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1 ORIGINAL ARTICLE

2

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

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22 **Running title:** Age and training effects on steroid hormones.

23

24 **Conflict of interest:** The authors declare no conflict of interest.

25

26 **Abstract**

27 **Purpose:** The aim of this study was to examine the effects of combined sprint and resistance training  
28 on serum total testosterone (TT), sex hormone-binding globulin (SHBG), and cortisol (C), at rest, and  
29 in response to the Wingate Anaerobic Test (WAnT) in younger (20 yrs) and middle-aged (40 yrs) men.

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

44

45 **Keywords:** Testosterone, cortisol, SHBG, stress, aging

46

47 **Introduction**

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

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

59           Physical inactivity has been shown to decrease testosterone concentrations [10], and well trained  
60 older individuals exhibit greater testosterone concentrations than sedentary males [11]. However, this  
61 consensus is not ubiquitous [12,13]. As such, whether long term exercise training increases testosterone  
62 remains a matter of debate. Likewise, exercise training interventions present homogeneity in results [13-  
63 15]. For example, Lovell et al. [15] observed no perturbation to TT, SHBG, or free testosterone (free-  
64 T) in an older cohort (~74 years) following resistance or aerobic training. Conversely, Hayes and  
65 colleagues [13] observed that although highly trained older adults displayed similar TT concentrations  
66 to that of sedentary older males, said sedentary participants increased TT following moderate aerobic  
67 exercise (150 min·wk<sup>-1</sup>). However, SHBG also increased, which rendered free-T unchanged. The same  
68 research group however, observed increased free-T following high intensity interval training (HIIT) in  
69 a later study (under review), which may suggest greater exercise intensity is required as a stimulus to  
70 increase free-T.

71           The body of literature concerning the influence of resistance exercise and testosterone generally  
72 report increased testosterone following resistance training [16,17]. For example, both Tremblay et al.  
73 [18] and Sato et al. [19] reported 12 weeks' resistance training increased basal free-T, 5-  
74 dihydrotestosterone (DHT) and dehydroepiandrosterone (DHEA) in young (26 yrs) and older (62 yrs)

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

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

83

## 84 **Methods**

### 85 **Participants**

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

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

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

103 fat:  $1064 \pm 12$  kcal·day<sup>-1</sup>, and carbohydrate:  $1773 \pm 50$  kcal·day<sup>-1</sup>). After the training period, these results  
104 remained stable and no differences were observed between groups: Young groups (protein:  $408 \pm 31$   
105 kcal·day<sup>-1</sup>, fat:  $1123 \pm 44$  kcal·day<sup>-1</sup>, and carbohydrate:  $1870 \pm 23$  kcal·day<sup>-1</sup>) and middle-aged groups  
106 (protein:  $487 \pm 24$  kcal·day<sup>-1</sup>, fat:  $1012 \pm 13$  kcal·day<sup>-1</sup>, and carbohydrate:  $1772 \pm 34$  kcal·day<sup>-1</sup>).

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

111

## 112 **Exercise training program**

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

118 Sprint running sessions entailed 3-5 sets of 3-5 short bouts at maximum velocity. A passive  
119 recovery of 2-3 min was permitted between each set. Sprint cycling sessions comprised 3-5 repetitions  
120 of 10-30 s. The 10-30 s trials were performed maximally. Subjects recovered actively (at a power output  
121 corresponding to 50%  $VO_{2max}$ ) for 3-5 min between each sprint. Resistance training sessions entailed 5-  
122 6 exercises targeting all major muscle groups (squat with Smith machine, machine leg extension,  
123 machine leg curl, calf raises over a step, triceps push down with cable machine, bicep preacher curl, and  
124 bench press. The load used during exercise was progressively increased from 40% to 65% of 1-repetition  
125 maximum (RM), [23,24]. To produce maximal power output (i.e. velocity  $\times$  load), the concentric phase  
126 of each exercise was performed as fast as possible [25]. Repetitions were maintained at 10-15 per sets  
127 and the number of sets increased from 3 to 4 during the training period. Hence, training volume increased  
128 progressively during the CSRT program. Rest periods between sets were 3-5 min for upper body  
129 muscles [26] and a minimum of 1 min for lower limbs [23]. To adjust load during resistance training

