European Journal of Endocrinology (2006) **154** 731–738 ISSN 0804-4643

CLINICAL STUDY

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

Fabio Lanfranco, Laura Gianotti, Andreea Picu, Roberta Giordano, Giovanni Abbate Daga[1], Valeria Mondelli[1], Giuseppe Malfi[2], Secondo Fassino[1], Ezio Ghigo and Emanuela Arvat

Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Turin, Corso Dogliotti 14, I-10126 Turin, Italy, [1]Division of Psychiatry, Department of Neurosciences, University of Turin, Turin, Italy and [2]Division of Clinical Nutrition, Azienda Ospedaliera San Giovanni Battista, Turin, Italy

(Correspondence should be addressed to E Arvat; Email: emanuela.arvat@unito.it)

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±S.E.M.: 23.9±2.3 years of age; body mass index (BMI): 14.9±0.6 kg/m^2) and seven normal women (NW; 25.6±2.3 years of age; BMI: 22.8±1.9 kg/m^2) 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.

European Journal of Endocrinology **154** 731–738

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

DOI: 10.1530/eje.1.02139
Online version via www.eje-online.org

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± S.E.M.: 23.9±2.3 years of age; body mass index (BMI): 14.9±0.6 kg/m^2; body surface: 1.38±0.04 m^2) took part in the study. All ANW patients were of the restricter 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±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/m^2; body surface: 1.64±0.04 m^2) 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 (mEq/l × 282 = 1 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 pg/ml × 0.22 = 1 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 (μg/dl; 1 μg/dl × 27.59 = 1 nmol/l) were measured in duplicate by RIA (CORT-CTK 125 RIA; Sorin Biomedica, Saluggia, Italy). Assay sensitivity was 0.4 μg/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 mg/dl × 0.05 = 1 mmol/l) were measured by a gluco-oxidase colorimetric method (GLUCOFIX; Menarini Diagnostici, Florence, Italy). Serum insulin levels (mU/l; 1 mU/l × 7.17 = 1 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±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±172.6 pg/ml per h, $P < 0.05$; cortisol: 3171.9±304.1 vs 1679.4±88.8 μg/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±181.6 mU/l per h, $P < 0.05$); glucose and FFA AUCs in ANW (111 29.9±386.7 mg/dl per h and 58.1±15.9 mEq/l per h) were similar to those in NW (111 83.2±360.8 mg/dl per h and 52.7±11.5 mEq/l per h) (data not shown).

