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THE AWAKENING CORTISOL RESPONSE AND BLOOD GLUCOSE LEVELS

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Summary

The hypothalamic-pituitary-adrenal axis is characterized by a marked circadian cycle with heightened activity in the morning. This is synchronized to awakening such that free cortisol increases two to three fold in the first thirty to forty five minutes following awakening - the awakening cortisol response. It has been suggested that this activity, by mobilizing energy reserves prepares the body for the metabolic demands of the day. Similar arguments are applied to the cortisol response to psychological challenge. Paradoxically the cortisol response to a psychosocial stressor is abrogated in fasted individuals with low blood glucose. Also cortisol response to a psychosocial stressor is positively correlated to blood glucose levels after glucose load. We examined if the same relationship applies to the awakening cortisol response. There was no correlation between the cortisol response and awakening blood glucose levels. Moreover a group with mean blood glucose at the bottom of the euglycemic range, identified by split at the median for glucose level upon awakening, showed no deficit in cortisol response. Hence the physiology of the awakening response differs to that of a psychological stress response. These data challenge the view that an oxidisable substrate for energy metabolism is permissive for cortisol responses. In addition the present findings do not support a predominantly gluconeogenic role for morning cortisol activation.

Key Words: cortisol, awakening response, blood glucose

A major feature of the hypothalamic-pituitary-adrenal (HPA) neuroendocrine axis is a burst of activity in the morning (1,2). The diurnal cortisol cycle is very much influenced by the process of awakening. Free cortisol, measured in saliva, increases two to three fold in most individuals during the first thirty to forty five minutes following awakening (3,4). This is centrally driven since it is subject to dexamethasone suppression (5). Cortisol in saliva is a valid index of free

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circulatory levels (6,7). The cortisol increase after awakening is independent of awakening time or whether awakening is induced by alarm or is spontaneous. It is not an orthostatic response since it is not influenced by morning activities - staying in bed or preparing for the day (3).

A major physiological role of cortisol is considered to be gluconeogenesis and maintenance of blood glucose (8). Hence the teleological argument has been proposed that this cortisol response is required for energy mobilization to prepare the body for the metabolic demands of the day, the shift from a resting to an active phase (3). A prediction would therefore be that the lower blood glucose at awakening time the greater the cortisol response. Similar arguments have also been advanced to explain HPA sensitivity to psychological stressors (see 9) the classic fight-flight adaptation. Paradoxically, the free salivary cortisol response to a psychosocial stressor in humans in relation to blood glucose behaves opposite to the gluconeogenic prediction. Individuals with blood glucose levels in the low euglycemic range, induced by a brief fast of 8-10 hours, fail to show a cortisol response to a psychosocial stressor. Elevation of blood glucose in fasted individuals, by an oral glucose load, one hour prior to the stressor results in a normal cortisol response. Moreover the cortisol response in all individuals (fasted and fasted plus glucose load) is highly positively correlated with blood glucose levels measured at the time of the stress procedure, Trier Social Stress Test (TSST) (10). These findings are not easy to explain. It is argued by these authors that oxidisable substrate availability for energy metabolism (glucose) may be permissive for the cortisol stress response, such that enhanced responses reflect times of increased substrate availability. This argument effectively reverses the classically assumed relationship.

We decided to further explore the glucose-cortisol relationship by examining the awakening response. The arguments proposed by Kirschbaum and co-workers (in 10) should also hold for the awakening cortisol response. If oxidisable substrate availability is a requirement for cortisol elevation, low blood glucose at time of awakening should be associated with a compromised cortisol response. In fact about 10% of individuals do fail to exhibit cortisol elevation following awakening (3). Further, a prediction of the substrate availability argument is that the awakening cortisol response should be positively correlated with blood glucose measured at the same time, as is the case with cortisol response to psychological stressor. Alternatively, if the principle role of awakening cortisol elevation is gluconeogenic and energy mobilization, to support the metabolic demands of the day, and if cortisol can be elevated in the face of low oxidative substrate availability, then the cortisol response should be negatively correlated with blood glucose level.

