

Glucose but Not Protein or Fat Load Amplifies the Cortisol Response to Psychosocial Stress

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We previously reported that glucose intake amplifies cortisol response to psychosocial stress and smoking in healthy young men, while low blood glucose levels prevented the stress-induced activation of the hypothalamus pituitary adrenal (HPA) axis. However, it remains unknown whether this modulation is specific for glucose load or a more common effect of energy availability. To elucidate this question, 37 healthy men, who fasted for at least 8 h before the experiment, were randomly assigned to four experimental groups, who received glucose ($n = 8$), protein ($n = 10$), fat ($n = 10$), and water ($n = 9$), one h before their exposure to the Trier Social Stress Test (TSST). Blood glucose levels were measured at baseline and following stress, while salivary cortisol was assessed repeatedly measured before after the TSST. The results show that both absolute cortisol levels and net cortisol increase were greater in the glucose group in comparison to the other groups ($F_{3,33} = 3.00$, $P < 0.05$ and $F_{3,33} = 3.08$, $P < 0.05$, respectively). No group differences were observed with respect to perceived stress and mood. Furthermore, the cortisol response was positively correlated with blood glucose changes ($r = 0.49$, $P < 0.002$). In conclusion, the results suggest a central mechanism responsible for regulation of energy balance and HPA axis activation, rather than peripheral mechanisms. We thus recommend controlling for blood glucose levels when studying HPA axis responsiveness.

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INTRODUCTION

HPA axis activity is associated with systems responsible for caloric flow in the organism (Akana, Strack, Hanson, and Dallman, 1994; Dallman, Akana, Strack, Hanson, and Sebastian, 1995; Dallman, Strack, Akana, Bradbury, Hanson, Scribner, and Smith, 1993; Tempel and Leibowitz, 1993). Hypoglycemic states after five days of fasting have been shown to increase cortisol secretion (Bergendahl, Iranmanesh, Evans, and Veldhuis, 2000; Bergendahl, Vance, Iranmanesh, Thorner, and Veldhuis, 1996) and alter pulsatile cortisol release (Vance and Thorner, 1989) in humans. On the other hand, caloric intake modulates cortisol secretion, since diurnal cortisol peaks are related to meals (Follenius, Brandenberger, and Hietter, 1982; Rosmond, Holm, and Bjorntorp, 2000). The fact that both fed and fasted states enhance the activity of the HPA axis, suggests different underlying mechanisms and/or timing in the interaction between caloric flow and profile of cortisol levels. In rats with a nocturnal cycle of activity, animals fed *ad libitum* during night showed lower basal activity of the adrenocortical system and a higher stress responsiveness in the morning than overnight fasted rats, which exhibited high basal corticosteroid levels and decreased responsiveness to stress (Choi, Horsley, Aguila, and Dallman, 1996).

In a previous study we could show that decreased energy availability after an eight-h fasting period in the morning, led to a blunted cortisol response to psychosocial stress and pharmacological stimulation, which could be restored by glucose administration (Kirschbaum, Gonzalez Bono, Rohleder, Gessner, Pirke, Salvador, and Hellhammer, 1997). However, the

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mechanism involved in this modulation of the stress-response remained unclear.

Several mechanisms are discussed to explain these results: On the one hand, glucose-specific or central mechanisms may account for modulation of the HPA axis response by caloric load. One possible explanation could be the stimulatory action of enhanced serotonin synthesis on the HPA axis. In nondiabetic subjects, glucose load produces an increase of insulin that, in turn, favors tryptophan transport into the central nervous system, which in turn increases serotonin synthesis. Choi *et al.* have proposed another mechanism for regulation at the hypothalamic level: high glucose and insulin levels, which are seen shortly after glucose load stimulate activity of the ventromedial nuclei (VMN). Activity of the VMN seems to be an important permissive input to the paraventricular nuclei (PVN), which mediate HPA activation, since inhibition of the VMN by colchicine is able to disrupt HPA axis responsiveness to fasting (Choi *et al.*, 1996; ter Horst and Luiten, 1986). Low glucose levels may also inhibit the VMN and consequently the PVN, and thereby mediate the attenuated HPA axis response.

On the other hand, if nonspecific or peripheral mechanisms were involved, the citric acid cycle as an energy provider to the organism could be an important candidate to modulate HPA responsiveness. In an acutely stressed organism, oxidative processes enhance ATP requirements and sugars, lipids and proteins all fuel the citric acid cycle efficiently. Thus, each of them might be able to modulate the stress-induced cortisol response.

