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No effect of free fatty acids on adrenocorticotropin and cortisol secretion in healthy young men

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Abstract

Free fatty acids (FFAs) affect anterior pituitary function. However, the effect of FFAs on corticotropin (ACTH) and cortisol in humans is controversial. Thus, we assessed the effect of a pronounced increase in circulating FFA levels induced by infusion of lipid/heparin on ACTH and cortisol secretion in young men. Eight healthy male volunteers who underwent a 10-hour overnight fast were investigated. A 20% lipid/heparin or saline/heparin infusion was given at a rate of 1.5 mL/min for 6 hours. A euglycemic hyperinsulinemic clamp was performed in 6 subjects 4 hours after the start of infusion. To assess steroid metabolism, we measured ACTH, cortisol, FFAs, and urinary steroids. Lipid infusion increased FFAs (6.06 ± 0.52 vs 0.70 ± 0.23 mmol/L; $P < .005$) and induced insulin resistance (glucose infusion rate, 4.08 ± 2.15 vs 6.02 ± 2.60 mg/kg per minute; $P < .005$). Serum cortisol and plasma ACTH decreased independent of lipid/heparin or saline/heparin infusion. In addition, we found no effect of hyperinsulinemia on ACTH and cortisol levels. There were no differences in urinary free cortisol, urinary free cortisone, 5β-tetrahydrocortisol, 5α-tetrahydrocortisol, and tetrahydrocortisone. In conclusion, FFAs had no effect on basal ACTH and cortisol secretion in normal-weight young men. In addition, no alterations in urinary glucocorticoid metabolites were detected, suggesting unchanged cortisol metabolism during lipid infusion.

1. Introduction

Free fatty acids (FFAs) are known to influence anterior pituitary function. Growth hormone secretion, for instance, is down-regulated by FFAs *in men and women* [1,2]. Several studies on *human* subjects demonstrated that a hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis is associated with an elevation of circulating FFA levels [3,4]. Thus, it was assumed that FFAs were causative for the activation of the HPA axis in the metabolic syndrome. A dose-related increase in corticotropin (ACTH) and corticosterone levels was found after the infusion of lipid emulsion or high-fat diet in rats [5-7], suggesting an activation of HPA axis by FFAs. Moreover, Kok and coworkers [8] demonstrated a blunted ACTH release in obese women after reduction of circulating FFAs by acipimox treatment. One action of FFAs *in rats* seems to be at the hypothalamic or pituitary level of the HPA axis [5]. In addition, FFAs, especially long-chain unsaturated FFAs, directly stimulated the steroidogenesis from cultured rat adrenocortical cells [9].

However, studies evaluating the effects of meal composition on the HPA axis showed that high-fat meals, as opposed to high-protein meals, did not modify spontaneous cortisol secretion in *men* [10]. Similarly, oral fat load did not modify the cortisol response to stress in healthy subjects [11].

In contrast to the direct stimulatory effect of FFAs on cultured *rat* adrenocortical cells, some data exist that suggest an inhibitory action of FFAs on the adrenocortical

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steroidogenic response to ACTH stimulation [9,12]. Furthermore, in vitro cultured *rat* pituitary cells were relatively unaffected by FFAs except at very high concentrations: neither basal ACTH secretion nor ACTH response to corticotropin-releasing hormone (CRH) was increased by FFAs [13]. In vivo, Lanfranco and coworkers [14] demonstrated decreased ACTH and cortisol levels in young lean female volunteers during lipid infusion compared with saline infusion. Because lipid load did not affect the ACTH and cortisol responses to human CRH (hCRH), a hypothalamic down-regulation of the HPA axis by FFAs was suggested.

In summary, the influence, if any, of FFA on HPA axis activity in humans remains controversial. Thus, this study assessed whether FFAs have an effect on basal ACTH and cortisol secretion and metabolism in vivo, suggesting the existence of a control of corticotrope function by lipids. We therefore studied the effect of a pronounced increase in circulating FFA levels, induced by a lipid infusion, on ACTH and cortisol secretion in normal-weight young men.