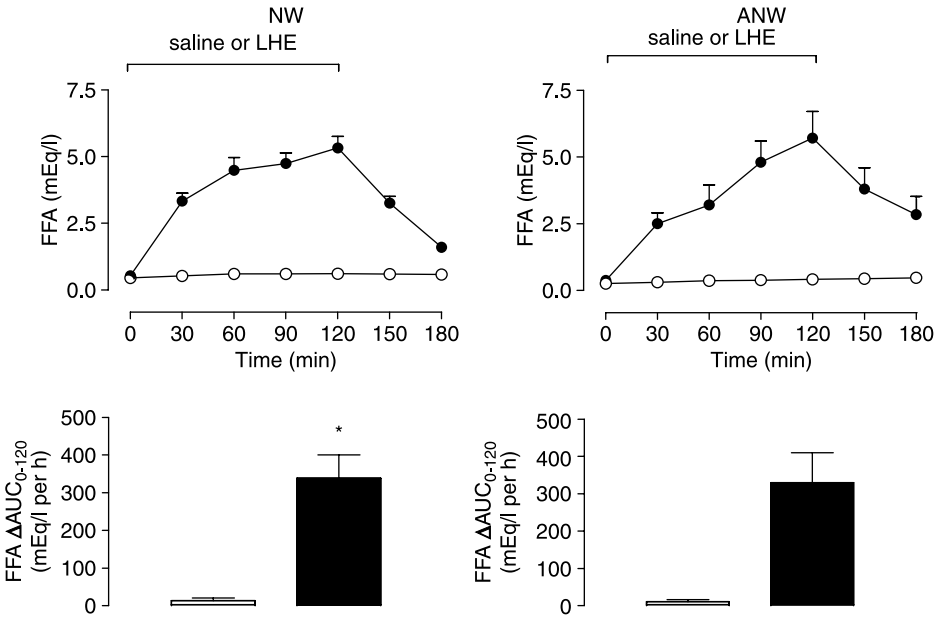

Figure 1 Mean±S.E.M. (top panels) FFA curves and (bottom panels) ΔAUC during i.v. saline (○ and open bars) or LHE infusion (Intralipid 10%; ● and solid bars) in NW and ANW. FFA, 1 mEq/l × 282 = 1 mg/l. *$P < 0.005$ vs saline.

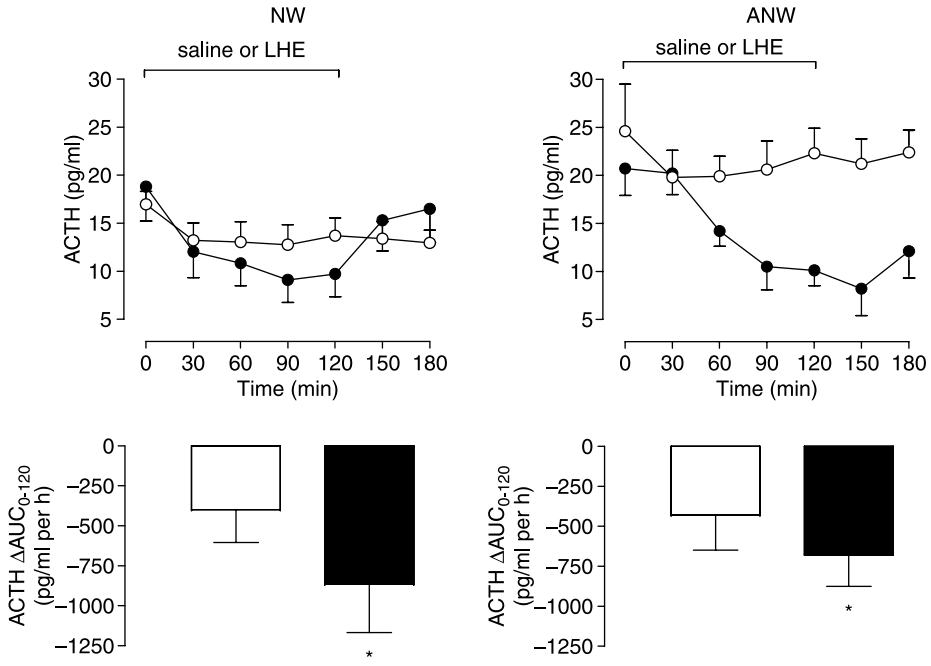

Figure 2 Mean±S.E.M. (top panels) ACTH curves and (bottom panels) ΔAUC during i.v. saline (○ and open bars) or LHE infusion (Intralipid 10%; ● and solid bars) in NW and ANW. ACTH, 1 pg/ml × 0.22 = 1 pmol/l. *$P < 0.05$ vs saline.

LHE infusion led to a prompt and sustained increase in FFA levels both in NW (ΔAUC_{0-120}: 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 (ΔAUC_{0-120}: −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 (ΔAUC_{0-120}: −4285.5±429.0 vs −2951.1±645.4 μg/dl per h, $P < 0.05$) (Fig. 3).

