Short Report

Free cortisol index as a surrogate marker for serum free cortisol

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Abstract

Background The biologically active component of a hormone is the unbound or free fraction. Changes in cortisol-binding protein could give misleading results if only total cortisol is measured for the interpretation of dynamic function tests.

Methods This study aimed to measure serum free cortisol using a steady-state gel-filtration method and then to evaluate the correlation between the serum free cortisol and the free cortisol index (FCI), defined as serum total cortisol/cortisol-binding globulin (CBG).

Results Forty-eight serum samples from healthy volunteers undergoing a short Synacthen test were analysed for total cortisol, free cortisol and CBG. The FCI correlated well with a previously established, but more complex, calculation of serum free cortisol ($R = 0{\cdot}98$, $P < 0{\cdot}001$) and with measured serum free cortisol ($R = 0{\cdot}90$, $P < 0{\cdot}001$).

Conclusion Free cortisol index is a reliable and user-friendly measure of serum free cortisol.

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Introduction

The biologically active component of a hormone is the unbound or free fraction. Cortisol is bound approximately 80% to cortisol-binding globulin (CBG) and 10% to albumin.[1,2] Changes in binding proteins may give misleading results if only total hormone concentrations are measured. Serum total cortisol is used for interpreting dynamic function tests of the hypothalamic–pituitary–adrenal axis, such as the short Synacthen test (SST). There are several techniques that have comparable results for the measurement of serum free cortisol, including ultrafiltration,[2,3] equilibrium dialysis[4,5] and gel filtration,[6] but the routine measurement of serum free cortisol remains too labour-intensive and costly. Previous attempts to establish an index for free cortisol[1,2] have not been further explored in clinically relevant situations and the complex mathematical calculation proposed[2] led to minimal uptake in clinical practice.

A chromatography method using steady-state gel filtration, previously applied to the measurement of free testosterone, demonstrated that free fractions of hormones can be measured with good accuracy and precision, with a percentage coefficient of variation (%CV) below 10.[7]

The aim of this study was to use a steady-state gel-filtration method to measure free cortisol and then to evaluate the correlation between serum free cortisol and the free cortisol index (FCI), which is defined as serum total cortisol/CBG.

Materials and methods

Six female and 17 male healthy volunteers, median age 52 (range 34–63) years underwent an SST, following approval from the local ethics committee. Women on exogenous oestrogen were excluded. All SSTs were performed between 08.00 and 09.00 h. Venous blood was taken on arrival; after 30 min of recumbency, another blood sample was taken followed by 250 μg of tetracosactrin (Synacthen[1] ; Norvatis Pharma, Stein, Switzerland) administered intravenously. Half an hour later a final venous sample was obtained to

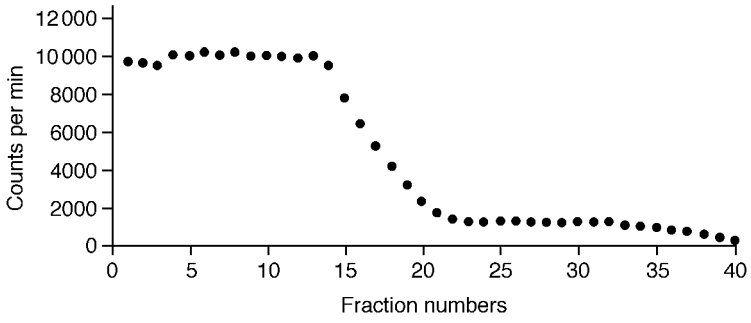

Figure 1. *Elution profile of protein-bound and apparent free cortisol during steady-state gel filtration of human serum on Sephadex G50 at 37°C.*

evaluate the maximum response of cortisol to Synacthen. Samples were stored at −20°C until assayed for serum total cortisol, CBG and serum free cortisol.

