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## RESEARCH LETTER

### Poor Levels of Agreement between Serum and Saliva Testosterone Measurement Following Exercise Training in Aging Men

LAWRENCE D HAYES<sup>1,2</sup>, NICHOLAS SCULTHORPE<sup>2</sup>, PETER HERBERT<sup>3</sup>, JULIEN S  
BAKER<sup>2</sup>, DAVID A HULLIN<sup>4</sup>, LIAM P KILDUFF<sup>5</sup>, and FERGAL M GRACE<sup>2</sup>

*<sup>1</sup>School of Human Sciences, London Metropolitan University, UK; <sup>2</sup>Institute of Clinical Exercise and Health Science, University of the West of Scotland, UK; <sup>3</sup>School of Sport, Health and Outdoor Education, Trinity Saint David, University of Wales, UK; <sup>4</sup>Department of Clinical Biochemistry, Royal Glamorgan Hospital, UK; <sup>5</sup>Applied Sports Technology, Exercise and Medicine, Swansea University, UK*

L. D. Hayes (✉)

School of Human Sciences,  
London Metropolitan University,  
Holloway Road, London, N7 8DB, UK  
020 7133 4686  
E-mail: L.Hayes@Londonmet.ac.uk

## **Abstract**

Testosterone (T) is a biologically important androgen that demonstrates a widely-known natural decline with advancing age. The use of salivary T (sal-T) as a determinant of systemic T has shown promise in recent years however the strength of the salivary-serum T relationship may be affected by measurement method and binding capacity with salivary proteins. The potential influence exercise may impact on this relationship is unstudied in aging men. Therefore, the aim of the present investigation was to examine the relationship of the delta change ( $\Delta$ ) in sal-T with  $\Delta$ serum T following six weeks exercise training. Fifteen sedentary (SED) males (aged  $60.4 \pm 5.0$  years of age) and twenty lifelong exercising (LE) males ( $60.4 \pm 4.7$  years of age) participated. Pearson's correlation coefficient revealed sal-T did not correlate with total testosterone (TT), sex hormone binding globulin (SHBG), bioactive T (bio-T), or free T (free-T) at week 0 or week 6.  $\Delta$ sal-T did not correlate with  $\Delta$ TT,  $\Delta$ SHBG,  $\Delta$ bio-T or  $\Delta$ free-T ( $r=0.271$ ,  $p=0.180$ ;  $r=0.197$ ,  $p=0.335$ ;  $r=0.258$ ,  $p=0.205$ ; and  $r=0.257$ ,  $p=0.205$  respectively). In conclusion, poor levels of agreement existed between saliva and serum measurements of T in response to exercise amongst aging men.

**Key words:** aging, exercise, saliva, sex hormone binding globulin, testosterone

**Dear Editor,** Testosterone (T) demonstrates a widely-known natural decline with advancing age [1], at a rate that ranges between 0.4 to 2.6% per year after the fourth decade [2, 3]. Androgenic status in aging men is further magnified by the gradual increase in sex hormone binding globulin (SHBG) producing pronounced declines in bioavailable (bio-T) and free testosterone (free-T) fractions [3].

Measurement of T in saliva was first described by Landman and colleagues [4] and the number of studies employing this technique to infer systemic T changes has gathered significant momentum in recent years [5]. Levels of agreement between saliva and serum derived T are equivocal, with reports of good [6], and poor comparability [7, 8]. Recent evidence suggests both analysis method and changes in salivary protein concentration may further impact the relationship between sal-T and free-T in a cohort of males and females of unreported age or training status [8].

Exercise *per se* is purported to positively affect circulating T, free-T, and SHBG, in aging men in coalition with improved insulin sensitivity, and T, free-T, and SHBG each correlate inversely with fasting insulin [9]. Moreover, obesity-mediated low total and free-T may result from be estradiol-regulated reduced luteinizing hormone (LH) and follicle stimulating hormone (FSH), suggesting suppression occurs at the hypothalamic-pituitary level [10]. We recently described poor agreement between sal-T and serum T in both lifelong trained (LE) and sedentary (SED) aging men [7]. However, given that exercise training has been promoted as a first line treatment for low serum T in aging men [11], it is important to elucidate whether a progressive exercise training programme can produce discernible changes in the salivary-serum T relationship. Therefore, the purpose of this study was to compare levels of agreement between serum and salivary steroid hormone measures in response to a

six-week supervised progressive exercise training intervention in SED and an age-matched cohort of LE aging males.

