Effects of Fasting and Glucose Load on Free Cortisol Responses to Stress and Nicotine*

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ABSTRACT

The availability of energy appears to exert important regulatory functions in pituitary-adrenal stress responses. In two studies, the effects of short-term fasting and subsequent glucose administration on the free cortisol response to psychological stress and nicotine consumption were investigated. Study 1: After fasting for 8–11 h, healthy young men ingested either 100 g glucose (n = 13) or water (n = 12). One hour later they were exposed to a psychosocial stress task (Trier Social Stress Test). A third group also ingested 100 g glucose, but they were not exposed to any additional treatment (n = 10). Capillary blood glucose levels were in the lower euglycemic range before and significantly elevated after the glucose load (64.9 ± 9.8 vs. 162.5 ± 43.5 mg/dL; F = 149.04, P < 0.001). Although glucose load per se did not affect free cortisol levels, psychosocial stress induced a large cortisol response in glucose-treated subjects. In contrast, fasted subjects who received tap water did not respond to the Trier Social Stress Test with significant changes in cortisol levels (F = 6.27, P < 0.001). Both groups responded with a similar increase in heart rates (F = 33.53,

P < 0.001) with no statistically significant difference between glucose and water-treated subjects. Study 2: Twelve habitual smokers received 100 g glucose or tap water after fasting for at least 8 h on two separate sessions (cross-over, random sequence). Forty-five min after glucose/water ingestion, they smoked two cigarettes with a nicotine content of 1.0 mg/cigarette. Subjects were euglycemic before smoking, with a significant rise of glucose levels after consumption of 100 g glucose (64.4 ± 8.3 vs. 143.5 ± 40.0 mg/dL; F = 40.25, P < 0.001). As in Exp 1, subjects showed a substantially larger free cortisol response to nicotine under glucose load compared with water load (F = 4.91, P < 0.001).

From these data we conclude that the free cortisol response to stimulation is under significant control of centers responsible for monitoring energy availability. Low glucose levels appear to inhibit adrenocortical responsiveness in healthy subjects. In agreement with results from animal studies, the present results suggest that ready access to energy is a prerequisite for hypothalamus-pituitary-adrenal stress responses. (*J Clin Endocrinol Metab* **82:** 1101–1105, 1997)

A DAPTATION to psychological and physical stress frequently involves activation of the hypothalamus-pituitary-adrenal (HPA) axis. Increases of CRH, ACTH, and cortisol levels in anticipation of or during stressful stimulation are interpreted as allostatic (1, 2) and/or homeostatic responses of the body (3). It is widely believed that in times of increased metabolic demands, such as stress, cortisol is required for energy mobilization (*e.g.* higher blood glucose concentrations) to fuel fight or flight reactions (*e.g.* see Ref. 4).

Although the insulin-antagonistic and gluconeogenetic actions of cortisol appear to favor such interpretation, there is only little experimental evidence available to date to support this idea. In a study of HPA responses to prolonged low-intensity physical exercise, Tabata and co-workers (5) could prevent the increase of ACTH and cortisol after 2.5 h of exercise by maintaining blood glucose concentrations at pre-

exercise levels. No comparable data are available on the role of glucose levels on more acute HPA responses in humans. In our first experiment we thus manipulated blood glucose levels to study the impact of glucose levels and caloric load on free cortisol levels in acutely stressed adults. A second experiment was performed to investigate whether similar effects of caloric load on free cortisol responses can be observed with pharmacological stimulation of the HPA axis.

Subjects and Methods

Study 1

Subjects. Thirty-five healthy, male, medication-free nonsmokers were randomly assigned to one of three experimental groups: 1) glucose load + stress (n = 13); 2) water load + stress (n = 12); or 3) glucose load, no treatment (n = 10). The groups were matched for age and body mass index (mean age 24.3 ± 3.4 yr SD; mean body mass index 23.7 ± 3.0 SD). Participants were required to fast for at least 8 h before the experiment; however, they were free to fast for a longer time interval. Subjects fasted for 9.3 ± 4.5 SD h before the experiment with no statistically significant group differences (one-way ANOVA). Written informed consent was obtained from the volunteers and, a compensation of 40,- DM was paid for participation.

Procedure. All tests were performed between 1600–2900 h. Upon arrival in the laboratory, baseline glucose levels were measured in capillary blood (puncture of finger tip; Reflolux S, Boehringer Mannheim, Mannheim, Germany). Five min later, subjects either ingested 100 g glucose dissolved in a total volume of 400 mL (Dextro OGTT, Boehringer Mannheim) or 400 ml water. One hour later, a second blood glucose reading

was obtained. Then subjects of groups 1 and 2 were exposed to the psychosocial stressor (see below) for 15 min followed by a final glucose measurement and a resting period (50 min). Subjects of group 3 rested for 65 min after the second glucose measurement. The Trier Social Stress Test (TSST) treatment was approved by the ethics committee of the University of Trier.

