Measuring the Salivary Testosterone and Cortisol Concentrations of Weightlifters Using an Enzyme-Immunoassay Kit

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- steroids
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- validation
- athletes

Abstract

This study assessed an enzyme-immunoassay (EIA) kit for measuring the salivary testosterone (T) and cortisol (C) concentrations of weightlifters. Saliva samples (n = 64) were collected from male and female weightlifters during normal training procedures and analysed for T and C using a commercial EIA kit and a criterion radioimmunoassay (RIA) method. Significant correlations were demonstrated between the EIA and RIA measurements of salivary T (r = 0.96) and C (r = 0.72) concentrations (P < 0.001). Further examination by sample and gender revealed similar relationships. The EIA concentrations of salivary T and C were found to be slightly greater (10–13 %) than the RIA values. Similar discrepancies were noted when gender comparisons were made, although the relative information on T (males > females) and C (males = females) were consistent for both assay methods. In conclusion, a commercially available EIA kit provided valid measures of the salivary T and C concentrations of male and female weightlifters. Factors to consider when using an EIA kit include the hormone(s) of interest, the magnitude of the correlations, as well as the descriptive information gained (e.g. absolute, relative) and its uses within sport.

Introduction

The monitoring of salivary testosterone (T) and cortisol (C) concentrations is gaining increasing acceptance within the wider community and in particular sport [6–9, 14, 18]. The use of saliva offers many benefits for assessing steroid hormones in athletes, because saliva collection is non-invasive, stress-free and enables real-time repeated sampling where blood collection is often undesirable or difficult [15]. Moreover, the salivary T and C concentration measures have correlated (r = 0.60–0.97) with the blood hormones, especially the free hormone that initiates the biological response at target tissue [3, 10, 24, 25] and the bioavailable hormone that is potentially available to tissue [3]. Thus, the monitoring of T and C in saliva could provide greater biological understanding of the effects of exercise, training and competition.

Radioimmunoassay (RIA) and enzyme-immunoassay (EIA) are two of the most common methods for analysing salivary hormones. Of these, EIA is perhaps the most practical and cost-effective option, because this method can be performed relatively quickly and without the greater laboratory costs associated with RIA. Furthermore, EIA kits can be purchased commercially and stored for many months, whereas RIA kits are more difficult to purchase and have only a limited (i.e. weeks) shelf life. The EIA measurement of salivary T and C concentrations have also correlated strongly (r = 0.96–0.99) with RIA in non-athletic populations [20, 23, 26]. However, we are unaware of any studies validating a commercially available EIA kit (v. RIA) for measuring these hormones in athletes.

Weightlifters and weight-trained athletes differ from non-athletes in their resting T concentrations [2], the acute T responses to exercise [1] and, potentially, the ability to use T in circulation [1]. Weightlifters can also differ from non-elite controls in the steroid receptor content of muscle [13], which further suggests differences in hormone uptake and utilisation. Similarly, correlations have been reported between the individual T and/or C concentrations of weightlifters or weight-trained athletes and functional measures of performance during training or competition [7, 18]. Given these apparently fundamental dif-

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ferences in the hormonal milieu between trained and untrained individuals, understanding if the EIA assessment of salivary hormones correlates well with RIA in an athletic sub-group is a prerequisite to validating athlete hormonal studies.

The aim of this study was to assess a commercially available EIA kit for measuring the salivary T and C concentrations of male and female weightlifters. For the adoption of research tools to be effective within sport, evidence must also show that the innovation is feasible and effective within the real-world setting. Consequently, the hormonal data in this study was collected within the normal training procedures of the study population.

Methods

Participants

Four male and four female weightlifters volunteered for this study. The respective mean (± standard deviation) age, height and body mass for male participants were 20.8 ± 3.5 years, 171.0 ± 5.2 cm, 79.3 ± 17.5 kg. The mean (± standard deviation) age, height and body mass for female participants were 22.8 ± 4.6 years, 158.6 ± 5.4 cm, 73.4 ± 17.1 kg. All participants were competitive weightlifters. This study was undertaken during the competitive season, at which time participants were training 1–2 times daily (between 45–120 min each session), 5 days a week. Ethical approval was granted by the Waikato Institute of Technology, Hamilton, New Zealand. This study has been performed in accordance with the ethical standards of the International Journal of Sports Medicine [12].