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

132

### 133 **Blood collection and biochemical analyses**

134 Upon arriving, a heparinized catheter (Insyte-W, 1.1 mm o.d. × 30 mm) was inserted into an  
135 antecubital vein, following 20 min sitting. Blood was drawn 8:00-9:00 h following overnight fasting.  
136 Venous blood samples were drawn at three times: rest ( $t_0$  [after 20 min sitting on the bike]), immediately  
137 post-WAnT ( $t_{end}$ ) and 10 min post-WAnT ( $t_{10}$ ). For each sample, 10 mL of blood was collected in tubes  
138 containing Ethylenediaminetetraacetic acid, (EDTA) to determine concentrations of serum TT, SHBG,  
139 and cortisol (C). Samples were centrifuged immediately for 15 min at 4°C (at 3,000 rpm), and the  
140 extracted serum was stored at - 80°C until analysis.

141 TT and SHBG were measured by electro-chemiluminescence immunoassay using the Elecsys  
142 2010 analyzer (Roche Diagnostics, Switzerland). Inter-assay coefficients of variation (CV) were 8.4-  
143 9.1% and intra-assay CVs were 7.8-9.6%. Assay sensitivity was 0.08 ng·ml<sup>-1</sup>. Cortisol was analyzed  
144 using a Gamma Coat Cortisol 125I RIA Kit (Diasorin, Inc., Stillwater, MN). The mean intra- and inter-  
145 assay coefficients of variation were 5.7% and 3.7% respectively. Free-T was calculated using the  
146 Vermueulen equation [27].

147

### 148 **Exercise testing**

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

158 On the first visit, subjects arrived at the laboratory 2 h postprandial, after a standardized  
159 breakfast recommended by a nutritionist. Breakfast comprised 10 kcal·kg<sup>-1</sup>, 55% carbohydrate, 33%  
160 lipids, and 12% protein.

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

167 On the third visit, subjects performed the WAnT on a mechanically braked Monark cycle  
168 ergometer (Monark 827E). The test commenced 5 min after warm-up (15 min at a power output  
169 corresponding to 50% VO<sub>2max</sub>). Subjects were asked to cycle maximally for 30 s. The highest value over  
170 1 s was considered peak power ( $W_{peak}$ ), and average power over 30 s was considered mean power  
171 ( $W_{mean}$ ).

172

### 173 **Statistical analysis**

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

184



## 185 **Results**

### 186 **Blood parameters**

187 There was a main effect of WAnT time in all groups for **TT** (table 1;  $P < 0.001$ ,  $\eta^2_p = 0.89$ ) i.e. we  
188 observed an increase from  $TT_0$  to  $TT_{10}$ . At P1, there was a significant age effect for  $TT_0$  ( $P = 0.041$ ,  
189 Cohen's  $d = 0.81$ ). CSRT induced an increase in YTT<sub>10</sub> ( $P < 0.001$ , Cohen's  $d = 0.38$ ), whilst MAT  
190 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.  
191 No change in TT was observed from P1 to P2 in control groups ( $P > 0.05$ ). TT AUC was not different  
192 between ages, nor was there was a change post-CSRT ( $P > 0.05$ ).

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

199 There was a main effect of WAnT time in all groups for **free-T** (table 3;  $P < 0.001$ ,  $\eta^2_p = 0.29$ ). At  
200 P1 there was a significant age effect for free-T ( $P = 0.031$ ,  $\eta^2_p = 0.22$ ). CSRT induced an increase only in  
201 MAT free-T<sub>0</sub>, ( $P = 0.039$ , Cohen's  $d = 1.60$ ). No difference in free-T was observed from P1 to P2 in control  
202 groups ( $P > 0.05$ ). Free-T AUC was not different between ages, nor was there was a change post-CSRT  
203 ( $P > 0.05$ ).