2. Subjects and methods

2.1. Subjects

In a prospective trial, 8 healthy male volunteers who underwent a 10-hour overnight fast were investigated. The subjects' anthropometric characteristics and metabolic parameters are shown in Table 1. None of the subjects had a history of hypertension, type 2 diabetes mellitus, renal or liver disease, dyslipidemia, heart failure, or a family history of diabetes or any other endocrine disorder, and none was taking any medication. All participants were initially screened to rule out any other systemic disease or biochemical evidence of impaired hepatic or renal function. Diabetes, impaired glucose tolerance, or impaired fasting glucose was ruled out by using an oral glucose tolerance test [15]. Volunteers' weight was stable for at least 2 months. Informed written consent was obtained from each participant after we explained the nature, purpose, and potential risks of these studies. The study protocol was approved by the institutional review board of the Charité Medical School, Campus Benjamin Franklin (Berlin, Germany).

Table 1
Study subjects

	Mean ± SEM
Age (y)	27.5 ± 1.3
Weight (kg)	87.1 ± 5.0
Height (cm)	181.0 ± 2.3
BMI (kg/m^2)	26.5 ± 1.1
WHR	0.95 ± 0.03
Cholesterol (mmol/L)	5.0 ± 0.3
HDL cholesterol (mmol/L)	1.4 ± 0.1
LDL cholesterol (mmol/L)	3.0 ± 0.2
Triglycerides (mmol/L)	1.4 ± 0.2
Protein (g/L)	65.6 ± 2.3
CRP (mg/L)	0.9 ± 0.4
Creatinine (μmol/L)	85.8 ± 3.0
Fasting insulin (mU/L)	6.1 ± 1.1
Fasting glucose (mmol/L)	5.3 ± 0.2
2-h Glucose (mmol/L)	4.7 ± 0.5
Systolic blood pressure (mm Hg)	130.6 ± 2.7
Diastolic blood pressure (mm Hg)	77.5 ± 3.7

Data are expressed as means ± SEM. BMI indicates body mass index; WHR, waist-to-hip ratio; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein.

2.2. Study design

Each subject was studied with a 2-day protocol in random order. The studies were performed at intervals of at least 2 days to avoid interactions between study procedures. After the volunteers had undergone a 10-hour overnight fast, a short polyethylene catheter was inserted at 8:00AM into an antecubital vein for infusion of test substances. Another catheter was placed into a contralateral forearm vein for blood sampling.

2.3. Euglycemic hyperinsulinemic clamp with lipid or saline infusion

On the first day, 0.9% saline infusion plus heparin (0.4 U/kg per minute) was infused at a rate of 1.5 mL/min for 6 hours. Four hours after the start of saline/heparin infusion, a euglycemic hyperinsulinemic clamp [16] was performed in 6 subjects. This started at noon and continued for at least 2.5 hours; 40 mIU/m^2 per minute human insulin (Actrapid, Novo Nordisk, Bagsvaard, Denmark) and a variable infusion of 10% glucose (Serag Wiessner, Naila, Germany) were used during the process. The priming dose of insulin was calculated as previously described [16]. Capillary glucose concentration was monitored every 5 minutes and was maintained between 4.0 and 4.9 mmol/L via variation of glucose infusion rate (GIR).

On the second day, the same procedure was carried out. However, instead of saline/heparin infusion, Abbolipid 20% (Abbott, Wiesbaden, Germany) plus heparin (0.4 U/kg per minute) was infused at a rate of 1.5 mL/min for 6 hours.

