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REVIEW

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Acute effect of HIIT on testosterone and cortisol levels in healthy individuals: A systematic review and meta-analysis

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To determine the acute effect of a single high-intensity interval training (HIIT) session on testosterone and cortisol levels in healthy individuals, a systematic search of studies was conducted in MEDLINE and Web of Science databases from inception to February 2020. Meta-analyses were performed to establish the acute effect of HIIT on testosterone and cortisol levels immediately after a single HIIT session; after 30 min and 60 min (primary outcomes); and after 120 min, 180 min, and 24 h (secondary outcomes, only for pre-post intervention groups). Potential effect-size modifiers were assessed by meta-regression analyses and analyses of variance. Study quality was assessed using the Cochrane's risk of bias tool and the Physiotherapy Evidence Database scale. The meta-analyses of 10 controlled studies (213 participants) and 50 pre-post intervention groups (677 participants) revealed a significant increase in testosterone immediately after a single HIIT session ($d = 0.92$ and 0.52, respectively), which disappeared after 30 min ($d = 0.18$ and -0.04), and returned to baseline values after 60 min ($d = -0.37$ and -0.16). Significant increases of cortisol were found immediately after ($d = 2.17$ and 0.64), after 30 min ($d = 1.62$ and 0.67) and 60 min ($d = 1.32$ and 0.27). Testosterone and cortisol levels decreased significantly after 120 min ($d = -0.48$ and -0.95, respectively) and 180 min ($d = -0.29$ and -1.08), and returned to baseline values after 24 h ($d = 0.14$ and -0.02). HIIT components and participant's characteristics seem to moderate the effect sizes. In conclusion, testosterone and cortisol increase immediately after a single HIIT session, then drop below baseline levels, and finally return to baseline values after 24 h. This meta-analysis provides a better understanding of the acute endocrine response to a single HIIT session, which would certainly be valuable for both clinicians and coaches in the prescription of exercise programs to improve health and performance. Testosterone and cortisol may be used as sensitive biomarkers to monitor the anabolic and catabolic response to HIIT.

KEYWORDS
aerobic interval training, steroid hormone, time-efficient training, training methodologies

1 | INTRODUCTION

Regardless of whether one is a non-trained individual or a professional athlete, one of the main challenges to physiological homeostasis is exercise, a component of the primitive "fight or flight" response.[1] It has been widely evidenced that significant changes in the endocrine system – which require certain physiological accommodations and adjustments – occurred in response to exercise.[1] These adaptations, in turn, are closely regulated by specific hormones, such as testosterone and cortisol.[1] Specifically, testosterone is one of the strongest naturally secreted androgenic-anabolic hormones with the main biological function of regulating the growth and maintenance of skeletal muscle, bone, and red blood cells.[2] Conversely, cortisol is a catabolic hormone that promotes energy substrate mobilization (i.e., carbohydrate, fat, and protein), and suppresses immune function.[3]

According to previous narrative reviews about testosterone[2,4,5] and cortisol[5] responses to resistance exercise, both hormone levels are increased following heavy resistance exercise. However, the available evidence in this field also suggests that the acute effect of exercise on testosterone and cortisol levels varies between exercise modalities. In this regard, a meta-analysis by Hayes et al.[6] certainly found significant differences in the standardized effect sizes of aerobic, resistance, and power exercise on salivary testosterone and cortisol. In addition, the intensity and duration of the exercise session, and fitness status of individuals, among others, also seem to be noteworthy factors with great impact on these specific hormones response to exercise.[2,4,7,8] Thus, a wide range of exercise variables need to be considered when modifying testosterone and cortisol levels in accordance with the objectives pursued.

High-intensity interval training (HIIT) involves repeated, short to long bouts of rather high-intensity exercise (i.e., equal or superior to maximal lactate steady-state velocity) interspersed with recovery periods (i.e., light exercise or rest).[9] This type of exercise has recently become a prime focus on research and clinical practice due to its effectiveness on triggering rapid adaptations in both central (i.e., cardiovascular) and peripheral (i.e., skeletal muscle) components linked to an enhanced health and sports performance.[10] Indeed, HIIT is the second worldwide fitness trend for 2020 according to the American College of Sport Medicine's annual survey.[11] Nonetheless, the acute effect of HIIT on testosterone and cortisol levels in both untrained and trained individuals remains unclear, mainly due to the reduced number of participants included in currently available studies. HIIT interventions are composed of several acute bouts of HIIT, which makes the study of a single HIIT session relevant for understanding the acute physiological responses that ultimately lead to positive adaptations.

To the best of our knowledge, there are no systematic review nor meta-analysis synthesizing the specific effects of HIIT on testosterone and cortisol levels. Furthermore, although previous systematic review and meta-analysis about the acute effects of exercise modalities on these hormones have been conducted, these studies only included men, saliva samples collected within 30 min after exercise, and did not distinguish between aerobic exercise and HIIT.[6] While aerobic exercise is characterized by low/moderate-intensity and high-volume, HIIT consists of high-intensity and short duration, thus significantly different responses of testosterone and cortisol are potentially expected. Hence, a comprehensive synthesis of the evidence regarding the acute effect of HIIT on testosterone and cortisol levels seems needed, which would potentially be highly valuable to both exercise clinicians, and coaches when prescribing exercise programs in order to enhance specific aspects of health and performance of individuals. For instance, testosterone and cortisol may be used as sensitive biomarkers to monitor the anabolic and catabolic response to HIIT in order to detect potential disorders before observing clinical symptoms (e.g., overtraining, anxiety, and depression).[12,13]

Our systematic review and meta-analysis aimed at synthesizing the evidence of the acute effect of HIIT on testosterone and cortisol levels —including both plasma and saliva samples— in healthy youth and adults, from non-trained to professional athletes. We also pursued to investigate the HIIT components and participant's characteristics which may drive the greatest responses in testosterone and cortisol.

2 | MATERIAL AND METHODS

This systematic review and meta-analysis protocol was registered in the International Prospective Register for Systematic Reviews (PROSPERO; registration number CRD42018108933). Recommendations of the Cochrane Collaboration Handbook[14] and relevant methodological references for the execution of systematic review and meta-analysis were strictly followed.[15-18] Findings were reported according to the Preferred Reporting Items for Systematic

Reviews and Meta-Analyses (PRISMA) guidelines[19] (Table S1 Online Resource).[20]

2.1 | Search strategy

A systematic search of eligible studies was conducted using MEDLINE (via PubMed) and Web of Science from inception to February 2020. Other sources were also manually screened for additional records (i.e., references from previous reviews or relevant studies). The set of search terms used were as follows: ((((“HIIT” or “HIT” or “HIIE” or "HIE") OR ((“high-intensity” or “high intensity” or “interval” or “intermittent” or “sprint” or “speed” or “aerobic” or “anaerobic”) AND (“training” or “exercise” or “sport” or “activity”))) AND ("testosterone" or “cortisol”))). The systematic search was only restricted by language, solely including those studies published in English or Spanish.

2.2 | Study selection criteria

Inclusion criteria were as follows: (i) participants: healthy youth and adults (12 to 75 years), from non-trained to professional athletes; (ii) intervention: any modality of HIIT performed in a single session (acute). HIIT is defined as repeated, short to long bouts of rather a high-intensity exercise (i.e., ≥90% of maximum oxygen consumption [VO_{2max}] or ≥to maximal lactate steady-state velocity) interspersed with recovery periods (i.e., light exercise or rest).[9] Furthermore, according to Buchheit and Laursen[9] HIIT can be categorized into repeated-sprint training (sprint lasting from 3–7 s at 120–160% of minimal running speed associated with VO_{2max}, interspersed with recovery periods ≤60 s), sprint interval training (30 s at 160–180% of minimal running speed associated with VO_{2max} or all-out efforts, interspersed with 2–4 min passive recovery periods), HIIT short intervals (10–60 s at 100–120% of minimal running speed associated with VO_{2max}, interspersed with variable recovery periods), and HIIT long intervals (≥60 s at 90–100% of minimal running speed associated with VO_{2max}, interspersed with variable recovery periods); (iii) study design: controlled studies (both randomized and non-randomized), crossover studies, and pre-post studies; (iv) outcome: change in total testosterone, free testosterone, and cortisol measured in plasma or saliva samples from baseline to the last available follow-up. The exclusion criteria were as follows: (i) patients with obesity (body mass index [BMI] ≥30) and/or chronic illness or cardiometabolic diseases; (ii) the combination of acute HIIT intervention with another type of exercise or sport (e.g., HIIT plus judo); (iii) HIIT intervention (long term effect); (iv) small-sided games, since the understanding of its oxygen volume response is limited;[21] (v) clinical case studies; and (vi) trials reported in languages other than English or Spanish.

