin is not clear. Since i.v. FFA (Coiro et al. 2007) as well as Aci inhibit lipolysis, the posibility that these conditions suppress ghrelin secretion *via* a shared sensor related to both antilipolysis and lipolysis. It is compatibile with the role of ghrelin as a lipogenic hormone (Ryber et al. 2006) and for an explanation of paradox that antilipolysis and food ingestion each are associated with ghrelin suppression during conditions of nutrient surplus. These data support that ghrelin is linked to energy metabolism rather than GH secretion and that the suppression of plasma ghrelin levels exerts lipolytic effects to provide the metabolic fuel needed for physical activity.

In fact, insulin resistance appears to be a negative determinant of ghrelin, reduced ghrelin levels were found in humans with insulin resistance (Pöykkö et al. 2003). Since both

GH induces insulin resistance and Aci administration influences FFA levels as well as insulin sensitivity, we cannot exclude that changes in insulin resistance during exercise alone may decrease ghrelin levels only in BN patients and that changes in insulin resistance during exercise together with Aci administration may suppress ghrelin levels in both groups when GH levels were supraphysiologically increased but plasma insulin were significantly decreased and insulin resistance seems to be lower in both groups in that condition (Vestergaard et al. 2005)

Interestingly, it was indicated that suppression of lipolysis *via* inhibition of HSL by Aci in combination with GH administration is associated with significantly reduced ghrelin levels (Vestergaard et al. 2005, Gormsen et al. 2006). These studies show for the first time that antilipolysis *per se* as well as GH suppress ghrelin secretion. These novel observations that the secretion and action of ghrelin indirectly or directly depend on alterations in nutritional background and/or on GH release. Similarly, there are investigated reciprocal changes in endogenous ghrelin and GH during fasting in healthy women. This is a new evidence of important physiological relationship of GH and ghrelin in responses to changes in caloric deprivation in healthy women, *i.e.* these data suggest that GH may exhibit feedback inhibition on ghrelin during prolonged fasting (Koutkia et al. 2005).

The important finding of the present study is that basal fasting NPY and GH plasma levels were increased and independent on BMI in BN patients, and were similarly increased after acute exercise (45 minute) with placebo in both groups. Anti-lipolytic drug Aci administered 60 minute before initiating short-term exercise induced further important increase of plasma NPY levels only in BN patients, and further increase of plasma GH levels in both groups.

Likewise, in the present study, basal fasting leptin plasma levels were decreased in BN patients, and were similarly decreased after acute exercise (45 minute) with placebo in both

groups. Pharmacological antilipolysis during short-term exercise not only prevented any falling of plasma leptin levels in both groups, but also increased plasma leptin in the post-exercise recovering phase (90 minute) with Aci administration, more in BN patients.

Orexigenic hypothalamic peptide NPY also participates in leptin, ghrelin and GH regulation pathways (Nogueiras et al. 2010). The molecular mechanisms by which acute exercise or anti-lipolytic drugs could cause an increase in plasma NPY and GH are not clear. Previously, it was demonstrated that ghrelin action can be modulated by NPY (Coiro et al. 2006). Thus, induced changes in NPY and GH may be working through divergent signal transduction pathways or receptor array. It has been reported that the GH release to low work loads is mainly mediated by an increased central cholinergic tone, which reduces hypothalamic somatostatin activity (de Vries et al. 2003), and that reduced somatostatinergic tone in AN patients rests on the presence of elevated GH levels (Pincelli et al. 2003). However, *in vitro* data shows that incorporation of FFA into the plasma membrane of GH cells disrupts signal transduction pathways that are pivotal for GHRH-induced GH release (Kok et al. 2004). Aci is a nicotinic acid derivative, and nicotinic acid can activate dopaminergic neurons, therefore, Aci may enhance both GH through lowering of FFA and can stimulate GH release through neural activation of dopamine receptors in humans (Kok et al. 2004). Indeed, Scacchi et al. (2010) observed the improvement in the GH response to GHRH in obese subjects treated with Aci and that elevated FFA levels and somatostatinergic hypertone might play a leading role (Lee et al. 1995). Furthermore, it was reported that i.v. administration of GH or synthetic GH secretagogues induce NPY messenger ribonucleic acid expression in NPY neurons of the arcuate nucleus (Chan et al. 1996, Shintani et al. 2001) where the GH receptor is expressed (Kamegai et al. 1996, Deltondo et al. 2008). It is suggested that NPY is released at the same time when SNS is activated and hypothalamic NPY levels show significant increases during intense physical exercise, thereby reducing

