CLINICAL STUDY

Effects of free fatty acids on ACTH and cortisol secretion in anorexia nervosa

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Abstract

Objective: Free fatty acids (FFAs) exert a stimulatory effect on the hypothalamic–pituitary–adrenal (HPA) axis in animals and inhibit spontaneous ACTH and cortisol secretion in humans. Patients with anorexia nervosa display concomitant HPA axis hyperactivity and increased lipolysis. We studied the effects of a lipid load on ACTH and cortisol secretion in patients with anorexia nervosa in comparison with normal subjects.

Design: Eight women with anorexia nervosa (ANW; means $\pm$ S.E.M.: 23.9 ± 2.3 years of age; body mass index (BMI): 14.9 ± 0.6 kg/m²) and seven normal women (NW; 25.6 ± 2.3 years of age; BMI: 22.8 ± 1.9 kg/m²) had FFA, ACTH, cortisol, glucose and insulin levels measured in the morning every 30 min for 180 min during i.v. saline or lipid-heparin emulsion (LHE) infusion.

Results: During saline infusion, ACTH and cortisol levels decreased spontaneously in both groups, ACTH and cortisol levels in ANW being higher than in NW. LHE infusion led to increased FFA levels in both groups ($P < 0.005$). The ACTH and cortisol decrease in NW was more marked than during saline infusion ($P < 0.05$). LHE infusion in ANW was associated with a more pronounced decrease in ACTH levels than during saline infusion ($P < 0.05$), while cortisol levels were unchanged. At the end of the LHE infusion, a progressive decrease in FFA levels was associated with an increase in ACTH and cortisol concentrations in NW ($P < 0.05$) but not in ANW in whom FFA levels decreased to a lesser extent ($P < 0.05$).

Conclusions: This study showed that corticotroph sensitivity to the inhibitory effect of an FFA load is preserved in patients with anorexia nervosa, in spite of persistent adrenal hyperactivity.

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Introduction

Free fatty acids (FFAs) have been shown to exert a modulatory action on anterior pituitary function. Their inhibitory effect on growth hormone (GH) secretion, for instance, has been well established both in man and other animals (1–3), and reflects a negative feedback mechanism on somatotroph function that is likely to take place both directly on somatotroph cells and via an indirect action at the hypothalamic level (1, 4–6).

FFAs can also modulate hypothalamic–pituitary–adrenal (HPA) axis function, although this effect is less clearly defined. Animal studies have shown that an increase in circulating FFA levels exerts a dose-dependent stimulatory effect on both corticotroph and adrenal secretion (7), suggesting the existence of a positive feedback of FFAs on the HPA axis. However, a dual effect of

lipids on adrenal activity has been reported (7–10). We have recently demonstrated that a lipid load-induced increase in circulating FFA levels in healthy adult subjects exerts an inhibitory rather than a stimulatory effect on spontaneous adrenocorticotropin (ACTH) and cortisol secretion (11), suggesting that there may be a negative feedback link between FFAs and the HPA axis in humans. A relationship between the HPA axis and lipid metabolism is inferred, based on the lipolytic effect of glucocorticoids. In fact, activation of this axis induces an increase in circulating FFAs and glycerol from adipose tissue (12–14). The lipolytic effect includes both a direct opposition of insulin anabolic action and a potentiation of adrenergic and GH catabolic effect on adipose tissue. Several pathophysiological conditions in humans (obesity, diabetes mellitus, fasting, anorexia nervosa) are accompanied by a concomitant

enhancement of the HPA axis activity and increased circulating FFA levels (15–22).

Anorexia nervosa is a complex psychiatric disorder characterized by a pathological fear of weight gain which results in an extremely disturbed and restricted eating pattern. Patients usually suffer all the medical effects of starvation. A variety of neuroendocrine abnormalities has been shown in anorexia nervosa, including sustained hypercortisolism (23–26). Changes in cortisol half-life and/or reduction of its clearance metabolic rate have been reported (24, 26–28). Moreover, primary neuroendocrine abnormalities in the control of the HPA axis have also been considered (29–32). Elevated corticotropin-releasing hormone (CRH) levels have been shown in the cerebrospinal fluid of anorectic patients (31, 33) and a cortisol resistance to the dexamethasone suppression test is quite common in these subjects, suggesting that the sensitivity to the glucocorticoid feedback action is impaired (34–36). HPA axis hyperactivity in anorexia nervosa is not quickly turned off by refeeding (29) which might indicate the existence of some primitive central HPA alterations strictly related to the pathogenesis of this illness and not simply to starvation.

