ORIGINAL ARTICLE

Six weeks of conditioning exercise increases total, but not free testosterone in lifelong sedentary aging men.

Lawrence D Hayes[1,2], Nicholas Sculthorpe[2], Peter Herbert[3], Julien S Baker[2], Roberto Spagna[4], and Fergal M Grace[2]

[1]School of Human Sciences, London Metropolitan University, UK; [2]Institute of Clinical Exercise and Health Science, University of the West of Scotland, UK; [3]School of Sport, Health and Outdoor Education, Trinity Saint David, University of Wales, UK; [4]Department of Clinical Biochemistry, Royal Glamorgan Hospital, 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

Introduction: Advancing age is associated with a gradual decline in circulating androgens and the putative role of exercise training on systemic androgens remain to be adequately defined.

Methods: The present investigation examined the impact of six weeks of supervised exercise training on resting levels of systemic hormones in a cohort of lifelong sedentary men (SED [*n*=28], 62.5 ± 5.3 years), compared with a positive control group of age-matched lifelong exercisers (LE [*n*=20], 60.4 ± 4.7 years, >30 years training history). Blood hormones were sampled pre- and post-intervention from an antecubital forearm vein and analysed using electrochemiluminescent immunoassay. Cardiorespiratory fitness ($\dot{V}O_{2peak}$) was determined via indirect calorimetry during an incremental cycle test to volitional exhaustion.

Results: Analysis of variance (ANOVA) revealed a lack of significant change in any parameter amongst LE whilst SED experienced a significant exercise-induced improvement in cardiorespiratory fitness, and total testosterone (all $p<0.05$). Concurrent increases in sex hormone-binding globulin (SHBG; $p<0.05$) resulted in a lack of change to either bioavailable or calculated free testosterone ($p>0.05$) amongst SED.

Conclusions: Although resting levels of systemic total testosterone increased in response to six weeks of exercise training, increases in SHBG negated any potential relationship between calculated-free or bioavailable testosterone. These findings indicate that increases in bioavailable testosterone fraction are not required for cardiorespiratory fitness improvements in aging men.

Key words: Aging, exercise, sex hormone-binding globulin, testosterone

INTRODUCTION

Advancing age is associated with a gradual decline in many physiological functions [1, 2] that accompany a concurrent reduction in time spent being physically active [3]. It is well established that both cardiorespiratory fitness (CRF) [1] and peak muscular power output [4] decline with advancing age. These declines are powerful predictors of both independence [2], and risk of morbidity [5]. Indeed, a major obstacle to achieving increased health span, reduced morbidity and optimal longevity in individuals is the decline in physiological function that accompanies advancing age [6]. Systemic testosterone (T) concentrations display an analogous pattern of age-associated decline to that of CRF and muscular power [7]. Available longitudinal studies demonstrate this decline in systemic-T to range between 0.4-2.6% per year after the age of 40 [8, 9], with an accelerated decline during the seventh decade [8]. Conversely, sex hormone-binding globulin (SHBG) is known to increase with age, resulting in a further decline in the biologically active 'free testosterone' (free-T) fraction [9]. This decrease in systemic-T is associated with musculoskeletal atrophy and decreased functional capacity [10]. Indeed, Folland et al. [11] have observed the age related decline in knee extensor strength to correlate with free-T, but not total testosterone (TT) in a cohort of aging men. Moreover, Hyde et al. [12] reported free-T, but not total T was independently associated with frailty in men aged 70-88 years leading to the suggestion that the decline in free-T might be of greater significance to the maintenance of adequate muscle function with advancing age.