energy stores (Chen et al. 2007). Interestingly, NPY exerts a facilitatory action on dopamine neurotransmission and that communicates with dopaminergic brain pathways to produce a complex brain network which regulates physical activity (Adewale et al. 2007, Teske et al. 2008).

It is known that ghrelin levels increase before meal and stimulate hypothalamic NPY which is the main orexigenic hormone of appetite regulation. Thus, ghrelin is an upstream regulator of the orexigenic peptide NPY and acts as a natural antagonist to leptin´s effect on NPY expressing neurons resulting in an increase in feeding and body weight (Dardennes et al. 2007). In our present study, exercise induced an increase in plasma NPY levels in the controls and in BN women in whom the NPY increase was higher, without exhibiting any significant dependence on plasma ghrelin and leptin levels and on plasma glycerol and FFA concentrations (Nedvídková et al. 2011). These results may indicate a disorder of the gut-hypothalamic-AT pathway in these patients in order to prevent energy losses. Hence, post-exercise decrease of leptin and increase of NPY are probably a part of adaptive mechanisms leading to conservation of energy storage in BN (Dostálová et al. 2007a, Nedvídková et al. 2011). In agreement with these reports, Sedlačková et al. (2010) documented that higher fasting NPY levels with anti-lipolytic properties and almost absent response to high-carbohydrate breakfast in BN patients might indicate protection of the organism against energy exhaustion. Interestingly, Karamouzis et al. (2002) reported that plasma leptin levels were significantly reduced, while plasma NPY, glycerol, and plasma FFA concentrations were increased after an intense prolonged exercise in man. Additionally, circulating leptin levels were more negatively correlated with plasma glycerol and NPY levels, respectively, after prolonged exercise-mediated energy expenditure.

Furthermore, we found even higher plasma glycerol levels after the exercise combined with Aci administration in the controls. These observations lead us to suggesting that glycerol

is not easily remetabolized, thus stays behind and its level therefore increases, and that the decrease of plasma FFA levels to basal values is exerted by facilitated turnover of FFA in BN. Unexpectedly, we did not confirm that increased plasma FFA levels inhibit lipolysis *via* a feedback mechanism (Coiro et al. 2007).

Thus, elevated NPY and GH levels induced by exercise together with Aci do not appear to be directly mediated *via* FFA, and to influence ghrelin secretion in both groups, concluding that FFA probably are not ghrelin enhancers (Smitka et al. 2008, Nedvídková et al. 2011). Interestingly, after 45 minute exercise with Aci plasma GH levels positively correlated with NPY only in BN patients and these observations lead us to suggestion that GH can be responsible on the increase of NPY production (Kamegai et al. 1994, Chan et al. 1996) and that the NPY exerts a co-feedback action with GH on suppression of ghrelin secretion.