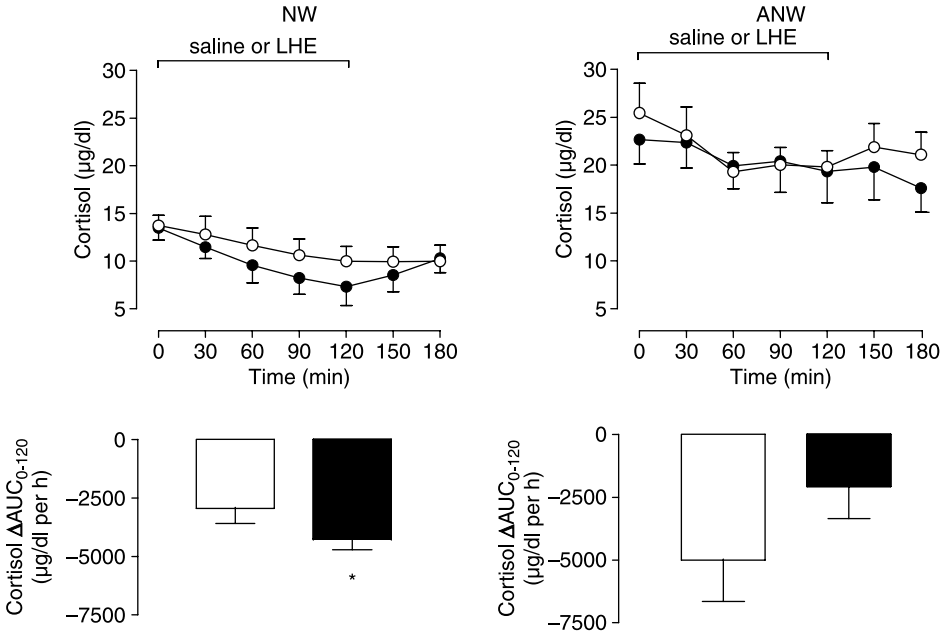

Figure 3 Mean±S.E.M. (top panels) cortisol curves (top panels) and (bottom panels) ΔAUC during i.v. saline (○ and open bars) or LHE infusion (Intralipid 10%; ● and solid bars) in NW and ANW. Cortisol, 1 μg/dl × 27.59 = 1 nmol/l. *$P < 0.05$ vs saline.

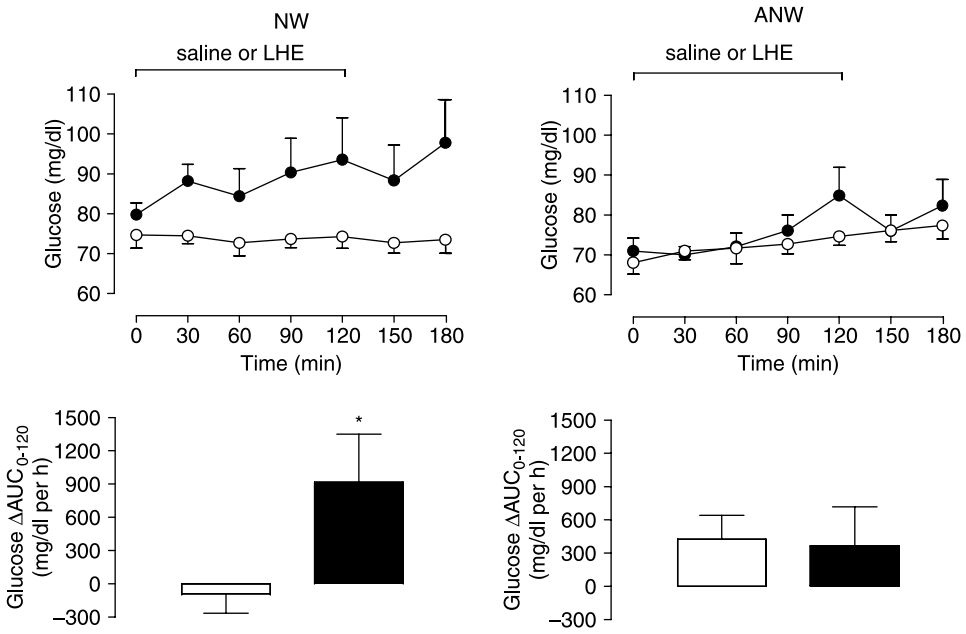

Figure 4 Mean±S.E.M. (top panels) glucose curves (top panels) and (bottom panels) ΔAUC during i.v. saline (○ and open bars) or LHE infusion (Intralipid 10%; ● and solid bars) in NW and ANW. Glucose, 1 mg/dl × 0.05 = 1 mmol/l. *$P < 0.05$ vs saline.

