ORIGINAL ARTICLE

The effect of combined sprint and resistance training on steroid hormones in middle-aged and young men: A randomized control trial

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Abstract

Purpose: The aim of this study was to examine the effects of combined sprint and resistance training on serum total testosterone (TT), sex hormone-binding globulin (SHBG), and cortisol (C), at rest, and in response to the Wingate Anaerobic Test (WAnT) in younger (20 yrs) and middle-aged (40 yrs) men. **Methods:** Thirty-two moderately trained men military soldiers participated in this study. After medical examination, subjects were randomly assigned to one of four groups: A young trained group (21±1 yrs, YT, n=8), a young control group (22±2 yrs, YC, n=8), a middle-aged trained group (41±3 yrs, MAT, n=8), and a middle-aged control group (40±2 yrs, MAC, n=8). Both YT and MAT participated in a combined sprint and resistance training program (CSRT) for 13 weeks. Before (P1), and after (P2) CSRT, all participants performed the WAnT. Blood samples were collected at rest, after warm-up (50% maximal oxygen uptake [VO_{2max}]), immediately post-WAnT, and 10 min post-WAnT. **Results:** At P1, higher C and lower TT was observed in middle-aged subjects compared to younger ones ($P<0.05$). At P2, this age difference was absent in basal TT between trained groups. After CSRT, C increased significantly ($P=0.014$) in MAT, only at the end of WAnT, whilst resting and post-WAnT TT increased significantly for both YT and MAT ($P<0.05$). Moreover, SHBG decreased significantly in YT at P2 at rest ($P=0.048$). Resting free testosterone was significantly higher in young compared to middle-aged groups at P1 ($P<0.05$), but after CSRT, this age-related effect disappeared between YT and MAT at rest ($P>0.05$). **Conclusions:** CSRT appears to counteract the negative effect of age on TT and C.

Keywords: Testosterone, cortisol, SHBG, stress, aging

Introduction

The age-related loss of anabolism is characterized by a decrease in muscle protein content and is attributable to an imbalance between muscle protein synthesis and breakdown. Numerous studies have observed alterations in contractile properties of muscle fibers, particularly fast-twitch fibers in older individuals [1,2], which leads to a decline in anaerobic performance [3].

Concomitant with this age-associated decline in muscular function exists a reduction in systemic testosterone concentrations [4]. Furthermore, sex hormone binding globulin (SHBG) increases with age, rendering the bioavailable fraction (i.e. the proportion available for interaction with the androgen receptor [AR]) of testosterone decreased [5]. Low testosterone has a number of adverse health consequences, such as loss of muscle mass, increased fat mass, reduced aerobic capacity, and increased cardiovascular disease risk [4,6-8]. Furthermore, significant correlations between testosterone and measures of physical performance in older adults have been observed [9].

Physical inactivity has been shown to decrease testosterone concentrations [10], and well trained older individuals exhibit greater testosterone concentrations than sedentary males [11]. However, this consensus is not ubiquitous [12,13]. As such, whether long term exercise training increases testosterone remains a matter of debate. Likewise, exercise training interventions present homogeneity in results [13-15]. For example, Lovell et al. [15] observed no perturbation to TT, SHBG, or free testosterone (free-T) in an older cohort (~74 years) following resistance or aerobic training. Conversely, Hayes and colleagues [13] observed that although highly trained older adults displayed similar TT concentrations to that of sedentary older males, said sedentary participants increased TT following moderate aerobic exercise (150 $min \cdot wk^{-1}$). However, SHBG also increased, which rendered free-T unchanged. The same research group however, observed increased free-T following high intensity interval training (HIIT) in a later study (under review), which may suggest greater exercise intensity is required as a stimulus to increase free-T.