Acute exercise-induced increases in T concentrations have been widely reported [13-17]. However, despite exercise being a commonly used first-line treatment for mild age-associated T decrements [18, 19], it remains unclear whether exercise can potentiate a reversal of the age-associated decline in systemic androgens. Ari and colleagues [20] reported higher basal TT in masters athletes compared with age-matched sedentary males. However, it

is unclear whether this represents a long term effect of exercise training on maintaining TT or whether those who sustain serum TT into old age (pro-androgenic phenotype) are also more able to undertake the rigours of exercise. Indeed, such observations are not consistent with similar investigations that demonstrate TT to be unaffected by exercise training in aging cohorts [21-23], and more recently in lifelong sedentary, but otherwise healthy, aging men [24].

Experimental studies have conveyed that individuals embarking on an exercise program from middle to older age typically experience improvements in CRF [21, 25, 26] in addition to improved muscle strength [21, 27] and power [28]. Modest increases in CRF are associated with lower mortality risk [29], such that one metabolic equivalent (MET) increase in peak oxygen uptake ($\dot{V}O_{2peak}$ [an increment achievable by most sedentary individuals, healthy or otherwise]) can produce considerable (10%–25%) improvement in survival [30]. Therefore, encouraging exercise participation during advancing age is a particularly pressing public health issue.

It is unclear whether the decline in sex steroid hormones with advancing age is due to biological aging *per se* or a secondary consequence of lifestyle changes such as reduced physical activity [21]. Cross-sectional studies have observed a positive correlation between physical fitness and serum T in older men [31, 32]. For example, using multiple regression analysis, Aguirre et al. [31] reported TT to be a consistent predictor of $\dot{V}O_{2peak}$, physical performance, and knee flexion and extension torque amongst frail, obese, older adults. Taken together, the tripartite relationship between exercise, sex steroid hormones and aging require further study. With this in mind, the present study examined the impact of six weeks exercise training programme on systemic sex steroid hormones in a group of otherwise healthy sedentary aging men (SED). These were compared with a positive control group of age-

matched lifelong exercisers (LE). We hypothesized that exercise training would induce a more favourable sex steroid hormone profile which would accompany an improved cardiorespiratory fitness in SED without change in LE.

METHODS AND MATERIALS

Participants

Prior to experimental data collection, participants were fully habituated and familiarized with procedures in order to minimise any learning effect. Following approval to exercise by their general practitioner, participants provided written informed consent prior to enrolment to the study which was approved by the University of the West of Scotland Ethics Committee. Twenty eight males (63 ± 5 years, with a stature of 175 ± 6 cm, and body mass of 91 ± 16 kg) participated in the SED group. Twenty males (60 ± 5 years, with a stature of 174 ± 6 cm, and body mass of 79 ± 10 kg) participated in the LE group and acted as a positive control group. As a result of high completion rates (87%), statistical power for $\dot{V}O_{2peak}$ was confirmed at 0.98 using a post hoc power calculation. Participants recruited for the SED group did not participate in any organized exercise program and had not done so for >30 years. The LE group were highly active exercisers and had been so for the previous >30 years. They consisted primarily of current masters competitors in sports including water-polo, triathlon, sprint cycling, road cycling and distance running. The non-competitive LE participants (*n*=7) were highly active cross-trainers and exercise enthusiasts. Whilst SED underwent the six week exercise intervention, LE were required to maintain their normal training practices. LE recorded their weekly exercise routines, which included the type and frequency of exercise, exercise intensity (recorded by heart rate telemetry) and duration thereof. A designated member of the research team recorded training data at the end of each

week. LE group weekly average time spent in low to medium intensity (<65% heart rate reserve [HRR]), and high-intensity training (>65% HRR) totalled 214 ± 131 mins·wk^{-1} and 67.2 ± 52 mins·wk^{-1} respectively. The LE group was confirmed by self-reported exercise time and affirmed by differences in $\dot{V}O_{2peak}$ from the SED group.