Procedures

Participants each provided whole saliva samples (2 ml) before and after four workouts, across a 4-week training period (n = 64 saliva samples) during the competitive season. Each workout was performed at the same time of day (4:00 p.m.–6:30 p.m.) and involved an identical warm-up routine followed by the same training exercises; squat, clean and jerk, and front squat. Participants were instructed not to eat, brush their teeth or drink hot fluids for 2 h before training to prevent saliva contamination [6]. The samples were collected in sterile containers (LabServe, New Zealand) by direct expectoration, after first using sugar-free gum (Extra-peppermint, Wrigley's, New Zealand) to increase saliva flow [11]. The saliva samples were aliquoted into three separate sterile containers (LabServe, New Zealand) and stored at −80 °C until assay.

Hormone analysis

The EIA samples were analysed in duplicate using commercial kits (Salimetrics LLC, USA) and the manufacturers' guidelines. The minimum detection limit for the T assay was 21 pmol·L^{-1} with intra- and inter-assay coefficients of variation (CV) of 1.3–13.3 %. The C assay had a detection limit of 0.33 nmol·L^{-1} with intra- and inter-assay CV of 3.0–10.4 %. The RIA samples were analysed in triplicate using diagnostic kits (Diagnostic Systems Laboratories, Inc. Texas) and the modifications described elsewhere [11,17]. The detection limit for the T assay was 3.5 pmol·L^{-1} with an intra-assay CV of 8.2–10.8 %. The C assay had a detection limit of 0.14 nmol·L^{-1} with an intra-assay CV of 0.8–3.1 %.

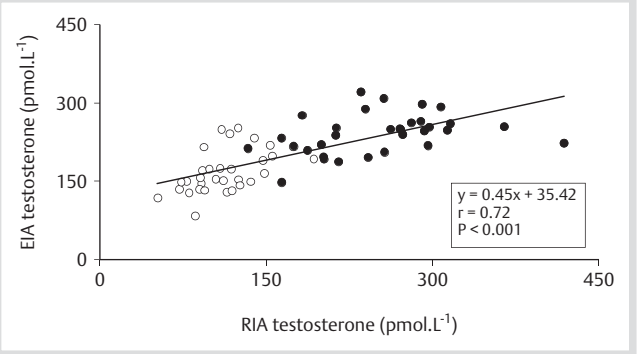

Fig. 1 Correlations between the salivary testosterone concentrations of weightlifters measured by an enzyme-immunoassay (EIA) and radioimmunoassay (RIA) method. The linear regression line for the pooled male (black) and female (white) data are presented.

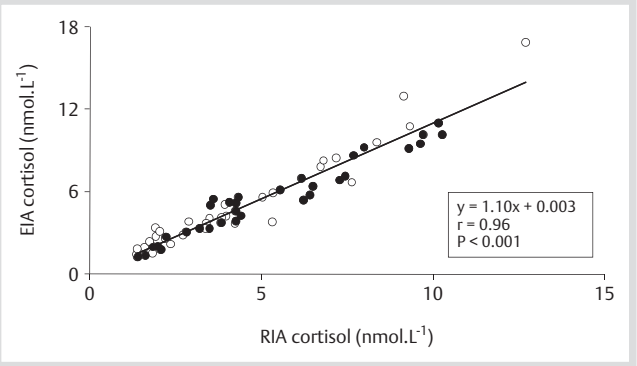

Fig. 2 Correlations between the salivary cortisol concentrations of weightlifters measured by an enzyme-immunoassay (EIA) and radioimmunoassay (RIA) method. The linear regression line for the pooled male (black) and female (white) data are presented.

Statistical analyses

Descriptive information for the hormonal variables were determined using standard methods. Relationships between the hormonal variables were assessed using Spearman's rank correlation coefficients (r) and linear regression. The hormonal data were examined as a pooled group, by gender and sample. The significance level was set at $P \leq 0.05$.