The lipid load in ANW was associated with a more pronounced decrease in ACTH levels compared with that recorded during saline infusion (ΔAUC_{0-120}: -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 (ΔAUC_{0-120}: -2083.3 ± 1266.7 vs -5006.3 ± 1647.4 μg/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

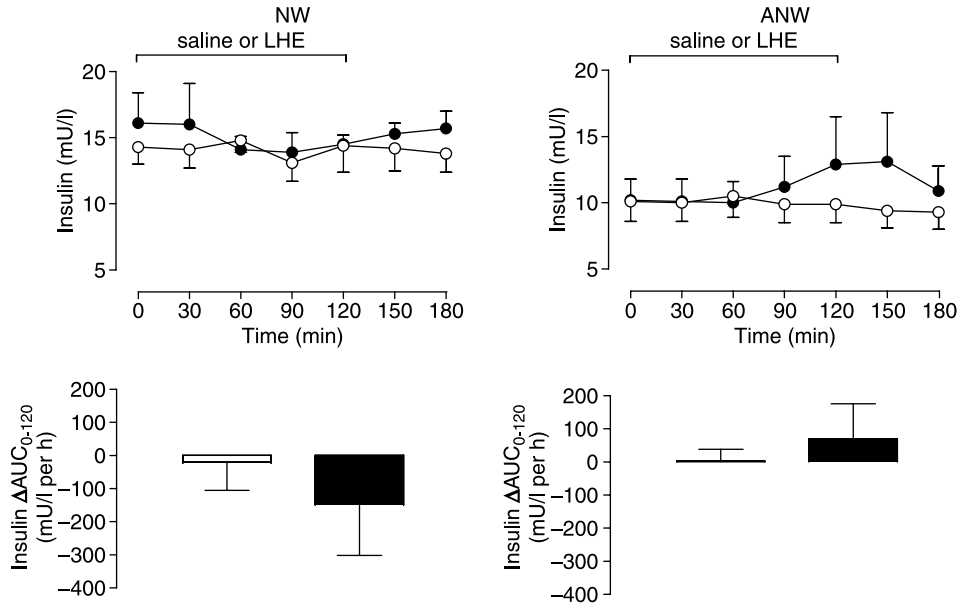

Figure 5 Mean±S.E.M. (top panels) insulin curves and (bottom panels) ΔAUC during i.v. saline (○ and open bars) or LHE infusion (Intralipid 10%; ● and solid bars) in NW and ANW. Insulin, 1mU/l × 7.17 = 1 pmol/l.

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.

Acknowledgements

The authors wish to thank Dr A Bertagna, A Barberis and M Taliano for technical assistance and Linda Massari for language editing of the manuscript. This work was supported by the University of Turin and the Foundation for the Study of Endocrinological and Metabolic Diseases.

References

1 Imaki T, Shibasaki T, Shizume K, Masuda A, Hotta M, Kiyosawa Y, Jibiki K, Demura H, Tsushima T & Ling N. The effect of free fatty acids on growth hormone (GH)-releasing hormone-mediated GH secretion in man. *Journal of Clinical Endocrinology and Metabolism* 1985 **60** 290–293.

2 Imaki T, Shibasaki T, Masuda A, Hotta M, Yamauchi N, Demura H, Shizume K, Wakabayashi I & Ling N. The effect of glucose and free fatty acids on growth hormone(GH)-releasing factor-mediated GH secretion in rats. *Endocrinology* 1986 **118** 2390–2394.

3 Maccario M, Procopio M, Loche S, Cappa M, Martina V, Camanni F & Ghigo E. Interaction of free fatty acids and arginine on growth hormone secretion in man. *Metabolism* 1994 **43** 223–226.