In coculture with adipocytes, sympathetic neurons secreted NPY, suggesting cross talk between the neural cells and adipocytes (Turtzo et al. 2001). Kos et al. (2007) demonstrated that NPY is expressed and secreted by human adipocytes. Therefore, AT has a great potential to contribute to the total AT-mediated NPY action, and AT-derived NPY could cause a significant rise of plasma NPY levels and may mediate reduction of leptin secretion. Morover, NPY is co-localizated with norepinephrine in perivascular sympathetic nerves, and it can be supposed that the exercise-induced increase in plasma NPY can be associated with increased norepinephrine in AT (Barták et al. 2004). Sympathetic neurotransmitter and NPY release increase in parallel with the intensity of the stressful stimuli (Taylor et al. 2008). This increase in sympathetic outflow could be exaggerated when two or more stressors are compounded, such as physical exercise and high anxiety in BN and AN. These findings are in accordance with previous and recent reports suggesting higher activity of SNS in AT and disrupted adrenergic regulation of lipolysis. observable in both receptor and postreceptor levels in sc abdominal AT in AN (Barták et al. 2003, Nedvídková et al. 2004). It has been shown by

numerous studies that SNS can exert tonic inhibitory action on leptin secretion, and that adrenergic regulation may contribute to rapid decrease both of plasma insulin and leptin levels during exercise (Gomez-Merino et al. 2002). In agreement with these studies, we showed that exercise-induced plasma insulin and leptin levels were significantly lower in AN patients compared to the controls (Dostálová et al. 2007a).

In the present study, we found significantly decreased both baseline plasma leptin and insulin levels in BN patients similarly as documented in AN patients (Dostálová et al. 2007a, b). Plasma leptin levels were significantly lower immediately after exercise (45 minute) and after the post-exercise recovering phase (90 minute) combined with placebo in both groups. On the other hand, plasma FFA levels were significantly increased immediately after exercise (45 minute), and after the post-exercise recovering phase (90 minute) associated with placebo the values approached the baseline ones in both groups. This may provide evidence that FFA are not involved in the exercise-induced leptin decrease. Furthermore, in the post-exercise recovering phase (90 minute) with placebo, a decrease in plasma insulin levels was observed in both groups.

The role of plasma leptin and FFA levels in exercise has not been defined. A negative correlation was found between plasma FFA and leptin levels (Duclos et al. 1999) and it was suggested that lipolysis may explain the rapid leptin decrease after the exercise, although Gomez-Merino et al. (2002) failed to find any correlation between plasma leptin levels and plasma FFA levels after physical activity in humans. It was found that leptin may reduce the response to many kinds of stress (Malendowicz et al. 2007); on the other hand, it was confirmed that leptin up-regulates the SNS activity and does not appear to respond to its above-mentioned anti-stress action (Malendowicz et al. 2007). Morover, treatment with Aci increased significantly plasma leptin levels in humans (Worm et al. 2000). These observations led us to suggesting that administration of Aci might prevent falling of plasma leptin levels

during short-term exercise in both groups; in addition, in the post-exercise recovering phase (90 minute) with Aci administration plasma leptin levels increased significantly more in BN patients. Consequently, we reveal that short-term exercise together with Aci administration resulted in opposite changes of plasma ghrelin and leptin in both groups (Vestergaard et al. 2005, Kalra et al. 2005).

After the 45-minute exercise, GH and blood glucose concentrations were more expressed in the presence of Aci *vs.* placebo in BN patients, and plasma insulin levels were lower after the post-exercise recovering phase (90 minute) combined with Aci administration, more in BN patients. Both FFA and insulin are known to inhibit blood glucose production; on the other hand, it has been shown that anti-lipolytic Aci may decrease insulin and increase blood glucose concentrations (Allick et al. 2004). Thus, GH and Aci administration during exercise promote glucose production, and increased plasma blood glucose may also stimulate leptin secretion (Worm et al. 2000). Likewise, Lissett et al. (2001) demonstrated that a single bolus of GH increases plasma leptin levels in humans. In our present study, plasma leptin levels correlated positively with blood glucose concentrations after 45 minute exercise with Aci administration in the controls.

