A comparison of capillary, venous and salivary cortisol sampling after intense exercise

Abstract

Venipuncture is expensive, invasive and impractical for many sport science and clinical based settings. Salivary free cortisol is often cited as a non-invasive practical alternative. However, when cortisol concentrations exceed the corticosteroid binding globulin (CBG) point of 500nmol·L^{-1} a lack of agreement between salivary and venous blood cortisol have been found. Alternatively, capillary blood may present a minimally invasive, cost effective and practical surrogate for determining cortisol concentration. Purpose: The aim of this study was to determine whether cortisol concentrations sampled from (a) capillary blood and (b) saliva accurately reflect those found in venous blood across a large range of concentrations following intense exercise. Methods: Eleven healthy aerobically trained male subjects were recruited. Capillary, salivary and venous blood samples were collected pre and post (immediately post and post 5, 10, 15 and 20 minutes) a treadmill $\dot{V}O_{2\ max}$ test. Total and free cortisol samples were analysed via an ELISA method using monoclonal antibodies. Results: Capillary and venous concentrations increased at a similar rate following exercise (Cohen's *d* between 0.14 – 0.33), increasing up to 15 minutes post before a decline was seen. Salivary cortisol values increased at a slower rate compared to venous and capillary cortisol, but continued to increase post 15 minutes (Cohen's *d* 0.19 – 0.47 and 0.09 – 0.72 respectively). Conclusions: Capillary and not salivary cortisol accurately reflects concentrations assayed from venous blood across a range of values below and above the CBG binding point. Capillary sampling provides a minimally invasive, cost effective practical surrogate for assessment of HPA function.

Introduction

Cortisol is the primary stress hormone used to assess function of the hypothalamic-pituitary-glad (HPA) axis, and is present in the body as both free and bound forms[1,2]. Cortisol is dispersed through all water spaces within the body[3], and can be sampled from urine, serum and saliva[4]. However, each technique can often be constrained by situational (sampling method) or analytical problems. Urinary analysis often fails to assess rapid changes in concentrations over time; salivary analysis represents only the biologically active free fraction of cortisol (cortisol which is not bound to the corticosteroid binding globulin (CBG),

or other proteins), and serum cortisol is normally sampled using venipuncture which can itself increase cortisol concentrations[5,6]. Several previous studies have investigated the differences between salivary (free) and serum (total) cortisol using a range of analytical methods including enzyme-linked immunosorbent assay (ELISA)[7,8] and radioimmunoassay (RIA)[9,10]. Although the use of saliva as a method of assaying cortisol concentration is a relatively new technique, its usefulness during high-intensity exercise may be limited. Previous studies have suggested that free cortisol accurately reflects HPA function when values are below the CBG point (<500nmol·L^{-1})[11]. However, above the CBG binding point a disproportionate rise in free cortisol has been observed[11-13]. During high-intensity exercise findings regarding the linearity and exponential fit of the cortisol response across a range of concentrations remain discordant[9,10].

Previously studies have reported that during bouts of intense exercise the adrenal cortex secretes high concentrations of cortisol above the CBG binding point[14,15]. Furthermore, cortisol is commonly known for being elevated during times of psychological stress. Recently, high risk sports which assessed intense physical exercise combined with psychological stress appear to have further increased HPA function, resulting in notably elevated cortisol concentrations above the CBG binding point[16-18]. Consequently, these elevated concentrations may result in disproportinately high free cortisol values. Although early literature has investigated the relationship between capillary and salivary cortisol during a graded exercise test, venous cortisol was not assessed[9].

Salivary free cortisol has often been suggested as a non-invasive and practical alternative for assessing cortisol concentration. Although its use may be limited during low to moderate intensity exercises i.e. exercise intensities or psychological stresses that are not likely to elicit cortisol concentrations above the CBG binding point[19]. Venipuncture for assessment of total cortisol does not have these associated issues with the CBG binding point, and is generally regarded as the gold standard for cortisol assay. Unfortunately this approach is invasive, expensive and often impractical, particularly when assessing the psychological stress response. Furthermore, the invasive nature of venipuncture may induce white coat syndrome, artificially elevating cortisol concentration[6]. However, the use of capillary finger prick sampling for subsequent cortisol analysis may present a practical surrogate for sampling cortisol concentration as a reflection of HPA function.