Blood samples for ACTH, cortisol, and FFAs were collected before, and 2 and 4 hours after start the lipid/heparin or saline/heparin *infusion and during* steady-state conditions of the euglycemic hyperinsulinemic clamp. All infusions were administered into an antecubital vein, whereas blood samples for analysis were drawn from a second antecubital vein at the contralateral arm. Blood potassium concentrations were controlled before and during the clamping procedure to avoid insulin-induced hypokalemia. Potassium substitution was not necessary in this study. Blood samples were centrifuged, and plasma and serum samples were frozen immediately at −80°C.

2.4. Urinary steroids

The balance of 5α- and 5β-reductases in vivo was assessed conventionally by calculating the urinary ratio of 5β-tetrahydrocortisol (THF)/5α-THF [17]. 5α and 5β reduction of cortisol was also assessed by the quotients 5α-THF/urinary free cortisol (UFF), 5β-THF/UFF, and 5β-tetrahydrocortisone/urinary free cortisone (THE/UFE)

[18,19]. Thus, urine was collected in 2 fractions in 6 subjects. The first batch was collected during infusion (saline/heparin or lipid/heparin) from 8:00AM to 8:00PM, and the second batch, after infusion, from 8:00PM until 8:00AM (the next day).

2.5. Laboratory tests

For measurement of ACTH, blood was sampled into iced EDTA tubes and centrifuged in a refrigerated centrifuge, then plasma was frozen immediately at −80°C. Plasma ACTH and serum cortisol were measured by sequential immunometric assays (Immulite Analyzer, Diagnostic Products, Los Angeles, CA). The analytic sensitivity of the assays was 9 pg/mL and 5.5 nmol/L, respectively.

Urinary free cortisol, UFE, 5β-THE, 5β-THF, and 5α-THF were measured by specific radioimmunoassays with the use of tritiated steroids (Amersham Pharmacia Biotech, Freiburg, Germany) and specific antibodies as previously described [20,21]. Before radioimmunoassay, UFF and UFE were extracted from urine with dichloromethane and chromatographically purified using Celite columns (Celite columns 545 AW; Sigma-Aldrich Chemie, Steinheim, Germany). Tetrahydrocortisone, THF, and 5α-THF were quantified after treatment with β-glucuronidase (Roche Diagnostics, Mannheim, Germany) in a final dilution of 1:1200 (vol/vol). Intra- and interassay coefficient of variation (CV) was less than 10% and less than 13%, respectively [20].

Capillary blood glucose was measured every 5 minutes during euglycemic hyperinsulinemic clamp by using the glucose oxidase method on a Dr Müller Super GL (Freital, Germany). Insulin was measured in plasma by enzyme-linked immunosorbent assay (DRG, Marburg, Germany). Interassay CV was 12% and intra-assay CV was 8%. Nonesterified fatty acids were quantified in serum by using a commercially available calorimetric assay (NEFA C, Wako, Neuss, Germany) performed on Cobas Mira (Roche, Basel, Switzerland). Interassay CV was 4.7%.

Potassium, sodium, serum creatinine, interleukin 6, triglycerides, cholesterol, high-density lipoprotein cholesterol, protein, C-reactive protein, urea, and glucose during glucose tolerance test were measured with standard laboratory methods. Low-density lipoprotein cholesterol was calculated by using the Friedewald formula [22].

2.6. Sample size

A type I error of 5% or less and a type II error of 20% or less were defined for sample size calculation. An SD of approximately 20% for ACTH and 23% for cortisol was observed in healthy normal-weight women by Lanfranco and colleagues [14], and differences of 27% in plasma ACTH and 25% in serum cortisol were detected between lipid/heparin and saline/heparin infusion [1]. If these parameters are assumed, the sample size needed to detect the presumed difference was 4.3 for ACTH and 6.6 for cortisol. Our trial was planned with 8 participants to detect differences at least in plasma ACTH of about 20% and in serum cortisol of about 23%.

2.7. Statistics

Statistical calculations were done with SPSS software version 11.5 from SPSS (Chicago, IL). Data were compared by paired Student t test for normally distributed data and Wilcoxon test for skewed data. In addition, profiles of FFAs, cortisol, ACTH, and insulin were compared via repeated-measures analysis of variance. Results were considered statistically significant if the 2-sided α was less than .05. Data are presented as mean ± SEM unless otherwise noted.