Serum total cortisol was measured by an automated competitive radioimmunoassay (RIA) (Roche ES700; Roche Diagnostics Ltd, Lewes, UK) with intra-assay %CV of 13·0 at 89 nmol/L, 3·2 at 498 nmol/L and 4·4 at 807 nmol/L. Serum CBG was measured using an RIA (Biosource Europe, Nivelles, Belgium) with intra-assay %CV of 6·0 at 24·2 μg/mL, 3·8 at 62·2 μg/mL and 4·9 at 112·4 μg/mL. The FCI was calculated as serum total cortisol/CBG (nmol/mg). In the insert to the CBG kit, the manufacturers recommend the mathematical formula of Coolens *et al.*[2] for the calculation of unbound cortisol:

$$\text{Unbound cortisol } (\mu\text{mol/L}) = [(0{\cdot}0167 + 0{\cdot}182(\text{CBG} - \text{total cortisol}))^2 + (0{\cdot}0122 \times \text{total cortisol})]^{0{\cdot}5} - [0{\cdot}0167 + 0{\cdot}182(\text{CBG} - \text{total cortisol})]$$

where both CBG and total cortisol are expressed in μmol/L.

Serum free cortisol was measured using a chromatographic steady-state gel-filtration method. Reagents and solvents for phosphate buffer were of Analar grade (BDH Ltd, Poole, UK). Tritiated cortisol (Amersham International Ltd, Amersham, UK) was purified by thin-layer chromatography on silica gel plates (Merck 60; BDH Chemicals Ltd, Poole, UK) using a solvent system of ethanol–benzene (50:50, v/v). Purified tracer was eluted with ethanol and made up to 20 mL to provide the working solution.

The equipment used and the preparation of Sephadex G50 (fine) columns were as described by Wheeler and Nanjee.[7] The whole system was maintained at 37°C in a waterbath fitted with a circulating pump. From a working stock of tracer, 120 μL was added to a pre-cleaned glass tube and dried in a fume cupboard. Thereafter, 1·0 mL of serum was added to the glass tube and mixed. The glass tube was placed in a waterbath and incubated for 30 min at 37°C. After incubation, 800 μL of serum was carefully applied to the top of the Sephadex column and allowed to penetrate into the Sephadex, then eluent flow was interrupted. The glass wall above the Sephadex was carefully washed three times with phosphate buffer, ensuring minimal disturbance to the top of the Sephadex column.

The column was refilled with buffer (37°C) and the flow restored. As the serum flows down the Sephadex column, free hormone rapidly diffuses into the interstices of the Sephadex beads. As more serum flows down the column an equilibrium is established between the free hormone in the beads and free hormone in the large spaces outside the beads. The state of equilibrium is seen as a plateau of radioactivity that represents protein-bound hormone plus free hormone in equilibrium. Once all the serum has passed through the column, the free hormone within the Sephadex beads is eluted out. This is seen as a second plateau of radioactivity. The ratio of the mean counts of the second plateau divided by the mean counts in the first gives the proportion of free hormone in the sample (*see* Fig. 1):

$$\text{Percentage free cortisol} = \frac{\text{Mean c.p.m. fractions in second plateau} - \text{Background c.p.m.}}{\text{Mean c.p.m. fractions in first plateau} - \text{Background c.p.m.}} \times 100$$

Fractions of three drops each were collected into mini-vials and 3 mL of scintillation fluid added to each vial. The radioactivity in each mini-vial was determined using a beta counter (Packard Instruments, Pangbourne, UK). The precision of the assay was $< 9{\cdot}2\%$ over the range of concentrations measured. The steady-state gel-filtration method does not disturb the free:bound equilibrium; this is an advantage over other methods for measuring free hormone, such as equilibrium dialysis. The assay is therefore free from interference unless the free:bound ratio is disturbed in the serum, a situation that would be the point of investigation.

