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REVIEW

Endogenous transient doping: physical exercise acutely increases testosterone levels—results from a meta-analysis

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Abstract

Purpose Although endogenous testosterone levels are demonstrated to be affected by both acute exercise and resistance training, the dynamic regulation of androgen production after physical activity is still a matter of debate. This meta-analysis was designed to assess whether physical exercise acutely affects testosterone levels in men.

Methods The literature search was conducted to identify longitudinal trials evaluating the acute change of both total testosterone (TT) and free testosterone (fT) after physical activity in adult men. Sensitivity analyses were performed considering the sample collected (blood or saliva), the intensity of the physical exercise and the interval between the end of the exercise and the sample collection.

Results Forty-eight studies were included in the analysis, accounting for 126 trials. A total of 569 patients were enrolled (mean age 29.7 ± 13.1 years). The physical activity increased acutely TT (standardized mean difference 0.74, 95%CI: 0.56, 0.91 nmol/L), considering both serum and saliva samples ($p < 0.001$). Testosterone increased after moderate ($p < 0.001$) and high-intensity ($p < 0.001$) exercises, but not after mild physical activity ($p = 0.19$). Moreover, the testosterone increase was evident when measured immediately at the end of the exercise and within 30 min ($p < 0.001$), but not after 30 min ($p = 0.930$). Similar significant results were obtained considering fT, while SHBG did not change after physical activity ($p = 0.090$).

Conclusion The comprehensive evaluation of the acute physical activity effect on testosterone levels identified a clear increase after exercise, irrespective of the sample collected. The main determinant of this fluctuation was the exercise intensity, with a mechanism that seems to be mostly SHBG independent. In particular, moderate/intense physical activity resulted able to increase endogenous androgenic production, albeit acutely and transitory.

Trial registration number PROSPERO registration ID: 157348

Keywords Testosterone · Exercise · SHBG · Physical activity · Androgen level · Free testosterone

Introduction

Testosterone is the prime suspect in sport medicine during anti-doping practice, considering its documented performance-enhancing properties. In particular, testosterone stimulates muscle mass, reduces body fat [1], improves physical resistance and increases aggression and motivation for competition [2, 3]. Thus, not surprisingly, many athletes tried to exploit the anabolic effect of exogenous testosterone or other androgens to maximize their physical performance. The testosterone use as a doping substance expanded in the 1950s and 1960s to then be banned from Olympic competitions since 1976 [4, 5]. However, the discrimination between the illicit exogenous testosterone use and the physiological endogenous hormones variation during and after physical activity is a long standing challenge in sport medicine [6]. To discriminate between the endogenous

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hormonal fluctuations and the illicit androgenic abuse, the accurate knowledge of how physical activity could physiologically influence the hormonal equilibrium becomes crucial. In recent years, the endocrine response to acute exercise bouts has received considerable attention [7–9]. In particular, both the acute cardiovascular exercise and the resistance training are able to affect testosterone concentrations, although several studies highlighted an increasing effect, whereas others did not [10–12]. In 2014, O'Leary et al. highlighted an acute testosterone raise after resistance exercise in nine clinical trials enrolling lean sedentary men, but not considering obese men [13]. This first systematic review evaluated only resistance exercises, which are known to increase muscle mass and to improve resting metabolic rate [14]. In 2015, Hayes et al. performed a second meta-analysis, showing a cortisol increase, without a significant testosterone change, probably due to the limited number of trials included [15]. Interestingly, this second meta-analysis considered trials in which hormonal measurements were performed using saliva samples instead blood ones. Indeed, the use of saliva for monitoring steroids, peptide hormones and markers of immune function has become more attractive to physicians involved in sport medicine, since it can be collected rapidly, frequently, without the stress induced by venepuncture, and outside of the laboratory setting. Additionally, a recent meta-analysis enrolling hypogonadal men demonstrated a significant total testosterone increase after physical exercise that was proportional to the amount and the duration of the exercise [16]. However, despite the available literature, which is the dynamic regulation of endogenous hormonal production during and after acute physical exercises is still a matter of debate in both men and women [6]. In particular, does testosterone acutely increase after physical exercise? Which exercise is able to affect testosterone levels in men? These two questions are still unanswered and specific evidences should be detected in this field.

With this in mind, the current meta-analysis was designed to shed new evidences on the physiological hormonal changes after physical activity, evaluating whether physical exercise acutely affects testosterone levels in men. Moreover, regarding the high heterogeneity of studies available in the literature, this study intended to identify the main regulatory factors of this suggested variation, considering the exercise intensity, the time elapsed between the end of exercise and the sample collection and the laboratory methodology used to measure testosterone.

Materials and methods

First, this meta-analysis was registered in the International Prospective Register of Systematic Reviews (PROSPERO; registration ID 157348) to ensure originality and transparency of the review process. Cochrane Collaboration and PRISMA statement were followed to perform the meta-analysis.

Data sources and search

Literature search was performed considering the following criteria in MEDLINE and Embase databases: ("testosterone"[All Fields] OR "testosterone serum level"[All Fields] OR "testosterone level"[All Fields] OR "androgens"[All Fields]) AND ("sport"[All Fields] OR "exercise"[All Fields] OR "physical activity"[All Fields]). All studies published until October 31th 2019 were considered.

Study selection and inclusion criteria

The following inclusion criteria were considered: (i) longitudinal design, both prospectively and retrospectively, (ii) male gender, (iii) adult subjects (age older than 18 years), (iv) testosterone levels reported before and after physical activity, (v) evaluation of an acute effect of physical activity, within 1 h of the end of the exercise, (vi) any physical activity, (vii) no other concomitant treatments possibly interfering with sexual hormones, such as hormones, diet, integration, etc. On the other hand, exclusion criteria were: (i) pre-pubertal boys or men younger than 18 years, (ii) chronic comorbidities, such as diabetes mellitus or genetic diseases, (iii) evaluation of hormonal changes after chronic, fractionated, physical activity (alternating periods of rest and activity), (iv) case–control studies in which the chronic physical activity effect on hormones was evaluated comparing trained with untrained subjects.

Data collection process and quality

Three authors performed separately the literature search and extracted the abstracts of studies of interest. All abstracts were evaluated for inclusion criteria and the data were extracted from each study considered eligible, with regard to the study design, year of publication and number of included subjects.

Data were extracted using testosterone levels before and after exercise as primary endpoint, considering sex hormone-binding globulin (SHBG) and free testosterone as secondary endpoints. All testosterone measurements were converted in nmol/L and free testosterone into pmol/L.

Moreover, for each work included, the following parameters were extracted: (i) type of exercise, (ii) duration of exercise, (iii) intensity of exercise, (iv) time elapsed between the end of the exercise and hormone measurements and (v) laboratory method used to measure testosterone levels, with particular attention to the specimen selected for the assay (i.e. serum and/or saliva).

To classify physical activity intensity, one of the more accurate measurements consist in the evaluation of the maximal intensity of the exercise. Among different methods available to obtain this parameter, the repetition maximum (1RM) [17, 18] or the maximum oxygen uptake (VO_{2max}) [19, 20] are the most used and reliable. We extracted all definition of exercise intensity reported in each included study, dividing the physical activity intensity in three categories (mild, moderate, high), considering either the intensity reported in the manuscript or the percentage of the maximal exercise intensity described. Thus, mild intensity was considered until the 60% of maximal exercise intensity, moderate between 60 and 80% and high above than 80%.

Data synthesis and analysis

Using the Review Manager (RevMan) 5.3 Software (Version 5.3.1 Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014), continuous variables were comprehensively evaluated as inverse variance of mean variables. The fixed model was initially used, whereas the random effect model was applied in case of I^2 higher than 60%. However, since the validity of heterogeneity tests can be limited with a small number of component studies, random effect model was used also when heterogeneity was lower than 60%. The heterogeneity degree among different studies was examined by inspecting both the scatter in the data points and the overlap in their confidence intervals (CIs), and by performing I^2 statistics. Weighted mean differences and 95% CIs were estimated for each endpoint. Standardized mean difference was considered when standard deviation showed higher heterogeneity among studies included in the analysis. Values of $p < 0.05$ were considered statistically significant.

The analysis was performed considering the overall group of studies included together and then subdivided according to the sample collected (i.e. saliva or blood). Indeed, since the potential surrogate role of salivary testosterone to measure serum testosterone remains to be completely determined, the evaluation of physical activity effect on testosterone could be different considering the assays used to detect hormone levels. Then, sensitivity analyses were performed first considering the methodologies used to measure testosterone. Indeed, the intra- and inter-assay coefficients of variation of radioimmunoassay (RIA) have been reported to be below 5%, showing greater sensitivity than enzyme-linked immunosorbent assay (ELISA). Second, sensitivity analyses were performed considering the intensity of the physical exercise as previously reported. Third, sensitivity analyses were performed considering the interval between the end of the physical activity and the samples collection. To this purpose, three categories were created, considering samples obtained (i) immediately after the activity (0–2 min), (ii) within 30 min (3–30 min), and (iii) within 60 min (31–60 min). Finally, since both professional and non-professional athletes have been enrolled in studies included, we performed a sensitivity analysis, excluding those trials in which professional men were considered. Meta-regression models were conducted to investigate the possible covariates that could affect the estimates.