The body of literature concerning the influence of resistance exercise and testosterone generally report increased testosterone following resistance training [16,17]. For example, both Tremblay et al. [18] and Sato et al. [19] reported 12 weeks' resistance training increased basal free-T, 5-dihydrotestosterone (DHT) and dehydroepiandrosterone (DHEA) in young (26 yrs) and older (62 yrs)

men. As such, resistance training has been considered an appropriate strategy to counteract the age-associated deterioration of muscle, and androgenic status [20].

There remains considerable ambiguity concerning the influence of exercise training on steroid hormones with age. Therefore, the aim of the present investigation was to compare steroid hormones at rest, and in response to anaerobic exercise, in younger (20 yrs), and middle-aged (40 yrs) men, after 13 weeks' combined sprint and resistance training. We hypothesized *a priori* that a) an age-affect in steroid hormones would exist pre-training, and b) said training period would ameliorate the age-affect in steroid hormones.

Methods

Participants

Thirty-two healthy, moderately trained men (military participants) were recruited for participation in the present study. Subjects reviewed and signed consent forms approved by the local Ethics Committee for Human Research (ECHR) of the General Direction of the Military Health of Tunisia in accordance with ethical standards of the 1964 Helsinki Declaration.

Training status was assessed using an adapted version of the Baecke questionnaire [21]. To identify those with a medical contraindication (exclusion) to performing specific assessments, participants completed medical history, and dietary, questionnaires. Inclusion criteria included no contraindications to maximal exercise testing such as cardiovascular or pulmonary risk factors, no history of chronic disease, illness, surgeries, hospitalizations, and musculoskeletal or joint injuries.

The conventional dietary survey was conducted by a sports nutritionist of the Department of Physical Education and Military Sport to monitor individual diet during the 13 weeks. Participants were asked to abstain from high glycemic loads, saturated and trans fatty acids, caffeine, alcohol, drugs, vitamins or supplements, and low fiber diets for the duration of the experimental period. Because participants belong to the same military school, they were offered the same menu component, which was suitable for "active" status. Before training period, estimated dietary energy intake was not significantly different between groups: Young groups (protein: 410±24 kcal·day^{-1}, fat: 1128±13 kcal·day^{-1}, and carbohydrate: 1879±34 kcal·day^{-1}) and middle-aged groups (protein: 387±14 kcal·day^{-1},

fat: 1064±12 kcal·day^{-1}, and carbohydrate: 1773±50 kcal·day^{-1}). After the training period, these results remained stable and no differences were observed between groups: Young groups (protein:408±31 kcal·day^{-1}, fat: 1123±44 kcal·day^{-1}, and carbohydrate: 1870±23 kcal·day^{-1}) and middle-aged groups (protein: 487±24 kcal·day^{-1}, fat: 1012±13 kcal·day^{-1}, and carbohydrate: 1772±34 kcal·day^{-1}).

Eligible participants were subsequently randomized to receive 13 weeks' combined sprint and resistance training (CSRT), or control. Thus, four groups existed: a young trained group (YT; 21±1 yrs, n=8), a young control group (YC; 22±2 yrs, n=8), a middle-aged trained group (MAT; 41±3 yrs, n=8) and a middle-aged control group (MAC; 40±2 yrs, n=8).

Exercise training program

Trained subjects (YT and MAT) participated in 13 weeks of CSRT as previously described [22]. Briefly, CSRT consisted of one sprint running, one sprint cycling, and one resistance training session per week, separated by a minimum of 48 h (13 sessions of each training unit). Sessions were performed during the morning and lasted no longer than 70 min, inclusive of 15 min warm-up (jogging and stretching) and 15 min cool-down (jogging and stretching).