The serum free cortisol was calculated from the percentage free cortisol and the measured total cortisol. The method required at least 1·0 ml of serum and only 48 samples had a sufficient volume of serum to allow analysis. Only those results for the samples that were analysed for all parameters were included.

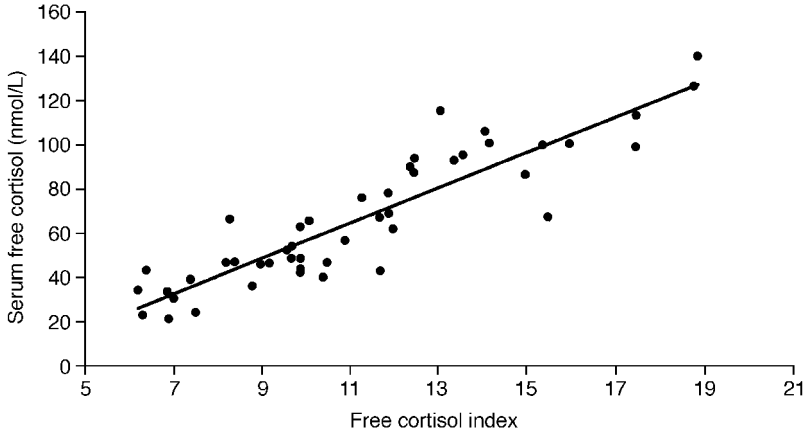

Figure 2. *Linear regression of the free cortisol index and serum free cortisol (*R = *0·9,* P < *0·001).*

Results

Forty-eight venous blood samples were used and the mean (standard deviation, absolute range) serum total cortisol was 488·5 (161, 218–974) nmol/L. The mean serum CBG was 45·7 (15·6, 26·7–66·3) mg/L. The mean serum free cortisol was 67·6 (28·8, 21·1–138·8) nmol/L and the mean FCI was 11·0 (3·24, 6·2–18·9) nmol/mg. The FCI correlated significantly with the serum free cortisol ($R = 0{\cdot}90$, $P < 0{\cdot}001$) (*see* Fig. 2). The FCI also correlated significantly with the value obtained from the complex calculation suggested by Coolens *et al.*[2] ($R = 0{\cdot}98$, $P < 0{\cdot}001$).

Following the intravenous Synacthen, the FCI increased from 9·0 (1·6, 6·3–11·7) nmol/mg to 15·2 (2·2, 11·9–18·9) nmol/mg and the measured free cortisol increased from 42·0 (11·2, 21·1–62·4) nmol/L to 99·2 (19·1, 66·0–138·9) nmol/L. The increment in FCI correlated significantly with the increment in the measured free cortisol ($R = 0{\cdot}89$, $P < 0{\cdot}001$).

Discussion

In the past, problems were encountered with the interpretation of serum total thyroxine due to significant changes in thyroxine-binding globulin; similar problems apply to serum total cortisol. Although the measurement of free cortisol remains the ideal, current methods cannot be used routinely because they are too labour-intensive and costly. Simple routine methods are, however, available for the measurement of CBG.

The strong correlation between serum free cortisol and FCI suggests that the FCI may be a useful surrogate marker for serum free cortisol. The FCI may be of particular use in patients with borderline serum total cortisol results and in situations where it is suspected that CBG is altered. These include patients on exogenous oestrogen replacement where CBG is raised[2] and patients with acute-phase responses such as burns or post surgery when CBG is low.[8]

Early morning and post-stimulation serum total cortisol has been used very successfully in the diagnosis and treatment of patients with cortisol deficiency, but in situations of altered CBG, serum total cortisol may not reflect the bioavailable free cortisol. In such cases, reliance on serum total cortisol measurements may result in incorrect clinical decisions.

In this paper, the free cortisol index has been validated as a reliable and user-friendly measure of serum free cortisol in healthy volunteers. The FCI should now be further validated in patients with suspected hypothalamic–pituitary–adrenal axis insufficiency.

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