Methods

Thirty healthy, medication free, adult subjects were recruited into the study. They were drawn from staff and students of the University of Westminster and offered no financial inducement for participation. Of these, twenty seven successfully completed the procedure and provided sufficient saliva for complete analysis. They were 14 males and 13 females. Only two males and one female were smokers and then only infrequently. Mean age was 30, range 20-66. Subjects were briefed in the laboratory and then given a study pack with written instructions and sampling equipment to take home for use the following morning. All gave informed consent.

Subjects were asked to provide a saliva sample immediately upon awakening and then three more samples at ten-minute intervals over the following thirty minutes. Sampling was by salivette (Sarstedt Ltd., Leicester UK.); the subjects were instructed to place the cotton sampling swab

under their tongue for a timed two minute period. Subjects were asked to take nil by mouth other than water during the sampling period and not to smoke or brush their teeth to avoid microcontamination with blood. Otherwise they could follow their normal morning routine. Glucose levels were measured in capillary blood (fingertip puncture; Accutrend Alpha, Boehringer Mannheim UK Diagnostics and Biochemicals Ltd.) on two occasions; at the beginning of the sampling procedure and again at the end. Glucose readings were recorded manually but also electronically in the memory facility of the glucose meter.

Saliva samples were immediately frozen in the subjects' domestic freezer and then returned to the laboratory in insulated cold packs. They were stored at -20 ^{0}C until time of assay. On return to the laboratory the glucose meters were checked for accurate calibration against glucose standards (Accutrend Control G). The memory facility was checked for accurate recording of glucose level; there were no discrepancies. The memory also could reveal any operational error. Meters were cleaned and sterilized for further use. The returned sample packs also contained used lances and test strips in sealed containers for safe disposal.

On thawing, saliva was recovered by centrifugation at 1,000g for 10 minutes. Cortisol was determined by solid phase radioimmunoassay (Corti-cote ICN Biomedicals Ltd., UK.) modified slightly to increase sensitivity (intra-assay %CV 7.9; inter-assay %CV 7.4).

Results

There was a clear elevation of cortisol over the thirty-minute period following awakening from a mean of 10.0 nmol/l in the first awakening sample to 19.4 nmol/l at thirty minutes (Fig 1).

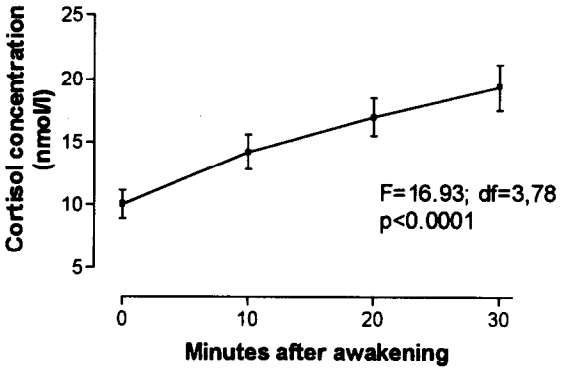

Fig. 1.

Mean ± SEM salivary cortisol concentration during the first 30 minutes after awakening (n=27). Mean blood glucose 4.4mmol/l for both 1st and 2nd measurement occasion.

Repeated measures ANOVA confirmed a highly significant difference between the four measurement occasions ($F=16.93$, $df=3,78$, $p<0.0001$, after Greenhouse Geisseer correction $p<0.001$). Age, gender, awakening time and saliva volume was each unrelated to the awakening