Based on the selection criteria, all studies were first independently screened for inclusion by title and abstract by two independent reviewers (MDM, FAG). Any discrepancies between reviewers were resolved by discussion and, if needed, a third reviewer's (ACB) final decision was required. Full texts of remaining studies were obtained and screened for the final inclusion and data extraction following the same protocol.

2.3 | Data extraction and study outcomes

A codebook and a data extraction protocol were developed specifically for this aim. The data extraction of the final selected studies was conducted in a standardized form by two independent authors (MDM, FAG), with advice from ACB on selection criteria. The primary outcomes of interest were changes in total testosterone and cortisol measured in plasma or saliva samples immediately after a single HIIT session, after 30 min, and 60 min.

In addition, as secondary outcomes in this meta-analysis, we also included changes in total testosterone and cortisol measured in plasma or saliva samples after 120 min, 180 min, and 24 h. Only pre-post intervention groups were included in the secondary outcomes analyses due to the reduced number of controlled studies including these outcomes. Free testosterone measured in plasma was also analyzed as a secondary outcome, but only immediately after a single HIIT session because of the reduced number of studies. Moreover, the standardized protocol contained the author's names, country, and year of publication (extrinsic variables); participants and HIIT characteristics (substantive variables); and methodological variables. The corresponding authors of the selected studies were contacted when the required data were not reported. If no response was received, means and standard deviations were estimated from figures using a computer software (WebPlotDigitizer Version 4.2),[22] which has been previously validated.[23]

2.4 | Risk of bias and quality assessment

All trials included in the meta-analysis were assessed for methodological quality using relevant items from the Cochrane's risk of bias tool[24] and the Physiotherapy Evidence Database (PEDro) scale.[25] The quality assessment of controlled studies consist of 9 items or criteria, each referring to a relevant methodological aspect of the study including (i) specification of eligibility criteria, (ii) random allocation to groups, (iii) concealed allocation, (iv) intergroup similarity in outcomes at baseline, (v) blinding (including outcome assessors, data analysts, participants, and researchers), (vi) sample dropout rate (less than or equal to 15%), (vii) intention to treat analysis, (viii) reported comparisons between groups, and (ix) report of effect size coefficients or other parameters, which make the calculation of them possible. Plausible scores were "no" (0 points) when the study did not meet the criteria; "unclear" (0 points) when the study reported no information on the scored item; 0.5 points when the study met the criteria for some outcomes but not all; "yes" (1 point) when the study met the criteria; and "not applicable" when the criteria were not applicable due to the study design. Pre-post intervention groups were similarly assessed for methodological quality adapting the items from the scale or tool, resulting in a total of four criteria. Crossover studies were evaluated as controlled studies when including a control group, and they were evaluated as pre-post intervention groups otherwise. No studies were excluded based on the quality appraisal. Details of the risk of bias and quality assessment can be found in Table S2 (Online Resource).[20]

2.5 | Statistical analysis

Controlled studies and pre-post intervention groups were separately analyzed, although all intervention groups from controlled studies were also included in the pre-post intervention groups meta-analysis. Crossover studies were considered as controlled studies when including a control group, they being evaluated as pre-post intervention groups otherwise.[15] A standardized mean difference effect-size coefficients from controlled studies were computed as the mean difference between the mean change in intervention and control groups, from baseline to post-intervention, divided by mean baseline standard deviation:[26] $d = c\left(df_{E,C}\right) \cdot [((\overline{X}_{pre,E} - \overline{X}_{pos,E}) - (\overline{X}_{pre,C} - \overline{X}_{pos,C}))/S_{pre}]$. Regarding pre-post intervention groups, standardized effect-size coefficients were calculated for each intervention group as the mean change from baseline to post-intervention divided by baseline standard deviation[27]: $d = c\,(df) \cdot [(\overline{X}_{pre,E} - \overline{X}_{pos,E})/S_{pre}]$. Both coefficients included $c(df_{E,C})$ and c(df), correction factors for small samples[28] (see all equations used in Table S3 in Online Resource).[20] The inverse variance method was used in all cases for the weighting of studies. Additionally, we calculated the raw (unstandardized) mean difference in percentage for controlled studies $[(((\overline{X}_{pos,E} \cdot 100)/\overline{X}_{pre,E}) - 100) - (((\overline{X}_{pos,C} \cdot 100)/\overline{X}_{pre,C}) - 100)]$ and pre-post intervention groups $((\overline{X}_{pos,E} \cdot 100)/\overline{X}_{pre,E}) - 100$ using the weights obtained in the standardized meta-analyses to estimate the pooled mean difference in each outcome.

Independent effect-size coefficients from studies and outcomes were combined and analyzed using the DerSimonian and Laird's random-effects model.[29] Weighted standardized mean change from baseline to post-intervention was the pooled effect size of each outcome with confidence interval (CI) set at 95%.

Heterogeneity among included studies was assessed using Cochran's Q test and I^2 statistic. Depending on I^2 statistic values, heterogeneity was classified as follows: might not be important (0–40%), may represent moderate (30–60%), substantial (50–90%), or considerable (75–100%) heterogeneity.[14] Given the heterogeneity among studies, potential effect-size modifiers were analyzed using meta-regression analyses for continuous variables and analyses of variance (ANOVAs) for the qualitative variables. Each effect-size modifier was analyzed individually due to the reduced number of groups in specific outcomes. Furthermore, analyses of effect-size modifiers were only performed for those testosterone and cortisol outcomes that included more than 10 study groups.[14] Additional sensitivity analyses were conducted to assess the influence of each individual study on the pooled effect sizes. Risk of publication bias was also analyzed using Egger's test[30] and Rosenthal method.[31] Rosenthal method (fail-safe N) calculates the number of additional studies with null results that would be needed to increase the P value for the meta-analysis to above an alpha level of 0.05. Assessment of risk of publication bias were exclusively performed for those testosterone and cortisol outcomes with more than 10 study groups.[14] Risk of bias/methodological quality of included primary studies was analyzed as a continuous (total quality score) effect-size modifier using meta-regression to assess its influence on effect sizes for primary testosterone and cortisol outcomes. All statistical analyses were performed using metaphor package[32] from R statistic program.[33]

3 | RESULTS

3.1 | Search results

The PRISMA flow diagram for the systematic search and study selection is shown in Figure 1. After exclusion of duplicate references and screening by title and abstract of the 5803 studies initially retrieved, 235 full-text studies were further evaluated for the final inclusion. The reasons for exclusion based on full-text documents were: (i) other exercise interventions or sports (142 studies); (ii) HIIT and other exercise (13 studies); (iii) HIIT intervention (11 studies); (iv) duplicate participants (7 studies); (v) high-intensity resistance training (6 studies); (vi) impossible to contact authors and extract data (1 article); (vii) full text not available (1 article); (viii) obese (1 article). Finally, 53 studies meeting our inclusion criteria were included in this systematic review: 13 controlled studies[34-46] and 55 pre-post intervention groups (13 intervention groups from controlled studies +42 pre-post intervention groups).[35,41,47-86] Two studies involved both controlled studies and pre-post intervention groups.[35,41] Forty-seven studies were finally included in the meta-analyses: 10 controlled studies and 49 pre-post intervention groups (i.e., 10 intervention groups from controlled studies plus 39 pre-post intervention groups). Six studies collected only one sample at 5 min,[42,54,77,78] 10 min,[44] and 300 min[43] after HIIT. Because of the reduced number of studies with samples collected at these minutes, a meta-analysis was not able to be conducted. Therefore, these 6 studies were not included in the meta-analysis.