This is the first randomized microdialysis study to evaluate the effect of antilipolysis on AT and plasma glycerol during short-term exercise in healthy women and patients with BN. The exercise induced a higher increase of glycerol concentrations in sc abdominal AT of BN patients, while exercise with Aci administration induced a higher decrease of extracellular glycerol in BN patients compared to the C group. The exercise induced similar increases in plasma glycerol levels in both groups. The exercise with Aci administration resulted in plasma glycerol decrease more in BN patients.

Furthermore, we observed lower both baseline AT and plasma glycerol levels in patients with BN when compared to age- and weight-matched healthy women. Some authors

did not observe changed baseline plasma and AT glycerol concentrations in AN patients with low BMI compared to the controls (Barták et al. 2004, Dostálová et al. 2007a) but the others found higher local glycerol concentrations in AT of underweight patients with AN (Nedvídková et al. 2004). These findings are in concordance with previous and recent reports suggesting the higher activity of SNS in AT and disrupted adrenergic regulation of lipolysis occuring both receptor and postreceptor levels in sc abdominal AT in AN (Krykorková et al. 2001, Nedvídková et al. 2003b, 2004, Barták et al. 2003). Likewise, we found that under *in vivo* conditions in patients with AN sensitivity beta-adrenergic receptors to norepinephrine in sc abdominal AT is decreased. This may be due to changed SNS in sc abdominal AT that results in down-regulation of beta-receptors and therefore to decreased lipolysis to protect fat stores from further depletion by increased sympathetic nervous activity (Barták et al. 2004). Indeed, Dostálová et al. (2007a) reported an extreme sensitivity of AN patients to energy imbalance and consequently to weight loss (*i.e.* relapse) and that we could not exclude the influence of excited activity of the SNS and of increased lipolysis in sc abdominal AT of patients with AN during short-term exercise. However, the mechanism of altered sympathetic activity in eating disorders is not entirely understood. In some studies, lower basal SNS activity was reported in AN patients compared to healthy volunteers (Kaye et al. 1985, Pirke 1996). Nevertheless, all these studies were based on measurements of plasma and cerebrospinal fluid norepinephrine levels but not local specific tissue catecholamine levels.

Importantly, we found a discrepancy between plasma glycerol and local (dialysate) glycerol levels in BN patients, and we determined a significantly higher dialysate glycerol level during exercise in BN compared to the controls. Currently, it is well known that local (tissue) lipolysis does not reflect plasma glycerol levels during exercise in BN patients (Barták et al. 2003, 2004). This discrepancy could be possibly explained by the fact that plasma glycerol concentration reflects the net amount of this parameter released from

different sources, whereas dialysate glycerol concentration determinates the quantity released in AT. Aci acutely received during the exercise led to much more abolished lipolysis in sc abdominal AT in BN than in the controls, which leads us to suggesting that altered lipolysis in BN may result from local modification of both adrenergic and NPY-ergic activities. Interestingly, the effect of Aci administration on higher epinephrine secretion was observed in obese and lean males (Allick et al. 2004) and that higher norepinephrine secretion was observed after exercise with Aci administration in non-insulin-dependent diabetic patients (Akanji et al. 1993), thus, Aci, catecholamines and NPY act *via* their inhibition on cAMP production in AT, rather than *via* alternative cAMP-independent pathways (Wang-Fisher et al. 2002, Soudijn et al. 2007), and up-regulation of receptor subtypes and/or their sensitivity or affinity are much more effective in abolishing lipolysis in BN. *In vitro* with isolated adipocytes, Wang-Fisher et al. (2002) showed that Aci stimulation was efficiently suppressed by norepinephrine, consistent with our hypothesis that Aci acts mainly *via* its inhibition on cAMP production. In another study performed *in vivo* (Flechtner-Mors et al. 2001), when norepinephrine was added to the perfusate, a rapid increase in dialysate glycerol concentration was observed in sc abdominal AT of Aci treated subjects. Furthermore, the increase in dialysate glycerol concentration after administration of norepinephrine was accompanied by a modest inhibition of local blood flow and vasoconstriction could have increased extracellular glycerol concentration in sc abdominal AT by decreasing local tissue drainage. This is likely to be only a minor effect, given the observed modest change in local blood flow because norepinephrine is the most important hormone regulating human AT lipolysis (Kurpad et al. 1994).