Sprint running sessions entailed 3-5 sets of 3-5 short bouts at maximum velocity. A passive recovery of 2-3 min was permitted between each set. Sprint cycling sessions comprised 3-5 repetitions of 10-30 s. The 10-30 s trials were performed maximally. Subjects recovered actively (at a power output corresponding to 50% VO_{2max}) for 3-5 min between each sprint. Resistance training sessions entailed 5-6 exercises targeting all major muscle groups (squat with Smith machine, machine leg extension, machine leg curl, calf raises over a step, triceps push down with cable machine, bicep preacher curl, and bench press. The load used during exercise was progressively increased from 40% to 65% of 1-repetition maximum (RM), [23,24]. To produce maximal power output (i.e. velocity × load), the concentric phase of each exercise was performed as fast as possible [25]. Repetitions were maintained at 10-15 per sets and the number of sets increased from 3 to 4 during the training period. Hence, training volume increased progressively during the CSRT program. Rest periods between sets were 3-5 min for upper body muscles[26] and a minimum of 1 min for lower limbs [23]. To adjust load during resistance training

session and monitor adaptation, we determined strength using a 1-RM for the six resistance exercises, pre-training (P1), during the sixth week, and post-training (P2).

Blood collection and biochemical analyses

Upon arriving, a heparinized catheter (Insyte-W, 1.1 mm o.d. × 30 mm) was inserted into an antecubital vein, following 20 min sitting. Blood was drawn 8:00-9:00 h following overnight fasting. Venous blood samples were drawn at three times: rest ($_0$ [after 20 min sitting on the bike]), immediately post-WAnT ($_{end}$) and 10 min post-WAnT ($_{10}$). For each sample, 10 mL of blood was collected in tubes containing Ethylenediaminetetraacetic acid, (EDTA) to determine concentrations of serum TT, SHBG, and cortisol (C). Samples were centrifuged immediately for 15 min at 4ºC (at 3,000 rpm), and the extracted serum was stored at - 80°C until analysis.

TT and SHBG were measured by electro-chemiluminescence immunoassay using the Elecsys 2010 analyzer (Roche Diagnostics, Switzerland). Inter-assay coefficients of variation (CV) were 8.4-9.1% and intra-assay CVs were 7.8-9.6%. Assay sensitivity was 0.08 ng·ml^{-1}. Cortisol was analyzed using a Gamma Coat Cortisol 125I RIA Kit (Diasorin, Inc., Stillwater, MN). The mean intra- and inter-assay coefficients of variation were 5.7% and 3.7% respectively. Free-T was calculated using the Vermueulen equation [27].

Exercise testing

Before training, subjects were familiarized with testing procedures to minimize learning effect. Participants avoided physical activity for 48 h preceding each test. Total energy and macronutrient intake per day during the previous three days was monitored to ensure consistency prior to exercise testing. The testing period was divided into two phases: before (P1), and after (P2) training. Each period lasted seven days and included three consecutive laboratory visits separated by 48 h. The second phase (P2) commenced 48 h after training cessation and finished seven days later. Anthropometric measurements were obtained at P1, and P2 using Haependen skinfold calipers and the Durnin & Wormersley [28] method. Fat free mass (FFM) was calculated by subtracted fat mass from total body mass.

On the first visit, subjects arrived at the laboratory 2 h postprandial, after a standardized breakfast recommended by a nutritionist. Breakfast comprised 10 $kcal \cdot kg^{-1}$, 55% carbohydrate, 33% lipids, and 12% protein.

On the second visit, subjects performed a repeated sprint cycling test on a cycle ergometer (Ergomeca, Bessenay, France). It consisted of five short trials (6 s) against increasing resistance (2 kg each sprint) until exhaustion. Recovery time between each trial was 5 min. The highest pedaling cadence recorded after each trial was collected from a photoelectric cell fixed on the wheel of the cycle ergometer and connected to a computer. The load which permitted the highest peak power output was used for the Wingate Anaerobic Test (WAnT).

On the third visit, subjects performed the WAnT on a mechanically braked Monark cycle ergometer (Monark 827E). The test commenced 5 min after warm-up (15 min at a power output corresponding to 50% VO_{2max}). Subjects were asked to cycle maximally for 30 s. The highest value over 1 s was considered peak power (W_{peak}), and average power over 30 s was considered mean power (W_{mean}).