Results

Study selection

Figure 1 shows the literature searching process. Of the 5120 publications initially identified, 4411 remained after duplicates removal. According to inclusion criteria, 158 potentially relevant studies have been extracted, based on the information given in the abstract. All trials were thoroughly appraised for eligibility in the meta-analysis and methodological quality. Among these, 110 studies were excluded for the following reasons: (i) in 72 studies, numerical data about testosterone levels before and after physical activity could not be extrapolated, (ii) 17 trials evaluated hormonal changes after chronic, fractionated, physical activity (alternating periods of rest and activity), (iii) 9 studies did not present an experimental study design (i.e. reviews of the literature), (iv) 4 studies did not consider any physical activity, (v) three studies enrolled boys younger than 18 years, (vi) 2 studies enrolled only female athletes, (vii) 2 studies involved animal models and (viii) 1 study considered the effect of testosterone replacement therapy on physical activity in hypogonadal patients. Finally, 48 studies were included in the analysis, accounting for 126 trials (Table 1, Fig. 1, Table 2).

Study characteristics

Table 1 reassumes study characteristics. Ten studies were randomized clinical trials, whereas the remaining 79.2% of included studies did not provide a randomization of patients enrolled. Only the 14.6% of included studies (7 trials), enrolled professional athletes (Table 1). A total of 569 patients were enrolled with a mean age of 29.7 ± 13.1 years.

Thirteen studies evaluated hormone levels on saliva samples, whereas the vast majority of studies considered serum samples (72.4%) (Table 1). All 126 trials included in the analysis reported total testosterone levels before and after physical activity (Table 2). SHBG measurements were available in 8 studies, for a total of 28 trials (21.4%). Free testosterone was reported in 12 studies, for a total of 26 trials (20.6%).

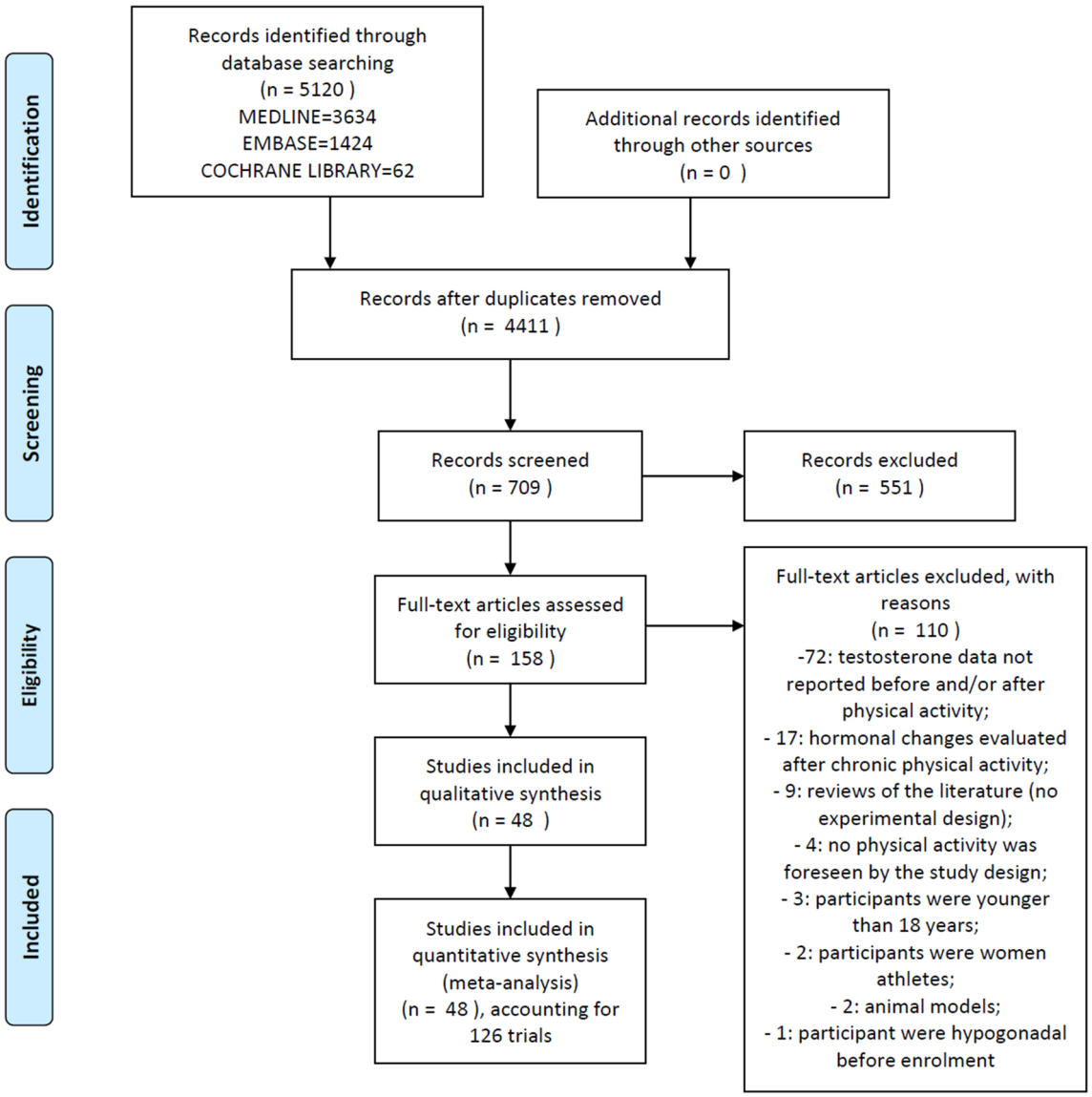

Fig. 1 Study flow diagram

Total Testosterone change after physical activity

Overall, 77 trials evaluated serum testosterone levels and 49 saliva for a total of 3194 subjects. The acute physical activity increased total testosterone levels ($p < 0.001$), considering both serum ($p < 0.001$) and saliva samples ($p < 0.001$) (Fig. 2). Considering the subjects age, seven studies for nine trials evaluated men older than 40 years. Excluding these trials, total testosterone remained significantly higher after physical exercise both in serum (0.71, 95%CI 0.48, 0.94 nmol/L, $p < 0.001$) and in saliva (0.79, 95%CI 0.48, 1.09 nmol/L, $p < 0.001$). Similarly, testosterone raise remained statistical significant ($p < 0.001$) also after exclusion of studies enrolling professional athletes.

Sensitivity analyses were performed and studies were divided according to the physical activity intensity. Testosterone levels significantly increased after moderate- ($p < 0.001$) and high-intensity ($p < 0.001$) exercises. Mild physical activity did not significantly change testosterone levels ($p = 0.190$) (Fig. 3). Testosterone raise remained significant after moderate (0.87, 95%CI 0.58, 1.15 nmol/L, $p < 0.001$) and high (0.73, 95%CI 0.47, 1.00, $p < 0.001$) intensity exercise also considering only subjects younger than 40 years.

Testosterone significantly increased after activity when measured 0–2 min after the exercise (0.90, 95%CI 0.66, 1.14 nmol/L, $p < 0.001$), but not after 3 min (3–30 min: 0.77, 95%CI 0.46, 1.09 nmol/L, $p < 0.001$ and 31–60 min: − 0.02,

Table 1 48 studies included in the meta-analysis

Author	Journal	Year	Subjects (*n*)	Age (years)	Professional or not-professional	Athletes description	Physical activity type (description)	Physical activity duration	Pshysical activity intensity (description)	Physical activity intensity (categorized)	Interval between exercise and examination	Sample	Laboratory method
Poderoso	Int J Environ Res Public Health	2019	17	20.2 ± 2.14	Not professional	Not specified	Maximal sprint at 09.00 h	NA	High	High	5 min	Saliva	ELISA
Ahmadi	Sport Sci Health	2017	11	22.3 ± 1.922.3 ± 1.9	Not professional	Not specified	Acute intermittent aerobic exercise-running on a treadmill with intensity alternating between 50% (2 min) and 80% (1 min) of reserve HR	40 min	High	High	0 min	Blood	ELISA
Hayes	Endocrine Connections	2017	17	20.2 ± 2.10	Not professional	Not specified	One RM squat at 17.00 h	NA	High	High	5 min	Saliva	ELISA
Rosety	Nutr Hosp	2017	17	20.2 ± 2.15	Not professional	Not specified	Maximal sprint at 09.00 h	NA	High	High	60 min	Saliva	ELISA
Hayes	The Aging Male	2015	17	20.2 ± 2.11	Not professional	Not specified	One RM squat at 17.00 h	NA	High	High	60 min	Saliva	ELISA
Budnar	J Strength Cond Res	2014	10	24 ± 4	Not professional	Recreational traine	16 kg kettlebell ball swings	30 min	70% of predictive HR_{max} for age	Moderate	0 min	Blood	ELISA
Di Blasio	J Sports Med Phys Fitness	2014	8	28.61 ± 3.51	Not professional	Trained men	Random workout (participants free to choose the order of exercises and number of consecutive repetitions)	NA	High	High	1 min	Saliva	RIA
Hayes	J Physiol Biochem	2014	18	23.2 ± 3.0	Not professional	Not specified	One RM squat at 09.00 h	NA	High	High	5 min	Saliva	ELISA
Lane and Hackney	Hormones	2014	12	22 ± 4.6	Not professional	Trained men	Cycling sessions at 40% of VO_{2max}	30 min	Low	Mild	30 min	Blood	ELISA