As patients with anorexia nervosa display concomitant alterations in HPA axis activity and lipolysis (20, 21), we studied the effects of the acute increase in FFA levels induced by an intravenous lipid load on ACTH and cortisol secretion in patients with anorexia nervosa in comparison with normal subjects.

Subjects and methods

Eight women with anorexia nervosa (ANW; means $\pm$ S.E.M.: 23.9 ± 2.3 years of age; body mass index (BMI): 14.9 ± 0.6 kg/m²; body surface: 1.38 ± 0.04 m²) took part in the study. All ANW patients were of the restrictive type in the acute phase of the illness and met the diagnostic criteria for anorexia nervosa according to the Diagnostic and Statistical Manual of Mental Disorders IV (37). Mean $\pm$ S.E.M. duration of the disease

was 2.4 ± 0.8 years (range: 1.0–8.0 years). None of the patients had a clinical history of depression or evidence of other diseases. None had received drugs interfering with the HPA axis activity for at least 1 month before the study. In particular, none was self-administering psychoactive drugs. None declared the use of laxatives and/or diuretics, or episodes of self-induced vomiting during the last month before the study. None presented with abnormal glucose or blood pressure levels. Body weight had been stable for at least 1 month prior to the study. Clinical details of ANW are reported in Table 1. Seven healthy age-matched normal women (NW; 25.6 ± 2.3 years of age; BMI: 22.8 ± 1.9 kg/m²; body surface: 1.64 ± 0.04 m²) were studied as controls; they were in their early follicular phase. All subjects gave their written informed consent to their participation in the study, which had been approved by an independent ethical committee of the University of Turin.

Each subject underwent the following tests: (a) i.v. saline infusion (100 ml/h 0.9% NaCl solution) from 0 to 180 min; (b) i.v. 10% lipid-heparin emulsion (LHE) infusion (100 ml/h Intralipid (Fresenius, Kabi, Italy), together with 10 U/ml heparin, corresponding to 214.4 kcal) from 0 to 120 min, followed by saline infusion (100 ml) from 120 to 180 min. Intralipid is a suspension of soybean oil and glycerol; 1000 ml Intralipid 10% contain purified soybean oil, 100 g; purified egg phospholipids, 12 g; glycerol anhydrous, 22 g; water for injection *quam sufficit ad*, 1000 ml. pH was adjusted with sodium hydroxide to approximately 8. During Intralipid infusion, an increase in circulating FFAs is generated from this suspension after the activation of lipoprotein lipase. To increase the lipoprotein lipase activity, heparin was added to the suspension. We chose an Intralipid dose equivalent to that used in many studies focusing on the effects of FFAs on GH secretion in normal subjects and anorectic patients (1, 3, 4, 38) and on HPA axis activity in normal subjects (11) and which showed a clear inhibitory effect on both somatotroph and corticotroph function. Blood samples were taken every 30 min from 0 to 180 min.

Table 1 Clinical details of patients with anorexia nervosa.

Case (n)	Age (years)	Weight (kg)	Height (m)	BMI (kg/m ²)	Body surface (m ²)	Duration of disease (years)
1	19	37.4	1.60	14.6	1.33	1.0
2	21	36.5	1.62	13.9	1.33	1.5
3	28	40.0	1.63	15.0	1.38	1.5
4	38	43.8	1.61	16.9	1.43	8.0
5	25	32.2	1.57	13.0	1.23	3.0
6	21	34.7	1.65	12.7	1.31	1.0
7	20	46.0	1.72	15.5	1.53	1.5
8	19	47.0	1.64	17.5	1.49	2.0
Mean	23.9	39.7	1.63	14.9	1.38	2.4
S.D.	6.5	5.4	0.04	1.7	0.10	2.3
S.E.M.	2.3	1.9	0.02	0.6	0.04	0.8