Statistical analysis

Data were analyzed using SPSS 23.0 for Windows (SPSS, Inc. Chicago, IL, USA). Means and standard deviations (SD) were calculated after verifying the normality of distributions using the Kolmogorov-Smirnov procedure. For anthropometric, physical performances indices, and area under the curve (AUC), data were analyzed using a multifactorial three-way (time [P1, P2] × age [young, middle-aged] × group [trained, control]) analysis of variance (ANOVA). Hormonal responses were analyzed using a four-factor ANOVA (time [P1, P2] × Wingate time [rest, immediately post-WAnT, and 10 min post-WAnT] × age [young, middle aged] × group [trained, control]). AUCs were calculated using trapezoidal integration. Bonferroni-adjusted pairwise post hoc comparisons were performed and effect size (η^2_P for main effects and Cohen's *d* for pairwise comparisons) is reported where appropriate. Statistical significance was set *a priori* at $P<0.05$.

Results

Blood parameters

There was a main effect of WAnT time in all groups for **TT** (table 1; P<0.001, η^2_P=0.89) i.e. we observed an increase from TT_0 to TT_{10}. At P1, there was a significant age effect for TT_0 (P=0.041, Cohen's *d*=0.81). CSRT induced an increase in YT TT_{10} (P<0.001, Cohen's *d*=0.38), whilst MAT increased TT_0, (P<0.015, Cohen's *d*=0.03), and TT_{10} (P<0.001, Cohen's *d*=0.28) at P2 compared to P1. No change in TT was observed from P1 to P2 in control groups (P>0.05). TT AUC was not different between ages, nor was there was a change post-CSRT (P>0.05).

There was no main effect of WAnT time in all groups for **SHBG** (table 2; P=0.881, η^2_P=0.004). At P1 and P2, there were no interaction observed between age and groups (P=0.338, η^2_P=0.026). No CSRT-induced SHBG perturbation was observed from P1 to P2 in any group (P>0.05), except YT who experienced an increase in $SHBG_0$ (P=0.01, Cohen's *d*=0.13). There was a main effect of age at P1 (P=0.047, Cohen's *d*=1.68) and P2 (P=0.007, Cohen's *d*=2.12) for SHBG AUC in experimental groups. Moreover, YT decreased SHBG AUC from P1 to P2 (P=0.001, Cohen's *d*=0.27).

There was a main effect of WAnT time in all groups for **free-T** (table 3; P<0.001, η^2_P=0.29). At P1 there was a significant age effect for free-T (P=0.031, η^2_P=0.22). CSRT induced an increase only in MAT free-T_0, (P=0.039, Cohen's *d*=1.60). No difference in free-T was observed from P1 to P2 in control groups (P>0.05). Free-T AUC was not different between ages, nor was there was a change post-CSRT (P>0.05).

There was a significant main effect of age, WAnT time, and group on **C** (table 4; P<0.001-0.01, η^2_P: 0.50-0.87) and a significant interaction between training phase, WAnT time, and group (P=0.007, η^2_P=0.13). At P1 and P2 younger groups exhibited lower C_0 (P=<0.001-0.002, Cohen's *d*=2.55-3.33) and C_{end} (P<0.001, Cohen's *d*=1.91-2.73) than middle-aged groups. C_{end} increased significantly (P=0.014, Cohen's *d*=2.02) at P2 compared to P1 in MAT. No other differences were observed between P1 and P2 for experimental groups (P>0.05). C AUC was lower in young groups compared to middle-aged groups at P1 (P<0.05), but after CSRT this main effect of age was not seen between YT and MAT (P>0.05).