To further investigate the mechanisms responsible for calorie-induced enhancement of HPA activity, we randomly administered glucose, protein, fat, and water to healthy men after an eight-h fasting period before exposure to a standardized psychosocial stress test (TSST). If the underlying mechanism responsible for HPA response modulation is located at a central level, modulation of the cortisol stress response should be limited to increases in blood glucose levels. Otherwise, if the citric acid cycle is responsible for HPA axis modulation by caloric load and fasting, other fuels like proteins or fat should also be able to produce this effect.

MATERIALS AND METHODS

Sample

Thirty-seven healthy men were randomly assigned to one of four experimental groups: glucose load ($n =$

8); protein load ($n = 10$); fat load ($n = 10$); or water ($n = 9$). All of them were nonsmokers and free from medication. The groups were matched for age and body mass index (BMI; mean age 23.22 ± 0.43 SEM; mean BMI 23.02 ± 0.32 SEM). Participants were required to fast for at least 8 h before the experiment started. The experimental procedure was approved by the Ethics Committee of the University of Trier and written informed consent was obtained from the volunteers.

Procedure

All tests were performed between 1600 and 1900 h. Subjects arrived at the laboratory after an eight-h fast, and baseline glucose levels were measured in capillary blood (puncture of finger tip; Reflolux S, Boehringer Mannheim, Mannheim, Germany). Five minutes later, subjects ingested the respective caloric load or water. The glucose group ingested 75 g dissolved in water in a total volume of 300 ml (Dextro OGTT, Boehringer Mannheim). The fat group ingested 200 grams of avocado, which has an estimated content of 80 g fat and less than 5 grams carbohydrates and proteins, respectively. The protein group drank 83 g of proteins dissolved in 300 ml of mineral water (Proteindrink, Formula 80+, Multipower, Hamburg, Germany). Finally, the water group drank 300 ml of mineral water. Forty-five minutes after intake, a second blood glucose reading was obtained. Immediately after this measurement, all subjects were exposed to the psychosocial stress test (TSST) and a third glucose determination was performed thereafter. Five saliva samples were collected before TSST and five samples afterward, all of them using the Salivette device (Sarstedt, Rommelsdorf, Germany). The aim of the first five samples was to obtain information about the response profile of cortisol after caloric/water uptake. Thus, saliva samples were obtained immediately before and 10, 20, 30, and 45 min after ingestion of glucose, fat, protein, or water (samples 1–5). Sample 5 was taken as baseline for the following stress-induced cortisol response. Samples 6–10 (after TSST) were used to assess the cortisol response to psychosocial stress, and were taken 1, 10, 20, 30, and 60 min after the TSST. In order to control for possible differences in the psychological parameters between groups, we evaluated perceived stress and mood changes using self-reports scales, which have been demonstrated to be sensitive to the TSST (Kudielka, Hellhammer, Hellhammer, Wolf, Pirke, Varadi, Pilz, and Kirschbaum, 1998).

Psychosocial Stressor

All groups were exposed to the “Trier Social Stress Test” (TSST). Briefly, the TSST consists of a 5-min speech task and a 5-min mental arithmetic task in front of an audience. This procedure has repeatedly demonstrated its efficiency in eliciting HPA activation with increases in salivary cortisol from two- to three-fold baseline levels in healthy men (Kirschbaum, Pirke, and Hellhammer, 1993).

Salivary Cortisol Analysis

After collection, samples were stored at -20°C before analysis. Immediately before assaying, samples were thawed and spun at 3000 rpm for 5 min to obtain clear saliva. One hundred microliters of saliva was removed in duplicate for analysis of cortisol levels using a time-resolved immunoassay with fluorescence detection (Dressendorfer, Kirschbaum, Rohde, Stahl, and Strasburger, 1992). The lowest detection limit of this assay is 0.43 nmol/l with inter- and intraassay coefficient of variance below 10%.

Psychological Self-Reports

Perceived stress was evaluated after TSST by six visual analog scales, as following: (1 = stressful, 2 = uncontrollable, 3 = new, 4 = unpredictable, 5 = ego-involvement, 6 = anticipation of negative consequences). Stress-induced mood changes were evaluated by the MDBF “Mehrdimensionaler Befindlichkeits-fragebogen” (Steyer, 1994). This questionnaire is composed of three scales: elevated vs depressed mood, wakefulness vs sleepiness, and calmness vs restlessness ranked on 5-point Likert scales from 1 = “not at all” to 5 = “very much,” which were assessed shortly before and directly after stress exposure.