3. Results

3.1. Free fatty acids

In the basal state (before the start of lipid/heparin or saline/heparin infusion), there was no difference in FFAs (0.70 ± 0.23 vs 0.91 ± 0.27 mmol/L; P = NS). Lipid infusion increased FFAs from 0.70 ± 0.23 to 5.65 ± 0.69 mmol/L at 2 hours, to 6.06 ± 0.52 mmol/L at 4 hours after the start of lipid infusion, and to 4.76 ± 0.77 mmol/L during clamp under steady-state conditions (P < .001, vs before start

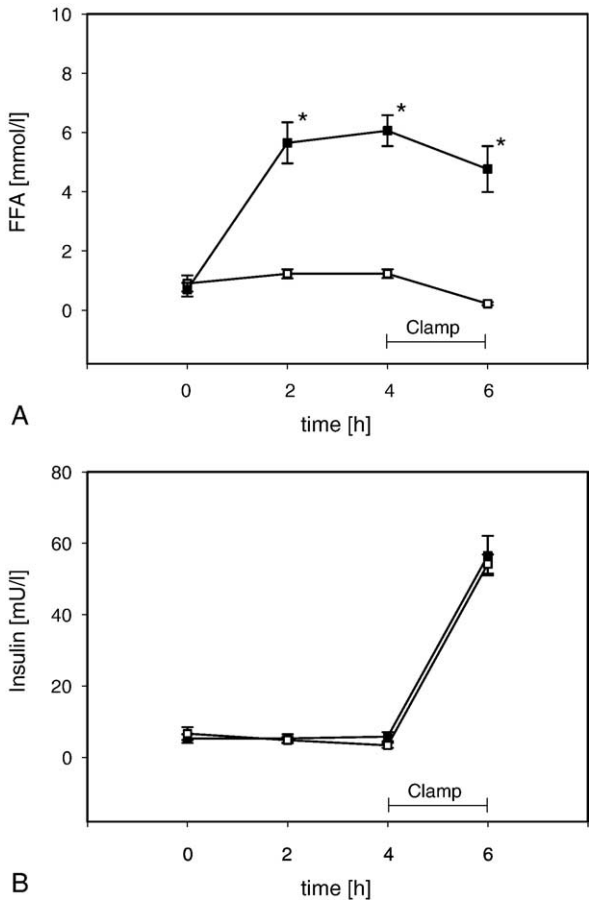

Fig. 1. FFA (A) and insulin (B) during lipid/heparin infusion (■) vs during saline/heparin infusion (□). *P < .05 vs saline/heparin infusion. Results are expressed as means ± SEM.

of lipid infusion). There was no increase in FFAs during saline/heparin infusion (0.91 ± 0.27, 1.23 ± 0.15, and 1.24 ± 0.15 mmol/L, respectively; P = NS). After insulin infusion (clamp under steady-state conditions), a decrease in FFAs was observed during saline/heparin infusion (1.24 ± 0.15 vs 0.22 ± 0.13 mmol/L; $P < .005$) (Fig. 1A).

3.2. Glucose

There were no differences in glucose levels during lipid or saline infusion both basally and 4 hours after start of infusion (5.5 ± 0.3 vs 5.3 ± 0.2 and 4.9 ± 0.2 vs 4.8 ± 0.1 mmol/L, respectively; P = NS).

3.3. Insulin

There were no significant differences between insulin levels during lipid and saline infusion before, and 2 and 4 hours after start of infusion (5.26 ± 1.21 vs 7.18 ± 1.97 mU/L; 5.31 ± 1.15 vs 4.85 ± 0.91 and 5.81 ± 1.26 vs 3.37 ± 0.70 mU/L, respectively; P = NS). During clamps, insulin levels increased significantly during both lipid and saline infusions (56.5 ± 5.6 vs 5.81 ± 1.26 and 54.2 ± 2.7 vs 3.37 ± 0.70 mU/L, respectively; $P < .001$); however, there were no differences in insulin levels during both lipid and saline infusion under steady-state conditions (Fig. 1B).