3.2 | Study characteristics

A detailed description of the included studies (i.e., controlled studies and pre-post intervention groups) is provided in Table 1. The total sample involved 1055 participants (154 control, 161 intervention from controlled studies, and 740 from pre-post intervention groups) in the systematic review and 890 participants (103 control, 110 intervention from controlled studies, and 677 from pre-post intervention groups) in the meta-analysis. The characteristics of the included studies in the systematic review were as follows: Women represented 9.1% of the total sample, the sample size ranging from 5[41] to 65[35] participants. Mean age was 24 years (standard deviation = 8 years), with a range from 14[63] to 69[79] years. Most of the studies included trained individuals (20 studies; 310 participants), followed by recreationally active individuals (14 studies; 299 participants), professional or semi-professional athletes (15 studies; 294 participants), non-trained individuals (3 studies; 120 participants), and only one reported data of non-trained, recreationally active, and trained individuals together (32 participants). The exercise modalities included were cycling (26 studies; 608 participants), running (26 studies; 458 participants), and swimming (2 studies; 19 participants). Most of the studies assessed the effects of HIIT long intervals (19 studies; 405 participants), followed by repeated-sprint training (15 studies; 393 participants), sprint interval training (17 studies; 359 participants), and HIIT short intervals (5 studies; 111 participants). Lastly, samples were collected in both plasma (37 studies; 675 participants) and saliva (16 studies; 380 participants).

3.3 | Acute effects of HIIT on primary outcomes

The acute effect of HIIT on testosterone at 0 min from 8 controlled studies (n = 99 participants; 0 women) and 41 pre-post intervention groups (n = 517 participants; 8 women) is displayed in Figure 2. Immediately after a single HIIT session, testosterone was significantly increased in controlled studies and pre-post intervention groups (both $p < 0.001$) with an overall pooled effect size of 0.92 (95% CI, 0.56 to 1.27) and 0.52 (95% CI, 0.35 to 0.69), respectively. According to the pooled raw mean differences, testosterone at 0 min increased ~28% and ~15% in controlled studies and pre-post intervention groups, respectively (Figure S1 Online Resource).[20] Heterogeneity was not found in controlled studies ($Q_{(df = 7)} = 10.93$, $p = 0.142$; $I^2 = 35.97\%$), whereas a substantial heterogeneity was observed in pre-post intervention groups ($Q_{(df = 40)} = 140.11$, $p < 0.001$; $I^2 = 71.45\%$).

The acute effect of HIIT on testosterone after 30 min from six controlled studies (n = 54 participants; 0 women) and 14 pre-post intervention groups (n = 136 participants; 0 women) are shown in Figure 3. Changes in testosterone after 30 min were not found in controlled studies nor pre-post intervention groups (both $p \geq 0.560$), with a mean effect size of 0.18 (95% CI, −0.41 to 0.76) and −0.04 (95% CI, −0.34 to 0.26), respectively. According to the pooled raw mean differences, testosterone after 30 min after HIIT increased ~12% in controlled studies whereas decreased ~ −7% in pre-post intervention groups (Figure S1 Online Resource).[20] There was substantial heterogeneity across controlled studies ($Q_{(df = 5)} = 14.59$, $p = 0.012$; $I^2 = 65.72\%$) and pre-post intervention groups ($Q_{(df = 13)} = 43.52$, $p < 0.001$; $I^2 = 70.13\%$).

Figure 4 depicts a forest plot for the acute effect of HIIT on testosterone after 60 min from five controlled studies (n = 46 participants; 0 women) and 27 pre-post intervention groups (n = 461 participants; 0 women). A trend toward significance decrease in testosterone after 60 min after HIIT was recorded in controlled studies ($p = 0.078$), which reach statistical significance in pre-post intervention groups ($p = 0.008$). Specifically, the mean effect size of HIIT on testosterone after 60 min was −0.37 (95% CI, −0.78 to 0.04) for controlled studies and −0.16 (95% CI, −0.28 to −0.04) for pre-post intervention groups. According to the pooled raw mean differences, testosterone after 60 min after HIIT did not vary (~0%) in controlled studies whereas decreased ~ −8% in pre-post intervention groups (Figure S1 Online Resource).[20] No heterogeneity was detected among controlled studies ($Q_{(df = 4)} = 5.11$, $p = 0.276$; $I^2 = 21.76\%$) nor pre-post intervention groups ($Q_{(df = 26)} = 34.80$, $p = 0.116$; $I^2 = 25.29\%$).

The acute effect of HIIT on cortisol at 0 min from six controlled studies (n = 66 participants; 0 women) and 43 pre-post intervention groups (n = 594 participants; 14 women) are shown in Figure 5. The meta-analyses of the acute effect of HIIT on cortisol at 0 min indicated a significant increase in controlled studies and pre-post intervention groups (both $p < 0.001$), showing an overall pooled effect size of 2.17 (95% CI, 1.4 to 2.94) and 0.64 (95% CI, 0.35 to 0.92), respectively. According to the pooled raw mean differences, cortisol at 0 min increased ~82% and ~28% in controlled studies and pre-post intervention groups, respectively (Figure S1 Online Resource).[20] There was substantial heterogeneity across controlled studies ($Q_{(df = 5)} = 15.94$, $p = 0.007$; $I^2 = 68.64\%$) and considerable heterogeneity across pre-post intervention groups ($Q_{(df = 42)} = 368.85$, $p < 0.001$; $I^2 = 88.61\%$).

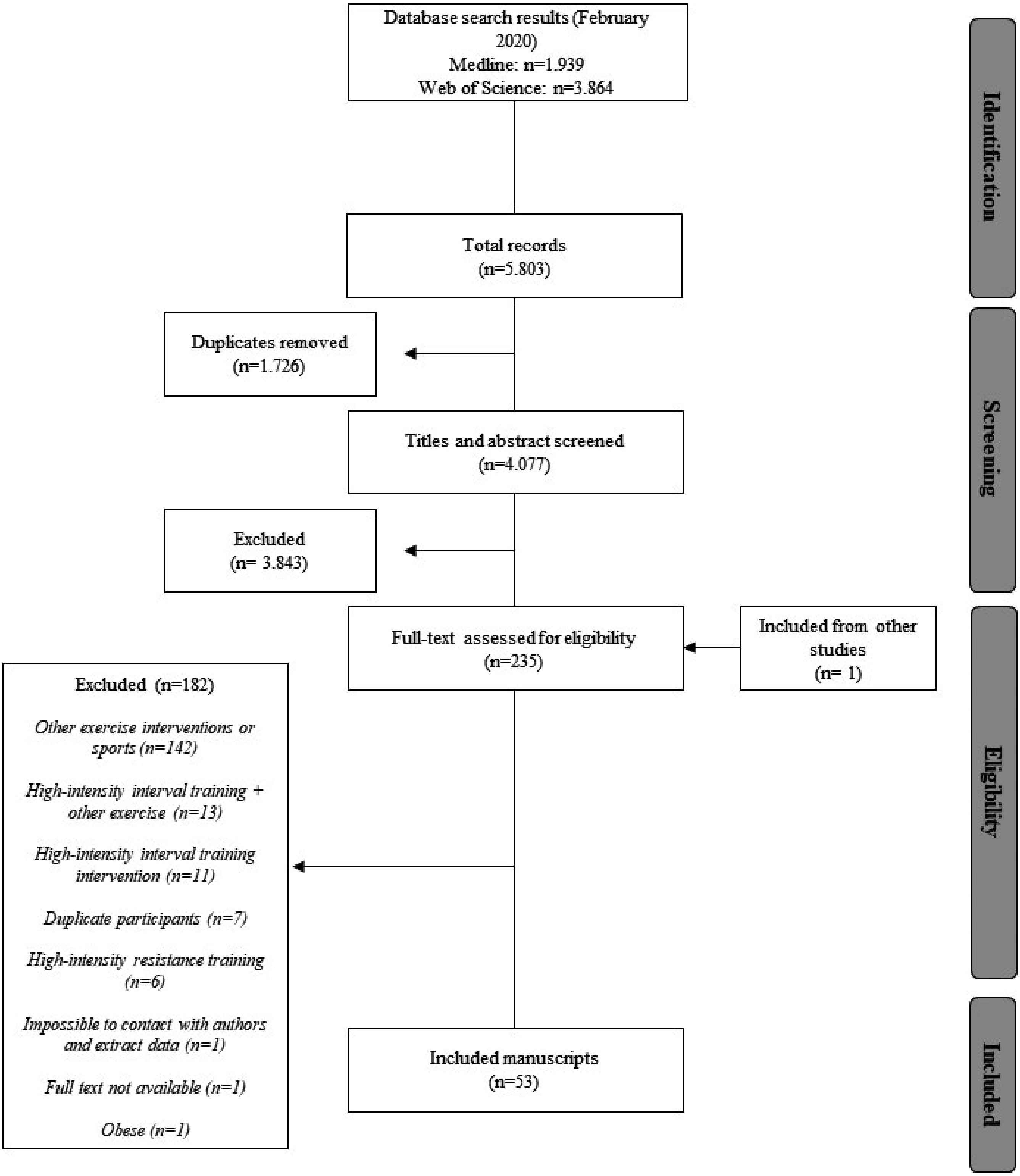


FIGURE 1 Preferred reporting items for systematic reviews and meta-analyses flow diagram

