RESEARCH LETTER

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

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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 (Δ) in sal-T with Δserum T following six weeks exercise training. Fifteen sedentary (SED) males (aged 60.4 ± 5.0 years of age) and twenty lifelong exercising (LE) males (60.4 ± 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. Δsal-T did not correlate with ΔTT, ΔSHBG, Δbio-T or Δfree-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 ± 5.5 years, stature 174.0 ± 5.9 cm, peak oxygen uptake [$\dot{V}O_{2peak}$] of 26.5 ± 4.9 ml·kg·min^{-1}, body mass of 92.5 ± 20.1 kg). The LE group consisted of twelve males (aged 60.4 ± 5.0 years, stature 173.2 ± 6.3 cm, $\dot{V}O_{2peak}$ 39.4 ± 5.6 ml·kg·min^{-1}, body mass 80.0 ± 13.6 kg). SED participants were not physically active, which was confirmed with by low $\dot{V}O_{2peak}$. LE status was determined by self-reported exercise time (>150 min·wk^{-1}) and confirmed by measurement of peak aerobic capacity ($\dot{V}O_{2peak}$).

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 ± 173 mins·wk^{-1}) 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 ± 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 $\dot{V}0_{2peak}$ 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). Δsal-T did not correlate with ΔTT, ΔSHBG, Δbio-T or Δfree-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 Δmass were not related to any hormonal parameters ($p>0.05$).

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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.

REFERENCES

1. Araujo AB, Dixon JM, Suarez EA, Murad MH, Guey LT, Wittert GA. Clinical review: Endogenous testosterone and mortality in men: a systematic review and meta-analysis. J Clin Endocrinol Metab 2011;96(10):3007-19.

2. Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. J Clin Endocrinol Metab 2001;86(2):724-31.

3. Feldman HA, Longcope C, Derby CA, Johannes CB, Araujo AB, Coviello AD, Bremner, WJ, McKinlay JB. Age trends in the level of serum testosterone and other hormones in middle-aged men: Longitudinal results from the Massachusetts Male Aging Study. J CLin Endocrinol Metab 2002;87(2):589-98.

4. Landman AD, Sanford LM, Howland BE, Dawes C, Pritchard ET. Testosterone in human saliva. Experientia 1976;32(7):940-1.

5. Hayes LD, Grace FM, Baker JS, Sculthorpe N. Exercise-induced responses in salivary testosterone, cortisol, and their ratios in men: A meta-analysis. Sports Med 2015; 1-14 doi: 10.1007/s40279-015-0306-y

6. Crewther BT, Lowe TE, Ingram J, Weatherby RP. Validating the salivary testosterone and cortisol concentration measures in response to short high-intensity exercise. J Sports Med Phys Fitness 2010;50(1):85-92.

7. Hayes LD, Sculthorpe N, Herbert P, Baker JS, Hullin DA, Kilduff LP, Grace FM. Resting steroid hormone concentrations in lifetime exercisers and lifetime sedentary males. Aging Male 2014: Epub ahead of print.

8. Fiers T, Delanghe J, T'Sjoen G, Van Caenegem E, Wierckx K, Kaufman J-M. A critical evaluation of salivary testosterone as a method for the assessment of serum testosterone. Steroids 2014;86(0):5-9.

9. Muller M, Tonkelaar I, Thijssen, JHH, Grobbee DE, van der Schouw YT. Endogenous sex horrmones in men aged 40-80 years. Eur J Endocrinol 2003;149:583-9.

10. Fui MNG, Dupuis P, Grossman M. Lowered testosterone in male obesity: mechanisms, morbidity and management. Asian J Androl 2014;16(2):223-31.

11. Khoo J, Tian HH, Tan B, Chew K, Ng CS, Leong D, Teo RC, Chen RY. Comparing effects of low- and high-volume moderate-intensity exercise on sexual function and testosterone in obese men. J Sex Med 2013;10(7):1823-32.

12. Hayes LD, Grace FM, Sculthorpe N, Herbert P, Ratcliffe JW, Kilduff LP, Baker JS. The effects of a formal exercise training programme on salivary hormone concentrations and body composition in previously sedentary aging men. SpringerPlus. 2013;2(1):18.

13. Nelson ME, Rejeski WJ, Blair SN, Duncan PW, Judge JO, King AC, Macera CA, Castaneda-Sceppa C. Physical Activity and Public Health in Older Adults: Recommendation from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc 2007;39(8):1435-45.

14. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84(10):3666-72.

15. Storer TW, Davis JA, Caiozzo VJ. Accurate prediction of VO2max in cycle ergometry. Med Sci Sports Exerc 1990;22(5):704-12.

16. Walker RF, Wilson DW, Read GF, Riad-Fahmy D. Assessment of testicular function by the radioimmunoassay of testosterone in saliva. Int J Androl 1980;3(2):105-20.

17. Morley JE, Perry HM, Patrick P, Dollbaum CM, Kells JM. Validation of salivary testosterone as a screening test for male hypogonadism. Aging Male 2006;9(3):165-169.

18. Goncharov N, Katsya G, Dobracheva A, Nizhnik A, Kolesnikova G, Herbst V, Westermann J. Diagnostic significance of free salivary testosterone measurement using a direct luminescence immunoassay in healthy men and patients with disorders of andrgenic status. Aging Male 2006;9(2):111-122.