Psychosocial stress protocol. Subjects of groups 1 and 2 were challenged with the TSST, which is described in detail elsewhere (6). The TSST mainly consists of a 5-min speech task and a 5-min mental arithmetic task in front of an audience. This stress protocol has been repeatedly shown to induce significant activation of the pituitary-adrenal axis, with 2- to 3-fold increases of free cortisol in healthy male nonsmokers.

Heart rate monitoring. Heart rate was continuously monitored before and after the TSST using wireless transmission with electrocardiogram precision at 1-min intervals (Sport Tester Profi, Polar Instruments, Groβ-Gerau, Germany).

Study 2

Subjects and protocol. Twelve male habitual smokers (age: 25.7 ± 3.8 yr; body mass index: 24.4 ± 3.7) who smoked for 7.6 ± 4.6 yr, were studied in a cross-over protocol on 2 different days. Subjects smoked at least 15 cigarettes a day at the time of study. The mean nicotine content of the cigarettes was 0.87 ± 0.28 mg.

Before reporting to the laboratory, subjects had to fast for at least 8 h. As in Study 1, they were free to fast for a longer period. After a first assessment of blood glucose levels, subjects drank 100 g glucose (day 1) or water (day 2) as in study 1. The sequence of glucose/water load was randomized. A second blood glucose level reading was obtained 45 min later. Then subjects smoked two cigarettes with a nicotine content of 1 mg nicotine/cigarette, deeply inhaling the smoke. Subjects were asked to consume the two cigarettes within 10–15 min. Written informed consent was obtained from the volunteers, and a compensation of 70,-DM was paid for participation. Except for glucose or water load, the experimental setup was identical for each subject on the 2 days. The stimulation protocol was approved by the ethics committee of the University of Munich Medical School.

Studies 1 and 2

Saliva sampling and biochemical analysis. Using the Salivette (Sarstedt, Rommelsdorf, Germany) device, the subjects collected 10 (study 1) and 12 (study 2) saliva samples before and after the TSST or smoking, respectively. The exact timing of saliva samples were: 70, 50, 35, 20, and 5 min before the TSST and 2, 10, 20, 35, and 50 min after the TSST, respectively (study 1). In study 2, samples were obtained 40, 30, 20, 10, and 1 min before and 1, 10, 20, 30, 45, and 60 min after smoking. After collection, samples were stored at −20 C before analysis. Briefly before assaying, samples were thawed and spun at 3000 rpm for 5 min to obtain samples with low viscosity. One hundred microliters clear saliva was removed for duplicate analysis of cortisol levels using a time-resolved immunoassay with fluorescence detection (7). The lower detection limit of this assay is 0.43 nmol/L with inter- and intraassay coefficients of variance of <10% across the expected range of cortisol levels (3–25 nmol/L).

Data analysis

ANOVAs for repeated measures were performed for glucose levels, cortisol concentrations, and heart rates. Degrees of freedom were adjusted with Greenhouse-Geisser correction where appropriate. Post hoc analyses were computed using Newman-Keuls tests. A response index following glucose load was obtained by deducting the baseline blood glucose level from the blood glucose level measured immediately before the TSST. The individual's area under the cortisol response curve (AUC) was computed using the trapezoid formula, aggregating the five post-stress cortisol levels (samples 6–10) relative to the individual baseline concentration (sample 5). Heart rates were aggregated into 5-min segments for a total of 40 min.

Spearman rank correlations were computed between glucose levels and cortisol responses. In study 2, one subject was removed from all statistical analyses because he showed cortisol levels that differed more than 3 SD from the group means under both glucose and water conditions for unknown reasons.

Results

Study 1

Before glucose or water load, blood glucose levels were in the low euglycemic range in all three experimental groups. Although glucose levels remained unchanged in subjects who drank water, both glucose groups showed the expected rise after ingestion of 100 g glucose (F = 29.05, $P < 0.001$; Fig. 1). Post hoc tests revealed no differences in baseline glucose levels between the three groups. Furthermore, no significant changes in glucose levels were observed in response to the TSST.

As shown in Fig. 2, free cortisol levels differed significantly between glucose and water-loaded subjects (F = 6.27, $P < 0.001$). With no differences between the three groups before stress, fasted treated with 100 g glucose showed a clear-cut increase of cortisol in response to the TSST ($P < 0.001$). However, stressed subjects preloaded with water after fasting failed to respond to the psychosocial stressor with a significant cortisol rise ($P > 0.1$). As indicated by continuously decreasing cortisol levels in the unstressed group, glucose load *per se* did not elevate cortisol levels. Post hoc tests revealed significantly enhanced cortisol levels at 10, 20, and 30 min after stress in the glucose-loaded subjects compared with the other two groups (all $P < 0.001$).