Results

The pooled concentrations of T (205.4 ± 53.5 pmol·L^{-1}) and C (5.24 ± 3.21 nmol·L^{-1}) were found to be slightly greater for the EIA method, than the measured values from the RIA method (182.1 ± 84.9 pmol·L^{-1}, 4.76 ± 2.80 nmol·L^{-1}), respectively. For both assay methods, the T concentrations of males were significantly ($P < 0.001$) greater than the females, but there were no gender differences in C concentrations ($P > 0.05$).

Significant ($P < 0.001$) moderate correlations ($r = 0.72$) were demonstrated between the pooled concentrations of T measured by EIA and RIA (**Fig. 1**). Significant ($P < 0.001$) strong correlations ($r = 0.96$) were also identified between the pooled concentrations of C (**Fig. 2**). Correlations between the pre- and post-workout samples for T ($r = 0.76$–0.78) and C ($r = 0.96$–0.97) were consistent with these results ($P < 0.001$). When examined by

gender, significant ($P < 0.01–0.001$) correlations were also identified between the T ($r = 0.48$, $r = 0.51$) and C ($r = 0.97$, $r = 0.96$) data for males and females, respectively.

Discussion

The measurement of the salivary T and C concentrations of weightlifters by a commercial EIA kit revealed moderate to strong relationships with a criterion RIA method, in agreement with previous research [19,20,23,26]. Our findings validate the use of EIA to assess these hormones in an athletic sub-group. However, the magnitude of these relationships suggests that the EIA measurement of C has strong validity, whereas T has only moderate validity. This could be explained by differences in absolute concentrations and the assay specifications (e.g. sensitivity, specificity) for each hormone, as well as hormone pharmacokinetics (e.g. diffusion rate, metabolism) within the salivary glands [4,16]. Other possible factors influencing these relationships include genetic variation [15], the population assessed [19] and the hormonal data range [19].

In contrast to the current findings, previous research has demonstrated gender variation in the magnitude of the T relationships, being much stronger for males than that observed for females using both EIA and RIA methods [21]. However, this finding was based on hormonal comparisons made between saliva and blood samples, whereas only saliva samples were taken in this study. It is important to emphasize that the primary aim of this study was to validate the salivary T and C concentration measures by an EIA assay (against a criterion RIA method) using the same biological medium. Still, we acknowledge some of the limitations of the current study, such as the small number of participants assessed, the limited range of hormonal values and the lack of control data.

The measurement of T and C concentrations by EIA produced values that were slightly larger (10–13%) than the RIA method. Further examination of the hormonal data by gender revealed similar findings, except for male T, which was similar between the two assay methods. These observations are supported by previous research [19,20,23,26]. The gender results were also consistent with other research, in terms of relative hormonal concentrations [5,11,14,21,24–26]. That is, female T concentrations represent a much lower proportion of male T concentrations, regardless of the biological medium collected (e.g. saliva, blood) and the assay method used. Conversely, C concentrations are generally no different between genders. Thus, in addition to the statistical results, the descriptive information gained (e.g. absolute, relative) is another consideration when deciding on the use of EIA to assess salivary hormones in sport.

It is noteworthy that the hormonal values in saliva still represent a wide physiological range in both athletic (T range 200–1200 $pmol \cdot L^{-1}$, C range 3–50 $nmol \cdot L^{-1}$) [6–9,14,18] and non-athletic (T range 20–800 $pmol \cdot L^{-1}$, C range 3–29 $nmol \cdot L^{-1}$) populations [3,5,11,24,26]. This variation could be explained by athletic factors relating to exercise, training and competition, along with other biological factors such as circadian rhythms, age and endocrine status (e.g. hypogonadism) [22]. As noted in this research, assay method and gender variation are other confounding variables when comparing hormonal data between studies. Therefore, where attempts are made to compare absolute concentrations or construct clinical or population range values, such differences have a marked impact that requires careful consideration.

In conclusion, a commercially available EIA kit provided valid measures of the salivary T and C concentrations of male and female weightlifters. Factors to consider when using an EIA kit include the hormone(s) of interest, the magnitude of the correlations, as well as the descriptive information gained (e.g. absolute, relative) and its uses within sport.

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