Participant blood was sampled in addition to body composition and $\dot{V}O_{2peak}$ being assessed at two points, separated by six weeks. A six week personalised and supervised preconditioning exercise programme that reflected the ACSM guidelines of 150 mins·wk^{-1} of moderate and vigorous exercise was prescribed to the SED group as described previously [34]. Participants were advised to achieve their weekly exercise prescription of a minimum of two sessions per week in accordance with the ACSM guidelines for older persons [35]. Light to moderate exercise was advised for the first two weeks reaching 130-150 mins·wk^{-1} by week three. Participants were given verbal instructions on the use of a Polar FT1 heart rate monitor (Polar, Kempele, Finland) and exercise intensities were self-monitored enabling the recording of exercise time, average and peak heart rate. The aim was to achieve an average HRR of approximately 55% for the first two weeks of the intervention. This was increased to 60% of HRR for the subsequent weeks including 5-10 s of increased intensity every 10 min. The final two weeks required vigorous periods of exercise every 5 min achieving a HRR of 60-65%. The mode of training was optional, and included walking, walk/jogging, jogging, cycling, (flat terrain) cycling, (hill terrain) adapted to suit participants' current physical status. Over the six week intervention, 160 ± 15 min·wk^{-1} exercise were achieved.

Blood Collection and Analysis

Blood samples from each participant were collected at week 0 and week 6 between 07:00-09:00 h and between 48-72 hours following the last exercise session by the same

investigator, in an attempt to control for biological variation and minimize inter-subject analytical variation. Samples were obtained from an antecubital forearm vein following an overnight fast and 20 min supine rest using a 20-gauge disposable needle equipped with a Vacutainer tube holder (Becton Dickinson, Oxford, UK). Approximately, 14 mL of blood was withdrawn into two 10 mL serum separator tubes and allowed to clot at room temperature prior to being centrifuged at 6,000 rpm at 15°C for 15 min. The resultant serum was divided into appropriate aliquots and stored at -80°C until subsequent analysis.

Serum concentrations of TT, SHBG, and cortisol (C) were measured by electrochemiluminescent immunoassay on the E601 module of the Roche Cobas 6000 (Burgess Hill, West Sussex, U.K.). The inter-assay CV over a 6 month period were 4.5%, 2.4% and 4.2% for TT, SHBG, and C respectively. All analyses were carried out in the Clinical Biochemistry Laboratory at Royal Glamorgan Hospital (Wales, UK). Free-T and bio-T were calculated using the equation of Vermueulen and colleagues [36] in Equation 1.

Equation 1: $\text{Free-T} = ([T]–(N \times [\text{free-T}])) \div (K_t(\text{SHBG}–[T] + N[FT]))$

Whereby K_t is the association constant of SHBG for T, and $N = K_aC_a + 1$ (K_a is 3.6×10^4 $L \cdot Mol^{-1}$ and albumin concentration (C_a) is assumed to be 43 $g \cdot L^{-1}$)

Body Composition

Height was measured to the nearest 0.1 cm using a stadiometer (Seca, Birmingham, UK). A multi frequency bioelectrical impedance analyzer (BIA [Tanita MC-180MA Body Composition Analyzer, Tanita UK Ltd.]) was used to measure body composition and body mass. Coefficient of variance (CV) of the impedance measure was 0.4%. GMON software (v1.7.0, Tanita UK Ltd.) was used to determine values for percentage body fat using Equation

2 and 3 and fat free mass (FFM) and using Equation 4. Details of the prediction equations were provided by the manufacturer. The prediction equation for men is derived against body density (BD) thus:

Equation 2: $BD = 1.100696 – 0.107903 \times Wt \times Z \div Ht^2 + 0.00017 \times Z$

Whereby Wt is weight (kg), Ht is height (m) and Z is impedance (Ω), and percentage fat is calculated from body density as:

Equation 3: $\%\ fat = (4.57/BD \div 4.142) \times 100$

Equation 4: $FFM = Wt - [(\%\ fat \div 100) \times Wt]$

Determination of Cardiorespiratory Fitness (CRF)