FFA, ACTH, cortisol, glucose and insulin levels were measured at each time-point. All tests were performed in the morning between 0830 and 0900 h, after overnight fasting and 30 min after insertion of an indwelling catheter in a forearm vein which was kept patent by slow infusion of isotonic saline. The two tests were performed in random order and at least 5 days apart. Plasma FFA levels ($\text{mEq/l} \times 282 = 1 \text{ mg/l}$) were measured by enzymatic analysis using the NEFA QUICK BMY kit (Roche Molecular Biochemicals, Yamanouchi, Tokyo, Japan). Plasma ACTH levels (pg/ml ; $1 \text{ pg/ml} \times 0.22 = 1 \text{ pmol/l}$) were measured in duplicate by immunoradiometric assay (Allegro HS-ACTH; Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Assay sensitivity was 1 pg/ml . Inter- and intra-assay variation coefficients ranged from 6.9 to 8.9% and from 1.1 to 3.0% respectively. Serum cortisol levels ($\mu\text{g/dl}$; $1 \mu\text{g/dl} \times 27.59 = 1 \text{ nmol/l}$) were measured in duplicate by RIA (CORT-CTK 125 RIA; Sorin Biomedica, Saluggia, Italy). Assay sensitivity was $0.4 \mu\text{g/dl}$. Inter- and intra-assay variation coefficients ranged from 6.6 to 7.5% and from 3.8 to 6.6% respectively. Plasma glucose levels (mg/dl ; $1 \text{ mg/dl} \times 0.05 = 1 \text{ mmol/l}$) were measured by a gluco-oxidase colorimetric method (GLUCOFIX; Menarini Diagnostici, Florence, Italy). Serum insulin levels (mU/l ; $1 \text{ mU/l} \times 7.17 = 1 \text{ pmol/l}$) were measured in duplicate by IRMA (INSIK-5; Sorin Biomedica). Assay sensitivity was 2.5 mU/l . Inter- and intra-assay variation coefficients ranged from 6.2 to 10.8% and from 5.5 to 10.6% respectively. All samples from an individual subject were measured in the same assay.

The hormonal response to saline or LHE infusion within each group was analysed using Wilcoxon

matched-pair signed rank test. Mann–Whitney U test was used to show differences between normal subjects and anorectic patients. Differences with a P value < 0.05 were considered statistically significant. All statistical analyses were performed with SPSS for Windows version 11.0 (SPSS, Chicago, IL, USA). Data are expressed as means $\pm$ S.E.M. of absolute values or of areas under the curves (AUC or ΔAUC) calculated by trapezoidal integration.

Results

Baseline cortisol levels in both sessions were higher in ANW than in NW ($P < 0.05$) while ACTH levels were not significantly different. Insulin levels were lower in ANW than in NW ($P < 0.05$); glucose and FFA levels were similar in the two groups (Figs. 1–5).

During saline infusion, ACTH and cortisol levels showed a spontaneous decrease both in NW and in ANW (Figs. 2 and 3). The ACTH and cortisol AUCs over 180 min of saline infusion were higher in ANW than in NW (ACTH: 3161.6 ± 303.5 vs $2012.1 \pm 172.6 \text{ pg/ml per h}$, $P < 0.05$; cortisol: 3171.9 ± 304.1 vs $1679.4 \pm 88.8 \mu\text{g/dl per h}$, $P < 0.01$) (data not shown). During saline infusion, insulin AUCs were lower in ANW than in NW (1509.3 ± 202.5 vs $2143.0 \pm 181.6 \text{ mU/l per h}$, $P < 0.05$); glucose and FFA AUCs in ANW ($11129.9 \pm 386.7 \text{ mg/dl per h}$ and $58.1 \pm 15.9 \text{ mEq/l per h}$) were similar to those in NW ($11183.2 \pm 360.8 \text{ mg/dl per h}$ and $52.7 \pm 11.5 \text{ mEq/l per h}$) (data not shown).

Figure 1 Mean $\pm$ S.E.M. (top panels) FFA curves and (bottom panels) ΔAUC during i.v. saline (○ and open bars) or LHE infusion (Intralipid 10%; ● and solid bars) in NW and ANW. FFA, $1 \text{ mEq/l} \times 282 = 1 \text{ mg/l}$. * $P < 0.005$ vs saline.

Figure 2 Mean $\pm$ S.E.M. (top panels) ACTH curves and (bottom panels) Δ AUC during i.v. saline (○ and open bars) or LHE infusion (Intralipid 10%; ● and solid bars) in NW and ANW. ACTH, 1 pg/ml $\times$ 0.22 = 1 pmol/l. * $P < 0.05$ vs saline.