19. Hayes LD, Grace FM, Sculthorpe N, Herbert P, Kilduff LP, Baker JS. Does chronic exercise attenuate age-related physiological decline in males? Red Sports Med 2013;21(4):343-354.

20. Cook CJ, Crewther BT. Changes in salivary testosterone concentrations and subsequent voluntary squat performance following the presentation of short video clips. Horm Behav 2012;61(1):17-22.

21. Selby C, Lobb PA, Jeffcoate WJ. Sex hormone binding globulin in saliva. Clin Endocrinol (Oxf). 1988;28(1):19-24.

22. Zhou X, Zhang X, Weng Y, Fang C, Kaminsky L, Ding X. High abundance of testosterone and salivary androgen-binding protein in the lateral nasal gland of male mice. J Steroid Biochem Mol Biol 2009;117(1-3):81-6.

23. Hayes LD, Sculthorpe N, Young JD, Baker JS, Grace FM. Critical difference applied to exercise-induced salivary testosterone and cortisol using enzyme-linked immunosorbent assay (ELISA): distinguishing biological from statistical change. J Physiol Biochem 2014;70(4):991-6.

24. Hayes LD, Sculthorpe N, Herbert P, Baker JS, Hullin DA, Kilduff LP, Reed D, Spagna R, Grace FM. Salivary testosterone measurement does not identify biochemical hypogonadism in aging men: a ROC analysis. Endocrine 2014. Epub ahead of print

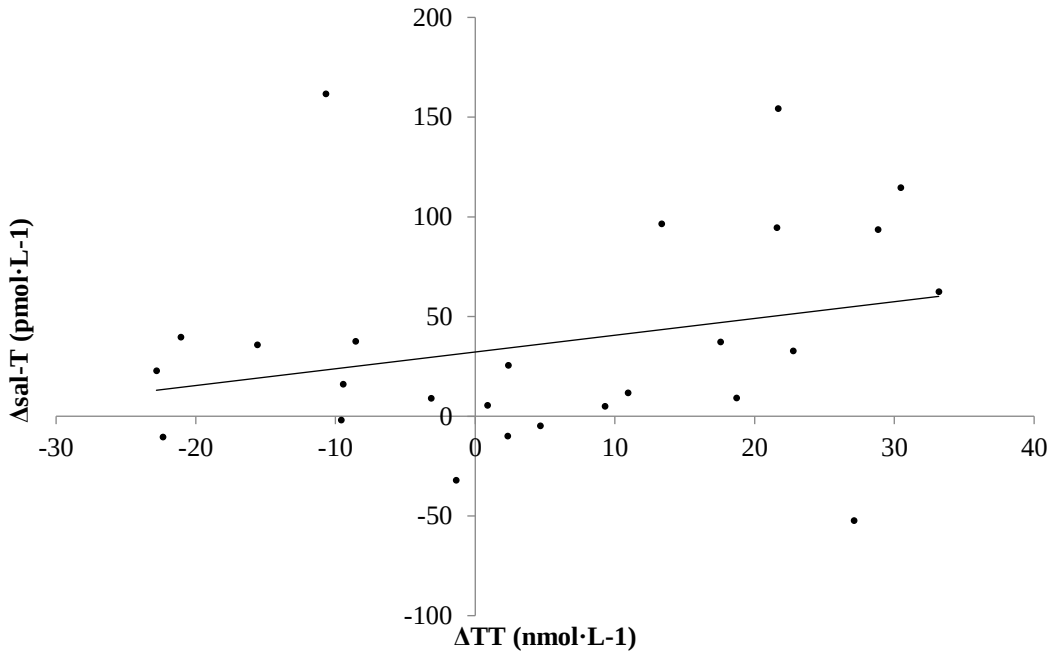

FIGURE 1

FIGURE 2

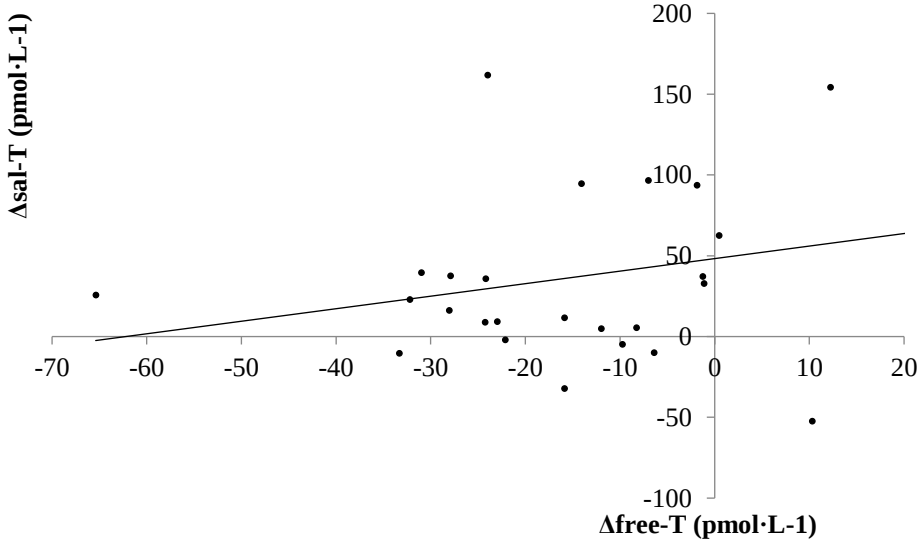

FIGURE LEGENDS

FIGURE 1 Correlation coefficient for delta change in (Δ) 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 (Δ) 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.