For assessment of insulin sensitivity, HOMA-IR (homeostatic model assessment) was calculated by the formula: fasting plasma glucose (mmol/L) x fasting plasma insulin (mU/L)/22.5 [23]. There was no difference in HOMA-IR before the start of lipid or saline infusion (1.23 ± 0.29 vs 1.68 ± 0.48; P = NS).

3.4. Glucose infusion rate

The hyperinsulinemic condition achieved during the steady-state level of the euglycemic hyperinsulinemic clamp with lipid infusion required a significantly lower mean GIR when compared with euglycemic hyperinsulinemic clamp with saline infusion (4.08 ± 2.15 vs 6.02 ± 2.60 mg/kg per minute; $P < .005$), showing decreased insulin sensitivity during lipid infusion (Fig. 2).

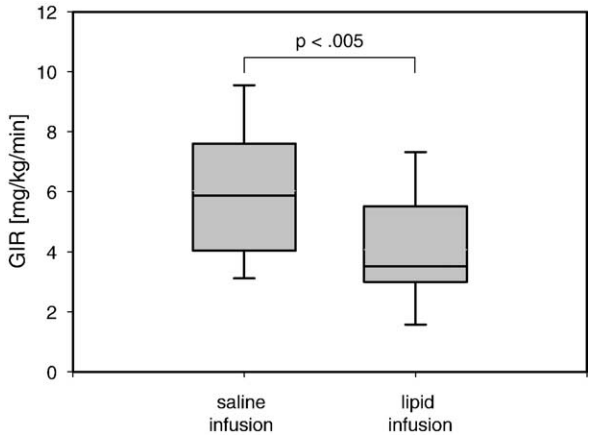

Fig. 2. Glucose infusion rate during steady-state level of the euglycemic hyperinsulinemic clamp with lipid/heparin infusion and saline/heparin infusion. Results are expressed as means ± SD.

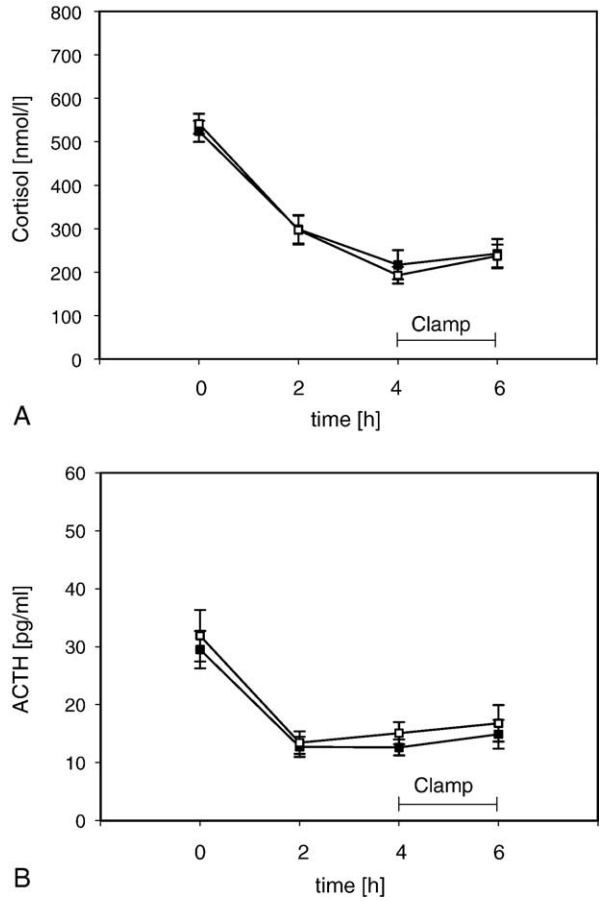

Fig. 3. Cortisol (A) and ACTH (B) during lipid/heparin infusion (■) and during saline/heparin infusion (□). Results are expressed as means ± SEM.