Table 1 (continued)

Author	Journal	Year	Subjects (*n*)	Age (years)	Professional or not-professional	Athletes description	Physical activity type (description)	Physical activity duration	Pshysical activity intensity (description)	Physical activity intensity (categorized)	Interval between exercise and examination	Sample	Laboratory method
Rosety-Rodriguez	International Braz J Urol	2014	9	25–35	Not professional	Not specified	Arm-crank exercise	30–45 min	Moderate	Moderate	NA	Blood	ELISA
Sgrò	J Endocrinol Invest	2014	12	25 ± 4	Not professional	Trained men	Sub-maximal exercise on cycle ergometer at individual anaerobic threshold	30 min	Moderate	Moderate	0 min	Blood	RIA
Cadore	J Strength Cond Res	2012	10	23.5 ± 0.9	Not professional	Not specified	Maximal incremental test with dynamic and aerobic protocols	NA	Medium (50 W)	Moderate	15 min	Blood	RIA
Caruso	J Strength Cond Res	2012	45	NA	Not professional	Not specified	Elbow flexor workouts on a novel inertial kinetic exercise		High speed	High	2 min	Saliva	RIA
Crewther	Physiol Behav	2012	12	20.0 ± 0.7	Professional	Rugby players	3 sets of power cleans, 3 sets of back squats and 3 sets of bench press	NA	Moderate with increasing range 40, 60, 70% of 1RM	Moderate	5 min	Saliva	ELISA
Lovell	Steroids	2012	12	74.1 ± 2.7	Not professional	Not specified	Sub-maximum exercise after resistance and aerobic training	30 min	High	High	30 min	Blood	RIA
Peeri	Middle East J Sci Res	2012	10	23.3 ± 1.5	Not professional	Active young men	Treadmil	30 min	70% of HR_{max}	Moderate	0 min	Saliva	ELISA
Simão	Appl. Physiol. Nutr. Metab	2012	20	22.4 ± 2.7	Not professional	Trained men	3 repetitions sets	NA	High	High	0 min	Blood	RIA

Table 1 (continued)

Author	Journal	Year	Subjects (*n*)	Age (years)	Professional or not-professional	Athletes description	Physical activity type (description)	Physical activity duration	Pshysical activity intensity (description)	Physical activity intensity (categorized)	Interval between exercise and examination	Sample	Laboratory method
Villanueva	J Strength Cond Res	2012	6	26 ± 2.4	Not professional	Not specified	Resistance training: 1) smith machine barbell back squat, 2) flat barbell bench press, 3) narrow/neutral grip lat pulldown, and 4) seated unilateral knee extension	NA	85% of 1RM	High	2 min	Blood	ELISA
Azarbayjani	Int J Exerc Sci	2011	10	24 ± 3.69	Not professional	Not specified	Elliptical instrument	25 min	Medium (70% of HR_{max})	Moderate	5 min	Saliva	ELISA
Blair	J Strength Cond Res	2011	30	23.1 ± 3.3	Professional	Rugby players	Push-ups (2 sets 3 10 repetitions, body-weight load), bench throws (BT; 2 sets 3 4 repetitions, 50-kg load), and a box squat (BS; 4 sets 3 2–10 repetitions, increasing loads)	30 min	647 + 69 W	Moderate	0 min	Saliva	RIA
Hough	J Strength Cond Res	2011	10	24 ± 3	Not professional	Not specified	Cycle to volitional fatigue	30 min	Moderate (75% of W_{max})	Moderate	0 min	Blood	ELISA
Meckel	J Strength Cond Res	2011	12	20.3 ± 1.0	Professional	Handball player	100 m run	NA	Medium (80% of maximum speed)	High	2 min	Blood	ELISA
Crewther	J Sports Med Phys Fitness	2010	9	29.8 ± 5.8	Not professional	Not specified	30-s Wingate test on friction-braked cycle ergometer (Monark Ergomedic 834E, Sweden)	30 s	NA	Moderate	1 min	Blood	RIA
Fry	Human Physiology	2010	4	24.5 ± 2.9	Not professional	Not specified	1 RM barbell squat [kg] = 129.3 ± 17.4; 10 sets of 5 speed squat repetitions	NA	70% of 1 RM	Moderate	5 min	Blood	RIA

Table 1 (continued)

Author	Journal	Year	Subjects (*n*)	Age (years)	Professional or not-professional	Athletes description	Physical activity type (description)	Physical activity duration	Pshysical activity intensity (description)	Physical activity intensity (categorized)	Interval between exercise and examination	Sample	Laboratory method
Migiano	J Strength Cond Res	2009	10	24 ± 1.2	Not professional	Not specified	Dumbbell (db) bench press, bent over db row, db military press, db bicep curl, and db triceps kickback	30 min	80% of 1RM	High	0 min	Blood	RIA
Ramson	J Strength Cond Res	2009	8	20.2 ± 1.6	Not professional	Rowers	Long-distance test 1	40 min	80% of the intensity of anaerobic threshold	High	5 min	Blood	RIA
Beaven	J Strength Cond Res	2008	23	25 ± 3	Professional	Rugby players	4 × 10–70% protocol consisted of four sets of 10 repetitions	NA	70% of 1RM	Moderate	0 min	Saliva	RIA
Cadore	J Strength Cond Res	2008	21	40.6 ± 6.4	Not professional	Trained men	Superset Strength Training Protocol—supersets, each consisting of 2 exercises, and each superset was repeated 4 times to total 16 sets	20 min	75% of the 1RM	Moderate	10 min	Blood	RIA
Crewther	J Strength Cond Res	2008	11	26.6 ± 6.7	Not professional	Not specified	8 sets of 6 reps	22 min	45% of 1RM	Mild	0 min	Saliva	RIA
Karkoulias	Eur J Intern Med	2008	11	56.3 ± 8.2	Not professional	Runners	Marathon race	4.4 h (3.3–5.23)	NA	Moderate	60 min	Blood	RIA
Vuorimaa	Scand J Med Sci Sports	2008	10	28 ± 4.4	Professional	Marathon runners	Marathon race	2:21:54 ± 0:04:30	VO_{2max} 75.7 + 3.6	Moderate	10 min	Blood	RIA
Smilios	Int J Sport Med	2007	8	69 ± 5	Not professional	Not specified	Seated chest press, pec deck, lateral pulldowns, biceps curls, leg extension and leg flexion	NA	60% of 1RM	Mild	0 min	Blood	RIA

Table 1 (continued)

Author	Journal	Year	Subjects (*n*)	Age (years)	Professional or not-professional	Athletes description	Physical activity type (description)	Physical activity duration	Pshysical activity intensity (description)	Physical activity intensity (categorized)	Interval between exercise and examination	Sample	Laboratory method
Daly	Eur J Appl Physiol	2005	22	24.6 ± 0.8	Professional	Endurance-trained men	Prolonged exercise run on the treadmill until reaching volitional fatigue	84.8 + 3.8 min	100% of 1RM	High	0 min	Blood	RIA
Trembley	Eur J Appl Physiol	2005	8	31.4 ± 9.7	Not professional	Not specified	Running	40 min	50% of 1RM	Mild	40 min	Blood	RIA
Väänänen	J Sport Med Phys Fitness	2004	10	34.8 ± 9.7	Not professional	Not specified	Finlandia Ski Race (50 km/day)	3:09:51 ± 0:35:7	Classical skiing technique	Moderate	60 min	Blood	RIA
Castellani	Eur J Appl Physiol	2001	4	36 ± 7	Not professional	Wheelchair athletes	Wheelchair ergometer	20 min	Ergometer resistance (0.03 kg/kg body mass); hand rim (pushing frequency 60 strokes/min)	Moderate	NA	Blood	RIA
Suay	Psychoneuroendocrinology	1999	26	18.32 ± 0.69	Not specified	Judo fighters	Judo fight	NA	NA	Moderate	10 min	Blood	RIA
Fahrner	Int J Sport Med	1998	10	26.5 ± 2.5	Not professional	Not specified	Running	45 min	70% of VO_{2max}	Moderate	0 min	Blood	RIA
Bonifazi	Eur J Appl Physiol	1996	9	35.4 ± 5.7	Not specified	Long-distance runners	Running	60 min	Running speed corresponding to 2 mmol' 1–1 lactate (v ~)	Moderate	0 min	Blood	RIA

Table 1 (continued)