LHE infusion led to a prompt and sustained increase in FFA levels both in NW (Δ AUC₀₋₁₂₀: 338.4 ± 61.6 vs 14.0 ± 6.7 mEq/l per h, $P < 0.005$) and in ANW (330.4 ± 79.9 vs 11.2 ± 5.8 mEq/l per h, $P < 0.005$) (Fig. 1). Under lipid load the ACTH decrease in NW was significantly higher than that recorded during

saline infusion (Δ AUC₀₋₁₂₀: -871.5 ± 295.4 vs -405.6 ± 198.9 pg/ml per h, $P < 0.05$) (Fig. 2). In the same subjects, the cortisol decrease under lipid load was also significantly higher than that recorded during saline infusion (Δ AUC₀₋₁₂₀: -4285.5 ± 429.0 vs -2951.1 ± 645.4 μ g/dl per h, $P < 0.05$) (Fig. 3).

Figure 3 Mean $\pm$ S.E.M. (top panels) cortisol curves (top panels) and (bottom panels) Δ AUC during i.v. saline (○ and open bars) or LHE infusion (Intralipid 10%; ● and solid bars) in NW and ANW. Cortisol, 1 μ g/dl $\times$ 27.59 = 1 nmol/l. * $P < 0.05$ vs saline.

Figure 4 Mean $\pm$ S.E.M. (top panels) glucose curves (top panels) and (bottom panels) Δ AUC during i.v. saline ($\circ$ and open bars) or LHE infusion (Intralipid 10%; $\bullet$ and solid bars) in NW and ANW. Glucose, 1 mg/dl $\times$ 0.05 = 1 mmol/l. * P < 0.05 vs saline.

The lipid load in ANW was associated with a more pronounced decrease in ACTH levels compared with that recorded during saline infusion (Δ AUC₀₋₁₂₀: -680.8 ± 195.1 vs -436.3 ± 212.2 pg/ml per h, P < 0.05) (Fig. 2) while cortisol levels were not modified by the lipid load-induced FFA increase (Δ AUC₀₋₁₂₀: -2083.3 ± 1266.7 vs -5006.3 ± 1647.4 μ g/dl per h) (Fig. 3).

The ACTH decrease during LHE infusion in NW was similar to that recorded in ANW, while cortisol decrease during LHE infusion was higher in NW than in ANW (P < 0.05) (Figs. 2 and 3).

At the end of lipid infusion, FFA concentrations decreased in NW (P < 0.05) and this was associated with an increase in ACTH and cortisol levels (P < 0.05), which reached values similar to those

Figure 5 Mean $\pm$ S.E.M. (top panels) insulin curves and (bottom panels) Δ AUC during i.v. saline ($\circ$ and open bars) or LHE infusion (Intralipid 10%; $\bullet$ and solid bars) in NW and ANW. Insulin, 1 mU/l $\times$ 7.17 = 1 pmol/l.

recorded during the saline session (Figs. 1–3). On the contrary, at the end of lipid infusion in ANW, FFA levels underwent a slower decrease and persisted at a higher level than at baseline ($P < 0.05$) (Fig. 1). Accordingly, ACTH levels persisted at a level lower than those recorded at the same time-points during saline infusion ($P < 0.05$) while cortisol levels showed no significant variations (Figs. 2 and 3). LHE infusion led to a progressive and significant increase in glucose levels in NW (ΔAUC_{0-120} : 915.0 ± 436.3 vs -100.0 ± 165.6 mg/dl per h, $P < 0.05$) but not in ANW (ΔAUC_{0-120} : 366.4 ± 351.0 vs 426.4 ± 214.3 mg/dl per h) (Fig. 4). On the other hand, LHE infusion did not significantly change insulin secretion either in NW or in ANW (Fig. 5).

Side-effects

No side-effects were observed during LHE infusion either in ANW or in NW.

Discussion

The results of the present study showed that the modulation of HPA axis by FFAs is at least partially preserved in anorexia nervosa. In fact, while an LHE-induced increase in FFA levels in NW negatively influenced both ACTH and cortisol secretion, it maintained its inhibitory effect on corticotroph secretion but did not affect cortisol hypersecretion in anorectic patients. Moreover, while ACTH and cortisol levels were restored to baseline values at the end of the lipid infusion in NW, the lipid load withdrawal in the ANW was followed by delayed FFA clearance and persistent inhibition of ACTH levels coupled with unchanged cortisol hypersecretion.