Peak aerobic capacity ($\dot{V}O_{2peak}$) was determined using a Cortex II Metalyser 3B-R2 (Cortex, Biophysik, Leipzig, Germany) utilizing the method previously described [34]. Expiratory airflow was achieved using a volume transducer (Triple V® turbine, digital) connected to an oxygen (O_2) analyzer. Expired gases were analyzed for O_2 with electrochemical cells and for carbon dioxide (CO_2) output with an infrared analyzer. Prior to each test, the Metalyser was calibrated according to manufacturers' guidelines. After a 60 min warm-up period, the CO_2 and O_2 sensors were calibrated against room air in addition to a reference gas of known composition (5% CO_2, 15% O_2, and 80% N_2) with volume calibrated by five inspiratory and expiratory strokes using a 3 L pump. Five minutes of warm-up exercise preceded a ramped protocol until volitional exhaustion on an air-braked cycle ergometer (Wattbike Ltd., Nottingham, UK). Saddle height was adjusted relative to the crank position and the foot was secured to a pedal with clips and participants knee joint at almost full extension (approx. 170-180°). Participant cadence during familiarization trial dictated the

cadence (either 70, 75, 80, or 85 rpm) to be maintained throughout the test. Participants warmed up on resistance setting Level 1 (75 rpm = 100 watts) at the cadence they would use in the test, which was conducted using a modified Storer Test [37]. During the ramped protocol, work-rate was increased each minute by raising the damper setting by one (equating to 18 W) until volitional exhaustion was achieved. Breath by breath oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}C0_2$) respiratory exchange ratio (RER), ventilation ($\dot{V}_E$) were displayed continuously. Heart Rate (HR) was recorded every 5 s using short-range telemetry (Polar T31, Kempele, Finland). Coefficient of variation (CV) for the determination of HR_{max} in our laboratory was <1.4% from reliability study. Participants rated perceived exertion (RPE) using the Borg scale [38], which was recorded during the last 10 s of each 1 min stage. Fingertip blood lactate (BLa) samples were collected into a portable automated lactate analyser (Lactate Pro, Arkray, Inc., Kyoto, Japan) within 45 s and again 5 min following the termination of the test. Time to $\dot{V}0_{2peak}$ was 8:26 ± 1:45 min (7:18 ± 1:25 min and 9:04 ± 1:52 min for SED and LE respectively). Breath-by-breath data were sampled and transferred to a PC for real-time display. The recorded data was saved to the internal database (Metasoft version 3.7.0) until analysis. CV for the determination of $\dot{V}0_{2peak}$ in our laboratory is <3.0% during reliability study. $\dot{V}O_{2peak}$ was confirmed when participants achieved a minimum of any three of the following criteria: RER >1.10, peak heart rate within 10 beats of age predicted maximum, BLa above 8 mmol•L^{-1}, final RPE >18 on Borg Scale. Order of measurements was blood sampling, body composition, and $\dot{V}O_{2peak}$ assessment.

Statistical analysis

Data were analyzed using SPSS (version 20; IBM North America, New York, USA). Analysis of variance (ANOVA) was used to analyze participant descriptive and experimental

data. Data were analyzed using a 2 x 2 (Group x Time) mixed design ANOVA to examine differences between groups and time points (week 0 and week 6). Pearson's correlation coefficient was used to determine relationship between steroid hormone status and CRF. Significance was set *a priori* at $p<0.05$ and 95% confidence intervals (CI) and effect size (ES) are reported for primary outcome measures.