To our knowledge, no research has compared venous blood and its agreement with samples collected from saliva and capillary blood, pre and post high-intensity exercise. Therefore, the purpose of this study was to examine differences in cortisol concentrations sampled via capillary and venous blood as well as saliva, both pre and post maximal exercise. We hypothesized that 1) salivary cortisol would rise at a similar rate as venous cortisol until ~500 nmol·L^{-1}, and 2) capillary cortisol would rise at a similar rate as venous cortisol, both above and below ~500 nmol·L^{-1}.

Method

Participants: Eleven non-smoking healthy males were tested. Mean average (± SD) age, height, weight, body fat percentage and maximal oxygen consumption ($\dot{V}O_2$) of the participants were 26.1 (5.3) yrs, 179.5 (5.28) cm, 77.5 (8.5) kg, 12.5 (4.4) % and 63.1 (7.4) mL·kg·min^{-1} respectively. All participants were free of disease and took part in regular aerobic exercise, training at least twice a week. All participants completed medical health history forms, gave informed consent prior to testing, and were excluded if they were currently or had been taking glucocorticiods or had an abnormal exercise stress test. In accordance with the Helsinki Declaration (Seoul, Korea, October 2008), ethical approval was obtained from the University of Canterbury Human Ethics Committee.

Procedures: All testing sessions were completed within a two week period between the hours of 1700 and 2000. Measurement of $\dot{V}O_2$ was conducted on a Woodway treadmill using the Athlete Led Protocol (ALP) as described by Draper & Marshall[20]. The ALP begins with the participant running at 8km/hr, this is then increased by 1km/hr every minute until the participant's maximal running speed is attained. From this point, the gradient is increased by 1% every minute until maximal volitional exhaustion is reached. The composition of expired air was measured using breath-by-breath analysis (K4b^2 Cosmed Rome, Italy). Salivary, capillary and venous blood samples were simultaneously collected pre, immediately post, and post 5, 10, 15 and 20 minutes of exercise.

Blood and salivary sampling: Total cortisol samples were collected from the first finger of the left hand and a left brachial vein. A Haemolance Plus (Haemedic, Poland) was used to puncture (1.6mm depth) the capillary bed of the fingertip, and then blood (300μL) was

extracted and collected using a lithium heparin CB300LH Microvette (Sarstedt Aktiengesellschaft & Co, Germany). Venous blood was sampled from a forearm vein using a Braun B19 cannula (Braun Medical, England). Both capillary and venous samples were immediately placed on crushed ice before being spun in a cr2000 centrifuge (Centurion Scientific, England) at 10,000 rpm for 10 min. Once the plasma and pack cell were separated, plasma was pipetted into Eppendorf microtubes (Sarstedt Aktiengesellschaft & Co, Germany) and stored at -20 °C for subsequent cortisol analysis. Participants provided 1mL of saliva (drooling) in a salivette tube (St.Louis, France) at the exact same time as both blood samples were taken. In accordance with González[21], participants were asked not to brush their teeth 30-min prior attendance, not to consume water 5-min before any sample, and not to consume food 2-hours pre arrival at the laboratory. After each salivary sample was taken it was immediately frozen and stored at -20 °C for subsequent cortisol analysis.

Total and free cortisol assays: Plasma and salivary cortisol samples were measured directly by ELISA technique (Dept of Clinical Biochemistry, Christchurch Hospital, New Zealand Lewis & Elder[22]) using monoclonal antibodies[23]. For saliva, cortisol was extracted from the matrix (to increase sensitivity) with dichloromethane prior to ELISA, whereas plasma cortisol was measured by direct ELISA in accordance with Ellis[24]. After extraction from the matrix, saliva cortisol recoveries were between 95 and 105% and had a limit of detection (LOD) of 3nmol·L. The interassay coefficient of variation (CV) was 12.6% for the 'low control' (mean cortisol value of 7nmol·L) and 7.4% for the 'high control' (mean cortisol value of 22nmol·L). Plasma cortisol assay had a LOD of 55nmol·L and interassay CV of 6.9-8.5% over the range of 98 to 1007nmol·L. All standards, controls and samples were analyzed in duplicate and on the same plates as previously described[24]. Intra and inter assay coefficients of variation were 7.6% and 8.6% respectively. Cortisol concentrations were expressed as $nmol \cdot L^{-1}$.