Anorexia nervosa is characterized by several endocrine abnormalities including HPA axis hyperactivity (23–26). In fact, urinary free cortisol excretion as well as serum cortisol levels in anorectic patients may be similar to those recorded in patients with Cushing's disease or severe depression (29). HPA axis hyperactivity in anorectic patients was also confirmed by our present study showing that ACTH and cortisol levels over 180 min in the morning were significantly higher than those in normal young women. Various mechanisms could explain HPA axis hyperactivity in anorexia nervosa. Changes in cortisol half-life and/or reduction of its clearance metabolic rate have been reported, suggesting alterations in peripheral glucocorticoid metabolism (24, 26–28). On the other hand, an increased frequency of cortisol secretory bursts leading to increased pulsatile and total cortisol secretion has been demonstrated (26), suggesting an adrenal hyperfunction that is likely to reflect a corticotroph hypersecretion. In fact, elevated cortisol levels in anorexia nervosa are coupled with ACTH concentrations that

are not inhibited, although an inverse relationship between cortisol and ACTH levels has been recorded in patients with this eating disorder (32, 39). This picture fits in with our present findings showing that elevated cortisol levels in ANW are coupled with relatively higher ACTH levels in comparison with NW. Overall, ACTH and cortisol hypersecretion in anorexia nervosa is likely to reflect a central HPA axis hyperactivation (25, 32). Hyperactivity of CRH- and/or arginine vasopressin-secreting neurons as well as derangement in the hypothalamic and/or supra-hypothalamic mechanisms of negative glucocorticoid feedback action should be considered in this context (25, 31, 32). Lipid metabolism is altered in anorexia nervosa besides many other malnutrition disorders in which lipolysis is generally hyperactivated (20, 40). Lipids, namely FFAs, are also known to play a major role also in controlling hypothalamus–pituitary function, which applies to somatotroph and gonadal axis control but also to the HPA axis (2, 3, 7, 41–43). Indeed, a stimulatory effect exerted by high FFA concentrations upon ACTH secretion from rat pituitary *in vitro* has been reported, suggesting a positive feedback link between lipids and the HPA axis (7). FFAs possess an electrophysiological effect on the central nervous system where they can be incorporated by central nervous system cells (44, 45). Indeed, we have demonstrated that a lipid load-induced increase in circulating FFA levels in healthy adult subjects exerts an inhibitory effect on spontaneous ACTH and cortisol secretion (11). Thus, we hypothesized some refractoriness of the enhanced HPA activity to lipid load in this pathological condition.

Surprisingly, our present findings have shown that the inhibitory effect of a lipid load-induced FFA increase on corticotroph secretion is clearly preserved in anorexia nervosa. Thus, this disorder is not accompanied by a refractoriness of the corticotroph function to the negative influence of elevated FFA levels. In fact, after lipid infusion withdrawal, a more persistent elevation in circulating FFA levels, coupled with a lack of ACTH recovery to baseline values, was present in anorectic patients. Interestingly, the inhibitory effect of the lipid load on ACTH secretion and the marked elevation of FFA levels are not connected to a significant inhibitory effect on cortisol levels in ANW. This observation is puzzling. On the one hand, it may be hypothesized that a long-lasting adrenal exposure to ACTH hypersecretion induced a chronic adrenal hyperactivity that was not promptly decreased after the acute reduction of ACTH levels. On the other hand, FFAs have been found able to directly stimulate corticosterone secretion from the adrenal glands (9). Thus, the possibility that FFA levels might positively influence peripheral glucocorticoid metabolism should also be considered. Cortisol hypersecretion refractory to the lipid load-induced inhibition of ACTH levels in anorexia nervosa could then paradoxically reflect the elevated lipid concentrations. On the other hand, circulating FFA levels in

our anorectic patients were only slightly and not significantly elevated and this could be due to the limited number of study subjects and/or to the severity of both their illness and starvation that would not allow further lipolysis given the lack of adipose tissue. Finally, some influence of heparin *per se* on ACTH secretion and/or assay has to be taken into account (46). Moreover, heparin has been shown to influence adrenal secretion and to alter corticosteroid-binding globulin binding (47–50). However, ACTH was reduced under LHE infusion in both anorectic patients and normal subjects, who also displayed concomitant decreases in cortisol levels. Furthermore, heparin concentrations were very low in the LHE solution used in our experimental model and lower than those able to influence an ACTH assay (46), making an influence of this compound on adrenal secretion unlikely.

In conclusion, the findings of our study have shown that, in patients with anorexia nervosa, the corticotroph sensitivity to the inhibitory effect of an FFA load is preserved, in spite of persistent adrenal hyperactivity. It is unlikely that the corticotroph hypersecretion of anorexia nervosa involves a reduced sensitivity to elevated FFA levels, while a somewhat adrenal effect of elevated FFA levels cannot be ruled out.

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