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Plasma and salivary concentrations of glucose and cortisol during insulin-induced hypoglycaemic stress in healthy Nigerians

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Summary

We measured cortisol levels in plasma and saliva samples obtained simultaneously from 10 fasting adult Nigerians at 0900 h and at 60 and 90 min of hypoglycaemia induced by intravenous insulin. Salivary glucose levels (fasting and after i.v. insulin) were unaffected by hypoglycaemia and did not correlate with plasma glucose at any time point. Cortisol levels in plasma and saliva increased by 50% and 120%, respectively, from fasting to 90 min values (both $P < 0.05$) after i.v. insulin. This increase was evident by 60 min (plasma 33% and saliva 40%, both $P < 0.05$ compared to fasting values). There was a significant positive correlation between the percentage increases in plasma and salivary cortisol ($r = 0.65$, $P < 0.05$). Salivary cortisol was always (0900 h and during hypoglycaemic stress) 15–20% of total plasma cortisol, a percentage similar to the reported values on the contribution of free plasma cortisol to total plasma cortisol. We conclude that increases in plasma cortisol are reflected in saliva, and salivary cortisol could be estimated as an alternative to free plasma cortisol in the dynamic assessment of adrenocortical function in humans.

Résumé

Nous avons mesuré les niveaux de cortisole qui se trouvent dans les prélèvements de la salive et du plasma pris simultanément de 10 Nigérians adultes qui étaient à une diète absolue, à 0900 h et après 60 min et 90 min d'hypoglycémie provoquée par l'insuline intraveineuse. Les niveaux de glucose salivaire (avant et après l'insuline intraveineuse) restaient inaltérables à l'hypoglycémie et n'étaient pas en corrélation avec le glucose du plasma à n'importe quel temps. Les niveaux de cortisole dans le plasma et la salive ont augmenté par 50% et 120%, respectivement des valeurs du début et celles d'après 90 min ($P < 0.05$ pour les deux) après l'insuline. Cette augmentation était évidente après 60 min (plasma 33% et salive 40%, $P < 0.05$ pour les deux, c'est-à-dire, par comparaison aux valeurs du début). Il y avait une corrélation positive significative entre les pourcentages d'augmentation dans la cortisole du plasma et celle de la salive ($r = 0.65$, $P < 0.05$). La cortisole salivaire était toujours (à 0900 h et pendant le stress d'hypoglycémie) 15–20% de la cortisole totale du plasma, un pourcentage qui est semblable aux valeurs rapportées sur la contribution de la cortisole libre du plasma à la cortisole totale du plasma. Notre conclusion est que les augmentations se sont reflétées dans la salive et la cortisole salivaire peut être estimée comme l'alternative de cortisole libre du plasma dans l'évaluation dynamique de la fonction adrénocortique des humains.

Introduction

Cortisol is predominantly bound in circulation to the plasma protein transcortin. The free form of plasma cortisol is the physiologically active component [1] and is usually estimated by various methods which are generally technically difficult [2,3]. It has, however, only been reported infrequently [4,5] that cortisol concentrations in saliva may reflect the free cortisol in

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plasma. For reasons that are unclear, however, salivary cortisol estimations have not been widely used in assessing adrenocortical function despite the obvious potential usefulness in clinical situations where repeated venepuncture is difficult or undesirable. There has certainly been no report on salivary cortisol levels in Africans to our knowledge. The need for such a study is accentuated by the fact that salivary cortisol may indicate plasma free cortisol, whose estimation is crucial in certain clinical situations (such as malnutrition) and which is difficult to measure in many African countries for lack of the requisite facilities.

We therefore simultaneously measured plasma and salivary cortisol levels in healthy young adult Nigerians, basally and during insulin-induced hypoglycaemic stress, attempting to investigate any relationships between the two analytes. Our results should additionally offer further information on endocrine responses in the African, in whom there are few similar studies.

Subjects and methods

Subjects

Ten healthy, young adult, non-obese male university students (aged 25.1 ± (s.e.m.) 0.5 years, with body-mass index, weight/height^2, of 22.4 ± 0.3 kg/m^2) were recruited into the study after voluntary informed consent. None had a family or personal history of endocrine disease. None was on any regular medication. The studies were performed in a metabolic ward at about 0900 h after a 10 h overnight fast (with water *ad libitum*). On the morning of the study, fasting blood and saliva (matched) samples were initially collected from an antecubital vein cannulated and kept patent by regular flushing with isotonic saline. Subsequently, 0.15 unit/kg body weight of soluble insulin (insulin BP, Novo Industrie, Copenhagen, Denmark) was intravenously injected as for a standard insulin tolerance test. Matched saliva and blood samples were collected thereafter at 30, 60 and 90 min in plastic tubes (saliva), lithium heparin tubes (plasma cortisol) and fluoride oxalate tubes (salivary and plasma glucose). The saliva samples were collected 15 min after gently rinsing the mouth with distilled water. The blood samples were centrifuged (2500 rpm) within 1 h of sample collection and the saliva and plasma samples stored at −20°C until estimation, within 4 weeks of sample collection.

Methods

Plasma and salivary glucose levels were determined by a specific glucose oxidase method [6] and cortisol levels in plasma and saliva measured by radioimmunoassay using a standard Amerlex cortisol RIA kit (Amersham International, Aylesbury, U.K.). This assay is specific for cortisol, well validated, and in our laboratory has an intra-assay coefficient of variation (CV) of 4.5% and inter-assay CV of 7.6%.

Statistics

Results are expressed as means ± s.e.m. Comparisons between the various parameters were made by paired and unpaired Student's *t*-tests as appropriate. The relationships between relative percentage increases in salivary and plasma cortisol were explored by Spearman rank-correlation coefficients (r_s). The level of statistical significance was $P < 0.05$.