Statistical analysis: Pearson product-moment correlation was used to determine agreement between sample sites. Differences between sample sites were compared using Cohen's *d*; in general, ≤ 0.20 is considered to be a small effect, >0.20 to ≤ 0.50 a moderate effect, and ≥ 0.60 a large effect. SPSS version 20.0 (SPSS, Inc., Chicago, Illinois) was used for data analysis. With measurements collected on an individual over-time, the effective sample size is reduced, due to the dependence of the measurements within the participant. However, in this case there is sufficient variation in each participant's measurements over-time, so this reduction will be small and will only slightly reduce the strength of the relationship.

Results

As seen in Table 1 mean (SD) venous and capillary cortisol concentrations increased throughout recovery, peaking at 15 minutes post, and showed a subsequent decline at 20 minutes post-exercise. Salivary cortisol concentrations rose continuously throughout the entire recovery period.

----------Table 1 near here----------

Figures 1, 2 and 3 suggest a strong relationship between capillary and venous cortisol samples (R^2= 0.80, p= <0.001), a moderate relationship between salivary and venous cortisol (R^2= 0.32, p= <0.001), as well as for salivary and capillary concentrations (R^2= 0.37, p= <0.001).

----------Figure 1, 2 and 3 near here----------

Capillary and venous cortisol concentrations increased at similar rates following exercise (Figure 4), peaking at 15 minutes post-exercise. Increases in salivary cortisol concentrations were smaller compared to venous and capillary cortisol (Figures 5 and 6) during all of the time periods post-exercise. No peak concentration was observed (peak representing the highest value before a subsequent decline). Between post 5 – 20 minutes salivary cortisol under-estimated the change in venous cortisol (d = 0.47) and capillary cortisol (d = 0.72).

----------Figures 4, 5 and 6 near here----------

Discussion

The aim of the current study was to examine concordance across a large range of cortisol concentrations sampled from saliva, the capillary bed (fingertip) and a forearm vein pre and post maximal exercise. As shown in Figure 1, capillary and venous cortisol responded similarly to high-intensity exercise, inclusive of values above the CBG binding point (500nmol.L^{-1}). However, Figures 2 and 3 demonstrate a much weaker liner association

between salivary and both venous and capillary cortisol across a range of concentrations. In accordance with previous studies, our results showed that following high-intensity exercise the concentration of total cortisol in the blood rose until a peak was reached at post 15 minutes, after which a decline was then observed (Table 1)[16,18,25,26]. Salivary cortisol did not elicit the same response. Data presented in Table 1 suggests a continued rise throughout the 20 minute recovery period, and consequently the peak capture point may not have been observed. This is likely due to the previously suggested delay in which cortisol takes to be detected in the saliva[5]. Furthermore, the similar cortisol fluctuations seen across the recovery period (Table 1) suggest the half life of cortisol sampled in capillary blood was not significantly affected by the time taken for cortisol to reach the body's vascular periphery.