RESULTS

No differences between groups were observed for TT, C, SHBG, bio-T, or free-T at week 0 ($p>0.05$). Significant increases in TT, and SHBG were observed in the SED group after six weeks ($p<0.05$). TT increased from 13.25 ± 6.15 (95% CI = 10.69 – 15.81) to 14.96 ± 6.89 (95% CI = 11.95 – 17.98) nmol•L^{-1} in the SED group ($p<0.05$, ES=0.716) but not the LE group (14.76 ± 3.17 and 14.45 ± 4.32 nmol•L^{-1} at week 0 and 6 respectively [$p>0.05$]). No effect of training status or intervention was observed for free-T, bio-T (Figure 1), or C ($p>0.05$). SHBG increased in the SED group from 42.99 ± 23.22 (95% CIs = 33.42 – 52.56) to 46.99 ± 25.70 (95% CIs = 35.78 – 58.19) nmol•L^{-1} ($p<0.01$, ES=0.914) following exercise training, whilst being unaltered amongst LE (48.64 ± 13.77 and 45.16 ± 16.91 nmol•L^{-1} at week 0 and week 6 respectively [$p>0.05$]).

Body mass and body fat percentage of the SED group were significantly higher than LE at week 0 and week 6 ($p<0.05$). Percentage body fat decreased in both groups ($p<0.001$), whilst FFM increased in the LE group ($p<0.001$) and body mass decreased in the SED group from week 0 to week 6 ($p<0.05$). Significant increases in $\dot{V}O_{2peak}$ were observed in the SED group after six weeks ($p<0.05$, ES=0.627). SED $\dot{V}O_{2peak}$ was significantly lower than LE ($p<0.01$) at week 0 and 6 (Table 1). No significant correlations existed between delta changes

in hormonal profile (TT, SHBG, bio-T, free-T, and C) and improvements in $\dot{V}O_{2peak}$ or body composition.

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DISCUSSION

The main findings of the present study were that six weeks of progressive exercise training improved TT and was mirrored by improvements in $\dot{V}O_{2peak}$ in otherwise healthy sedentary aging men. However, concomitant increases in SHBG contrived to prevent exercise inducing effect on bio-T or free-T in SED. These findings support the contention that exercise-induced improvements in physiological functioning in sedentary aging men are achieved without concomitant increases in bioavailable or free-T fractions.

The present $\dot{V}O_{2peak}$ increase in SED participants is in line with previous findings that moderate exercise (approximately 75% HR_{max}) can increase $\dot{V}O_{2peak}$ of older adults [25]. It was evident that cardiorespiratory adaptations occurred as *absolute* values increased by ~5% as well as the ~6% *relative* increase in $\dot{V}O_{2peak}$. The magnitude of improvement is similar to that reported by Niederseer et al. [25] and Lira et al. [39] who both report ~7% improvements in relative $\dot{V}O_{2peak}$ in cohorts of older adults following 12 weeks of alpine skiing and moderate treadmill running respectively. This increase, although modest, would still represent a significant reduction in mortality risk [30] as it approximates 0.6 METs in older males [40]. Similarly, the ~2% decrease in body fat percentage observed in SED is in line

with a previous investigation [25] which reported a of 2.3% reduction following 12 weeks of alpine skiing. SED body mass decreased 1.3% in the present investigation whereas previous investigations have reported a 1.6% decrease following 18 months of combined aerobic and resistance training [41] and no change following 12 weeks of alpine skiing and moderate treadmill running [25, 39]. The relatively high body mass loss compared to the previous longer duration study may be attributed to the incorporation of resistance exercise into their exercise training [41], known to cause muscular hypertrophy and therefore attenuate total body mass losses [42].

The present study is in agreement with data from Khoo et al. [18] which indicated increased TT after an exercise program in middle-aged obese men (~44 years) over a 24 week period. Similar to the findings of the present investigation, SHBG also increased although to a lesser extent than in the present study, which resulted in a small but statistically change in free-T. In contrast, Lovell et al. [21] reported exercise (resistance or aerobic) training performed three times per week over 16 weeks did not increase resting TT or free-T in older men. There are few comparable studies on the sex hormone response to exercise in aging men. However, the available literature suggests a potential mechanism. The present study and that of Khoo et al. [18] both report increases in TT and SHBG in sedentary and obese older men. Khoo et al. [18] reported small but significant increase in free-T which was not the case in the present study. In contrast, Lovell et al. [21] found no change to TT, SHBG or free-T in an older cohort than the present study (~74 years) but critically, this cohort were already moderately active rather than the sedentary cohort of the present study and that of Khoo et al. [18]. Taken together, this suggests participants training status may affect outcomes of subsequent exercise training interventions.