Statistical Analysis

ANOVAs for repeated measures were carried out for cortisol, glucose levels, and mood scales. Greenhouse-Geisser corrections for degree of freedom were applied where appropriate. Post hoc analyses as well as between-groups comparisons of perceived stress scales were performed by means of one-way ANOVAs. A glucose response index was computed by deducting the baseline blood glucose levels from blood glucose levels measured immediately before TSST (blood samples 1 and 2). The area under the cortisol response curve (AUC) was computed for each

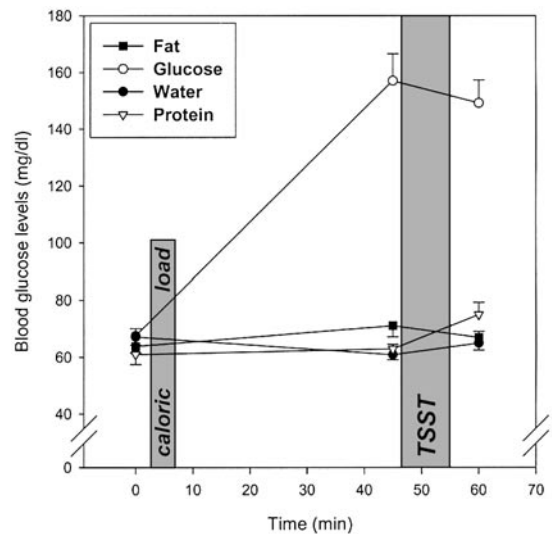


FIG. 1. Blood glucose levels (means \pm SEM; mg/dL) before and after caloric/water load and TSST in the four experimental groups, after ingestion of either fat ($n = 10$), glucose ($n = 8$), water ($n = 9$), or protein ($n = 10$) diets.

subject using the trapezoid formula aggregating the five post-stress cortisol levels (samples 6 to 10) relative to the individual baseline concentration (sample 5). Relationships between cortisol and glucose were examined by Spearman rank correlations.

RESULTS

Blood Glucose Changes

Before caloric/water intake, blood glucose levels were in the low euglycemic range in all subjects, with nonsignificant differences between groups. As expected, blood glucose levels increased from 60 to 160 mg/dl in the glucose group, which remained elevated throughout the remainder of the experiment. All other groups showed no significant changes in blood glucose levels and remained euglycemic until the end of the experiment (group effect: $F_{3,33} = 144.17$, $P < 0.0001$; time effect: $F_{1,7,57.4} = 41.86$, $P < 0.0001$; group by time interaction: $F_{5,2,57.4} = 30.62$, $P < 0.0001$, respectively). Post-hoc tests for the group factor revealed a highly significant difference between the glucose group and the other three groups (Scheffe, Bonferroni, LSD, all $P < 0.001$; Fig. 1).

Psychological Self-Reports

Exposure to the TSST induced a worsening in the subscales mood and calmness in all groups ($F_{1,33} =$

TABLE 1Mean \pm SEM of Scores of Perceived Stress and Mood Scales in Glucose, Protein, Fat, and Water Group

	Glucose <i>n</i> = 8	Protein <i>n</i> = 10	Fat <i>n</i> = 10	Water <i>n</i> = 9
Perceived stress				
Stressfulness	61.00 \pm 5.17	70.90 \pm 5.63	56.40 \pm 8.19	63.44 \pm 5.46
Uncontrollability	58.38 \pm 10.74	40.60 \pm 10.35	37.90 \pm 11.03	34.56 \pm 10.62
Novelty	68.75 \pm 9.64	60.10 \pm 11.28	73.10 \pm 10.40	56.89 \pm 12.84
Unpredictability	63.12 \pm 12.74	42.60 \pm 10.25	68.80 \pm 10.88	69.44 \pm 10.33
Ego-involvement	48.13 \pm 11.46	66.20 \pm 7.73	44.90 \pm 11.55	44.78 \pm 10.77
Anticipation of adverse outcome	27.50 \pm 12.69	20.00 \pm 7.41	27.40 \pm 9.37	16.33 \pm 6.50
Elevated mood				
Pre ^a	30.13 \pm 1.20	32.00 \pm 1.26	29.20 \pm 1.68	30.67 \pm 1.56
Post	28.88 \pm 1.01	28.30 \pm 1.94	25.60 \pm 1.59	27.56 \pm 1.71
Wakefulness				
Pre	13.38 \pm 1.21	14.40 \pm 0.87	13.50 \pm 1.19	11.33 \pm 0.97
Post	14.25 \pm 1.00	14.00 \pm 0.91	12.60 \pm 1.06	12.67 \pm 1.26
Calmness				
Pre ^a	14.88 \pm 1.04	15.70 \pm 1.51	15.10 \pm 0.89	15.33 \pm 1.55
Post	10.50 \pm 0.06	13.20 \pm 0.70	11.90 \pm 0.67	11.89 \pm 0.70

^a Significant decrease after stress; $p < 0.0001$.