Furthermore, we found even higher plasma glycerol levels after the exercise combined with Aci administration in the controls. These observations lead us to suggesting that glycerol is not easily remetabolized and the decrease of plasma glycerol after the exercise associated

with Aci administration is exerted by altered activity of SNS in BN, and/or by facilitated turnover of plasma glycerol which would reflect metabolic status in this eating disorder. However, Gianotti et al. (2000) studied effect of Aci (Aci 250 mg p.o. at – 60 minutes) on basal plasma lipolysis (plasma FFA levels) in AN and that overall lipolysis was inhibited by Aci in both groups but persisted higher in AN than in healthy women, they used a lower dose of Aci (by approximately 50%) than our study.

The significant changes in plasma NPY as a relevant biomarker for sympathetic tone contributing to an increase in systolic blood pressure in BN patients and maximal values of heart rate attained during the exercise with Aci administration, correlated positively with the systolic and diastolic blood pressure, respectively. These findings suggest that an imbalance of sympatho-adrenal activation in BN patients (Dostálová et al. 2007a) during short-term exercise is hypersensitive to alterations of endogenous NPY levels as a potent vasoconstrictor (Karamouzis et al. 2002, Cleary et al. 2007).

Endocrine perturbations and a dysfunction within the FFA-leptin-ghrelin-NPY-GH system may also take part in the etiopathogenesis of either bulimia or AN. Better understanding of the role of ghrelin, NPY and leptin agonists or inhibitors and their interactions with adipocyte lipolysis and the ghrelin-GH neuroendocrine axis may provide an entirely new therapeutic approach in treatment of BN and AN patients who poorly respond to various pharmacological therapies.

12. CONCLUSIONS

Taken together, we concluded and supported the hypothesis that after acute Aci received during and post-exercise may be consistent with a negative feedback of GH on ghrelin secretion in both groups but more in BN. We observed hypersensitivity to negative caloric balance during exercise and administration of Aci in BN patients and these data established ghrelin as a potential discriminator of eating disorders but not in healthy women.

Our results support the hypothesis that exercise and Aci-induced GH released are not mediated by ghrelin. Thus, changes in FFA levels did not respond to changes in GH and ghrelin levels.

Thus, it can be concluded based on this randomized, placebo-controlled, single-blind microdialysis study that antilipolysis during exercise (45 minute) further increases plasma NPY, GH and leptin levels (90 minutes after post-exercise recovering phase) in BN patients and leads to lipolysis abolished in a much higher extent in sc abdominal AT in BN using the *in situ* and *in vivo* microdialysis technique. Thus, it appears that bulimic patients are very sensitive to negative caloric balance and acute administration of Aci, and show hyperreactive responses both in GH and NPY; this data establish GH, NPY, ghrelin and leptin as important biomarkers of BN (Coiro et al. 1992, Dostálová et al. 2007a, Smitka et al. 2008, Sedláčková et al. 2010, Nedvídková et al. 2011). Importantly, we observed reciprocal changes in plasma ghrelin and leptin levels (Vestergaard et al. 2005), *i.e.* that Aci administration during exercise decreases plasma ghrelin levels and increases plasma leptin levels in both groups.

Our results support the hypothesis that elevated NPY and GH levels induced by the exercise together with Aci administration thus do not appear to be directly mediated *via* FFA and that exercise- and Aci-induced leptin releases are not mediated by FFA. Thus, these observations lead us to suggesting that Aci exerts an effect on the FFA-independent

mechanism. In addition, the exercise induced similar increases in plasma glycerol levels in both groups, but the exercise with Aci administration resulted in plasma glycerol decrease more in BN patients, thus, plasma glycerol is not easily remetabolized, and that the decrease of plasma glycerol after the exercise associated with Aci administration could be exerted by altered activity of SNS in BN, and/or by facilitated turnover of plasma glycerol which would reflect abnormal metabolic status in this eating disorder.