## **METHODS AND MATERIALS**

### *Participants*

Following approval to exercise by their general practitioner, participants provided written informed consent prior to the study which was approved by the University of the West of Scotland Ethics Committee. SED consisted of fifteen males (aged  $64.2 \pm 5.5$  years, stature  $174.0 \pm 5.9$  cm, peak oxygen uptake [ $\dot{V}O_{2\text{peak}}$ ] of  $26.5 \pm 4.9$  ml·kg·min<sup>-1</sup>, body mass of  $92.5 \pm 20.1$  kg). The LE group consisted of twelve males (aged  $60.4 \pm 5.0$  years, stature  $173.2 \pm 6.3$  cm,  $\dot{V}O_{2\text{peak}}$   $39.4 \pm 5.6$  ml·kg·min<sup>-1</sup>, body mass  $80.0 \pm 13.6$  kg). SED participants were not physically active, which was confirmed with by low  $\dot{V}O_{2\text{peak}}$ . LE status was determined by self-reported exercise time ( $>150$  min·wk<sup>-1</sup>) and confirmed by measurement of peak aerobic capacity ( $\dot{V}O_{2\text{peak}}$ ).

### *Exercise Training*

SED participants underwent 6 weeks of previously described supervised progressive exercise training [12], which met recommended guidelines for physical activity in older adults [13]. The LE group maintained their current training habits (equating to  $269 \pm 173$  mins·wk<sup>-1</sup>) for the duration of the study which incorporated both resistance and endurance training.

### *Serum and Saliva Sampling*

Methods have been previously described elsewhere [7]. Briefly, blood and saliva samples were collected at week 0 and week 6 between 07:00 – 09:00 h following an overnight fast and 30 min supine rest. Saliva was collected using passive drool, and analysed in duplicate (without separation or extraction) for T using commercially available immunoassay protocols (Salimetrics, State College, PA). Intra- and inter-assay coefficients of variation were less than 7% and 10% respectively. Blood was sampled from an antecubital forearm vein and duplicate serum concentrations of total testosterone (TT) and SHBG were measured by electrochemiluminescent immunoassay on the E601 module of the Roche Cobas 6000 (Burgess Hill, West Sussex, U.K.), with the mean value used as criterion. Inter-assay CVs over a 6 month period were 4.5% and 2.4% for TT and SHBG respectively. Free-T and bio-T were calculated using the equation outlined by Vermueulen and colleagues [14].

### *Determination of peak aerobic capacity ( $\dot{V}O_{peak}$ )*

Methods have been detailed elsewhere [7]. Briefly, five minutes of warm-up preceded a ramped protocol until volitional exhaustion on an air-braked cycle ergometer (Wattbike Ltd., Nottingham, UK) using a modified Storer Test [15]. Work-rate was increased each minute by raising the damper setting by one (equating to 18 W) until volitional exhaustion was achieved. Based on prior pilot study, the test was expected to elicit  $\dot{V}O_{2peak}$  in  $10 \pm 2$  mins. Peak aerobic capacity was determined using open circuit spirometry using a Cortex II Metalyser 3B-R2 (Cortex, Biophysik, Leipzig, Germany). Prior each test, the Metalyser was calibrated according to manufacturers' guidelines. CV for the determination of  $\dot{V}O_{2peak}$  in our laboratory is <3.0%.

### *Statistical analysis*

Data were analyzed using SPSS (version 20; IBM North America, New York, NY, USA). Pearson's correlation coefficient was used to observe relationships between parameters. Significance was set *a priori* at  $p < 0.05$ .