3.5. Cortisol and ACTH

Basally, there were no differences in cortisol and ACTH levels (Fig. 3A and B). During saline/heparin infusion, both serum cortisol and plasma ACTH showed a progressive decline. During lipid/heparin infusion, the increase in FFAs was also associated with a significant decrease in serum cortisol and plasma ACTH; however, this decrease was not different compared with the decline observed during saline/heparin infusion (cortisol: area under the curve [AUC] 110 660 ± 8863 vs 107 150 ± 8828 nmol/L per minute; ACTH: AUC 6081 ± 605 vs 6886 ± 924 pg/mL per minute). Levels of ACTH and cortisol at 2 and 4 hours after the start of infusion were not different from ACTH during steady state of euglycemic hyperinsulinemic clamp both during saline infusion and lipid infusion.

3.6. Urinary steroids

No significant differences in UFF, UFE, THF, 5α-THF, and THE could be observed during lipid and saline infusion, both in urine samples collected from 8:00AM to 8:00 PM and those collected from 8:00 PMto 8:00 AM. No differences in ratios of 5α and 5β reduction of cortisol were observed at any time interval.

4. Discussion

To our knowledge, this is the first study to evaluate the effect of FFAs on ACTH and cortisol secretion in healthy lean men. The levels of ACTH and cortisol declined during both lipid and saline infusion, which reflects the circadian rhythm in HPA activity. However, no difference was found between saline and lipid infusion. In addition, no alterations in urinary glucocorticoid metabolites were detected, suggesting no increase or decrease in cortisol metabolism during lipid infusion in healthy young men.

In human beings, an activation of the HPA axis was described in different states of elevated FFAs, such as obesity [8,24,25] and diabetes mellitus [26,27]. This hyperactivity of the HPA axis with enhanced responsiveness to stimulation with CRH or arginine vasopressin and to suppression with dexamethasone was particularly observed in individuals with abdominal obesity [24,25,28,29]. The higher sensitivity of the HPA axis was also associated with insulin resistance [25]. In accordance with these findings, the response to meals, which are associated with elevated FFAs and insulin levels, seems to indicate a much more rapid cortisol response after lunch in women with visceral obesity and a reduced activation of the HPA axis after dinner in women with subcutaneous obesity [30]. These data suggest that the HPA axis is modulated by FFAs in insulin-resistant individuals with visceral obesity, although data in healthy individuals are rare.

The GIR in our volunteers, measured in euglycemic hyperinsulinemic clamp, was comparable to the GIR measured in healthy human subjects by other groups [31-33], showing that the participants were not insulin-resistant. This might explain why, in our study group of healthy lean men with no signs of impaired insulin sensitivity, no effect of FFAs on ACTH or cortisol was observed.

Recently, Kok and coworkers [8] demonstrated reduced FFAs and ACTH secretion in obese premenopausal women after treatment with acipimox, suggesting a FFA-mediated effect on ACTH secretion. However, there was no correlation between the reduction in FFA levels and the decrease in ACTH production after acipimox treatment, suggesting an FFA-independent mechanism of acipimox. In addition, the effect of acipimox on HPA activity was independent of body composition parameters such as size of various fat areas (including visceral and subcutaneous fat depots). However, as previously noted, the effect of FFAs on HPA activity seems to depend on the obesity phenotype. Unfortunately, the study did not include treatment of lean women. Therefore, the effect of reduced FFA on HPA in healthy women could not be assessed.

Surprisingly, in contrast to these findings, a suprapituitary inhibition of FFAs on ACTH and cortisol secretion was recently shown in 6 young lean women [14]. This inhibitory effect of FFAs, observed by Lanfranco et al, cannot be explained based on published data.