A forest plot of the acute effect of HIIT on cortisol after 30 min from four controlled studies ($n = 35$ participants; 0 women) and 16 pre-post intervention groups ($n = 207$ participants; 0 women) is displayed in Figure 6. Cortisol after 30 min after HIIT was significantly increased in controlled studies and pre-post intervention groups (both $p < 0.001$), showing an overall pooled effect size of 1.62 (95% CI, 1.02 to 2.22) and 0.67 (95% CI, 0.28 to 1.06), respectively. According to the pooled raw mean differences, cortisol after 30 min after HIIT increased ~84% and ~50% in controlled studies and pre-post intervention groups, respectively (Figure S1 Online Resource).[20] There was no heterogeneity across controlled studies ($Q_{(df = 3)} = 3.57$, $p = 0.311$; $I^2 = 16.04\%$), whereas differences among pre-post intervention groups yielded considerable heterogeneity ($Q_{(df = 15)} = 96.73$, $p < 0.001$; $I^2 = 84.49\%$).

The acute effect of HIIT on cortisol after 60 min from four controlled studies ($n = 39$ participants; 0 women) and 19 pre-post intervention groups ($n = 244$ participants; 0 women) are shown in Figure 7. HIIT significantly increased cortisol after 60 min in controlled studies ($p < 0.001$), whereas no changes were found in pre-post intervention groups ($p = 0.101$). The mean effect size of HIIT on cortisol after 60 min was 1.32 (95% CI, 0.84 to 1.80) for controlled studies, and 0.27 (95% CI, −0.05 to 0.59) for pre-post intervention groups. According to the pooled raw mean differences, cortisol after 60 min after HIIT increased ~51% and ~18% in controlled studies and pre-post intervention groups, respectively (Figure S1 Online Resource).[20] Heterogeneity was not found in controlled studies ($Q_{(df = 3)} = 3.11$, $p = 0.375$; $I^2 = 3.49\%$), whereas a considerable heterogeneity in pre-post intervention groups was found ($Q_{(df = 18)} = 108.59$, $p < 0.001$; $I^2 = 83.42\%$).

Raw data of each included study on primary outcomes can be found in Tables S4 and S5 (Online Resource).[20]

3.4 | Acute effects of HIIT on secondary outcomes

The meta-analyses on the acute effects of HIIT on secondary testosterone, free testosterone, and cortisol outcomes are presented in Table 2.

The effect of HIIT on testosterone after 15 min was non-significant ($p = 0.559$), with a mean effect size of 0.14 (95% CI, −0.34 to 0.62) and a slight increase of ~3% in line with the pooled raw mean differences (Figure S1 Online Resource).[20] Testosterone after 120 min and 180 min after HIIT decreased ($p < 0.001$ and $p = 0.011$, respectively), showing an overall pooled effect size of −0.48 (95% CI, −0.70 to −0.27) and −0.29 (95% CI, −0.51 to −0.06), respectively. According to the pooled raw mean differences, testosterone after 120 min and 180 min after HIIT decreased ~ −12% and ~ −10% (Figure S1 Online Resource).[20] Testosterone after 24h after HIIT did not vary ($p = 0.267$), with an overall pooled effect size of −0.15 (95% CI, −0.42 to 0.12) and a slight decrease of ~ −5% in line with the pooled raw mean differences (Figure S1 Online Resource).[20] The effect of HIIT on free testosterone at 0 min was also non-significant ($p = 0.160$), with a mean effect size of 0.43 (95% CI, −0.17 to 1.03) and an increase of ~15% in line with the pooled raw mean differences (Figure S1 Online Resource).[20]

The meta-analyses of the acute effect of HIIT on cortisol after 15 min indicated a significant increase in this outcome ($p < 0.001$), showing a mean effect size of 1.63 (95% CI, 0.97 to 2.29) and an increase of ~64% in line with the pooled raw mean differences (Figure S1 Online Resource).[20] HIIT significantly decreased cortisol after 120 min and 180 min ($p < 0.001$ and $p = 0.009$, respectively), with an overall pooled effect size of −0.95 (95% CI, −1.45 to −0.45) and −1.08 (95% CI, −1.90 to −0.26), respectively. According to the pooled raw mean differences, cortisol after 120 min and 180 min after HIIT decreased ~ −23% and ~ −36% (Figure S1 Online Resource).[20] No changes were found in cortisol after 24h after HIIT ($p = 0.890$), with a mean effect size of −0.02 (95% CI, −0.29 to 0.25) and a slight decrease of ~ −1% in line with the pooled raw mean differences (Figure S1 Online Resource).[20]

Heterogeneity varied across outcomes and studies (Table 2).

Raw data of each included study on secondary outcomes can be found in Table S6 (Online Resource).[20]

3.5 | Analyses of potential effect-size modifiers

Due to the significant heterogeneity found in some meta-analyses, we considered HIIT and participant characteristics of the studies, as well as the type of measurement (plasma or saliva), as potential modifiers of the variability found in effect sizes. Concretely, we analyzed statistically controlled whether the type of HIIT (i.e., repeated-sprint training, sprint interval training, HIIT short intervals, and HIIT long intervals) modulates the effect size on primary outcomes. Regarding participant's characteristics, we also considered fitness status (i.e., non-trained, recreationally active, trained, and professional or semi-professional athletes) and BMI as potential effect-size modifiers. Because individuals included in the present meta-analysis were mostly young adults, age was not incorporated in these analyses; merely significant results are mentioned in this section.

Concerning qualitative effect-size modifiers, HIIT long intervals produced a greater increase in cortisol at 0 min ($p < 0.001$) and after 30 min ($p = 0.047$) in pre-post intervention groups. The largest increase in cortisol at 0 min was detected in recreationally active and trained individuals in pre-post intervention groups ($p < 0.001$). Similarly, a greater increase in cortisol after 30 min was observed in recreationally active individuals and professional or semi-professional athletes in pre-post intervention groups ($p = 0.005$). Type of measurement (plasma or saliva) moderated the acute effects of HIIT on testosterone after 30 min in pre-post intervention groups ($p = 0.045$). Concretely, testosterone decreased in plasma samples, whereas it remained increased in saliva samples.

With regards to continuous effect-size modifiers, the acute effects of HIIT on testosterone after 30 and 60 min