## **RESULTS**

Correlation coefficient and alpha level are reported for pooled analysis. **Sal-T** did not correlate with TT, SHBG, bio-T or free-T at week 0 ( $r=0.040$ ,  $p=0.813$ ;  $r=-0.106$ ,  $p=0.531$ ;  $r=0.133$ ,  $p=0.432$ ; and  $r=0.134$ ,  $p=0.431$  respectively). At week 6, sal-T did not correlate with TT, SHBG, bio-T or free-T ( $r=-0.195$ ,  $p=0.301$ ;  $r=-0.131$ ,  $p=0.492$ ;  $r=-0.204$ ,  $p=0.279$ ; and  $r=-0.204$ ,  $p=0.279$  respectively).  $\Delta$ sal-T did not correlate with  $\Delta$ TT,  $\Delta$ SHBG,  $\Delta$ bio-T or  $\Delta$ free-T ( $r=0.271$ ,  $p=0.180$ ;  $r=0.197$ ,  $p=0.335$ ;  $r=0.258$ ,  $p=0.205$ ; and  $r=0.257$ ,  $p=0.205$  respectively) when pooled, or when LE and SED were analysed separately.  $\Delta\dot{V}O_{2peak}$  and  $\Delta$ mass were not related to any hormonal parameters ( $p > 0.05$ ).

INSERT FIGURE 1 NEAR HERE

INSERT FIGURE 2 NEAR HERE

## DISCUSSION

The main finding of this study was that sal-T did not correspond to serum T parameters (TT, bio-T, and free-T) in aging men prior to, or following a supervised progressive exercise training programme. To our knowledge, this is the first comparison of saliva-serum relationship of T in response to an exercise-training programme in aging men.

The lack of saliva-serum agreement pre, post, and delta change within the present investigation is at odds with earlier validation of sal-T [16-18]. A possible explanation may be different analytical methods as Walker et al. [16] used radioimmunoassay (RIA) and Goncharov et al. [18] used luminescent immunoassay (LIA) for the determination of sal-T whereas the present investigation utilised enzyme-linked immunosorbent assay (ELISA). Whilst Morley and colleagues [17] reported a strong correlation between sal-T and bio-T, free-T, and TT, the training status of participants was not described. Chronic exercise may influence the saliva-serum relationship as increased sal-T [19], but not TT [7] has been reported in a group of highly active older men. Moreover, although sal-T is commonly cited to reflect free-T due to the inability of SHBG to enter saliva [20], SHBG has been detected in saliva [21] and therefore, age related increases in SHBG may have contrived to influence salivary-serum T agreement in the present investigation. Furthermore, a recent investigation suggested specificity and matrix related problems limit the application of assay derived sal-T [8]. It is also possible salivary proteins compromised salivary-serum agreement. High abundance of T and salivary-androgen binding protein has been observed in the lateral nasal gland of mice [22], which lead these authors to suggest organ specific T expression. As the influence of age and exercise on salivary gland androgen receptor expression is currently unknown in humans, further research is required to delineate these issues. These



uncertainties, in addition to recent reporting of large (>90%) sal-T critical difference (clinically relevant threshold) [23], **and inability of sal-T to detect serum-defined biochemical hypogonadism [24]** implies that sal-T, whilst an appealing surrogate of serum collection, is unsuitable to determine androgen status in aging men.

In conclusion, the present study highlights poor levels of agreement between saliva and serum measurements of T in response exercise training amongst aging men. Our findings indicate that sal-T has limited application for tracking exercise-induced changes in T amongst aging male cohorts.

Authors declare they have no conflict of interest.