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

### 212 **Body composition and performance**

213 At P1, there was a significant main effect of age for body mass ( $P=0.004$ ,  $\eta^2_p=0.21$ ), whereby  
214 YT and YC ( $74.8\pm 4.0$  kg and  $73.7\pm 4.7$  kg respectively) were significantly lighter than MAT and MAC  
215 ( $78.1\pm 4.4$  kg and  $77.4\pm 2.5$  kg respectively). YT body mass decreased at P2 ( $72.3\pm 2.9$  kg) compared to  
216 P1 ( $P<0.001$ , Cohen's  $d=0.44$ ), as did MAT body mass ( $76.9\pm 4.8$  kg;  $P=0.002$ , Cohen's  $d=0.28$ ). After  
217 training, the body mass measurements for MAC ( $77.3\pm 2.6$  kg;  $P=0.774$ , Cohen's  $d=0.04$ ) and YC  
218 ( $73.80\pm 4.80$  kg;  $P=0.796$ , Cohen's  $d=0.02$ ) were not significantly different from P1.

219 At P1, there was no main effect of age for **body fat percentage** ( $11.6\pm 1.3\%$ ,  $11.2\pm 1.7\%$ ,  
220  $12.5\pm 0.5\%$ , and  $12.0\pm 2.2\%$  for YT, YC, MAT, and MAC respectively;  $P=0.061$ ,  $\eta^2_p=0.09$ ). YT body  
221 fat percentage decreased from P1 to P2 ( $10.3\pm 0.8\%$ ;  $P=0.010$ , Cohen's  $d=1.20$ ), as did MAT body fat  
222 percentage ( $11.1\pm 1.3\%$ ;  $P=0.005$ , Cohen's  $d=1.42$ ). At P2, MAC ( $12.2\pm 2.2\%$ ;  $P=0.683$ , Cohen's  
223  $d=0.09$ ) and YC ( $11.5\pm 1.3\%$ ;  $P=0.648$ , Cohen's  $d=0.20$ ) body fat percentage was unchanged from P1.

224 At P1, no significant main effect of age was observed for **FFM** ( $65.1\pm 5.0$  kg,  $63.7\pm 5.6$  kg,  
225  $62.2\pm 5.8$  kg, and  $61.3\pm 2.3$  kg for YT, YC, MAT, and MAC respectively;  $P=0.111$ ,  $\eta^2_p=0.07$ ). YT FFM  
226 was unaltered at P2 ( $66.2\pm 6.7$  kg) compared to P1 ( $P=0.285$ , Cohen's  $d=0.18$ ), as was MAT ( $63.1\pm 6.4$   
227 kg;  $P=0.332$ , Cohen's  $d=0.15$ ). At P2, MAC ( $61.5\pm 2.2$  kg;  $P=0.830$ , Cohen's  $d=0.08$ ) and YC ( $64.2\pm 7.6$   
228 kg;  $P=0.651$ , Cohen's  $d=0.07$ ) FFM was not significantly different from P1.

229 We observed a significant main effect of age for  **$W_{peak}$**  at P1 ( $1037\pm 127$  W,  $955\pm 258$  W,  $896\pm 70$   
230 W, and  $872\pm 122$  W for YT, YC, MAT, and MAC respectively  $P=0.040$ ;  $\eta^2_p=0.11$ ).  $W_{peak}$  at P2 in YT  
231 ( $1093\pm 202$  W;  $P=0.067$ , Cohen's  $d=0.33$ ), and MAT ( $950\pm 350$  W;  $P=0.076$ , Cohen's  $d=0.21$ ) was not  
232 significantly increased at P2 compared to P1 ( ), despite small effect sizes. At P2, YC ( $944\pm 246$  W;  
233  $P=0.606$ , Cohen's  $d=0.04$ ) and MAC ( $874\pm 111$  W;  $P=0.958$ , Cohen's  $d=0.03$ )  $W_{peak}$  was not  
234 significantly different from P1. There was an age effect for  **$W_{mean}$**  at P1 ( $P=0.009$ ;  $\eta^2_p=0.18$ ). YT  $W_{mean}$   
235 was  $575\pm 58$  W and  $581\pm 71$  W at P1 and P2 respectively ( $P=0.792$ , Cohen's  $d=0.09$ ). MAT  $W_{mean}$  was  
236  $508\pm 95$  W and  $543\pm 79$  W at P1 and P2 respectively ( $P=0.141$ , Cohen's  $d=0.40$ ), meaning the age effect  
237 was not present in trained groups at P2 ( $P=0.268$ ).