No differences in cortisol levels between water-loaded stressed subjects and glucose-loaded unstressed controls were observed at any time point.

Neither body mass index nor duration of fasting correlated with the individual's cortisol response (r = −0.24, $P = 0.25$; r = 0.20, $P = 0.33$). Likewise, glucose levels before glucose/water load showed only weak association with the cortisol response to the TSST (r = −0.34, $P = 0.10$). In contrast, both
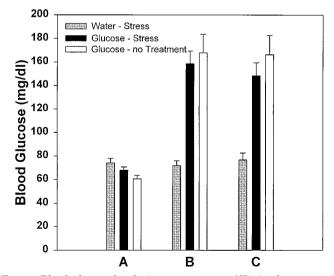

FIG. 1. Blood glucose levels (means ± SE; mg/dL) in three experimental groups: before water/glucose load (A), 60 min after water/glucose load (B), and immediately following psychosocial stress task (C); the nonstress group was measured after same time interval following second glucose measurement.
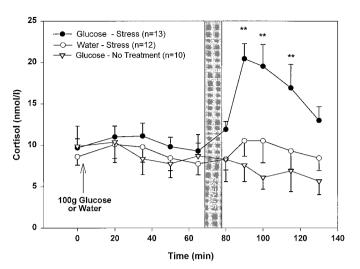
FIG. 2. Salivary cortisol levels (means ± SE; nmol/L) before and after water/glucose load and after exposure to psychosocial stressor (TSST; indicated by *shadowed bar*; **, $P < 0.001$, glucose/stress *vs.* water/stress and *vs.* glucose/no treatment).
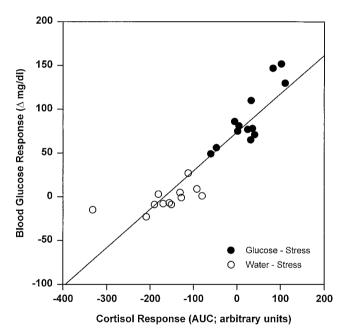

FIG. 4. Heart rates (means ± SE; bpm) before, during, and following psychosocial stress task in water- and glucose-loaded subjects. Heart rates sampled every minute were collapsed over 5-min intervals.

FIG. 3. Scattergram of cortisol responses (AUC) plotted against increase (△) in blood glucose levels after water/glucose load ($r = 0.93$, $P < 0.001$; Spearman rank correlation).

FIG. 5. Blood glucose levels (means ± SE) before and after water/glucose load (*, $P < 0.01$; **, $P < 0.001$).

Study 2

Fasting and glucose/water load led to similar blood glucose levels as in study 1. Glucose consumption raised blood glucose levels significantly, whereas they remained unchanged after water load ($F = 40.25$, $P < 0.001$; Fig. 5). The cortisol response pattern was also similar to study 1, with a significant difference between the glucose and the water-loaded subjects ($F = 4.91$, $P < 0.001$; Fig. 6). Although baseline cortisol levels did not differ between conditions, smoking two cigarettes with a nicotine content of 1 mg/cigarette led to a significant rise only when subjects ingested glucose before smoking (all $P < 0.003$); consumption of water before smoking resulted in a nonsignificant change in cortisol levels (all $P > 0.2$). Unlike in study 1, there were no statistically significant correlations between the rise in glucose levels and the individual cortisol response.

the absolute glucose levels before and after TSST as well as the change in glucose levels after glucose/water load yielded very high correlation coefficients ($r = 0.85$, $r = 0.89$, $r = 0.93$; all $P < 0.001$). Figure 3 depicts the scattergram for the correlation between changes in glucose levels and the cortisol responses (AUC) for all subjects exposed to the TSST.

Heart rates were significantly elevated during TSST in both groups ($F = 33.53$, $P < 0.001$). In contrast to cortisol levels, glucose and water-loaded subjects showed similar response patterns and magnitude (Fig. 4). Differences in heart rate responses between the two groups were not statistically significant ($F < 1$).
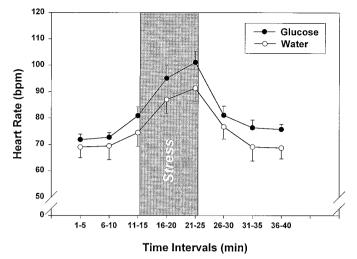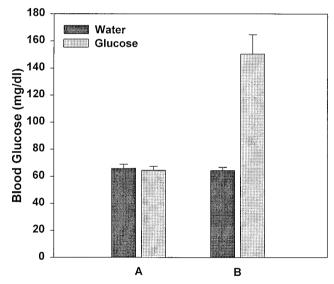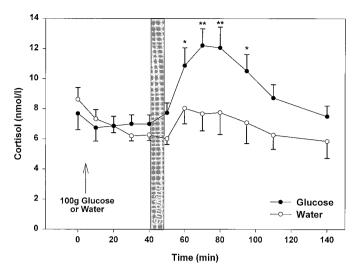
FIG. 6. Salivary cortisol levels (means ± SE; nmol/L) before and after water/glucose load and following smoking of two cigarettes with a nicotine content of 1 mg each (indicated by *shadowed bar*). Note different scale for y-axis compared with Fig. 2.