Author	Journal	Year	Subjects (*n*)	Age (years)	Professional or not-professional	Athletes description	Physical activity type (description)	Physical activity duration	Pshysical activity intensity (description)	Physical activity intensity (categorized)	Interval between exercise and examination	Sample	Laboratory method
Zmuda	Metabolism	1996	7	70 ± 4	Not professional	Not specified	Four consecutive bouts of exercise on a model 818E cycle ergometer	60 min	Exercises designed to approximate 50%, 60%, 70% and 80% of HR_{max}	Moderate	0 min	Blood	RIA
Marinelli	Horm Res	1994	6	32.0 ± 7.6	Not specified	Long-distance runners	Marathon race	5:39:00–8:34:00	NA	Moderate	0 min	Blood	RIA
Gray	Eur J Appl Physiol	1993	8	31.5 ± 4.5	Not professional	Not specified	Treadmill	To exhaustion	VO_{2max} 64.3 ± 3.8	Moderate	0 min	Blood	RIA
Hakkinen	J Appl Physiol	1993	10	29.7 ± 8.0	Professional	Strength athletes (power lifters, body builders, and weight lifters)	Squat-lift 10 × 10	NA	70% of 1RM	Moderate	0 min	Blood	RIA
Jensen	Eur J Appl Physiol	1991	7	25.0 ± 2.5	Not professional	Not specified	Running-endurance training	90 min	VO_{2max} 68.4 ± 4.6	Moderate	0 min	Blood	RIA
Pestel	Clin Exp Pharmacol Physiol	1989	2	45	Not professional	Long-distance runners	1000 km Sydney to Melbourne Ultra-marathon foot race (1986)	NA	NA	Moderate	15 min	Blood	RIA
Vogel	Med Sci Sports Exerc	1985	10	25.9 ± 1.7	Not professional	Not specified	Bycicle ergometer	45 min	50% of VO_{2max}	Mild	15 min	Blood	RIA
Guglielmini	Int J Sport Med	1984	7	31 (26–37)	Not specified	Competitive walkers	20-km race	1:30:00 (1:24–1:34)	NA	High	0 min	Blood	RIA

Table 1 (continued)

Author	Journal	Year	Subjects (*n*)	Age (years)	Professional or not-professional	Athletes description	Physical activity type (description)	Physical activity duration	Pshysical activity intensity (description)	Physical activity intensity (categorized)	Interval between exercise and examination	Sample	Laboratory method
Kuoppasalmi	Scand J Clin Lab Invest	1980	5	20–26	Not specified	Not specified	120 m run	15 s	Maximal speed	High	0 min	Blood	RIA

ELISA enzyme-linked immunosorbent assay, HR_{max} maximum heart rate, *NA* not available, *RIA* radioimmunoassay, *RM* repetition maximum, VO_{2max} maximal oxygen uptake, W_{max} peak power output

95%CI − 0.41, 0.38 nmol/L, $p = 0.930$) (Supplementary Fig. 1). Similarly, testosterone remained significantly higher after exercise collecting sample 0–2 min (0.91, 95%CI 0.66, 1.16 nmol/L, $p < 0.001$) and 3–30 min (0.70, 95%CI 0.37, 1.03 nmol/L, $p < 0.001$) after activity, but not 31–60 min (0.03, 95%CI − 0.37, 0.44 nmol/L, $p = 0.860$) considering subjects younger than 40 years. Meta-regression analysis showed that increase in testosterone level after exercise was inversely associated with the time elapsed between the end of the exercise and hormone assessments ($\beta = -0.015$; $p = 0.02$), meaning that each minute from the end of physical activity resulting in 0.015 nmol/L decrease of total testosterone level.

Moreover, sensitivity analyses were performed considering the laboratory methodology used to measure testosterone levels. Thus, two groups were created, considering ELISA and RIA methods. Testosterone levels significantly increased after physical exercise considering both ELISA (0.83, 95%CI 0.42, 1.03 nmol/L, $p < 0.001$) and RIA (0.64, 95%CI 0.42, 0.87 nmol/L, $p < 0.001$). Trials enrolling subjects older than 40 years used only RIA methodology. Thus, sensitivity analysis considering subjects younger than 40 years was performed only for RIA methods, confirming the same significant testosterone raise after physical activity (0.64, 95%CI: 0.40, 0.88 nmol/L, $p < 0.001$). Furthermore, meta-regression analysis showed a positive and significant association with the duration of physical activity ($\beta = 0.004$, $p = 0.002$), but not significant relationship with age of enrolled subjects ($p = 0.55$) was found.

Free testosterone change after physical activity

Twenty-six trials reported free testosterone measurement for a total of 582 subjects. All trials used RIA to measure free testosterone on serum sample. Free testosterone serum levels significantly increased after physical activity (1.09, 95%CI 0.59, 1.59 pmol/L, $p < 0.001$) (Supplementary Fig. 2). Considering subjects younger than 40 years, free testosterone remained significantly higher after exercise (0.90, 95%CI − 0.42, 1.39, pmol/L, $p < 0.001$). Similarly, free testosterone significantly increased ($p < 0.001$) also after exclusion of studies enrolling professional athletes.

Sensitivity analysis confirmed. Sensitivity analysis confirmed a significant free testosterone increase after exercise only when moderate ($p = 0.002$) or high intensity has been applied (< 0.001). On the contrary, free testosterone did not change after mild ($p = 0.270$) physical activity (Fig. 4). Similar results were maintained considering subjects younger than 40 years. In particular, free testosterone remained significantly high after physical exercise for moderate (0.68, 95%CI 0.20, 1.16 pmol/L, $p = 0.006$) and high-intensity exercise (1.73, 95%CI 0.25, 3.21 pmol/L, $p = 0.020$).

Table 2 126 trials included in the meta-analysis with regard to physical activity intensity (described and categorized)

Author	Journal	Year	Physical activity duration	Pshysical activity intensity (description)	Physical activity intensity (categorized)	Interval between exercise and examination
Poderoso	Int J Environ Res Public Health	2019	NA	High	High	5 min
Ahmadi (A)	Sport Sci Health	2017	40 min	High	High	0 min
Ahmadi (B)	Sport Sci Health	2017	40 min	High	High	0 min
Hayes	Endocr Connect	2017	NA	High	High	5 min
Rosety	Nutr Hosp	2017	NA	High	High	60 min
Hayes	The aging male	2015	NA	High	High	60 min
Budnar (A)	J Strength Cond Res	2014	30 min	70% of predictive HR_{max} for age	Moderate	0 min
Budnar (B)	J Strength Cond Res	2014	30 min	70% of predictive HR_{max} for age	Moderate	15 min
Budnar (C)	J Strength Cond Res	2014	30 min	70% of predictive HR_{max} for age	Moderate	30 min
Di Blasio (A)	J Sports Med Phys Fitness	2014	NA	High	High	1 min
Di Blasio (B)	J Sports Med Phys Fitness	2014	NA	High	High	1 min
Di Blasio (C)	J Sports Med Phys Fitness	2014	NA	High	High	1 min
Hayes (A)	J Physiol Biochem	2014	NA	High	High	5 min
Hayes (B)	J Physiol Biochem	2014	NA	High	High	5 min
Hayes (C)	J Physiol Biochem	2014	NA	High	High	5 min
Hayes (D)	J Physiol Biochem	2014	NA	High	High	5 min
Lane and Hackney (A)	Hormones	2014	30 min	Low	Mild	30 min
Lane and Hackney (B)	Hormones	2014	30 min	Low	Mild	60 min
Lane and Hackney (C)	Hormones	2014	30 min	Moderate	Moderate	30 min
Lane and Hackney (D)	Hormones	2014	30 min	Moderate	Moderate	60 min
Lane and Hackney (E)	Hormones	2014	30 min	High	High	30 min
Lane and Hackney (F)	Hormones	2014	30 min	High	High	60 min
Lane and Hackney (G)	Hormones	2014	30 min	Low	Mild	30 min
Lane and Hackney (H)	Hormones	2014	30 min	Low	Mild	60 min
Lane and Hackney (I)	Hormones	2014	30 min	Moderate	Moderate	30 min
Lane and Hackney (L)	Hormones	2014	30 min	Moderate	Moderate	60 min
Lane and Hackney (M)	Hormones	2014	30 min	High	High	30 min
Lane and Hackney (N)	Hormones	2014	30 min	High	High	60 min
Rosety-Rodriguez	International Braz J Urol	2014	30–45 min	Moderate	Moderate	NA
Sgrò (A)	J Endocrinol Invest	2014	30 min	Moderate	Moderate	0 min
Sgrò (B)	J Endocrinol Invest	2014	30 min	Moderate	Moderate	15 min
Sgrò (C)	J Endocrinol Invest	2014	30 min	Moderate	Moderate	30 min
Sgrò (D)	J Endocrinol Invest	2014	30 min	Moderate	Moderate	60 minues
Sgrò (E)	J Endocrinol Invest	2014	NA	High	High	0 min
Sgrò (F)	J Endocrinol Invest	2014	NA	High	High	15 min

Table 2 (continued)