Taken together, our results support the hypothesis that higher sensitivity of SNS to anti-lipolytic drug Aci in sc abdominal AT exists in BN patients, and that Aci influences the same signal transduction pathway as norepinephrine, the major representative of SNS, *i.e.* that Aci acts *via* its inhibition on cAMP production, rather than *via* alternative cAMP-independent pathways (Wang-Fisher et al. 2002, Villena et al. 2004, Soudijn et al. 2007). Likewise, it can be concluded based on this randomized, placebo-controlled, single-blind microdialysis study that pharmacological antilipolysis in sc abdominal AT during short-term exercise is much higher in patients with BN. Simultaneously, we found facilitated turnover of plasma glycerol after short-term exercise together with Aci administration which would reflect abnormal metabolic status in BN. Lower basal lipolysis in AT in BN patients may be due to the protective mechanism before the exhaustion of energy reserves.

The present microdialysis study has a high impact on understanding of mechanisms that may contribute to altered functions of the AT in patients with BN. The results of our study should contribute further to the development of a new generation of drugs, such as leptin, anti-lipolytic NPY and ghrelin synthetic analogues that could alter synaptic cleft concentrations of norepinephrine and epinephrine and therefore lipid mobilization and energy expenditure. Endocrine perturbations and a dysfunction within sc abdominal AT may also take part in the etiopathogenesis of either bulimia or AN. Thus, we envision that results from this study may advance the understanding of hormone-induced regulations of AT metabolism

and introduce more specific and effective interventions because current long-term pharmacological therapy of bulimic patients is almost unsuccessful.

Therefore, our observations may contribute to the disruption of gut-brain-AT signalling system in BN. In recent years, knowledge in the field of food behavior has widely increased, leading to the design of molecules targeted for pharmacological correction of eating disorders and weight control (Capasso et al. 2009). At present, leptin, ghrelin and potentially NPY or their synthetic analogues as well as selective serotonin reuptake inhibitors (SSRI) and serotonin norepinephrine reuptake inhibitors (SNRI) may be useful agents for the modulation of food intake. In the treatment of eating disorders, modified blood-brain barrier in BN and AN is a therapeutic target for delivery of any therapeutics to the central nervous system (Banks 2010). Further research is required to investigate the gut-brain-AT orexigenic/anorexigenic agonists or antagonists and the modifications of their patways with receptor antagonists and agonists for potential treatment of eating disorders such as BN and AN in clinical practice. Taken together, more data are needed to clarify the etiopathogenesis and pathophysiology of BN and AN.

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14. LIST OF TABLES AND FIGURES

14.1. Tables

Table 1. Anthropometric and major laboratory characteristics of the study subjects (means ± S.E.M.). C = controls; BN = bulimia nervosa; BMI = body mass index; % BF = percentage of body fat; neuropeptide Y (NPY); growth hormone (GH); free fatty acids (FFA); NS = not significant; $^{\$}P < 0.05$ BN *vs.* control subjects (C); *n* = the number of subjects.

Table 2. Effect of exercise (45 min, 2 W/kg of lean body mass [LBM]) alone or together with Acipimox (Aci) administration on plasma gut-brain-adipose tissue (AT) peptides in the controls (C) (*n* = 7) and bulimia nervosa (BN) patients (*n* = 7).