TABLE 1 Main characteristics of studies included in the systematic review

Study reference	Country	Design	*N*	Sex	Age (SD)	Fitness level	BMI (kg/m^2)
Abedelmalek et al., 2013	Tunisia	Pre-post	13	Males	21.1 (1.25)	Trained	22.6
Bonato et al., 2017	Italy	Pre-post	23	Males	22.0 (4.2)	Trained	23.0
Broodryk et al., 2017	South Africa	Pre-post	47	Females	22.0 (2.7)	Athletes	22.2
Cofré-Bolados et al., 2019	Chile	Controlled study	13	Males	20.2 (2.1)	Active	25.1
Crewther et al., 2017	United Kingdom	Pre-post and Controlled study	65 15	Males	22.6 (4.9)	Active	24.3
Cui et al., 2015	China	Pre-post	18	Males	20.2 (1.0)	Active	22.4
Eryilmaz et al., 2019	Turkey	Pre-post	9	Males	23. 3(3.6)	Trained	23.3
Erylmaz et al., 2019	Turkey	Pre-post	12	Males	24.0 (3.5)	Trained	23.0
Esbjornsson et al., 2009	Sweden	Pre-post	18	Both	24.0 (7.2) and 21.0 (6.7)	Trained	24.0 and 22.9
Gravisse et al., 2018	France	Pre-post	11	Females	20.6 (1.7)	Active	22.2
Gray et al., 1993	Australia	Pre-post	8	Males	31.5 (4.5)	Trained	-
Hackney et al., 2012	USA	Controlled study	15	Males	27.2 (4.6)	Trained	23.0
Hackney et al., 1995	USA	Controlled study	9	Males	30.6 (3.8)	Trained	24.0
Hermann et al., 2018	Germany	Pre-post	32	Males	24.3 (3.4)	Mixed	23.6
Hoffman et al., 1997	Israel	Pre-post	8	Males	25.0 (3.0)	Active	23.2
Hough et al., 2015	United Kingdom	Pre-post	7	Males	19.0 (1.0)	Trained	22.1
Hough et al., 2013	United Kingdom	Controlled study	12	Males	25.0 (4.0)	Active	24.2
Hough et al., 2011	United Kingdom	Controlled study	10	Males	24.0 (3.0)	Active	23.7
Johnston et al., 2016	United Kingdom	Pre-post	15	Males	21.0 (1.0)	Athletes	29.4
Johnston et al., 2015	United Kingdom	Pre-post	18	Males	20.5 (1.2)	Athletes	28.7
Jurimae et al., 2004	Estonia	Pre-post	10	Males	College students	Trained	20.4
Kargotich et al., 1997	Australia	Pre-post	8	Males	19.9 (2.3)	Trained	23.6
Kilian et al., 2016	Germany	Pre-post	12	Males	14.4 (0.8)	Athletes	19.3
Kraemer et al., 2003	USA	Controlled study	7	Males	28.7 (7.7)	Trained	22.9
Kuoppasalmi et al., 1976	Finland	Pre-post and Controlled study	5	Males	22.0 (0.0)	Trained	-
Lee et al., 2014	Taiwan	Pre-post	12	Males	20.4 (1.1)	Active	23.4
Liu et al., 2013	Taiwan	Pre-post	16	Males	21.4 (0.3) and 49.3 (2.4)	Non-trained	-
Loures et al., 2019	Brazil	Pre-post	11	Both	15.0 (1.5)	Athletes	23.0
Macdonald et al., 2017	Australia	Pre-post	14	Males	32.0 (11.0)	Active	25.1
Meckel et al., 2011	Israel	Pre-post	12	Males	20.3 (3.5)	Athletes	23.1
Neek et al., 2011	Iran	Pre-post	8	Males	-	Athletes	21.1
Nemet et al., 2009	Israel	Pre-post	12	Males	20.3 (3.5)	Athletes	23.1
Paton et al., 2010	New Zealand	Pre-post	9	Males	24.1 (7.3)	Trained	23.9
Peake et al., 2014	Australia	Pre-post	10	Males	33.2 (6.7)	Trained	23.4
Pullinen et al., 2005	Finland	Pre-post	10	Males	24.0 (3.0)	Athletes	22.3
Rooijackers et al., 2017	Netherlands	Controlled study	10	Both	25.2 (5.5)	Active	22.5
Russell et al., 2020	United Kingdom	Pre-post	14	Males	18.0 (1.0)	Athletes	29.4
Russell et al., 2017	United Kingdom	Pre-post	14	Males	18.0 (2.0)	Athletes	23.5

Exercise modality	HIIT protocol	Time of HIIT	Hormone	Sample	Minute (s) of sample measurement	Diet control after HIIT
Running	Sprint interval training	8:00	TT and C	Plasma	0	N/A
Running	HIIT long intervals	8:00 and 20:00	C	Saliva	0, 15, 30, 45, and 60	AFI
Running	Sprint interval training	-	C	Saliva	15	N/A
Running	HIIT short intervals	-	TT	Plasma	0 and 720	SDR
Cycling	Repeated-sprint training	10:00–15:00	TT and C	Saliva	15	RFI
Cycling	Sprint interval training	10:00–11:30	TT and C	Plasma	0	N/A
Running	Repeated-sprint training	-	C	Plasma	0 and 1440	-
Running	Repeated-sprint training	-	C	Plasma	0 and 1440	-
Cycling	Sprint interval training	07:00–10:00	TT and C	Plasma	0, 9, and 18	N/A
Running	Repeated-sprint training	9:30–10:00	TT	Saliva	5	N/A
Running	HIIT short intervals	08:00–10:00	TT	Plasma	0, 60, 360, and 1440	-
Running	HIIT long intervals	18:00–19:01	FT and C	Plasma	0 and 720	RFI
Cycling	HIIT long intervals	07:00–08:00	TT and C	Plasma	0, 60, 120, 180, 240, 300, 360, 420, and 480	SM
Cycling	Sprint interval training	-	C	Plasma	0, 5, 10, 20, 30, 45, and 60	NFI
Cycling	Sprint interval training	-	TT and C	Plasma	0, 15, 30, 45, and 60	-
Cycling	HIIT long intervals	-	TT and C	Saliva	0 and 30	NFI
Cycling	HIIT long intervals	12:00–12:30	TT and C	Saliva	0 and 30	NFI
Cycling	HIIT short intervals	-	TT and C	Plasma	0, 10, 20, 30, 40, 50, and 60	NFI
Running	Repeated-sprint training	-	TT and C	Plasma	0, 120, and 1440	SDP
Running	Repeated-sprint training	-	TT and C	Plasma	0, 120, and 1440	SM
Running	Sprint interval training	10:00–12:00	TT and C	Plasma	0 and 30	-
Swimming	HIIT long intervals	05:00–06:00	TT and C	Plasma	0, 30, 60, and 120	NFI
Cycling	HIIT long intervals	23:00–01:00	TT and C	Saliva	0, 30, 60, and 180	SM
Running	HIIT long intervals	09:10–09:40	TT	Plasma	0,15, 30, 45, and 60	-
Running	Sprint interval training	11:00–12:00	TT and C	Plasma	0, 30, 60, 180, and 360	-
Cycling	Repeated-sprint training	-	TT and C	Plasma	0	N/A
Cycling	HIIT long intervals	09:00–09:15	TT, FT, and C	Plasma	0, 15, and 1440	-
Swimming	HIIT long intervals	-	C	Plasma	0 and 1440	-
Cycling	Sprint interval training	-	C	Saliva	15	N/A
Running	Sprint interval training	-	TT and C	Plasma	0 and 60	-
Cycling	HIIT long intervals	-	TT and FT	Plasma	0	N/A
Running	Sprint interval training	-	TT and C	Plasma	0 and 60	-
Cycling	Sprint interval training	-	TT and C	Saliva	0	N/A
Cycling	HIIT long intervals	07:00–8:00	C	Plasma	0, 60, and 120	NFI
Running	Repeated-sprint training	-	TT, FT, and C	Plasma	0	N/A
Cycling	Sprint interval training	-	C	Plasma	5	N/A
Running	Repeated-sprint training	-	TT and C	Saliva	0	N/A
Running	Repeated-sprint training	-	TT and C	Saliva	0, 120, and 1440	SM

(Continues)

TABLE 1 (Continued)

Study reference	Country	Design	N	Sex	Age (SD)	Fitness level	BMI (kg/m^2)
Russell et al., 2016	France	Controlled study	15	Males	24.0 (3.0)	Athletes	28.4
Suay et al., 1999	Spain	Controlled study	26	Males	18.0 (0.0)	Athletes	23.0
Tacey et al., 2019	Australia	Pre-post	9	Males	27.8 (5.1)	Active	24.4
Tanner et al., 2014	United Kingdom	Controlled study	10	Males	39.3 (6.6)	Trained	24.2
Thomas et al., 2010	United Kingdom	Pre-post	19	Females	15.5 (0.6)	Active	20.1
Thomas et al., 2009	United Kingdom	Pre-post	17	Males	15.5 (0.4)	Active	22.0
Velasco-Orjuela et al., 2018	Colombia	Controlled study	26	Males	24.6 (3.6)	Non-trained	28.1
Venckunas et al., 2019	Lithuania	Pre-post	31	Males	22.3 (4.6), 69.9 (6.3), and 26.4 (9.4)	Non-trained and trained	26.4, 25.0, and 24.0
Vitale et al., 2019	Italy	Pre-post	15	Males	18.3 (1.0)	Athletes	20.4
Vuorimaa et al., 2008	Finland	Pre-post	20	Males	24.6 (0.0)	Trained	-
Vuorimaa et al., 1999	Finland	Pre-post	10	Males	22.0 (3.0)	Trained	21.0
Wahl et al., 2013	Germany	Pre-post	12	Males	24.7 (3.4)	Trained	22.9
Wahl et al., 2010	Germany	Pre-post	11	Males	26.5 (5.6)	Active	23.0
Williams et al., 2018	United Kingdom	Pre-post	24	Males	21.8 (3.0)	Athletes	28.3
Zinner et al., 2014	Germany	Pre-post	13	Males	15.8 (1.8)	Trained	19.5

Abbreviations: AFI, subjects were asked to avoid food intake during the sampling period; C, cortisol; FT, free testosterone; HIIT, high-intensity interval training; N/A, not applicable; NFI, no food intake was allowed during the sampling period; RFI, subjects were asked to replicate food intake on each day of testing; SDP, standardized diet was provided; SDR, standardized diet was recommended; SM, standardized meal was consumed at a set time; TT, total testosterone.

were higher in those individuals with higher BMI in pre-post intervention groups ($p = 0.006$ and $p = 0.009$, respectively).