Author	Journal	Year	Physical activity duration	Pshysical activity intensity (description)	Physical activity intensity (categorized)	Interval between exercise and examination
Sgrò (G)	J Endocrinol Invest	2014	NA	High	High	30 min
Sgrò (H)	J Endocrinol Invest	2014	NA	High	High	60 min
Cadore (A)	J Strength Cond Res	2012	NA	Medium (50 W)	Moderate	15 min
Cadore (B)	J Strength Cond Res	2012	NA	Medium (50 W)	Moderate	15 min
Caruso	J Strength Cond Res	2012	NA	High speed	High	2 min
Crewther (A)	Physiol Behav	2012	NA	Moderate with increasing range 40, 60, 70% of 1RM	Moderate	5 min
Crewther (B)	Physiol Behav	2012	NA	Moderate with increasing range 40, 60, 70% of 1RM	Moderate	5 min
Lovell (A)	Steroids	2012	30 min	High	High	30 min
Lovell (B)	Steroids	2012	30 min	High	High	30 min
Peeri (A)	Middle East J Sci Res	2012	30 min	70% of HR_{max}	Moderate	0 min
Peeri (B)	Middle East J Sci Res	2012	30 min	70% of HR_{max}	Moderate	60 min
Simão (A)	Appl. Physiol. Nutr. Metab	2012	NA	High	High	0 min
Simão (B)	Appl. Physiol. Nutr. Metab	2012	NA	High	High	0 min
Villanueva (A)	J Strength Cond Res	2012	NA	85% of 1RM	High	2 min
Villanueva (B)	J Strength Cond Res	2012	NA	85% of 1RM	High	15 min
Azarbayjani (A)	Int J Exerc Sci	2011	25 min	Medium (70% of HR_{max})	Moderate	5 min
Azarbayjani (B)	Int J Exerc Sci	2011	25 min	Medium (70% of HR_{max})	Moderate	5 min
Azarbayjani (C)	Int J Exerc Sci	2011	25 min	Medium (70% of HR_{max})	Moderate	5 min
Azarbayjani (D)	Int J Exerc Sci	2011	25 min	Medium (85% of HR_{max})	Moderate	5 min
Azarbayjani (E)	Int J Exerc Sci	2011	25 min	Medium (85% of HR_{max})	Moderate	5 min
Azarbayjani (F)	Int J Exerc Sci	2011	25 min	Medium (85% of HR_{max})	Moderate	5 min
Blair (A)	J Strength Cond Res	2011	30 min	647 + 69 W	Moderate	0 min
Blair (B)	J Strength Cond Res	2011	30 min	2398 + 345 W	Moderate	0 min
Hough (A)	J Strength Cond Res	2011	30 min	Moderate (75% of W_{max})	Moderate	0 min
Hough (B)	J Strength Cond Res	2011	30 min	Moderate (75% of W_{max})	Moderate	0 min
Hough (C)	J Strength Cond Res	2011	30 min	Moderate (alternating 60% to 90% of W_{max})	Moderate	0 min
Hough (D)	J Strength Cond Res	2011	30 min	Moderate (alternating 60% to 90% of W_{max})	Moderate	0 min
Hough (E)	J Strength Cond Res	2011	30 min	Moderate (alternating 55% to 80% of W_{max})	Moderate	0 min
Hough (F)	J Strength Cond Res	2011	30 min	Moderate (alternating 55% to 80% of W_{max})	Moderate	0 min
Meckel (A)	J Strength Cond Res	2011	NA	Medium (80% of maximum speed)	High	2 min

Table 2 (continued)

Author	Journal	Year	Physical activity duration	Pshysical activity intensity (description)	Physical activity intensity (categorized)	Interval between exercise and examination
Meckel (B)	J Strength Cond Res	2011	NA	Medium (80% of maximum speed)	High	2 min
Meckel (C)	J Strength Cond Res	2011	NA	Medium (80% of maximum speed)	High	2 min
Meckel (D)	J Strength Cond Res	2011	NA	Medium (80% of maximum speed)	High	2 min
Crewther (A)	J Sports Med Phys Fitness	2010	30 s	NA	Moderate	1 min
Crewther (B)	J Sports Med Phys Fitness	2010	30 s	NA	Moderate	10 min
Crewther (C)	J Sports Med Phys Fitness	2010	30 s	NA	Moderate	20 min
Crewther (D)	J Sports Med Phys Fitness	2010	30 s	NA	Moderate	30 min
Crewther (E)	J Sports Med Phys Fitness	2010	30 s	NA	Moderate	60 min
Crewther (F)	J Sports Med Phys Fitness	2010	30 s	NA	Moderate	1 min
Crewther (G)	J Sports Med Phys Fitness	2010	30 s	NA	Moderate	10 min
Crewther (H)	J Sports Med Phys Fitness	2010	30 s	NA	Moderate	20 min
Crewther (I)	J Sports Med Phys Fitness	2010	30 s	NA	Moderate	30 min
Crewther (L)	J Sports Med Phys Fitness	2010	30 s	88% of 1 RM	Moderate	60 min
Fry	Human Physiology	2010	NA	70% of 1 RM	Moderate	5 min
Migiano (A)	J Strength Cond Res	2009	30 min	80% of 1 RM	High	0 min
Migiano (B)	J Strength Cond Res	2009	30 min	80% of 1 RM	High	5 min
Ramson (A)	J Strength Cond Res	2009	40 min	80% of the intensity of anaerobic threshold	High	5 min
Ramson (B)	J Strength Cond Res	2009	40 min	80% of the intensity of anaerobic threshold	High	5 min
Ramson (C)	J Strength Cond Res	2009	40 min	80% of the intensity of anaerobic threshold	High	5 min
Beaven (A)	J Strength Cond Res	2008	NA	70% of 1 RM	Moderate	0 min
Beaven (B)	J Strength Cond Res	2008	NA	70% of 1 RM	Moderate	30 min
Beaven (C)	J Strength Cond Res	2008	NA	85% of 1 RM	High	0 min
Beaven (D)	J Strength Cond Res	2008	NA	85% of 1 RM	High	30 min
Beaven (E)	J Strength Cond Res	2008	NA	55% of 1 RM	Mild	0 min
Beaven (F)	J Strength Cond Res	2008	NA	55% of 1 RM	Mild	30 minnutes
Beaven (G)	J Strength Cond Res	2008	NA	40% of 1 RM	Mild	0 min
Beaven (H)	J Strength Cond Res	2008	NA	40% of 1 RM	Mild	30 min
Cadore (A)	J Strength Cond Res	2008	20 min	75% of the 1 RM	Moderate	10 min
Cadore (B)	J Strength Cond Res	2008	20 min	75% of 1 RM	Moderate	10 min
Crewther (A)	J Strength Cond Res	2008	22 min	45% of 1 RM	Mild	0 min
Crewther (B)	J Strength Cond Res	2008	22 min	75% of 1 RM	Moderate	0 min
Crewther (C)	J Strength Cond Res	2008	22 min	100% of 1 RM	High	0 min
Karkoulias	Eur J Intern Med	2008	4.4 h (3.3–5.23)	NA	Moderate	60 min

Table 2 (continued)

Author	Journal	Year	Physical activity duration	Pshysical activity intensity (description)	Physical activity intensity (categorized)	Interval between exercise and examination
Vuorimaa	Scand J Med Sci Sports	2008	2:21:54 (0:04:30)	VO_{2max} 75.7 + 3.6	Moderate	10 min
Smilios (A)	Int J Sport Med	2007	NA	60% of 1 RM	Mild	0 min
Smilios (B)	Int J Sport Med	2007	NA	60% of 1 RM	Mild	0 min
Daly	Eur J Appl Physiol	2005	84.8 + 3.8 min	100% of 1 RM	High	0 min
Trembley (A)	Eur J Appl Physiol	2005	40 min	50% of 1 RM	Mild	40 min
Trembley (B)	Eur J Appl Physiol	2005	120 min	55% of 1RM	Mild	0 min
Väänänen (A)	J Sport Med Phys Fitness	2004	3 h, 9 min, 51 s ± 35 min, 7 s	Classical skiing technique	Moderate	60 min
Väänänen (B)	J Sport Med Phys Fitness	2004	2 h, 44 min, 24 s ± 33 min, 22 s	Free skiing technique	Moderate	60 min
Castellani (A)	Eur J Appl Physiol	2001	20 min	Ergometer resistance set at 0.03 kg/kg body mass; hand rim pushing frequency set by a metronome at 60 strokes/minute	Moderate	NA
Castellani (B)	Eur J Appl Physiol	2001	20 min	Ergometer resistance set at 0.03 kg/kg body mass; hand rim pushing frequency set by a metronome at 60 strokes/minute	Moderate	NA
Suay (A)	Psychoneuroendocrinology	1999	NA	NA	Moderate	10 min
Suay (B)	Psychoneuroendocrinology	1999	NA	NA	Moderate	30 min
Fahrner	Int J Sport Med	1998	45 min	70% of VO_{2max}	Moderate	0 min
Bonifazi (A)	Eur J Appl Physiol	1996	60 min in the morning	Running speed corresponding to 2 mmol' 1–1 lactate (v ~), calculated by extrapolation or interpolation	Moderate	0 min
Bonifazi (B)	Eur J Appl Physiol	1996	60 min in the afternoon	Running speed corresponding to 2 mmol' 1–1 lactate (v ~), calculated by extrapolation or interpolation	Moderate	0 min
Zmuda	Metabolism	1996	60 min	Each exercise was designed to approximate 50%, 60%, 70% and 80% of HR_{max}	Moderate	0 min
Marinelli	Horm Res	1994	5 h, 39 min to 8 h, 34 min	NA	Moderate	0 min
Gray	Eur J Appl Physiol	1993	to exhaustion	VO_{2max} 64.3 ± 3.8	Moderate	0 min
Hakkinen	J Appl Physiol	1993	NA	70% of 1RM	Moderate	0 min
Jensen (A)	Eur J Appl Physiol	1991	90 min	VO_{2max} 68.4 ± 4.6	Moderate	0 min
Jensen (B)	Eur J Appl Physiol	1991	90 min	80% of 1RM	High	0 min
Pestel	Clin Exp Pharmacol Physiol	1989	NA	NA	Moderate	15 min