15.96, $P < 0.0001$ and $F_{1,33} = 25.23$, $P < 0.0001$), while no differences between groups could be found before and after the stress test (all $P > 0.10$; Table 1). Also, perceived stress, as measured by the visual analogue scales after TSST, did not reveal group differences either (all $P > 0.10$).

Salivary Cortisol

Unlike the psychological measures, cortisol levels showed a significant group by time interaction ($F_{5,9,64.7} = 3.31$, $P < 0.01$) and time effect ($F_{2,0,64.7} = 46.02$, $P < 0.0001$). As shown in Fig. 2, cortisol levels of the glucose group were greater after stress than those of protein, fat or water groups. These differences were significant in the samples collected 10 and 30 min after TSST ($F_{3,33} = 2.86$, $P < 0.05$ and $F_{3,33} = 3.15$, $P < 0.05$; one-way ANOVA). The cortisol response to stress, estimated as AUC, was also greater in the glucose group compared to the other three groups ($F_{3,33} = 3.08$, $P < 0.05$). Additionally, this stress-induced cortisol response correlated significantly with glucose changes found before TSST in the total sample ($r = 0.49$, $P < 0.002$). Figure 3 depicts the scatterplot for this correlation.

DISCUSSION

The present study suggests that the acute cortisol response to stress is under significant control of cen-

ters responsible for glucose regulation. Subjects who fasted for 8 h had significantly decreased cortisol responses to a psychosocial stress paradigm, compared to those who received a glucose substitution shortly before the stress test. Extending the results from a previous study (Kirschbaum *et al.*, 1997), we show here, that other food components such as fat and protein were unable to restore the fasting-induced blunted cortisol response to stress. Subjects who received fat or protein did not show higher cortisol

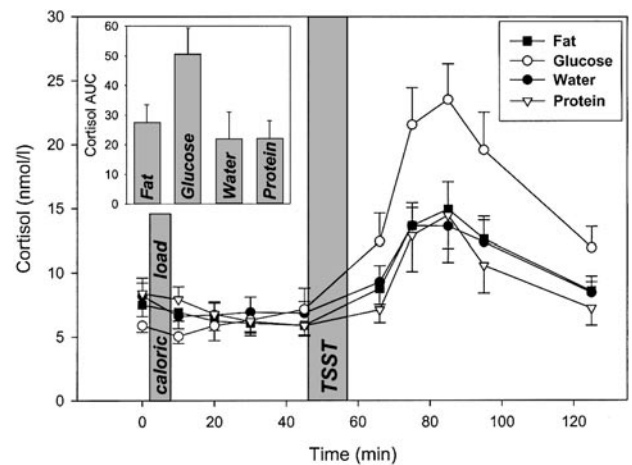


FIG. 2. Salivary cortisol levels (means \pm SEM; nmol/l) before and after caloric/water load and TSST in the four experimental groups, after ingestion of either fat ($n = 10$), glucose ($n = 8$), water ($n = 9$), or protein ($n = 10$) diets.

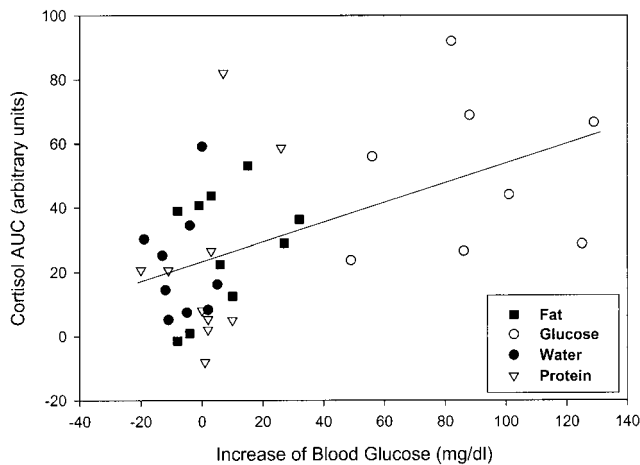


FIG. 3. Scattergram of cortisol AUC plotted against increase in blood glucose levels in the total sample.

levels than those who received water only. Furthermore, a close association was found between the increase of cortisol levels and the increase in blood glucose.