3.6 | Sensitivity analysis and assessment of the risk of bias

Sensitivity analysis revealed that only two studies[45,46] influenced the pooled effect size of HIIT on testosterone after 0 and 30 min in controlled studies. Yet, these differences in the pooled effect size were not statistically significant and both studies were methodologically correct, thus they were included in the total effect-size calculation. Conversely, two articles[38,46] affected considerably the pooled effect size for cortisol at 0 and 30 min in controlled studies, hence they were excluded from the total effect size calculation.

Egger's test showed publication bias in testosterone at 0 and 60 min in pre-post intervention groups ($p < 0.001$ and $p = 0.001$, respectively). However, Rosenthal method (fail-safe N) revealed that 1679 and 91 additional studies, respectively, with null results would be needed to increase the p value for the meta-analysis to above an alpha level of 0.05. Egger's test also indicated publication bias in cortisol at 0, 30, and 60 min in pre-post intervention groups ($p < 0.001$, $p < 0.001$, and $p = 0.002$, respectively); nonetheless, the fail-safe N was relatively large (1214, 210 and 32, respectively).

The analyses of methodological quality as a potential effect-size modifier showed that higher methodological quality of studies resulted in lower increases in cortisol at 0 min in pre-post intervention groups ($p = 0.002$).

4 | DISCUSSION

This systematic review and meta-analysis synthesizes the acute effect of HIIT on testosterone and cortisol levels, including both plasma and saliva samples, in healthy youth and adults, from non-trained to professional athletes. The findings indicate that testosterone increases immediately after a single HIIT session, returns to baseline levels between 15–30 min, drops below baseline levels between 60–180 min, and returns to baseline levels again after 24h. HIIT-induced cortisol acute elevations may last longer, since cortisol increases between 0–60 min, drops below baseline levels between 120–180 min, and returns to baseline levels after 24 h. In addition, HIIT long intervals (≥60 s) seem to be the HIIT modality, which produces a greater increase in cortisol. This

Exercise modality	HIIT protocol	Time of HIIT	Hormone	Sample	Minute (s) of sample measurement	Diet control after HIIT
Cycling and running	Repeated-sprint training	-	TT	Saliva	300	AFI
Cycling	HIIT short intervals	09:00–13:30	TT and C	Plasma	10	N/A
Cycling	HIIT long intervals	-	TT	Plasma	0, 60, and 180	-
Running	HIIT long intervals	15:00–18:00	TT and C	Saliva	0, 15, 30, and 60	NFI
Cycling	Repeated-sprint training	-	TT and C	Saliva	5	N/A
Cycling	Repeated-sprint training	-	TT and C	Saliva	5	N/A
Running	HIIT long intervals	6:00–9:00	TT, FT, and C	Plasma	0	N/A
Cycling	Repeated-sprint training, sprint interval training and HIIT long intervals	-	TT	Plasma	5 and 60	-
Running	Sprint interval training	17:00–17:30	C	Plasma	15	N/A
Running	HIIT long intervals	09:00–09:40	TT and C	Plasma	0, 10, and 90	-
Running	HIIT short intervals	09:00–09:30	TT and C	Plasma	120 and 1440	-
Cycling	Sprint interval training and HIIT long intervals	08:00–08:45	TT and C	Plasma	0, 30, 60, and 180	SM
Cycling	Sprint interval training	-	C	Plasma	10, 60, and 240	-
Running	Repeated-sprint training	-	TT and C	Saliva	0	N/A
Cycling	HIIT long intervals	15:00–17:00	TT and C	Plasma	0, 30, and 60	-

meta-analysis provides a better understanding of the endocrine response to a single HIIT session, which could certainly be highly valuable for the exercise prescription for both clinicians and coaches.

The acute increase in testosterone levels immediately following a non-exhaustive high-intensity exercise bout is a well-known phenomenon[2,4-6] and concurs with our results (i.e., ~28% and ~15% in controlled studies and pre-post intervention groups, respectively). According to the available evidence, acute exercise may increase, decrease, or fail to change plasma luteinizing hormone concentrations.[5,12] Furthermore, those studies that have shown an increase in luteinizing hormone levels in response to acute exercise have also observed that testosterone levels increase more rapidly than luteinizing hormone.[12] Therefore, the acute rise in testosterone levels immediately following a single HIIT session seems not to be mediated by luteinizing hormone.[4,5,12,87] In men, HIIT may increase testosterone production from the testis by direct (luteinizing hormone independent) stimulatory mechanisms,[5] such as sympathetic stimulation of the testis[88] and lactate-stimulated secretion via increases in testicular cAMP production.[89] Acute elevations of testosterone levels in response to a single HIIT session could also be explained by a reduction in plasma volume, hepatic clearance, and degradation rates.[5,62] Following this increase, it has been reported that testosterone typically returns to baseline levels within 15–30 min and, subsequently, drops below them.[2,4] Our results exactly follow the same pattern, since testosterone returns to baseline levels after 15 and 30 min and drops below baseline levels after 60 (~ −8%), 120 (~ −12%), and 180 min (~ −10%). It has been proposed that this combination of responses may represent the transition of testosterone from the blood to the skeletal muscle to execute its androgenic-anabolic effects.[4] Specifically, testosterone promotes protein synthesis (anabolic effect) and suppresses protein degradation (anti-catabolic effects) leading to skeletal muscle hypertrophy and, consequently, increasing muscle strength.[2,4] These androgenic-anabolic effects may occur through two different pathways: genomic and non-genomic androgen action. In the genomic androgen action, only free testosterone diffuses through the membrane into the cell cytoplasm to bind the intracellular androgen receptor which increases expression of the target genes and inducing protein synthesis. This process is known as "slow action" of testosterone due to the larger time required to observe a measurable response (from half an hour to hours or days).[4] Conversely, in non-genomic androgen action, bound testosterone can bind to a membrane receptor that triggers intracellular signaling cascades resulting in measurable biological response within seconds.[4,90] It appears that the transition of testosterone from the blood to the skeletal muscle may be related to the non-genomic androgen action and, although its not completely required, may contribute to muscle hypertrophy and greater muscle strength.[2,4] Currently, although testosterone supplementation has shown a wide range of benefits such as an increase in muscle hypertrophy and muscle strength, endurance and power performance, sexual function, bone mineral density, and decrease in fat mass,[91] the biological roles of the acute increase in testosterone levels in response to exercise remain somewhat uncertain.[4]