cortisol response, thus confirming previous reports (3,4). Blood glucose (mean ± SEM) was, on the first occasion 4.4±0.16 mmol/l and on the second occasion 4.4±0.14 mmol/l. Individual blood glucose levels (mean for both measures) ranged from 2.3 to 5.7 mmol/l and was stable across the two measurement occasions ($r=0.79$, $p<0.0001$, $n=27$). The blood glucose did correlate to some degree with awakening time; for blood glucose immediately upon awakening ($r=-0.27$) and 30 minutes after awakening ($r=-0.45$, $p=0.02$, $n=27$). Hence individuals who awoke later in the morning tended to lower blood glucose. There was no correlation between the cortisol response (difference between base at time 0 and peak at time 30 minutes.) and blood glucose measured at the beginning of the awakening cortisol response ($r=-0.04$) or blood glucose measured at the end of the sampling period ($r=-0.17$). Similarly no correlation was detected between the cortisol response calculated as area under the cortisol curve (AUC, calculated with first measure as zero) with either the first or second glucose measurement ($r=0.09$ and $r=0.14$ respectively, $n=27$; see Fig 2 and 3). All correlational analysis was by Pearson product moment.

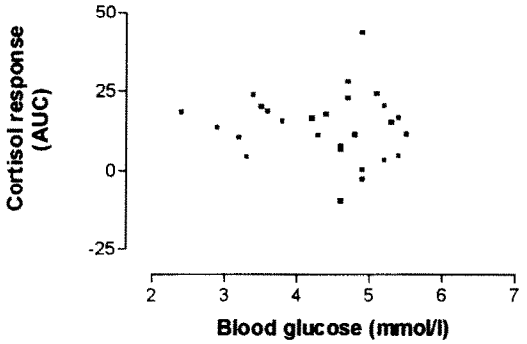

Fig. 2.

Scatterplot of the cortisol response (AUC) against blood glucose at time of awakening.

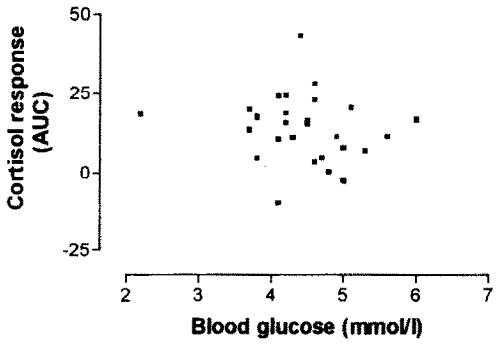

Fig. 3.

Scatterplot of the cortisol response (AUC) against blood glucose at time 30 minutes after awakening.

A median split of the population by the first blood glucose level produced two populations distinguished for blood glucose: mean ± SEM: 3.8±0.18 mmol/l for the low group and 5.1±0.08 for the high group. The low glucose population, with mean blood glucose at the bottom of the euglycemic range, exhibited a cortisol response to awakening from a mean of 11.7 nmol/l at first awakening to 19.6 nmol/l 30 minutes later. Repeated measures ANOVA showed that the cortisol response for this group was highly significant ($F=7.89$, df 3, 39, $p< 0.001$, after Greenhouse Geissser correction $p<0.01$; see Fig 4).

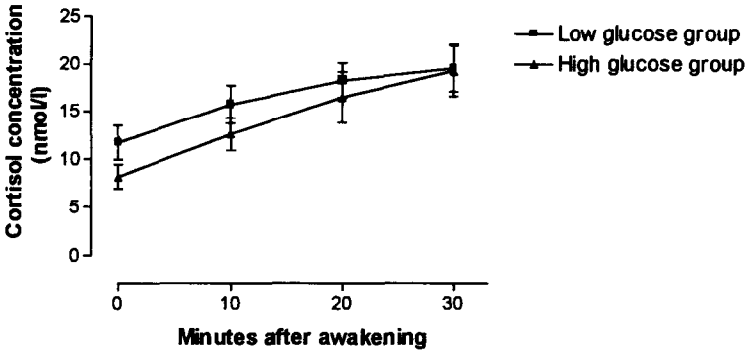

Fig. 4.

Mean ± SEM salivary cortisol concentration during the first 30 minutes after awakening in the low and high glucose groups as determined by median split of the first blood glucose level (n=14 and 13 respectively).