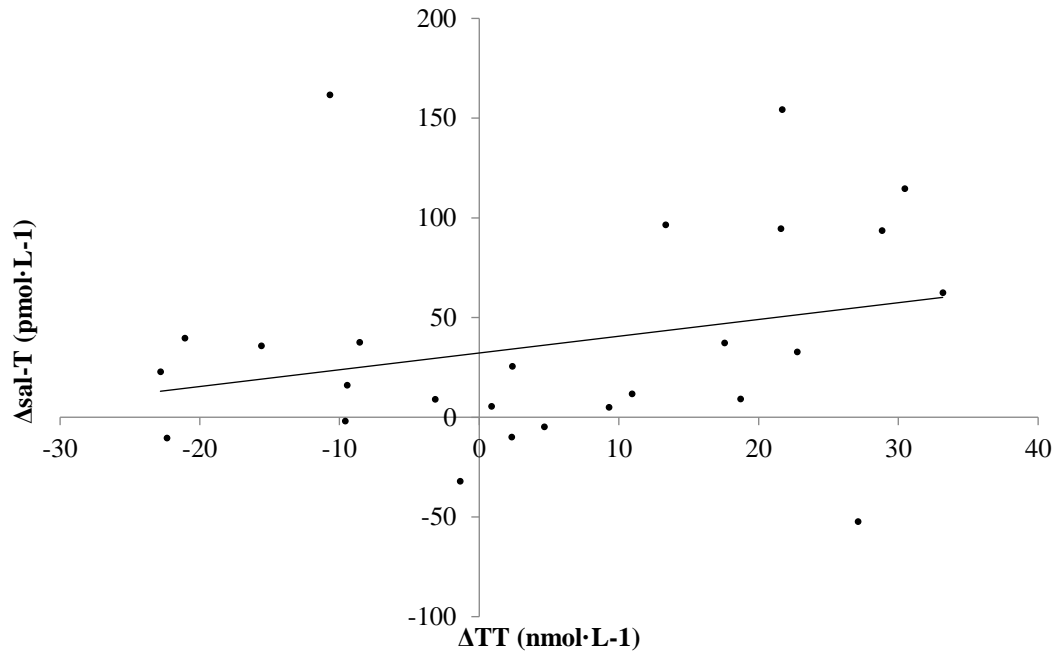
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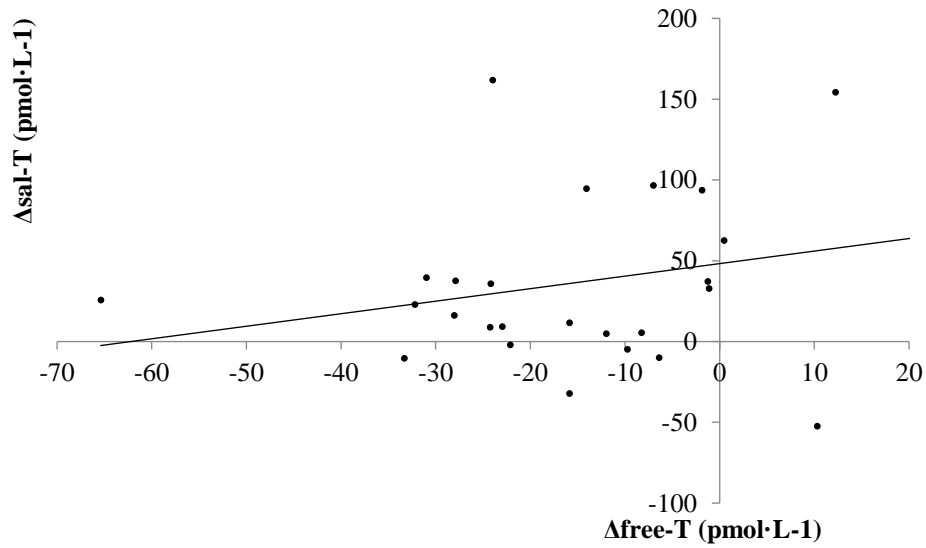
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**FIGURE 1**

**FIGURE**

**2**



## FIGURE LEGENDS

FIGURE 1 Correlation coefficient for delta change in ( $\Delta$ ) salivary testosterone (sal-T) and total testosterone (TT) in aging men following a six week aerobic training intervention. **X-axis = change in TT from week 0 to week 6. Y-axis = change in sal-T from week 0 to week 6.**  $r=0.271$ ;  $p=0.180$ .

FIGURE 2 Correlation coefficient for delta change in ( $\Delta$ ) salivary testosterone (sal-T) and free testosterone (free-T) in aging men following a six week aerobic training intervention. **X-axis = change in free-T from week 0 to week 6. Y-axis = change in sal-T from week 0 to week 6.**  $r=0.257$ ;  $p=0.205$ .