238 **Discussion**

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

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

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

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

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

277

## 278 **Conclusion**

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

283

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286

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288 All persons designated as authors qualify for authorship, and all those who qualify for authorship are  
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414 **Table 1.** Serum total testosterone (TT; nmol·l<sup>-1</sup>) at rest (TT<sub>0</sub>), at the end of a Wingate Anaerobic  
 415 Test (TT<sub>end</sub>), during recovery (TT<sub>10</sub>), and area under the curve (AUC) in young trained (YT),  
 416 young control (YC), middle-aged trained (MAT), and middle-aged control (MAC) participants,  
 417 before training (P1), and after training (P2).

		<b>TT<sub>0</sub></b>	<b>TT<sub>end</sub></b>	<b>TT<sub>10</sub></b>	<b>TT AUC</b>
<b>YT</b>	P1	33.9±3.9 <sup>a</sup>	42.44±6.0	40.61±5.0 <sup>e</sup>	488.1±93.4
<b>(n=10)</b>	P2	34.5±4.2 <sup>g</sup>	41.33±5.9 <sup>h</sup>	42.53±5.2	477.3±95.2
<b>YC</b>	P1	31.5±4.9	38.79±2.3	40.32±3.8	473.6±232.5
<b>(n=10)</b>	P2	31.7±4.7 <sup>g</sup>	39.26±2.3 <sup>h</sup>	40.82±3.8	471.0±227.2
<b>MAT</b>	P1	25.7±13.7 <sup>e</sup>	34.56±16.2	33.73±11.3 <sup>e</sup>	540.3±90.2
<b>(n=10)</b>	P2	26.1±13.4 <sup>g</sup>	37.63±16.2 <sup>h</sup>	36.93±11.2	526.4±91.5
<b>MAC</b>	P1	24.2±8.6	33.32±4.1	33.40±2.2	530.5±245.2
<b>(n=10)</b>	P2	24.7±8.6 <sup>g</sup>	30.09±4.3 <sup>h</sup>	34.13±2.1	523.5±231.3

418 Data are presented as mean±SD.<sup>a</sup>Significant difference (p<0.05) between YT and MAT,  
 419 <sup>b</sup>Significant difference (p<0.05) between YC and MAC, <sup>c</sup>Significant difference (p<0.05)  
 420 between YT and YC, <sup>d</sup>Significant difference (p<0.05) between MAT and MAC, <sup>e</sup>Significant  
 421 difference (p<0.05) from before and after training, <sup>f</sup>Significant difference (p<0.05) between “0”  
 422 and “end”, <sup>g</sup>Significant difference (p<0.05) between “0” and “10”, <sup>h</sup>Significant difference  
 423 (p<0.05) between “end” and “10”.

424

425 **Table 2.** Serum Sex hormone binding globulin (SHBG; nmol·l<sup>-1</sup>) at rest (SHBG<sub>0</sub>), at the end  
 426 of a Wingate Anaerobic Test (SHBG<sub>end</sub>), during recovery (SHBG<sub>10</sub>), and area under the curve  
 427 (AUC) in young trained (YT), young control (YC), middle-aged trained (MAT), and middle-  
 428 aged control (MAC) participants, before training (P1), and after training (P2).