Associations between HPA axis regulation and brain centers that regulate hunger and feeding are well documented in animals. The circadian rhythm and feedback sensitivity of the HPA axis could be experimentally changed in rats by altered feeding paradigms (Akana *et al.*, 1994; Dallman *et al.*, 1993). For example, Ruiz-Gayo found increased ACTH and corticosterone levels in food-deprived rats (Ruiz-Gayo, Garrido, and Fuentes, 2000). In humans, few data are available from studies with rather long fasting periods up to 72 h, where fasting leads to increases in cortisol levels (Berga, Loucks, and Cameron, 2001; Fichter, Pirke, and Holsboer, 1986) and blunted cortisol responses to insulin-induced hypoglycemia (Adamson, Lins, and Grill, 1989). Shorter fasting periods during the night increased cortisol responses to low-grade exercise (Tabata, Ogita, Miyachi, and Shibayama, 1991). Recently, Kasckow found decreased ACTH and cortisol levels in the first three hs of fasting, while higher levels were reported after longer fasting periods (Kasckow, Hagan, Mulchahey, Baker, Ekhtor, Strawn, Nicholson, Orth, Loosen, and Geraciotti, 2001). On the other hand, ingestion of meals leads to significant increases of ACTH and cortisol during the circadian rhythm in the absence of stress and exercise (Follenius *et al.*, 1982; Gibson, Checkley, Papadopoulos, Poon, Daley, and Wardle, 1999; Rosmond *et al.*, 2000). Our results do not indicate such a meal-related cortisol increase, which should have been visible in

the cortisol samples before stress, given that the meal-related peaks reported above were found 15–30 min after meal ingestion. The reason for these inconsistencies might be found in the different nutritional contents. The carbohydrate contained in the standard meals used in the studies cited above may have been sufficient to increase blood glucose levels, while our diets contained very little glucose. Another possible explanation is provided in the results of Follenius *et al.* (1982), which show that cortisol increases to the same standard meal are significantly lower at 1000 and 1900 h compared to the peak at noon. Our study was conducted between 1600 and 1900 h, so that a lower meal related peak would be expected.

The present results do not support the notion that peripheral mechanisms like the citric acid cycle are significantly involved in HPA regulation in response to acute stimulation since administration of protein or fat did not restore the HPA axis response to stress. Since only glucose, but not fat or protein administration restored the HPA axis response to stress in fasted subjects, these data suggest the involvement of central mechanisms. As a prime candidate for CNS structures involved in glucose-mediated HPA regulation, hypothalamic nuclei stand out. Choi *et al.* showed that high glucose and insulin levels (which are seen shortly after glucose load, but not after fat or protein ingestion) stimulate activity of the VMN, which in turn permits stimulation of the PVN which results in HPA axis activation. In contrast, low glucose levels may inhibit activation of the PVN (Choi *et al.*, 1996).

However, we cannot exclude other central pathways like increased synthesis of serotonin or involvement of neuropeptides like neuropeptide Y (NPY), which also participate in the complex neural network that regulates energy balance (Dallman *et al.*, 1995). Furthermore, the cortisol responses in the nonglucose conditions are slightly higher than those reported before for fasted subjects after water load (Kirschbaum *et al.*, 1997). Since we did not include a nonfasted control group in the present study, we can only speculate if the results found here are higher for glucose, or lower for the nonglucose groups relative to the nonfasted response. Data from other studies using the TSST suggest that the response of the glucose group is within the normal range for healthy young men, while the other groups' responses are rather attenuated (Rohleder, Schommer, Hellhammer, Engel, and Kirschbaum, 2001).

In conclusion, the present study again showed that HPA responsiveness is under significant control of centers sensing or regulating blood glucose levels. The

fact that glucose, but not fat or protein administration restored the HPA axis response after fasting, supports the hypothesis that central regulatory sites, presumably in the hypothalamus, are involved. Along with our previous observation that the glucose effect is not limited to HPA responses to psychosocial stress, it suggests a general mode of action and control for the HPA axis. However, since we studied a rather short time period, one should also take into account that possible effects of protein or fat consumption might present well beyond the one-h period studied here. Despite this shortcoming, we again stress the importance of controlling glucose levels in studies of HPA responses.

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