4 Casanueva FF, Villanueva L, Dieguez C, Diaz Y, Cabranes JA, Szoke B, Scanlon MF, Schally AV & Fernandez-Cruz A. Free fatty acids block growth hormone (GH) releasing hormone-stimulated GH secretion in man directly at the pituitary. *Journal of Clinical Endocrinology and Metabolism* 1987 **65** 634–642.

5 Alvarez CV, Mallo F, Burguera B, Cacicedo L, Dieguez C & Casanueva FF. Evidence for a direct pituitary inhibition by free fatty acids of *in vivo* growth hormone responses to growth hormone-releasing hormone in the rat. *Neuroendocrinology* 1991 **53** 185–189.

6 Perez FR, Camina JP, Zugaza JL, Lage M, Casabiell X & Casanueva FF. cis-FFA do not alter membrane depolarization but block Ca2 + influx and GH secretion in KCl-stimulated somatotroph cells. Suggestion for a direct cis-FFA perturbation of the Ca2 + channel opening. *Biochimica et Biophysica Acta* 1997 **1329** 269–277.

7 Widmaier EP, Rosen K & Abbott B. Free fatty acids activate the hypothalamic-pituitary-adrenocortical axis in rats. *Endocrinology* 1992 **131** 2313–2318.

8 Goodfriend TL, Ball DL, Elliott ME, Morrison AR & Evenson MA. Fatty acids are potential endogenous regulators of aldosterone secretion. *Endocrinology* 1991 **128** 2511–2519.

9 Sarel I & Widmaier EP. Stimulation of steroidogenesis in cultured rat adrenocortical cells by unsaturated fatty acids. *American Journal of Physiology* 1995 **268** R1484–R1490.

10 Matthys LA & Widmaier EP. Fatty acids inhibit adrenocorticotropin-induced adrenal steroidogenesis. *Hormone and Metabolic Research* 1998 **30** 80–83.

11 Lanfranco F, Giordano R, Pellegrino M, Gianotti L, Ramunni J, Picu A, Baldi M, Ghigo E & Arvat E. Free fatty acids exert an inhibitory effect on adrenocorticotropin and cortisol secretion in humans. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 1385–1390.

12 Bjorntorp P. Neuroendocrine perturbations as a cause of insulin resistance. *Diabetes/Metabolism Research and Reviews* 1992 **15** 427–441.

13 Ottosson M, Vikman-Adolfsson K, Enerback S, Olivecrona G & Bjorntorp P. The effects of cortisol on the regulation of lipoprotein lipase activity in human adipose tissue. *Journal of Clinical Endocrinology and Metabolism* 1994 **79** 820–825.

14 Dimitriadis G, Leighton B, Parry-Billings M, Sasson S, Young M, Krause U, Bevan S, Piva T, Wegener G & Newsholme EA. Effects of glucocorticoid excess on the sensitivity of glucose transport and metabolism to insulin in rat skeletal muscle. *Biochemical Journal* 1997 **321** 707–712.

15 Casper RC, Pandey G, Jaspan JB & Rubenstein AH. Eating attitudes and glucose tolerance in anorexia nervosa patients at 8-year followup compared to control subjects. *Psychiatry Research* 1988 **25** 283–299.

16 Akana SF, Strack AM, Hanson ES & Dallman MF. Regulation of activity in the hypothalamopituitary-adrenal axis is integral to a larger hypothalamic system that determines caloric flow. *Endocrinology* 1994 **135** 1125–1134.

17 Casper RC. Carbohydrate metabolism and its regulatory hormones in anorexia nervosa. *Psychiatry Research* 1996 **62** 85–96.

18 Bjorntorp P. Body fat distribution, insulin resistance, and metabolic diseases. *Nutrition* 1997 **13** 795–803.

19 Pasquali R. Is the hypothalamic-pituitary-adrenal axis really hyperactivated in visceral obesity? *Journal of Endocrinological Investigation* 1998 **21** 268–271.