		<b>SHBG<sub>0</sub></b>	<b>SHBG<sub>end</sub></b>	<b>SHBG<sub>10</sub></b>	<b>SHBG AUC</b>
<b>YT(n=10)</b>	P1	28.7±7.4 <sup>e</sup>	31.7±5.5	29.4± 6.1	6492.8±494.6 <sup>a,c,e</sup>
	P2	27.7±8.1	31.2±6.2	28.9±7.0	6328.8±712.3 <sup>a,c</sup>
<b>YC (n=10)</b>	P1	28.0±8.7	31.0±7.7	28.9±6.5	5148.0±1080.8 <sup>b</sup>
	P2	27.6±8.6	30.7±7.7	28.5±6.5	5313.0±970.4 <sup>b</sup>
<b>MAT (n=10)</b>	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
<b>MAC (n=10)</b>	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

429 Data are presented as mean±SD.<sup>a</sup>Significant difference (p<0.05) between YT and MAT,  
 430 <sup>b</sup>Significant difference (p<0.05) between YC and MAC, <sup>c</sup>Significant difference (p<0.05)  
 431 between YT and YC, <sup>d</sup>Significant difference (p<0.05) between MAT and MAC, <sup>e</sup>Significant  
 432 difference (p<0.05) from before and after training, <sup>f</sup>Significant difference (p<0.05) between “0”  
 433 and “end”, <sup>g</sup>Significant difference (p<0.05) between “0” and “10”, <sup>h</sup>Significant difference  
 434 (p<0.05) between “end” and “10”.

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436

437 **Table 3.** Free testosterone (Free-T; nmol·l<sup>-1</sup>) at rest (Free-T<sub>0</sub>), at the end of a Wingate Anaerobic  
 438 Test (Free-T<sub>end</sub>), during recovery (Free-T<sub>10</sub>), and area under the curve (AUC) in young trained  
 439 (YT), young control (YC), middle-aged trained (MAT), and middle-aged control (MAC)  
 440 participants, before training (P1), and after training (P2).

		<b>Free-T<sub>0</sub></b>	<b>Free-T<sub>end</sub></b>	<b>Free-T<sub>10</sub></b>	<b>Free-T AUC</b>
<b>YT</b> (n=10)	P1	0.71±0.25 <sup>a</sup>	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
<b>YC</b> (n=10)	P1	0.59±0.24	0.85±0.17 <sup>b,g</sup>	0.66±0.16	12.57±3.51 <sup>b</sup>
	P2	0.68±0.26	0.74±0.17	0.68±0.13	13.66±5.53
<b>MAT</b> (n=10)	P1	0.38±0.12 <sup>c</sup>	0.66±0.35	0.68±0.28 <sup>d</sup>	9.85±3.79
	P2	0.58±0.13	0.76±0.24	0.77±0.32 <sup>d</sup>	11.70±3.21
<b>MAC</b> (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

441 Data are presented as mean±SD.<sup>a</sup>Significant difference (p<0.05) between YT and MAT,  
 442 <sup>b</sup>Significant difference (p<0.05) between YC and MAC, <sup>c</sup>Significant difference (p<0.05)  
 443 between YT and YC, <sup>d</sup>Significant difference (p<0.05) between MAT and MAC, <sup>e</sup>Significant  
 444 difference (p<0.05) from before and after training, <sup>f</sup>Significant difference (p<0.05) between “0”  
 445 and “end”, <sup>g</sup>Significant difference (p<0.05) between “0” and “10”, <sup>h</sup>Significant difference  
 446 (p<0.05) between “end” and “10”.

447

448 **Table 4.** Serum cortisol (C; ng·ml<sup>-1</sup>) at rest (C<sub>0</sub>), at the end of a Wingate Anaerobic Test (C<sub>end</sub>),  
 449 during recovery (C<sub>10</sub>), and area under the curve (AUC) in young trained (YT), young control  
 450 (YC), middle-aged trained (MAT), and middle-aged control (MAC) participants, before  
 451 training (P1), and after training (P2).