Indeed, in vitro studies suggest that FFAs could have a positive- or negative-feedback action on HPA axis, depending on the administered dose [5,13]. However, the FFA levels, reported by Lanfranco and coworkers [14] during lipid infusion, were comparable to our findings. Consequently, the different effects could not be attributable to lower FFA levels.

It is conceivable that the sensitivity of HPA axis to FFAs depends on HPA activity. Indeed, we could not exclude an effect of FFAs on HPA activity if infusions were performed in the afternoon or in the early morning, when HPA axis was approaching its peak. However, Lanfranco and colleagues [14] performed lipid and saline infusions in the morning, the same time as we did. Therefore, the different effects of FFAs, as measured by Lanfranco et al [14] and by our group, do not seem to be caused by differences in HPA activity.

On the other hand, we compared the effects of a saline/heparin and a lipid/heparin infusion. Lanfranco and coworkers [14] did not add heparin to the saline infusion. There exist some hints for an inhibitory effect of heparin per se on adrenal function [34,35]. Therefore, an inhibitory effect of heparin is conceivable during lipid/heparin infusion and could not be definitely ruled out, although we used low doses of heparin.

Another potentially interacting factor may be the degree of insulin resistance of the participants. Indeed, individuals in the study of Lanfranco et al [14] were more insulin-resistant, as depicted by a higher HOMA value (3.02), compared with the individuals (1.10) in our study. Although HOMA values of different population groups are not directly comparable, the increasing glucose levels during lipid infusion in the study of Lanfranco et al are remarkable. In contrast, the glucose levels did not change during lipid infusion in our study group. These data are suggestive of substantially impaired insulin sensitivity in the study group of Lanfranco et al, whereas our participants were lean and insulin-sensitive. Clearly, insulin resistance might modulate the effect of FFAs on HPA axis, as previously noted. However, a stimulatory effect of FFAs on HPA activity would then be expected.

On the other hand, differences in HPA axis response to psychological stress [36,37], infusion of interleukin 6 [38], hCRH [39-41], cholinergic stimulation [42], hCRH/arginine vasopressin [43], dexamethasone [41], and differences in adrenal sensitivity to ACTH in men and women [43] are well known. The sex differences in HPA sensitivity may be responsible for the observed modification of ACTH and cortisol secretion by FFAs only in women. Therefore, further investigations have to be performed to evaluate sex differences in modification of HPA activity by FFAs.

Because the ACTH secretory response of cultured pituitary cells was relatively unaffected by FFAs, an indirect action of FFAs on HPA axis was discussed [13]. In fact, it is conceivable that FFAs influence HPA activity only during hyperinsulinemia, as observed in obese and insulin-resistant subjects. Elevated insulin secretion, induced by

FFAs [44-46], may be responsible for the increased HPA activity during lipid infusion (Fig. 2). Therefore, we performed a hyperinsulinemic clamp in 6 subjects, which led to moderately enhanced insulin levels, to achieve physiologic hyperinsulinemic conditions. We found no differences in HPA activity compared with saline infusion. Indeed, "high-dose" insulin infusion increased plasma ACTH [47,48]. However, in accordance with our findings, no stimulatory effect of insulin on HPA axis was observed during the more physiologic "low-dose" insulin infusion [47,48], which was comparable to the conditions during euglycemic hyperinsulinemic clamp. Thus, these findings did not support the theory of FFA-induced changes in ACTH and cortisol secretion during hyperinsulinemia.

Because there was no change in urinary cortisol and cortisone metabolites, an effect of FFAs was not masked by a compensatory increase in glucocorticoid metabolism.

Taken together, FFAs seem to have no effect on basal ACTH and cortisol secretion in normal-weight young men. Given the well-known sex and age effects on HPA activity [41], one has to take into account that our results in men are not necessarily applicable to women. Therefore, further investigations have to be performed to clarify the role of FFAs on HPA activity in obese, insulin-resistant men and women.

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