The current findings support previous research suggesting that the free fraction of cortisol (salivary) may have a biphasic response, exponentially increasing once the CBG point has been saturated[3,9,27]. Previous research has shown that when the stress response is moderate and therefore concentrations remain below the CBG binding point, free cortisol appears to be representative of total cortisol[28-30]. However, previous studies which used different exercise intensities to assess the relationship between free and total cortisol have found differing results[9 10]. These discordant findings may be in part due to the different exercise modalities and intensities used. After sub-maximal cycle ergometry (75% of $\dot{V}O_2$ max), O'Connor[10] reported a strong linear relationship (r = 0.60-0.93, p <0.01) between salivary and total cortisol, whereas after a maximal cycle ergometry test Port[9] suggested that an exponential fit (r = 0.86, p <0.001) better represented the free/total cortisol relationship. In the two aforementioned studies the elicited concentrations were approximately 500 nmol·L^{-1} and 800 nmol·L^{-1} respectively, suggesting that an exercise intensity which elicits a response above the CBG binding point may in-part explain the exponential relationship between free and total cortisol concentrations. In further support of this notion, Gozansky[3] reported that salivary free cortisol samples which exceeded the CBG binding point had an exponential fit with total serum samples when participants exercise on a treadmill at 90% of heart rate max for a 10-min period. Findings from the current study builds upon the belief that free cortisol may be misrepresentative in its biphasic response during maximal exercise stress tests, or protocols which are expected to elicit greater quantities of both psychological and physiological stress (>500 nmol·L^{-1}). Furthermore, the linear correlation (r = 0.89, p <0.001) presented in Figure 1 suggests that the use of capillary sampling may be an alternative to venous sampling when

assessing total serum cortisol. Interestingly, the current study showed that the mean difference between capillary and venous cortisol became even smaller (3.5%) during high levels of stress (when the cortisol response is >388nmol·L^{-1}), unlike that seen in the salivary response.

In agreement with previous research[5,31], Figures 4,5 and 6 suggest that the activation of the HPA axis maybe detected later in the saliva compared to both venous and capillary serum. A peak was observed in the venous sample at 15min with capillary concentrations showing a slight delay, likely due to the time taken for the steroid to reach the vascular periphery during recovery (passive recovery). However, salivary cortisol does not appear to peak within the 20 minutes post-exercise, and the capture point may well have occurred later. Therefore, unlike saliva, both venous and capillary cortisol may be of greater value when assessing peak, rate and temporal changes after an intense acute stress.

Cortisol concentration has been shown to increase as a consequence of psychological stress resulting from venipuncture[6]. Anecdotally, participants in the current study reported less discomfort with the capillary sampling technique as opposed to venipuncture. This is in agreement with previous research that reported less pain with capillary blood sampling during its use in a diagnostic setting[32]. Although psychological stress and pain responses were not directly measured in the current study, it can be assumed that capillary sampling would have elicited a far smaller cortisol response compared to venipuncture. Capillary sampling is not as invasive as venipuncture and is therefore less likely to cause increases in psychological stress, which may interfere with exercise science or clinical based assessments. Furthermore, capillary cortisol sampling may prove particularly useful when venipuncture is considered problematic, typically with respect to sporting contexts where movement may inhibit the safe use of a cannula, or in some paediatric populations.

In conclusion, cortisol sampled from the capillary bed of the fingertip accurately reflects cortisol values seen in venous blood across a broad range of concentrations over the same time period in healthy individuals. In agreement with earlier research, salivary cortisol appears to have a biphasic response and rise disproportionately when concentrations exceed the CBG binding point. Capillary cortisol sampling may represent a minimally invasive, cost-effective, practical and valid approach for assessing the HPA axis in response to acute

situations which may elicit high concentrations of cortisol such as maximal exercise or situations which involve both high levels of psychological and physiological stress.

Table 1. Mean (SD) cortisol concentrations assayed from samples taken from venous blood, capillary blood and saliva.

Time	Venous		Capillary		Saliva	
min	*nmol·L^{-1}*		*nmol·L^{-1}*		*nmol·L^{-1}*	
Pre	312.7	(118.2)	287.7	(127.3)	11.9	(5.8)
0	300.0	(153.6)	271.5	(134.5)	12.7	(7.4)
5	415.5	(145.3)	362.9	(118.9)	14.4	(5.5)
10	483.4	(113.8)	480.4	(100.7)	14.2	(5.0)
15	534.4	(109.4)	511.3	(88.7)	15.2	(5.1)
20	518.3	(101.2)	509.9	(97.0)	17.8	(7.1)

NB: All samples were analysed in duplicate, values reported in Table 1 represent the mean value

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