20 Stoving RK, Hangaard J, Hansen-Nord M & Hagen C. A review of endocrine changes in anorexia nervosa. *Journal of Psychiatric Research* 1999 **33** 139–152.

21 Douyon L & Schteingart DE. Effect of obesity and starvation on thyroid hormone, growth hormone, and cortisol secretion. *Endocrinology and Metabolism Clinics of North America* 2002 **31** 173–189.

22 Alrefai H, Allababidi H, Levy S & Levy J. The endocrine system in diabetes mellitus. *Endocrine* 2002 **18** 105–119.

23 Seed JA, Dixon RA, McCluskey SE & Young AH. Basal activity of the hypothalamic-pituitary-adrenal axis and cognitive function in anorexia nervosa. *European Archives of Psychiatry and Clinical Neuroscience* 2000 **250** 11–15.

24 Stoving RK, Hangaard J & Hagen C. Update on endocrine disturbances in anorexia nervosa. *Journal of Pediatric Endocrinology and Metabolism* 2001 **14** 459–480.

25 Lanfranco F, Gianotti L, Destefanis S, Arvat E, Ghigo E & Camanni F. Endocrine abnormalities in anorexia nervosa. *Minerva Endocrinologica* 2003 **28** 169–180.

26 Misra M, Miller KK, Almazan C, Ramaswamy K, Lapcharoensap W, Worley M, Neubauer G, Herzog DB & Klibanski A. Alterations in cortisol secretory dynamics in adolescent girls with anorexia nervosa and effects on bone metabolism. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 4972–4980.
27 Boyar RM, Hellman LD, Roffwarg HP, Katz J, Zumoff B, O'Connor J, Bradlow HL & Fukushima DK. Cortisol secretion and metabolism in anorexia nervosa. *New England Journal of Medicine* 1977 **296** 190–193.
28 Vierhapper H, Kiss A, Nowotry P, Wiesnagrotzki S, Monder C & Waldhausl W. Metabolism of cortisol in anorexia nervosa. *Acta Endocrinologica* 1990 **122** 753–758.
29 Gold PW, Gwirtsman HE, Avgerinos PC, Nieman LK, Gallucci WT, Kaye W, Jimerson D, Ebert M, Rittmaster R, Loriaux DL & Chrousos GP. Abnormal hypothalamic-pituitary-adrenal function in anorexia nervosa: pathophysiologic mechanisms in underweight and weight-corrected patients. *New England Journal of Medicine* 1986 **314** 1335–1342.
30 Cavagnini F, Invitti C, Passamonti M & Polli EE. Response of ACTH and cortisol to corticotropin-releasing hormone in anorexia nervosa. *New England Journal of Medicine* 1986 **314** 184–185.
31 Kaye WH, Gwirtsman HE, George DT, Ebert MH, Jimerson DC, Tomai TP, Chrousos GP & Gold PW. Elevated cerebrospinal fluid levels of immunoreactive corticotropin-releasing hormone in anorexia nervosa: relation to state of nutrition, adrenal function, and intensity of depression. *Journal of Clinical Endocrinology and Metabolism* 1987 **64** 203–208.
32 Licinio J, Wong ML & Gold PW. The hypothalamic-pituitary-adrenal axis in anorexia nervosa. *Psychiatry Research* 1996 **62** 75–83.
33 Hotta M, Shibasaki T, Masuda A, Imaki T, Demura H, Ling N & Shizume K. The responses of plasma adrenocorticotropin and cortisol to corticotropin-releasing hormone (CRH) and cerebrospinal fluid immunoreactive CRH in anorexia nervosa patients. *Journal of Clinical Endocrinology and Metabolism* 1986 **62** 319–324.
34 Estour B, Pugeat M, Lang F, Lejeune H, Broutin F, Pellet J, Rousset H & Tourniaire J. Rapid escape of cortisol from suppression in response to i.v. dexamethasone in anorexia nervosa. *Clinical Endocrinology* 1990 **33** 45–52.