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$ *vs*. resting (baseline) values

$ = $P < 0.05$ BN *vs.* control subjects (C)

+ = $P < 0.05$ exercise together with Aci administration *vs.* exercise alone, 45 minute

++ = $P < 0.01$ exercise together with Aci administration *vs.* exercise alone, 45 minute

+++ = $P < 0.001$ exercise together with Aci administration *vs.* exercise alone, 45 minute

= $P < 0.05$ post-exercise together with Aci administration *vs.* exercise alone, 90 minute

Table 3. Effect of exercise (45 min, 2 W/kg of lean body mass [LBM]) alone (placebo) or together with Acipimox (Aci) administration on plasma glycerol, free fatty acids (FFA) and

blood glucose levels in the controls (C) (n = 7) and bulimia nervosa (BN) patients (n = 7). Values are means ± S.E.M.; n = the number of subjects.

** = $P < 0.01$, **** = $P < 0.0001$ *vs.* resting (baseline) values

$ = $P < 0.05$ BN *vs.* control subjects (C)

++ = $P < 0.01$ exercise together with Aci administration *vs.* exercise alone, 45 minute

+++ = $P < 0.001$ exercise together with Aci administration *vs.* exercise alone, 45 minute

= $P < 0.05$ post-exercise recovering phase together with Aci administration *vs.* post-exercise recovering phase alone, 90 minute

Table 4. Dialysate glycerol concentration in subcutaneous (sc) abdominal adipose tissue (AT) during basal conditions and during exercise (45 min, 2W/ kg of lean body mass [LBM] alone or together with Acipimox (Aci) administration in the controls (C) (n = 7) and bulimia nervosa patients (BN) (n = 7). Values are means ± S.E.M.; n = the number of subjects.

** = $P < 0.01$, **** = $P < 0.0001$ *vs.* resting (baseline) values

$ = $P < 0.05$ BN *vs.* control subjects (C)

$$ = $P < 0.01$ BN *vs.* control subjects (C)

++ = $P < 0.01$ exercise together with Aci administration *vs.* exercise alone, 45 minute

++++ = $P < 0.0001$ exercise together with Aci administration *vs.* exercise alone, 45 minute

= $P < 0.05$ post-exercise recovering phase together with Aci administration *vs.* post-exercise recovering phase alone, 90 minute

Table 5. Circulatory response of the study subjects to the exercise during Acipimox (Aci) and placebo treatment; the controls (C) (n = 7) and bulimia nervosa (BN) patients (n = 7).

Exercise results are maximal values attained during the investigation (45 min, 2 W/kg of lean body mass [LBM]). Values are means ± S.E.M., C = controls, BN = bulimia nervosa, n = number of subjects are in brackets, p.o., per os.

*$P < 0.05$, ***$P < 0.001$ *vs.* resting (baseline) values, $^{\$}P < 0.05$ *vs.* control subjects (C), $^{+}P <$ 0.05 exercise together with Aci administration *vs.* exercise alone, 45 minute

14.2. Figures

Fig. 1. Effect of exercise (45 min, 2 W/kg of lean body mass, LBM) alone or together with Acipimox (Aci) administration on plasma growth hormone (GH) levels (means ± S.E.M.) in the controls (C) (n=7) and bulimia nervosa (BN) patients (n=7).

$^{\$}$ = $P < 0.05$ *vs.* control subjects (C)

*** = $P < 0.001$, **** = $P < 0.0001$ *vs.* resting (basal) values

Fig. 2. Effect of exercise (45 min, 2 W/kg of lean body mass, LBM) alone or together with Acipimox (Aci) administration on plasma ghrelin levels (means ± S.E.M.) in the controls (C) (n=7) and bulimia nervosa (BN) patients (n=7).

$ = $P < 0.05$ BN *vs.* control subjects (C)

* = $P < 0.05$, ** = $P < 0.01$ *vs.* resting (basal) values

Fig. 3. Effect of exercise (45 min, 2 W/kg of lean body mass, LBM) alone or together with Acipimox (Aci) administration on plasma neuropeptide Y (NPY) levels (means ± S.E.M) in the controls (C) (n=7) and bulimia nervosa (BN) patients (n=7).