Body composition and performance

At P1, there was a significant main effect of age for body mass (P=0.004, η^2_P=0.21), whereby YT and YC (74.8±4.0 kg and 73.7±4.7 kg respectively) were significantly lighter than MAT and MAC (78.1±4.4 kg and 77.4±2.5 kg respectively). YT body mass decreased at P2 (72.3±2.9 kg) compared to P1 (P<0.001, Cohen's *d*=0.44), as did MAT body mass (76.9±4.8 kg; P=0.002, Cohen's *d*=0.28). After training, the body mass measurements for MAC (77.3±2.6 kg; P=0.774, Cohen's *d*=0.04) and YC (73.80±4.80 kg; P=0.796, Cohen's *d*=0.02) were not significantly different from P1.

At P1, there was no main effect of age for **body fat percentage** (11.6±1.3%, 11.2±1.7%, 12.5±0.5%, and 12.0±2.2% for YT, YC, MAT, and MAC respectively; P=0.061, η^2_P=0.09). YT body fat percentage decreased from P1 to P2 (10.3±0.8%; P=0.010, Cohen's *d*=1.20), as did MAT body fat percentage (11.1±1.3%; P=0.005, Cohen's *d*=1.42). At P2, MAC (12.2±2.2%; P=0.683, Cohen's *d*=0.09) and YC (11.5±1.3%; P=0.648, Cohen's *d*=0.20) body fat percentage was unchanged from P1.

At P1, no significant main effect of age was observed for **FFM** (65.1±5.0 kg, 63.7±5.6 kg, 62.2±5.8 kg, and 61.3±2.3 kg for YT, YC, MAT, and MAC respectively; *P*=0.111, η^2_P=0.07). YT FFM was unaltered at P2 (66.2±6.7 kg) compared to P1 (P=0.285, Cohen's *d*=0.18), as was MAT (63.1±6.4 kg; P=0.332, Cohen's *d*=0.15). At P2, MAC (61.5±2.2 kg; P=0.830, Cohen's *d*=0.08) and YC (64.2±7.6 kg; P=0.651, Cohen's *d*=0.07) FFM was not significantly different from P1.

We observed a significant main effect of age for $\mathbf{W_{peak}}$ at P1 (1037±127 W, 955±258 W, 896±70 W, and 872±122 W for YT, YC, MAT, and MAC respectively P=0.040; η^2_P=0.11). W_{peak} at P2 in YT (1093±202 W; P=0.067, Cohen's *d*=0.33), and MAT (950±350 W; P=0.076, Cohen's *d*=0.21) was not significantly increased at P2 compared to P1 (),despite small effect sizes.At P2, YC (944±246 W; P=0.606, Cohen's *d*=0.04) and MAC (874±111 W; P=0.958, Cohen's *d*=0.03) W_{peak} was not significantly different from P1. There was an age effect for $\mathbf{W_{mean}}$ at P1 (P=0.009; η^2_P=0.18). YT W_{mean} was 575±58 W and 581±71 W at P1 and P2 respectively (P=0.792, Cohen's *d*=0.09). MAT W_{mean} was 508±95 W and 543±79 W at P1 and P2 respectively (P=0.141, Cohen's *d*=0.40), meaning the age effect was not present in trained groups at P2 (P=0.268).

Discussion

The main finding of the present investigation is that a programme of CSRT can attenuate the effect of age on TT, free-T, and C evident in middle-aged men compared to young men. Moreover, CSRT appears to increase the sensitivity of TT and free-T to a WAnT in experimental groups.

This study demonstrated a small increase in mean power output during supramaximal exercise in MAT. Previous longitudinal studies observed increased anaerobic performances in 20 yr old subjects after sprint training [29] or after 21 week of heavy resistance training in younger (25 yrs) and older (65 yrs) trained subjects [16]. However, after combined sprint and strength training, few studies have reported increased anaerobic performance in young and older trained subjects [22,30]. This anaerobic performance potentiation was accompanied by increased anabolic hormone concentrations during study, providing a possible explanation for the increase in power production, as previously hypothesized (https://www.ncbi.nlm.nih.gov/pubmed/28178145).