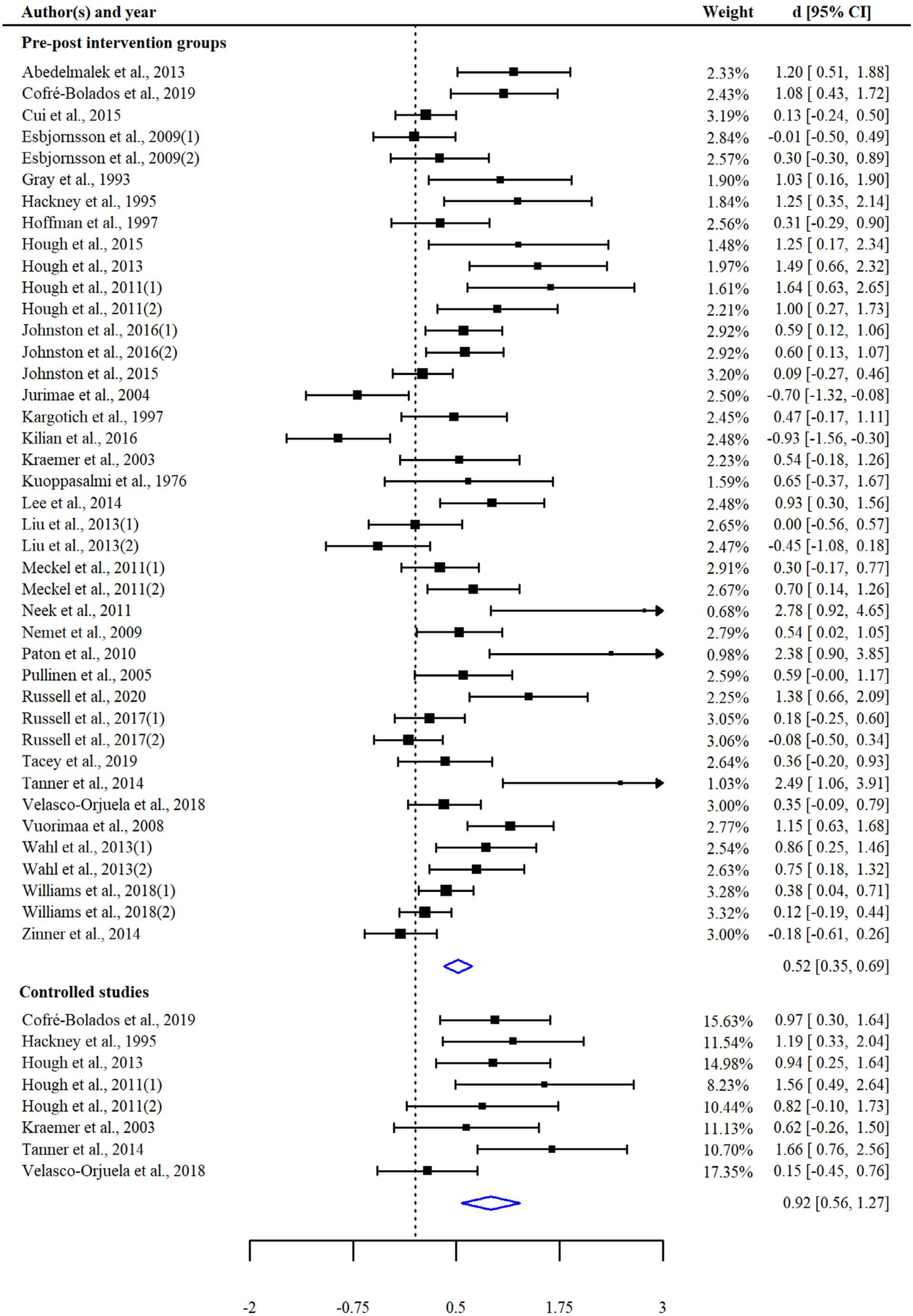
Similarly, there is mounting evidence showing an acute increase in cortisol levels following a single session of both short-term high-intensity exercise or prolonged moderate-intensity exercise,[3,5,6] which is in line with our results (i.e., ~82% and ~28% in controlled studies and pre-post intervention groups, respectively). Stress stimulus (e.g., exercise) activates the hypothalamic-pituitary-adrenal axis, which results in the synthesis of cortisol.[87,92] Similar to testosterone, acute rise in cortisol levels can also be the consequence of the above-mentioned factors (i.e., a reduction in plasma volume, hepatic clearance, and degradation rates).[5,62] Nonetheless, these non-specific mechanisms may not completely explain the huge increase in cortisol levels observed in response to a single HIIT session. For instance, other studies have shown that cortisol concentrations still remain elevated following HIIT and resistance exercise even after adjusting for plasma volume changes.[62,93] Cortisol concentrations linearly increase with exercise intensity,[3] hence a longer time is needed for cortisol to return to baseline values after high-intensity exercises, such as HIIT. This could explain that cortisol levels remain elevated 60 min after a single HIIT bouts (i.e., ~51% and ~18% in controlled studies and pre-post intervention groups, respectively). However, it appears that, independently of exercise duration and intensity, cortisol levels return to baseline values, or even drop below, 120–150 min after a HIIT bout.[3,94] Our results further support these findings, since we observed that cortisol decreased below baseline levels after 120 and 180 min (i.e., ~ −23% and ~ −36%, respectively), although the mechanism(s) inducing this decrease has not been fully elucidated yet.[3,94] Cortisol plays several roles in coping with metabolic stress caused by exercise: (i) it increases activeness and alertness;[95] (ii) it suppresses immune function[3] and may even increase the risk of upper respiratory tract infections after prolonged high-intensity exercise;[96] (iii) it furthers energy substrate mobilization (i.e., carbohydrate, fat, and protein) and hence inhibits muscle protein synthesis;[3] and (iv) it may also influence neuromuscular function (e.g., neuronal activity and muscle force) through various short-term mechanisms.[97] Lastly, testosterone and cortisol return to baseline levels at 24 h after HIIT, indicating that 24 h may be the enough time to recover from a single session of HIIT. However, other biomarkers should be also considered to determine between-sessions recovery.[13] Moreover, a greater recovery period may be needed after several HIIT sessions (chronic effect).

Regarding potential modifiers of the effect of HIIT in these hormones:

HIIT long intervals (≥60 s at 90–100% minimal running speed associated with VO_{2max} or maximal lactate steady state) appear to produce a greater increase in cortisol at 0 min and after 30 min. These findings seem plausible since exercise intensity and duration are the two major factors that modulate the cortisol response to exercise.[3] Therefore, although repeated-sprint training and sprint interval training are high-intensity exercises, they may not be long enough to induce a robust increase in cortisol levels.

It has been documented that exercise-induced cortisol secretion is independent of fitness status.[3] When exercise is performed at similar relative intensity, the exercise intensity and the duration needed to increase cortisol levels are similar between non-trained and trained individuals.[3] Nonetheless, it is also accepted that endurance athletes develop a reduced cortisol sensitivity to protect muscle tissue and other cortisol-sensitive tissues against the increased cortisol secreted during and after exercise.[3,98] Indeed, the response to cortisol increments is regulated not only by its own concentration but also by the sensitivity of the target tissue.[3,98] These adaptations may explain the capacity of endurance athletes to achieve effectively a second exercise session separated by a short recovery period.[3] We observed the largest increase in cortisol in recreationally active and trained individuals at 0 min while higher increments were noted in recreationally active individuals and professional or semi-professional athletes after 30 min. This could be due to the fact that non-trained individuals are less likely to achieve and maintain the exercise intensity prescribed, particularly when it is high-intensity, such as HIIT. This raises a debate about the effectiveness of HIIT in non-trained individuals. Some studies indicate that HIIT has low implementation and maintenance due to the psychologically aversive nature of HIIT,[99] whereas a scoping review has shown that enjoyment of, and preferences for HIIT are equal or greater than those obtained by moderate-intensity continuous training.[100] Similarly, a previous meta-analysis has indicated that HIIT is a tolerable and acceptable intervention for non-trained individuals, presenting usually lower dropout rates than commonly reported for traditional exercise programs.[101]

FIGURE 2 Forest plot of the standardized mean differences (d) for testosterone at 0 min, grouped by pre-post intervention groups and controlled trials. A negative value means a reduction of the outcome after high-intensity interval training, whereas a positive value means an increase of the outcome after high-intensity interval training. Abbreviations: CI, confidence interval