Results

Table 1 shows the mean plasma and salivary glucose levels basally and during insulin-induced hypoglycaemic stress. Clinical (symptomatic) and biochemical (plasma glucose < 2.2 mmol/l) hypoglycaemia was always established in the subjects within 30 min of insulin injection. Plasma and salivary glucose levels were not significantly correlated, either fasting (r_s = 0.4, $P > 0.05$) or during the insulin tolerance test up to 90 min (r_s = 0.11, $P > 0.05$).

Table 2 indicates the plasma and salivary cortisol levels before and after induction of hypoglycaemia. The mean salivary cortisol level was about 15–20% of the value for total plasma cortisol either basally or with changing cortisol levels accompanying the hypoglycaemic stress. The cortisol measurements were continued up to 90 min after insulin administration for both saliva and plasma, as this time corresponds to the point of peak plasma cortisol secretion in

Table 1. Plasma and salivary glucose levels during insulin-induced hypoglycaemic stress

	Time (min)			
	Fasting	30	60	90
Plasma glucose (mmol/l)	4.17 ± 0.21	1.39 ± 0.15*	2.25 ± 0.26*	2.77 ± 0.29*
Salivary glucose (mmol/l)	0.26 ± 0.04	0.27 ± 0.03	0.25 ± 0.03	0.26 ± 0.03

Values represent means ± s.e.m.
*$P < 0.05$ compared to fasting value.

Table 2. Plasma and salivary cortisol levels during insulin-induced hypoglycaemic stress

	Time (min)			Percentage increase (0–90 min)
	0	60	90	
Plasma cortisol (nmol/l)	478 ± 34	622 ± 24*	687 ± 24*	50.2 ± 13.0
Salivary cortisol (nmol/l)	73 ± 11	97 ± 10*	136 ± 10*	119.1 ± 32.8†
Ratio of salivary to plasma cortisol × 100 (%)	15.3	15.5	19.8	—

Values represent means ± s.e.m.
*$P < 0.05$ compared to 0 min value (plasma or saliva).
†$P < 0.05$ compared to plasma value.

response to hypoglycaemia as reported in a previous study on Nigerians [7].

Figure 1 shows a scattergram of the percentage increase from fasting to 90 min after induction of hypoglycaemia for the salivary and total plasma cortisol levels. These two parameters demonstrated a significant positive correlation ($r_s = 0.65$, $P < 0.05$). The percentage increase in cortisol level in saliva by 90 min (120%) was about double that in plasma (50%) ($P < 0.05$).

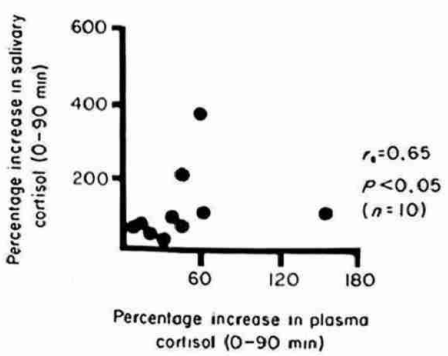

Fig. 1. The relationship between percentage increases in plasma and salivary cortisol levels during insulin-induced hypoglycaemia.

Discussion

The insulin tolerance test is useful in the assessment of the hypothalamic–pituitary–

adrenal axis. Here, we confirmed that insulin-induced hypoglycaemia produced a significant increase in plasma and salivary cortisol levels. Glucose was also present in measurable amounts in saliva, but the small levels detected did not significantly correlate with levels in plasma. This agrees with some earlier observations [8] but not others [9]. This study also confirmed earlier reports [4] that the percentage increase in salivary cortisol after hypoglycaemia is greater than in total plasma cortisol.

The mean salivary cortisol level was 15–20% of total plasma cortisol. This is similar to the value of 10–20% of total plasma cortisol reported for the free cortisol fraction [4,7] and may support the belief that salivary cortisol is equivalent to the dialysable (free) form of plasma cortisol, especially as saliva is an ultrafiltrate of plasma. It also raises the possibility of using either measurement (salivary or free plasma cortisol) as an index of the physiologically active component of cortisol. Saliva offers an easier alternative because its use avoids the problems of dialysis and elaborate sample preparation necessary for free cortisol estimation; saliva is also easily obtainable even by paramedical personnel and can be repetitively obtained non-invasively during the insulin tolerance test.

There was a significant correlation between the percentage increase in plasma and salivary cortisol levels, confirming earlier reports [5,10]. This observation, coupled with the finding of a greater increase in salivary than plasma cortisol with hypoglycaemia suggests that salivary cortisol levels may reflect more sensitively the stress response of the hypothalamic–pituitary–adrenal axis than plasma cortisol levels. However, as reported elsewhere [11], and observed here, the salivary cortisol response is highly variable between individuals and may lag significantly behind plasma cortisol secretion. This discrepancy has been ascribed to delay in the secretion of the free fraction into saliva after release of cortisol from the adrenal cortex [11], adsorption of cortisol by oral debris or epithelial cells [12], partial conversion of cortisol to cortisone during passage through the salivary gland [5] and significant binding of cortisol to erythrocytes delaying secretion into saliva [4]. Nonetheless, there was a linear response up to 90 min after insulin administration in both salivary and plasma cortisol levels in this study, and this time period corresponds to the peak plasma cortisol levels observed during hypoglycaemic stress in another similar group of Nigerians [7].

We therefore conclude that saliva offers an alternative to plasma samples for measuring cortisol levels in the assessment of adrenocortical function. Salivary cortisol, furthermore, reflects the free plasma cortisol fraction and thus offers an indication of the physiologically active component of the circulating cortisol level.

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