Study duration beyond six weeks does not appear to influence the testosterone response, as increases in TT and SHBG in the present study could be considered as a

transient physiological response that accompanies initiation of an exercise training programme. Considering the lack of change observed by Lovell and colleagues [21] over 16 weeks, in contrast to the increases reported by Khoo et al. [18], suggests that increases in TT might not be transitory. Future studies should address this lack of clarity by employing sampling points that include days prior to and following an exercise bout in addition to capturing multiple TT and SHBG measurements during the hours that follow a single exercise bout.

It is further possible that training volume may influence exercise induced T response. Lovell and colleagues [21] trained participants for 75-135 $min \cdot wk^{-1}$, whereas Khoo et al. [18] trained participants for 90-150 $min \cdot wk^{-1}$ (low volume) and 200-300 $min \cdot wk^{-1}$ (high volume) after which only the high volume group increased TT. In the present investigation, SED group exercised for 130-190 $min \cdot wk^{-1}$, so it appears that exercise volumes at approximately the recommended 150 $min \cdot wk^{-1}$ may be necessary to achieve an exercise-induced T response in aging men. However, it is unclear whether this volume threshold may be altered as a result of higher intensity training in aging males.

The present study has some important limitations that should be considered. Firstly, the short duration of the present six week study may not have been of sufficient duration to achieve increases in free-T. Furthermore, moderate aerobic type exercise may not have been of sufficient intensity to induce a hormonal response that may have been observed with more vigorous exercise. However, our objective was to specifically examine moderate exercise, as this is the most likely and feasible in an aging male population and our findings support the contention that increased free-T is not necessary to achieve increased CRF. Another limitation relates to the limited number of blood hormone sampling points and the lack of any post-exercise (bout) measurements that may have induced an acute hormonal response that may be of biological significance. We are some way from fully understanding the hormonal

response to exercise and the relative importance of sex hormones in determining physiological function in aging men. This field of research would benefit from more large randomized controlled trials incorporating multiple sampling points of during various exercise modes and intensities to better understand interactions between physiological functions and sex hormones in aging men.

Conclusion

In conclusion, six weeks of exercise training significantly elevated TT in previously sedentary aging men. However, concomitant increases in SHBG abrogated alterations to basal bio-T or free-T. However, this lack of significant change to bio-T and free-T did not preclude improvements in $\dot{V}O_{2peak}$ and therefore suggest changes in bio-T are not required for CRF improvements in aging men. These data further emphasize that caution should be exercised when interpreting TT in isolation during training intervention studies.

Authors declare they have no conflict of interest.

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TABLE & FIGURE LEGENDS

TABLE 1 Participant aerobic capacity, steroid hormone profile, and body composition for sedentary (SED) and lifelong exercising (LE) aging males prior to, and following six weeks of supervised aerobic exercise. C = cortisol; $\dot{V}O_{2peak}$ = peak aerobic capacity; FFM = fat free mass. Data are presented as mean $\pm$ SD.

FIGURE 1 (A); Total testosterone (TT) in the sedentary (SED) group and lifelong exercising (LE) group at week 0 and week 6 (B); Free testosterone (Free-T) in the SED group and LE group at week 0 and week 6 (C); Bioavailable testosterone (Bio-T) in the SED group and LE group at week 0 and week 6 (D); and sex hormone binding globulin (SHBG) in the SED and LE group at week 0 and week 6. Data are presented as mean ± SD. *Denotes significant differences from week 0 ($p<0.05$). **Denotes significant difference from week 0 ($p<0.01$).