Our hormonal data are in agreement with some [12,14], but not all [15] previous investigations reporting increased basal testosterone in older males following exercise training. In the present investigation, free-T and TT was increased in MAT which contradicts some of our previous work [12] which observed increased TT but not free-T following moderate aerobic conditioning. However, the addition of a high intensity exercise phase did promote an increase to free-T (Hayes et al., 2017 – Under review) suggesting that augmented free-T may be intensity-dependent. However, Hakkinen et al.[31] reported that during, and following, a 24-week strength training period, TT and free-T was unchanged, despite a considerably higher relative load than in the present investigation being used (4-6-RM utilized periodically throughout the investigation). In the present study, there was a CSRT-induced increase in free-T, which would suggest a greater amount of the biologically active hormone was available for interaction with the AR. This is further supported by positive alterations to body composition observed in training groups.

Our data conflict those of Hakkinen et al. [32] in that we observed increased reactivity of TT and free-T to a single WAnT post-CSRT. Hakkinen et al. [32] observed that although a single resistance exercise session resulted in significant increased TT and free-T, this response was not augmented, or dampened by exercise training in middle-aged (~42 yrs), or older (~72 yrs), men. A similar finding was later replicated by the same research group [31] in older men and women (~65 yrs). Whether transient

exercise-induced changes in ostensibly anabolic hormones occur or not, the physiological significance of this remains equivocal [33-35]. For example, West et al. [36] investigated the addition of subsequent leg exercise (included to potentiate increases in anabolic hormones) during 15 weeks' elbow flexion training. These authors observed no difference in strength or hypertrophy gains between the group that experienced acute exercise-induced TT and free-T elevations, and the group that did not . Similarly, Mitchell and colleagues [37] reported no relationship between the magnitude of exercise-induced changes in serum free-T, growth hormone, or insulin-like growth factor (IGF)-I,and muscle hypertrophy following 16 weeks' resistance training. As such, the importance of acute exercise-induced hormonal increases are questionable, and therefore, the result of increased basal TT and free-T are likely more physiologically pertinent.

Conclusion

Thirteen weeks' combined sprint and resistance training increased basal serum TT, and free-T, in middle-aged trained subjects, which abrogated the age-effect on steroid hormones post-training. This training type also appears to promote small improvements in anaerobic performance in middle-aged men.

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Authors' contributions

All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed. All authors have approved the final version to be submitted and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

All authors had revised and approved the final version to be submitted.

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Table 1. Serum total testosterone (TT; $nmol \cdot l^{-1}$) at rest (TT_0), at the end of a Wingate Anaerobic Test (TT_{end}), during recovery (TT_{10}), and area under the curve (AUC) in young trained (YT), young control (YC), middle-aged trained (MAT), and middle-aged control (MAC) participants, before training (P1), and after training (P2).

		TT_0	TT_{end}	TT_{10}	TT AUC
YT (n=10)	P1	33.9±3.9[a]	42.44±6.0	40.61±5.0[e]	488.1±93.4
	P2	34.5±4.2[g]	41.33±5.9[h]	42.53±5.2	477.3±95.2
YC (n=10)	P1	31.5±4.9	38.79±2.3	40.32±3.8	473.6±232.5
	P2	31.7±4.7[g]	39.26±2.3[h]	40.82±3.8	471.0±227.2
MAT (n=10)	P1	25.7±13.7[e]	34.56±16.2	33.73±11.3[e]	540.3±90.2
	P2	26.1±13.4[g]	37.63±16.2[h]	36.93±11.2	526.4±91.5
MAC (n=10)	P1	24.2±8.6	33.32±4.1	33.40±2.2	530.5±245.2
	P2	24.7±8.6[g]	30.09±4.3[h]	34.13±2.1	523.5±231.3

Data are presented as mean±SD.[a]Significant difference ($p<0.05$) between YT and MAT, [b]Significant difference ($p<0.05$) between YC and MAC, [c]Significant difference ($p<0.05$) between YT and YC, [d]Significant difference ($p<0.05$) between MAT and MAC, [e]Significant difference ($p<0.05$) from before and after training, [f]Significant difference ($p<0.05$) between "0" and "end", [g]Significant difference ($p<0.05$) between "0" and "10", [h]Significant difference ($p<0.05$) between "end" and "10".