The repeated measures analysis for cortisol over time was repeated including the median split by first glucose as a between subject factor. No overall difference was found between the groups in levels of cortisol nor was the interaction term significant. Thus cortisol rise over time was similar for both groups ($F<1$ for both the overall difference and interaction; see Fig. 4).

Discussion

Morning blood glucose levels reported here, before food or any calorific drink, encompass the entire euglycemic range (3.9-5.6 mmol/l (11)). Overnight fasting during sleep in humans is physiologically different to daytime fasting. Whereas an 8-9 hour fast during the day results in glucose decline to the bottom of the euglycemic range, blood glucose is much better maintained during sleep fasting at night (12). In the present study, mean glucose for both the first and second samples was 4.4 mmol/l and is a little lower than data presented in Van Couter et al., (12) - 4.8 mmol/l at the end of night sleep fast, although the variance is quite large (see 12). In the present study also it is of interest that those individuals with later awakening times tended to lower blood glucose, particularly when measured 30 minutes after awakening. The range and distribution allowed exploration of correlations with the awakening cortisol response. None was

evident. This contrasts with the findings of Kirschbaum and co-workers (10) that absolute glucose levels measured both before and after psychosocial stressor (TSST) are highly positively correlated with the cortisol response (area under the cortisol curve) to the stressor ($r=0.85$, $r=0.89$ respectively, both $p<0.0001$).

The median split low glucose population had a mean glucose level of 3.8 mmol/l, which is at the bottom of the euglycemic range and comparable to the level reported by Kirschbaum and co-workers in daytime fasted subjects (10). Such a population showed complete failure of cortisol response to TSST. By contrast, the cortisol response to awakening reported here was not compromised in the low euglycemic group.

The current findings suggest that readily available oxidisable substrate for energy metabolism (blood glucose) is not in some way a necessary condition for cortisol responses as was argued by Kirschbaum and co-workers (10). At least this does not seem to be the case for awakening activation. An alternative hypothesis is that brain tryptophan availability is the key variable. Serotonergic input to the paraventricular nucleus (PVN) is important in HPA responses to stressors (for reviews see 13 and 14). Reductions in circulating tryptophan and/or transport into the brain can compromise 5-hydroxytryptamine (5HT) synthesis (see 15). Insulin promotes brain tryptophan uptake (16). Hence low insulin in fasted-low glucose individuals may adversely influence brain tryptophan uptake so as to compromise indolamine signaling to the PVN. If this is the case the present data suggests that central 5HT availability is not important in the awakening HPA activation. Since brain tryptophan can be depleted either by administering a tryptophan-free amino acid load, to lower circulating tryptophan, or a large neutral amino acid such as valine, which competes for brain transporter (see 15), this hypotheses can be explored. Alternatively the glucose–insulin–tryptophan uptake relationship may be different after night sleep fasting.

Finally, the present study provided no evidence that the opposite relationship obtains. The awakening cortisol response wasn't exaggerated in the low blood glucose group and there was no negative correlation between blood glucose and the awakening cortisol response. Thus the teleological argument that awakening cortisol plays an important role in mobilizing energy to move from a resting to an active phase receives no support from this study. An intriguing speculation is that morning cortisol activation is more to do with modulating a shift in the immune system from nighttime Th1 to daytime Th2 domination. Th1 inflammatory-cell mediated activity is heightened at night, during rest. The balance is shifted in the morning under neuroendocrine regulation (17). Cortisol is thought to play a key role since it downregulates Th1 domination in favor of Th2 (18). This circadian oscillation in Th1 - Th2 balance is important in directing immune responses appropriate to rest, compared with activity and also prevents polarization since Th1 and Th2 are counteregulatory.

Thus we have shown that the awakening cortisol response differs from the cortisol response to psychosocial stress in as much as the awakening response is independent of blood glucose level. We speculate that the morning cortisol activation is related to immune Th1/Th2 shift rather than to metabolic demands. Consideration of neuroendocrine-immune relationships indicates a new perspective to the physiology of the awakening cortisol response.

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