		C <sub>0</sub>	C <sub>end</sub>	C <sub>10</sub>	C AUC
<b>YT</b>	P1	251±28 <sup>a,f,g</sup>	421±50 <sup>a,c</sup>	471±75	1.66±0.20 <sup>a</sup>
<b>(n=10)</b>	P2	254±22 <sup>a,f,g</sup>	412±88 <sup>a,c</sup>	451±89	1.70±0.47
<b>YC</b>	P1	247±21 <sup>b,f</sup>	344±77 <sup>b</sup>	331±67 <sup>b</sup>	1.88±0.47 <sup>b,e</sup>
<b>(n=10)</b>	P2	201±18 <sup>b,f,g</sup>	350±66 <sup>b</sup>	382±61	2.79±2.79 <sup>b</sup>
<b>MAT</b>	P1	364±56 <sup>f,g</sup>	512±45 <sup>e</sup>	585±67	1.08±0.45
<b>(n=10)</b>	P2	374±46 <sup>d,f,g</sup>	602±44 <sup>d</sup>	581±52	1.10±0.29
<b>MAC</b>	P1	363±53 <sup>f,g</sup>	544±67	524±90	1.00±0.43
<b>(n=10)</b>	P2	291±81 <sup>f,g</sup>	512±66	525±96	1.02±0.56

452 Data are presented as mean±SD.<sup>a</sup>Significant difference (p<0.05) between YT and MAT,  
 453 <sup>b</sup>Significant difference (p<0.05) between YC and MAC, <sup>c</sup>Significant difference (p<0.05)  
 454 between YT and YC, <sup>d</sup>Significant difference (p<0.05) between MAT and MAC, <sup>e</sup>Significant  
 455 difference (p<0.05) from before and after training, <sup>f</sup>Significant difference (p<0.05) between “0”  
 456 and “end”, <sup>g</sup>Significant difference (p<0.05) between “0” and “10”, <sup>h</sup>Significant difference  
 457 (p<0.05) between “end” and “10”.

458

459 **Table 5.** Serum total testosterone:cortisol ratio at rest (TT:C<sub>0</sub>), at the end of a Wingate  
 460 Anaerobic Test (TT:C<sub>end</sub>), during recovery (TT:C<sub>10</sub>), and area under the curve (AUC) in young  
 461 trained (YT), young control (YC), middle-aged trained (MAT), and middle-aged control  
 462 (MAC) participants, before training (P1), and after training (P2).

		<b>TT:C<sub>0</sub></b>	<b>TT:C<sub>end</sub></b>	<b>TT:C<sub>10</sub></b>	<b>TT:C AUC</b>
<b>YT</b>	P1	0.13±0.03 <sup>a,g</sup>	0.10±0.02 <sup>a</sup>	0.09±0.02 <sup>c</sup>	1.66±0.22 <sup>a</sup>
<b>(n=10)</b>	P2	0.14±0.03	0.10±0.03	0.09±0.04	1.70±0.47
<b>YC</b>	P1	0.13±0.04 <sup>b</sup>	0.12±0.04 <sup>b</sup>	0.11±0.02 <sup>b</sup>	1.88±0.47 <sup>b,e</sup>
<b>(n=10)</b>	P2	0.46±0.71 <sup>b,f,g</sup>	0.12±0.08 <sup>b</sup>	0.10±0.03 <sup>b</sup>	2.80±2.80 <sup>b</sup>
<b>MAT</b>	P1	0.07±0.02	0.07±0.04	0.06±0.04	1.09±0.45
<b>(n=10)</b>	P2	0.08±0.01	0.07±0.02	0.07±0.03	1.11±0.31
<b>MAC</b>	P1	0.07±0.04	0.06±0.04	0.06±0.02	1.01±0.43
<b>(n=10)</b>	P2	0.09±0.06	0.06±0.03	0.06±0.03	1.03±0.57

463 Data are presented as mean±SD.<sup>a</sup>Significant difference (p<0.05) between YT and MAT,  
 464 <sup>b</sup>Significant difference (p<0.05) between YC and MAC, <sup>c</sup>Significant difference (p<0.05)  
 465 between YT and YC, <sup>d</sup>Significant difference (p<0.05) between MAT and MAC, <sup>e</sup>Significant  
 466 difference (p<0.05) from before and after training, <sup>f</sup>Significant difference (p<0.05) between “0”  
 467 and “end”, <sup>g</sup>Significant difference (p<0.05) between “0” and “10”, <sup>h</sup>Significant difference  
 468 (p<0.05) between “end” and “10”.

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