35 Schweitzer I, Szmkler GI, Maguire KP, Harrison LC, Tuckwell V & Davies BM. The dexamethasone suppression test in anorexia nervosa: the influence of weight, depression, adrenocorticotrophic hormone and dexamethasone. *British Journal of Psychiatry* 1990 **157** 713–717.
36 Duclos M, Corcuff J-B, Roger P & Tabarin A. The dexamethasone-suppressed corticotrophin-releasing hormone stimulation test in anorexia nervosa. *Clinical Endocrinology* 1999 **51** 725–731.
37 American Psychiatric Association. *DSM-IV Diagnostic and Statistical Manual of Mental Disorders*, ed. 4, p. 539. Washington DC: American Psychiatric Press, 1994.
38 Gianotti L, Fassino S, Abbate Daga G, Lanfranco F, De Bacco C, Ramunni J, Arvat E, Maccario M & Ghigo E. Effects of free fatty acids and acipimox, a lipolysis inhibitor, on the somatotroph responsiveness to GHRH in anorexia nervosa. *Clinical Endocrinology* 2000 **52** 713–720.
39 Gwirtsman HE, Kaye WH, George DT, Jimerson DC, Ebert MH & Gold PW. Central and peripheral ACTH and cortisol levels in anorexia nervosa and bulimia. *Archives of General Psychiatry* 1989 **46** 61–69.
40 Coppack SW, Patel JN & Lawrence VJ. Nutritional regulation of lipid metabolism in human adipose tissue. *Experimental and Clinical Endocrinology and Diabetes* 2001 **109** S202–S214.
41 Senaris RM, Lewis MD, Lago F, Dominguez F, Scanlon MF & Dieguez C. Effects of free fatty acids on somatostatin secretion, content and mRNA levels in cortical and hypothalamic fetal rat neurones in monolayer culture. *Journal of Molecular Endocrinology* 1993 **10** 207–214.
42 Widmaier EP, Margenthaler J & Sarel I. Regulation of pituitary-adrenocortical activity by free fatty acids *in vivo* and *in vitro*. *Prostaglandins, Leukotrienes, and Essential Fatty Acids* 1995 **52** 179–183.
43 Archer ZA, Rhind SM, Findlay PA, Kyle CE, Thomas L, Marie M & Adam CL. Contrasting effects of different levels of food intake and adiposity on LH secretion and hypothalamic gene expression in sheep. *Journal of Endocrinology* 2002 **175** 383–393.
44 Dhopeshwarkar GA & Mead JF. Fatty acid uptake by the brain. II. Incorporation of [I-14C] palmitic acid into the adult rat brain. *Biochimica et Biophysica Acta* 1969 **187** 461–467.
45 Love JA, Saum WR & McGee R Jr. The effects of exposure to exogenous fatty acids and membrane fatty acid modification on the electrical properties of NG108-15 cells. *Cellular and Molecular Neurobiology* 1985 **5** 333–352.
46 Dupouy JP, Godaut M & Chatelain A. Influence of heparin on the radioimmunological assay of ACTH. *Annales d'Endocrinologie* 1986 **47** 429–434.
47 Kloppenborg PW, Casparie AF, Benraad TJ & Majoor CL. Inhibition of adrenal function in man by heparin or heparinoid Ro 1-8307. *Acta Medica Scandinavica* 1975 **197** 99–108.
48 Jokay I, Karczag E, Kelemenics K & Foldes I. Inhibition of cortisone action in mice by heparin. *Endokrinologie* 1979 **73** 199–208.
49 O'Kelly R, Magee F & McKenna TJ. Routine heparin therapy inhibits adrenal aldosterone production. *Journal of Clinical Endocrinology and Metabolism* 1983 **56** 108–112.
50 Haourigui M, Martin ME, Thobie N, Benassayag C & Nunez EA. Stimulation of the binding properties of adult rat corticosteroid-binding globulin by a lipolysis-induced rise in plasma free fatty acids. *Endocrinology* 1993 **133** 183–191.

Received 19 November 2005
Accepted 9 February 2006