Table 2. Serum Sex hormone binding globulin (SHBG; $nmol \cdot l^{-1}$) at rest ($SHBG_0$), at the end of a Wingate Anaerobic Test ($SHBG_{end}$), during recovery ($SHBG_{10}$), and area under the curve (AUC) in young trained (YT), young control (YC), middle-aged trained (MAT), and middle-aged control (MAC) participants, before training (P1), and after training (P2).

		$SHBG_0$	**$SHBG_{end}$**	**$SHBG_{10}$**	**SHBG AUC**
YT(n=10)	P1	28.7±7.4[e]	31.7±5.5	29.4± 6.1	6492.8±494.6[a,c,e]
	P2	27.7±8.1	31.2±6.2	28.9±7.0	6328.8±712.3[a,c]
YC (n=10)	P1	28.0±8.7	31.0±7.7	28.9±6.5	5148.0±1080.8[b]
	P2	27.6±8.6	30.7±7.7	28.5±6.5	5313.0±970.4[b]
MAT (n=10)	P1	31.7±4.5	35.0±4.7	33.0±4.9	8114.1±1269.9
	P2	31.6±5.7	34.6±5.3	32.2±5.3	8499.2±1261.6
MAC (n=10)	P1	30.0±5.7	34.5±5.2	32.9± 4.9	8061.0±1544.6
	P2	29.8±5.7	34.0±5.5	32.5±4.7	7594.0±1233.5

Data are presented as mean±SD.[a]Significant difference ($p<0.05$) between YT and MAT, [b]Significant difference ($p<0.05$) between YC and MAC, [c]Significant difference ($p<0.05$) between YT and YC, [d]Significant difference ($p<0.05$) between MAT and MAC, [e]Significant difference ($p<0.05$) from before and after training, [f]Significant difference ($p<0.05$) between "0" and "end", [g]Significant difference ($p<0.05$) between "0" and "10", [h]Significant difference ($p<0.05$) between "end" and "10".

Table 3. Free testosterone (Free-T; $nmol \cdot l^{-1}$)at rest (Free-T_0), at the end of a Wingate Anaerobic Test (Free-T_{end}), during recovery (Free-T_{10}), and area under the curve (AUC) in young trained (YT), young control (YC), middle-aged trained (MAT), and middle-aged control (MAC) participants, before training (P1), and after training (P2).

		Free-T_0	Free-T_{end}	Free-T_{10}	Free-T AUC
YT (n=10)	P1	0.71±0.25[a]	0.77±0.23	0.80±0.28	12.28±3.36
	P2	0.70±0.34	0.87±0.26	0.73±0.18	12.72±3.13
YC (n=10)	P1	0.59±0.24	0.85±0.17[b,g]	0.66±0.16	12.57±3.51[b]
	P2	0.68±0.26	0.74±0.17	0.68±0.13	13.66±5.53
MAT (n=10)	P1	0.38±0.12[e]	0.66±0.35	0.68±0.28[d]	9.85±3.79
	P2	0.58±0.13	0.76±0.24	0.77±0.32[d]	11.70±3.21
MAC (n=10)	P1	0.45±0.09	0.57±0.19	0.47±0.10	8.24±1.95
	P2	0.59±0.29	0.64±0.23	0.51±0.12	10.49±4.32

Data are presented as mean±SD.[a]Significant difference ($p<0.05$) between YT and MAT, [b]Significant difference ($p<0.05$) between YC and MAC, [c]Significant difference ($p<0.05$) between YT and YC, [d]Significant difference ($p<0.05$) between MAT and MAC, [e]Significant difference ($p<0.05$) from before and after training, [f]Significant difference ($p<0.05$) between "0" and "end", [g]Significant difference ($p<0.05$) between "0" and "10", [h]Significant difference ($p<0.05$) between "end" and "10".