Table 2 (continued)

Author	Journal	Year	Physical activity duration	Pshysical activity intensity (description)	Physical activity intensity (categorized)	Interval between exercise and examination
Vogel	Med Sci Sports Exerc	1985	45 min	50% of VO_{2max}	Mild	15 min
Guglielmini (A)	Int J Sport Med	1984	1 h, 30 min (1:24–1:34)	NA	High	0 min
Guglielmini (B)	Int J Sport Med	1984	1 h	NA	High	0 min
Guglielmini (C)	Int J Sport Med	1984	2 h, 33 min (2:19–2:52)	NA	High	0 min
Guglielmini (D)	Int J Sport Med	1984	14 h (7:45–19:36)	NA	High	0 min
Kuoppasalmi	Scand J Clin Lab Invest	1980	15 s	Maximal speed	High	0 min

HR_{max} maximum heart rate, *NA* not available, *RM* repetition maximum, *VO_{2max}* maximal oxygen uptake, *W_{max}* peak power output

The free testosterone increases after physical activity remained when blood sample has been obtained 0–2 min after exercise (1.77, 95%CI 0.75, 2.80 pmol/L, $p < 0.001$) and 3–30 min after (0.95, 95%CI 0.44, 1.46 pmol/L, $p < 0.001$). On the other hand, no differences were obtained when blood samples were collected 31–60 min after the physical exercise (0.06, 95%CI − 0.69, 0.82 pmol/L, $p = 0.870$) (Supplementary Fig. 3). Similarly, free testosterone was significantly higher after exercise in subjects younger than 40 years when measured 0–2 min (1.48, 95%CI: 0.50, 2.46 pmol/L, $p = 0.003$) and 3–30 min (0.66, 95%CI: 0.29, 1.03, pmol/L, $p < 0.001$) after exercise, but nor when measured 31–60 min after (0.28, 95%CI − 0.52, 1.08 pmol/L, $p = 0.490$).

SHBG change after physical activity

Twenty-three trials reported SHBG levels, for a total of 531 subjects. All trials measured SHBG on serum sample. SHBG did not change after physical activity (0.19, 95%CI − 0.03, 0.40 nmol/L, $p = 0.090$) (Supplementary Fig. 4). Similar results were obtained considering only subjects younger than 40 years ($p = 0.090$) and after exclusion of professional athletes ($p = 0.092$).

SHBG serum levels were significantly higher after physical activity when high-intensity exercise was performed ($p = 0.020$). On the contrary, SHBG did not change after physical activity considering moderate-intensity exercise ($p = 0.530$) (Fig. 5). However, when only subjects younger than 40 years were considered, SHBG did not change after exercise, considering both moderate (0.17, 95%CI − 0.08, 0.42 nmol/L, $p = 0.190$) and high (0.30, 95%CI − 0.04, 0.64, nmol/L, $p = 0.090$) intensity exercise.

SHBG did not change after exercise, neither 0–2 min after (0.25, 95%CI − 0.22, 0.72, nmol/L, $p = 0.290$) nor 3–30 min (0.13, 95%CI − 0.13, 0.38 nmol/L, $p = 0.340$), or 31–60 min after the exercise (0.00, 95%CI − 0.48, 0.49 nmol/L, $p = 0.990$) (Supplementary Fig. 5). Similar results remained considering only subjects younger than 40 years (data not shown).

Discussion

The comprehensive evaluation of the acute physical exercise influence on androgens serum levels clearly describes a quick and transient testosterone increase. Here, we confirm previous suggestion that physical activity increases testosterone levels in animal models [21, 22] and humans [13, 15]. Moreover, our systematic revision of the literature highlights those factors potentially able to influence this hormonal raise. First, testosterone increases immediately after physical activity and, in any case, within 30 min since the end of the acute exercise. As a confirm, no differences with baseline levels are detected after 1 h from the end of the physical activity and meta-regression analysis showed an inverse relationship between testosterone level and time from the end of physical activity. This detection suggests that physical exercise rapidly influences the anabolic/catabolic balance in the human body, with a potential stronger effect on anabolic hormones. However, at the end of physical activity, this effect is rapidly lost, returning the hormonal balance to its basal state. In this setting, the physical exercise intensity seems to be the most relevant influencing factor. Indeed, only moderate- and high-intensity exercises (defined as higher than 80% of the maximal exercise) are able to increase testosterone levels, as opposed to mild exercise, which shows no effect. In details, when a high physical intensity is performed, both total and free testosterone increase, together with a SHBG serum levels raise. Thus, the acute physical high/moderate-intensity exercise stimulates the anabolic function of the body, with also an increased protein production by the liver. Similar alterations in serum SHBG levels are already reported in different trials, although there is no unanimous consensus on the overall trend [23]. Here, since an increase in free

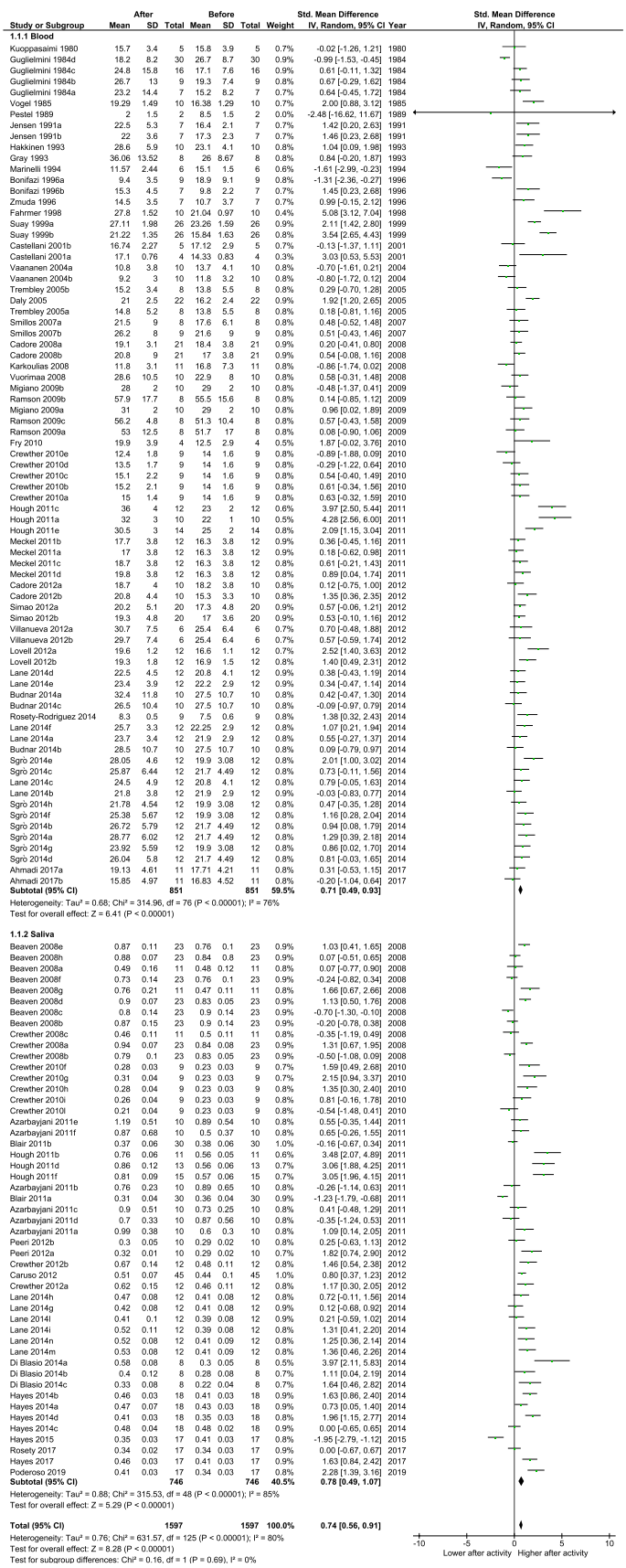

Fig. 2 Forest plots depicting the standardized mean difference in serum levels of total testosterone between before and after acute physical exercise. Diamonds indicate the overall summary estimates for the analyses (width of the diamonds represents the 95% CI); boxes indicate the weight of individual studies in the pooled analyses. Serum total testosterone levels are reported in nmol/l. *CI* confidence interval, *df* degrees of freedom, *IV* inverse variance, *M–H* Mantel–Haenszel, *SD* standard deviation