Discussion

The present studies support the view that the HPA axis is closely associated with systems responsible for caloric flow in the body (8, 9). Although a rather brief period of fasting (8–10 h) did not alter baseline cortisol levels, it abolished adrenocortical stress responses in healthy normal weight men.

Only recently have animal studies revealed evidence for fasting-induced attenuation of pituitary-adrenal responses to stress. A prolonged fast of 4 days decreased cortisol responses to stress in sheep isolated from the flock compared with stressed sheep fed *ad libitum* (10). Interestingly, administration of synthetic ACTH in 4-day fasted sheep resulted in an overshooting cortisol response (11), suggesting differential effects of fasting on central *vs.* peripheral sites of the HPA axis. Also, shorter periods of fasting have been shown to reduce HPA responsiveness. Rats fasted for 14–24 h showed a blunted corticosterone response to novelty (12) and reduced ACTH levels following restraint stress compared with controls (13, 14). However, despite lower ACTH levels, restraint stress after fasting was associated with an increased corticosterone response in the latter experiments (14). As these authors note, a fast of 14–24 h in young, quickly growing rats is a major metabolic insult. Besides its metabolic consequences, removal of food (*i.e.* fasting) will inevitably induce an acute ACTH and corticosterone stress response in laboratory animals raised on a standard feeding schedule (*ad libitum* access to food). In humans, only one study reported fasting-induced changes in adrenocortical responsiveness (15). Fasting for 3 days led to reduced cortisol increases following insulin-induced hypoglycemia; a 24-h fast was without effect.

In light of these findings, the results of the present experiments were rather surprising. After a brief fast of 8–10 h, healthy young nonsmokers with blood glucose levels in the low euglycemic range no longer respond to psychosocial stress (study 1) or smoking (study 2) with an

increase in cortisol levels. This elimination of the adrenocortical stress response in the fasted, water-loaded subjects is noteworthy.

Having employed the TSST protocol for induction of psychosocial stress in more than 20 studies in our laboratory, we have never observed a comparable cortisol nonresponse in an unselected group of male nonsmokers. The fact that these subjects showed similar increases in heart rates as glucose-loaded individuals is suggestive of a comparable stress level in both experimental groups. No subjective stress ratings were obtained from the subjects in the present study, because in previous experiments with manipulations of endocrine or physiological parameters subjective ratings could not explain the observed changes in hormonal responsiveness (16).

Caloric load with glucose 45–60 min before stress or smoking was able to reverse the effect of fasting. Subjects with high blood glucose levels showed the well-established response pattern of a 2-fold increase in free cortisol levels following the TSST (17, 18). Choosing nicotine as a stimulus for the HPA axis in study 2 not only extended the findings to alteration of adrenocortical responses to pharmacological agents, it also enabled us to investigate the effect of fasting and caloric load in the same individuals, which essentially rules out the possibility that the different cortisol levels after smoking merely reflect interindividual differences in adrenocortical response dispositions.

Which mechanisms could be responsible for the observed effects of fasting and glucose load on the adrenocortical responsiveness? Glucose load leads to a rapid rise in insulin levels in nondiabetics, and thereby to an increased transport of tryptophan into the central nervous system. This is followed by an increased synthesis of serotonin, which is known to have a stimulatory influence on the HPA axis at the hypothalamic level (19). Although this mechanism might explain a normal or even an exaggerated cortisol stress response, it can hardly account for the nonresponse in fasted, water-loaded subjects. An alternative interpretation of the present findings includes the Krebs cycle and its prime role for energy generation. An increased demand for ATP in the acutely stressed organism can usually be well met by increasing the oxidation of food components, such as sugars, lipids, and protein. The citric acid cycle provides a rapid and highly energy efficient way to generate the fuel needed for cellular processes such as synthesis and secretion of hormones. Thus, one consequence of increased availability of rapidly oxidizable substrates could be a permissive effect on endocrine responses to external stimulation with enhanced responses at times of increased substrates availability. If this hypothesis is true, then the observed effects of glucose load in restoring a normal cortisol response after fasting are not specific to glucose. Rather, fat or protein-rich diets would exert similar effects. Current studies in our laboratory investigate this hypothesis, and the physiological and psychological consequences of lowered/abolished HPA stress responses following fasting. In conclusion, the present data are in agreement with animal studies (10, 13, 14) suggesting that ready availability of energy is a prerequisite for significant acute stress responses of the HPA axis.

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