$ = $P < 0.05$ BN *vs.* control subjects (C), p.o., per os

** = $P < 0.01$, *** = $P < 0.001$ *vs.* resting (baseline) values

+ = $P < 0.05$ exercise together with Aci administration vs. exercise alone, 45 minute

Fig. 4. Effect of the exercise (45 min, 2 W/kg of lean body mass [LBM]) alone or together with Acipimox (Aci) administration on plasma leptin levels (means ± S.E.M.) in the controls (C) ($n = 7$) and bulimia nervosa (BN) patients ($n = 7$).

$ = $P < 0.05$ *vs.* control subjects (C), p.o., per os

* = $P < 0.05$ *vs.* resting (baseline) values

+ = $P < 0.05$ exercise together with Aci administration *vs.* exercise alone, 45 minute

= $P < 0.05$ post-exercise recovering phase together with Aci administration *vs.* post-exercise recovering phase alone, 90 minute

Fig. 5. Effect of the exercise (45 min, 2 W/kg of lean body mass [LBM]) alone or together with Acipimox (Aci) administration on plasma insulin levels (means ± S.E.M.) in the controls (C) ($n = 7$) and bulimia nervosa (BN) patients ($n = 7$).

$ = $P < 0.05$ BN *vs.* control subjects (C), p.o., per os

* = $P < 0.05$, ** = $P < 0.01$ *vs.* resting (baseline) values

$^{+}$ = $P < 0.05$ exercise together with Aci administration *vs.* exercise alone, 45 minute

$^{\#}$ = $P < 0.05$ post-exercise recovering phase together with Aci administration *vs.* post-exercise recovering phase alone, 90 minute

Fig. 6. Effect of the exercise (45 min, 2 W/kg of lean body mass [LBM]) alone or together with Acipimox (Aci) administration on plasma free fatty acids (FFA) levels (means ± S.E.M.) in the controls (C) ($n = 7$) and bulimia nervosa (BN) patients ($n = 7$).

**** = $P < 0.0001$ *vs.* resting (baseline) values, p.o., per os

$^{+}$ = $P < 0.05$ exercise together with Aci administration *vs.* exercise alone, 45 minute

$^{\#}$ = $P < 0.05$ post-exercise recovering phase together with Aci administration *vs.* post-exercise recovering phase alone, 90 minute

Fig. 7. Effect of the exercise (45 min, 2 W/kg of lean body mass [LBM]) alone or together with Acipimox (Aci) administration on plasma glycerol levels (means ± S.E.M.) in the controls (C) ($n = 7$) and bulimia nervosa (BN) patients ($n = 7$).

$^{\$}$ = $P < 0.05$ BN *vs.* control subjects (C), p.o., per os

** = $P < 0.01$, **** = $P < 0.0001$ *vs.* resting (baseline) values

$^{++}$ = $P < 0.01$ exercise together with Aci administration *vs.* exercise alone, 45 minute

$^{+++}$ = $P < 0.001$ exercise together with Aci administration *vs.* exercise alone, 45 minute

$^{\#}$ = $P < 0.05$ post-exercise recovering phase together with Aci administration *vs.* post-exercise recovering phase alone, 90 minute

Fig. 8. Effect of the exercise (45 min, 2 W/kg of lean body mass [LBM]) alone or together with Acipimox (Aci) administration on microdialysate glycerol levels (means ± S.E.M.) in the controls (C) (n = 7) and bulimia nervosa (BN) patients (n = 7).

$ = P < 0.05 BN *vs.* control subjects (C), p.o., per os

$$ = P < 0.01 BN *vs.* control subjects (C)

** = P < 0.01, **** = P < 0.0001 *vs.* resting (baseline) values

++ = P < 0.01 exercise together with Aci administration *vs.* exercise alone, 45 minute

++++ = P < 0.0001 exercise together with Aci administration *vs.* exercise alone, 45 minute

= P < 0.05 post-exercise recovering phase together with Aci administration *vs.* post-exercise recovering phase alone, 90 minute