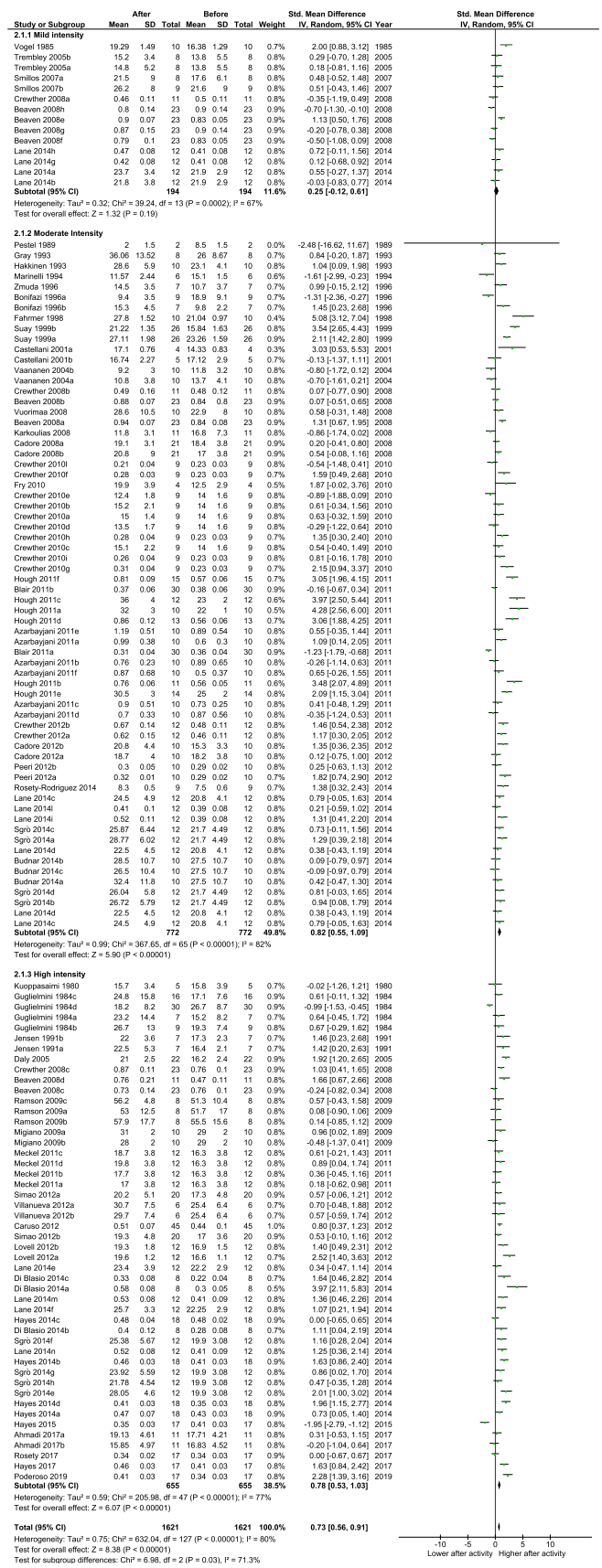

Fig. 3 Forest plot including the results of sub-group analysis on total testosterone serum level by exercise intensity. Diamonds indicate the overall summary estimates for the analyses (width of the diamonds represents the 95% CI); boxes indicate the weight of individual studies in the pooled analyses. Serum total testosterone levels are reported in nmol/l. *CI* confidence interval, *df* degrees of freedom, *IV* inverse variance, *M–H* Mantel–Haenszel, *SD* standard deviation

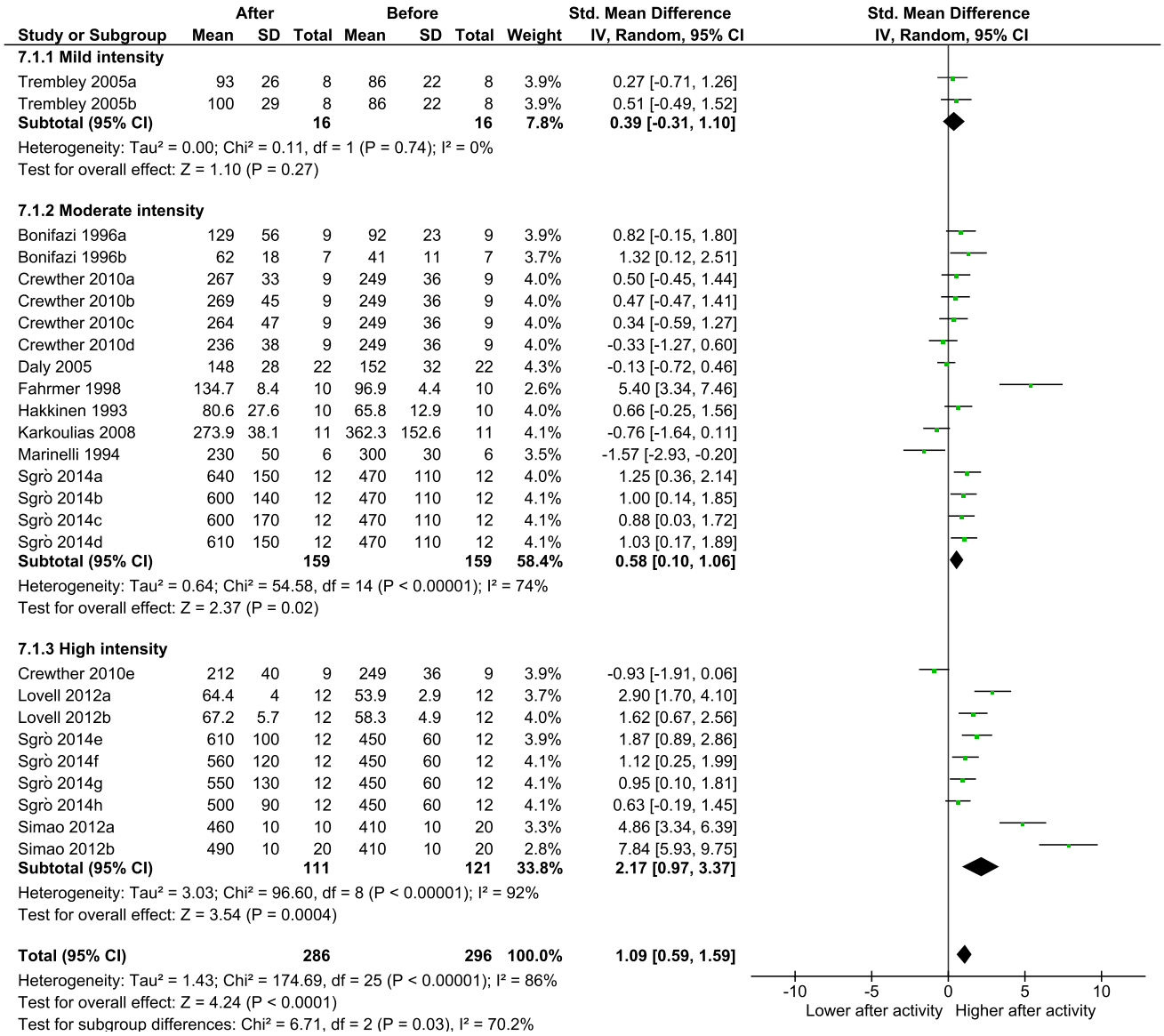

Fig. 4 Forest plot including the results of sub-group analysis on free testosterone level by exercise intensity. Diamonds indicate the overall summary estimates for the analyses (width of the diamonds represents the 95% CI); boxes indicate the weight of individual studies in the pooled analyses. Free total testosterone levels are reported in pmol/l. *CI* confidence interval, *df* degrees of freedom, *IV* inverse variance, *M–H* Mantel–Haenszel, *SD* standard deviation

testosterone remains evident after physical activity albeit in the presence of an exercise-induced SHBG raise, we could speculate that the hypothalamic–pituitary–gonadal (HPG) axis is more stimulated by intense activity, compared to the induction of protein synthesis by the liver. This mechanism is confirmed in animal models in which diabetic rats have been evaluated after physical exercise, showing a significant increase in luteinizing hormone (LH) serum levels and consequently in testosterone production [22]. Although the HPG involvement after physical exercise is clear in animal models, conflicting results are evident for humans. Indeed, Tremblay et al. detected an LH reduction after endurance training in men [24], while Hackney et al. highlighted a LH increase after exercise [25]. Thus, evidences available in the literature suggested so far that physical activity could change testosterone and gonadotropins serum levels. Here, we speculate that the testosterone raise observed after physical exercise could be the consequence of the HPG axis activation.