Table 4. Serum cortisol (C; ng·ml^{-1}) at rest (C_0), at the end of a Wingate Anaerobic Test (C_{end}), during recovery (C_{10}), and area under the curve (AUC) in young trained (YT), young control (YC), middle-aged trained (MAT), and middle-aged control (MAC) participants, before training (P1), and after training (P2).

		C_0	C_{end}	C_{10}	C AUC
YT (n=10)	P1	251±28[a,f,g]	421±50[a,c]	471±75	1.66±0.20[a]
	P2	254±22[a,f,g]	412±88[a,c]	451±89	1.70±0.47
YC (n=10)	P1	247±21[b,f]	344±77[b]	331±67[b]	1.88±0.47[b,e]
	P2	201±18[b,f,g]	350±66[b]	382±61	2.79±2.79[b]
MAT (n=10)	P1	364±56[f,g]	512±45[e]	585±67	1.08±0.45
	P2	374±46[d,f,g]	602±44[d]	581±52	1.10±0.29
MAC (n=10)	P1	363±53[f,g]	544±67	524±90	1.00±0.43
	P2	291±81[f,g]	512±66	525±96	1.02±0.56

Data are presented as mean±SD. [a]Significant difference ($p<0.05$) between YT and MAT, [b]Significant difference ($p<0.05$) between YC and MAC, [c]Significant difference ($p<0.05$) between YT and YC, [d]Significant difference ($p<0.05$) between MAT and MAC, [e]Significant difference ($p<0.05$) from before and after training, [f]Significant difference ($p<0.05$) between "0" and "end", [g]Significant difference ($p<0.05$) between "0" and "10", [h]Significant difference ($p<0.05$) between "end" and "10".

Table 5. Serum total testosterone:cortisol ratio at rest ($TT:C_0$), at the end of a Wingate Anaerobic Test ($TT:C_{end}$), during recovery ($TT:C_{10}$), and area under the curve (AUC) in young trained (YT), young control (YC), middle-aged trained (MAT), and middle-aged control (MAC) participants, before training (P1), and after training (P2).

		$TT:C_0$	**$TT:C_{end}$**	**$TT:C_{10}$**	**TT:C AUC**
YT (n=10)	P1	0.13±0.03[a,g]	0.10±0.02[a]	0.09±0.02[c]	1.66±0.22[a]
	P2	0.14±0.03	0.10±0.03	0.09±0.04	1.70±0.47
YC (n=10)	P1	0.13±0.04[b]	0.12±0.04[b]	0.11±0.02[b]	1.88±0.47[b,e]
	P2	0.46±0.71[b,f,g]	0.12±0.08[b]	0.10±0.03[b]	2.80±2.80[b]
MAT (n=10)	P1	0.07±0.02	0.07±0.04	0.06±0.04	1.09±0.45
	P2	0.08±0.01	0.07±0.02	0.07±0.03	1.11±0.31
MAC (n=10)	P1	0.07±0.04	0.06±0.04	0.06±0.02	1.01±0.43
	P2	0.09±0.06	0.06±0.03	0.06±0.03	1.03±0.57

Data are presented as mean±SD.[a]Significant difference ($p<0.05$) between YT and MAT, [b]Significant difference ($p<0.05$) between YC and MAC, [c]Significant difference ($p<0.05$) between YT and YC, [d]Significant difference ($p<0.05$) between MAT and MAC, [e]Significant difference ($p<0.05$) from before and after training, [f]Significant difference ($p<0.05$) between "0" and "end", [g]Significant difference ($p<0.05$) between "0" and "10", [h]Significant difference ($p<0.05$) between "end" and "10".