The role of HPG axis underlying the testosterone raise detected immediately after physical activity remains not completely understood. Indeed, alongside the acute effect of physical activity, it is well known that long-term exercise, especially the so-called over-training exercise, negatively affects testicular function [26]. Animal studies show

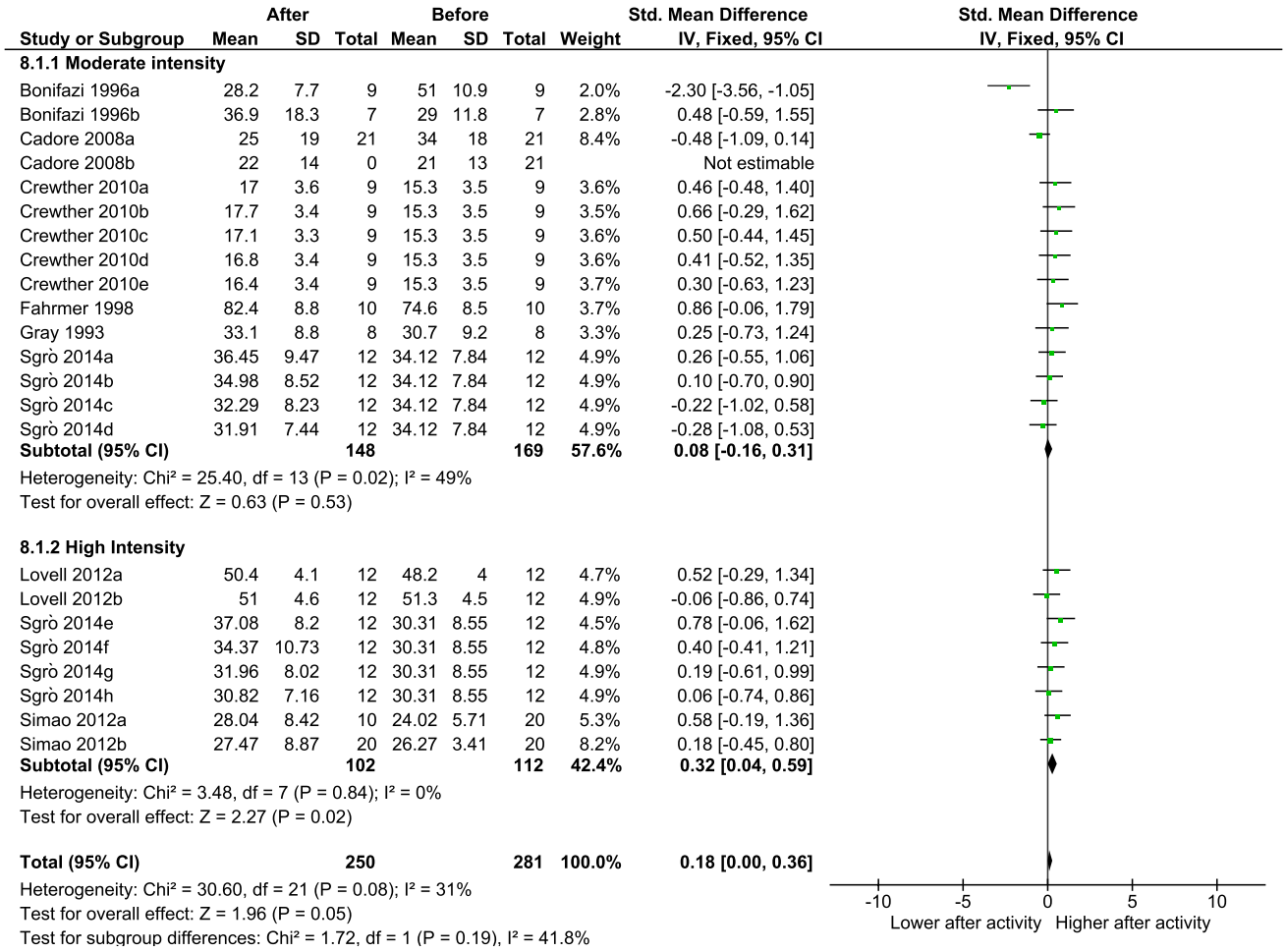

Fig. 5 Forest plot including the results of sub-group analysis on SHBG level by exercise intensity. Diamonds indicate the overall summary estimates for the analyses (width of the diamonds represents the 95% CI); boxes indicate the weight of individual studies in the pooled analyses. Free total testosterone levels are reported in pmol/l. *CI* confidence interval, *df* degrees of freedom, *IV* inverse variance, *M–H* Mantel–Haenszel, *SD* standard deviation

a negative relationship between prolonged intense training and hormones and seminal parameters [27–29]. Recently, mice models highlighted that high-intensity physical exercise chronically performed leads to a reduction of transcriptional levels of kisspeptin (Kiss) and gonadotropin releasing hormone (GnRH) [30], suggesting that chronic intense activity could smooth the HPG axis activation. As a confirm, a randomized controlled trial in humans showed that 60 weeks of high-intensity exercise ($VO_2max > 80\%$) was associated with decreased total testosterone levels, impaired seminal parameters and decreased gonadotropins levels [31]. The hypothesis that the intense activity-mediated suppression of gonadal function is of central origin is similarly supported also in females. Indeed, the functional hypothalamic amenorrhea possibly detected in exercising women seems to be due to energy deficiency and suppressed hypothalamic central drive [32, 33]. However, the exact mechanism by which chronic high-intensity physical activity is able to depress gonadal function remains largely unknown [23]. Interestingly, moderate-intensity exercise chronically performed is conversely associated with increased androgen values, showing that endocrine testicular function benefits from moderate-intensity aerobic or endurance exercise [34–37]. These studies suggested that physical training upregulates the HPG axis, increasing LH and follicle-stimulating hormone (FSH) production and consequentially stimulating functions of both Leydig and Sertoli cells. Moreover, the exercise influence on hormone levels could be exerted also in others testosterone-sensible tissue, such as muscles. Indeed, recent animal studies suggested that physical activity is able to induce both muscle steroidogenic enzymes and muscle testosterone production, to obtain a sort of paracrine effect [38–40]. Here, we confirm that moderate-to-high-intensity acute exercise increase testosterone production within 30 min from the end of the activity, contributing to hormonal changes previously observed after chronic exercise. These oscillations, which

we reported clearly for the first time, could contribute with a feedback mechanism to the action of the entire HPG axis, although the exact mechanism remains unexplored.

The testosterone raise is evident in both saliva and serum samples, resulting independent from the laboratory method applied. The reliability of this result confirms the overlap in the measurement of testosterone levels in saliva and blood samples, confirming that salivary testosterone levels reflect its circulating levels [41, 42]. Since saliva sampling is easier to be collected compared to blood, it could be considered a non-invasive, stress-free, self-sampling procedure facilitating frequent monitoring of hormones changes that could be required in sport medicine [43, 44]. Moreover, the acute testosterone raise depicted in our analysis is confirmed when only young subjects (< 40 years) are considered. Therefore, in our analysis, the subject age is irrelevant to androgen oscillations after acute physical exercise. The testicular function is strongly age-related, as demonstrated by several longitudinal studies [45, 46]. Moreover, several trials have shown that elderly men initiated to motor activity programs show an improvement in androgen levels [47, 48]. In "aged" animal models, physical activity modulates oxidative stress and the levels of intratesticular proinflammatory cytokines [49–51], which, in turns, seems to be responsible for the Leydig cells function reduction with age. However, these mechanisms require time, and are probably involved in hormonal changes after chronic, rather than acute, physical activity. The mechanisms causing variations in testosterone levels after acute exercise are probably different, justifying the age irrelevance emerging from our results.

This meta-analysis shows some limitations. First, a high heterogeneity rate is evident in selected studies, and both the division in subgroups and the application of sensitivity analyses were not able to reduce the source of heterogeneity. Indeed, the complexity of study designs and the variability of physical exercises applied could be not deleted or adjusted. In addition, both randomized clinical trials and uncontrolled interventional studies are included in the overall analysis, increasing the sample size but also the heterogeneity source. Moreover, sensitivity analyses reduced the number of studies included in each analysis, probably limiting the statistical power of these results. Furthermore, enrolled studies recruited both professional and non-professional subjects. With this approach, we are not able to discriminate whether the basal training status of subjects enrolled could impact on testosterone change after physical exercise. However, the non-professional subjects were exercise-trained individuals, balancing potential difference about the training status of each man. We performed the sensitivity analysis excluding professional athletes, to reduce this potential confounding factor. Finally, in our study, gonadotropins serum levels are not available, limiting the possibility to evaluate the HPG functionality as a whole and to develop new hypotheses about the mechanism by which physical activity influences hormonal levels. Similarly, free testosterone was measured using RIA, which is a known inaccurate methodology.

In conclusion, acute exercise increases free and total testosterone levels. This increase is detectable in both blood and salivary samples within 30 min after the end of physical exercise. The main determinant of this increase is the intensity of exercise, with a mechanism independent of SHBG. Specifically, moderate and intense physical exercise induces a transitory increase in testosterone levels. Further proper designed studies are needed to clarify the exact mechanism by which HPG axis is modulated by physical activity and the possible biological significance of such variations.

Author contributions Conceptualization: DS; literature search and data analysis: SD'A, GS, DS; draft the article: SD'A, GS, DS; critical revision: SD'A, GS, AB, DS.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent No informed consent.

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