Author(s) and year
Weight
d [95% CI]
Pre-post intervention groups
Abedelmalek et al., 2013 2.33% 1.20 [0.51, 1.88]
Cofré-Bolados et al., 2019 2.43% 1.08 [0.43, 1.72]
Cui et al., 2015 3.19% 0.13 [-0.24, 0.50]
Esbjornsson et al., 2009(1) 2.84% -0.01 [-0.50, 0.49]
Esbjornsson et al., 2009(2) 2.57% 0.30 [-0.30, 0.89]
Gray et al., 1993 1.90% 1.03 [0.16, 1.90]
Hackney et al., 1995 1.84% 1.25 [0.35, 2.14]
Hoffman et al., 1997 2.56% 0.31 [-0.29, 0.90]
Hough et al., 2015 1.48% 1.25 [0.17, 2.34]
Hough et al., 2013 1.97% 1.49 [0.66, 2.32]
Hough et al., 2011(1) 1.61% 1.64 [0.63, 2.65]
Hough et al., 2011(2) 2.21% 1.00 [0.27, 1.73]
Johnston et al., 2016(1) 2.92% 0.59 [0.12, 1.06]
Johnston et al., 2016(2) 2.92% 0.60 [0.13, 1.07]
Johnston et al., 2015 3.20% 0.09 [-0.27, 0.46]
Jurimae et al., 2004 2.50% -0.70 [-1.32, -0.08]
Kargotich et al., 1997 2.45% 0.47 [-0.17, 1.11]
Kilian et al., 2016 2.48% -0.93 [-1.56, -0.30]
Kraemer et al., 2003 2.23% 0.54 [-0.18, 1.26]
Kuoppasalmi et al., 1976 1.59% 0.65 [-0.37, 1.67]
Lee et al., 2014 2.48% 0.93 [0.30, 1.56]
Liu et al., 2013(1) 2.65% 0.00 [-0.56, 0.57]
Liu et al., 2013(2) 2.47% -0.45 [-1.08, 0.18]
Meckel et al., 2011(1) 2.91% 0.30 [-0.17, 0.77]
Meckel et al., 2011(2) 2.67% 0.70 [0.14, 1.26]
Neek et al., 2011 0.68% 2.78 [0.92, 4.65]
Nemet et al., 2009 2.79% 0.54 [0.02, 1.05]
Paton et al., 2010 0.98% 2.38 [0.90, 3.85]
Pullinen et al., 2005 2.59% 0.59 [-0.00, 1.17]
Russell et al., 2020 2.25% 1.38 [0.66, 2.09]
Russell et al., 2017(1) 3.05% 0.18 [-0.25, 0.60]
Russell et al., 2017(2) 3.06% -0.08 [-0.50, 0.34]
Tacey et al., 2019 2.64% 0.36 [-0.20, 0.93]
Tanner et al., 2014 1.03% 2.49 [1.06, 3.91]
Velasco-Orjuela et al., 2018 3.00% 0.35 [-0.09, 0.79]
Vuorimaa et al., 2008 2.77% 1.15 [0.63, 1.68]
Wahl et al., 2013(1) 2.54% 0.86 [0.25, 1.46]
Wahl et al., 2013(2) 2.63% 0.75 [0.18, 1.32]
Williams et al., 2018(1) 3.28% 0.38 [0.04, 0.71]
Williams et al., 2018(2) 3.32% 0.12 [-0.19, 0.44]
Zinner et al., 2014 3.00% -0.18 [-0.61, 0.26]
0.52 [0.35, 0.69]
Controlled studies
Cofré-Bolados et al., 2019 15.63% 0.97 [0.30, 1.64]
Hackney et al., 1995 11.54% 1.19 [0.33, 2.04]
Hough et al., 2013 14.98% 0.94 [0.25, 1.64]
Hough et al., 2011(1) 8.23% 1.56 [0.49, 2.64]
Hough et al., 2011(2) 10.44% 0.82 [-0.10, 1.73]
Kraemer et al., 2003 11.13% 0.62 [-0.26, 1.50]
Tanner et al., 2014 10.70% 1.66 [0.76, 2.56]
Velasco-Orjuela et al., 2018 17.35% 0.15 [-0.45, 0.76]
0.92 [0.56, 1.27]
-2
-0.75
0.5
1.75
3

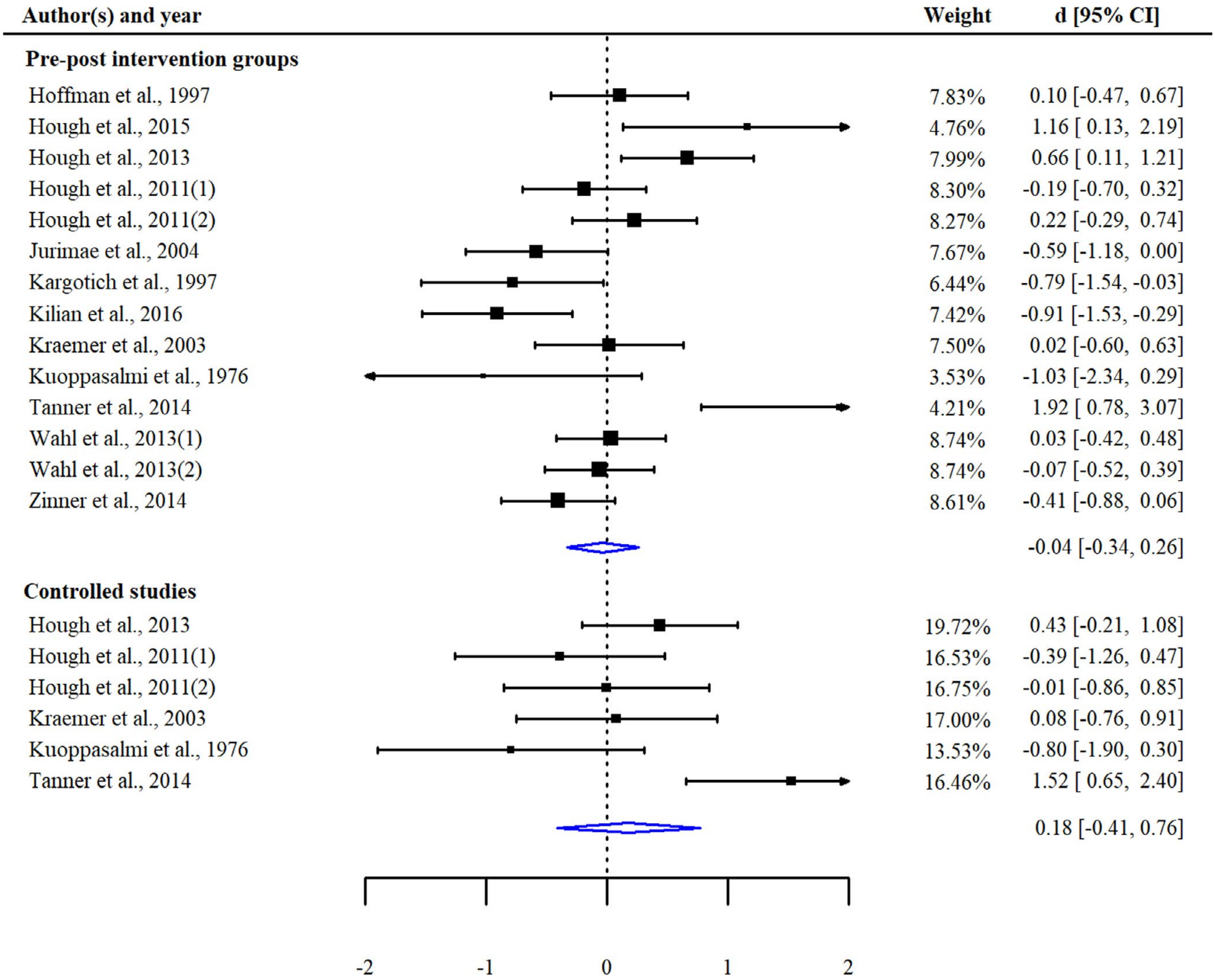


FIGURE 3 Forest plot of the standardized mean differences (d) for testosterone at 30 min, grouped by pre-post intervention groups and controlled trials. A negative value means a reduction of the outcome after high-intensity interval training, whereas a positive value means an increase of the outcome after high-intensity interval training. Abbreviations: CI, confidence interval

Type of measurement (i.e., plasma or saliva) only moderate the acute effects of HIIT on testosterone after 30 min Specifically, testosterone starts to decrease below baseline values in plasma samples, whereas it remains increased in saliva samples. This difference is reasonable because it takes some time for hormones to diffuse into saliva, hence salivary testosterone levels are likely to occur later than in plasma.[39] Our findings indicate that similar results are obtained with plasma and saliva samples, thus both methods are appropriate to assess the hormone response to HIIT. Future studies should choose plasma or saliva samples according to the research aims. Saliva sampling is a rapid and noninvasive method that can be used in large sample sizes and in the playing field, whereas plasma sampling is considered the reference method to assess hormone concentrations. Regarding the question of whether the total or free hormone levels should be measured, clinicians/researchers should also decide it according to the research aims and resources. The "free hormone hypothesis" postulates that only the free or unbound hormone in the circulation is biologically active;[102] conversely, recent evidence has suggested that bound hormone can also exert biologic effects (non-genomic androgen action).[4] The major obstacle is commonly practical since the free hormone assessment is costly and not routinely available.[103,104] Hence, free hormone concentrations are usually calculated using data for association constants between the hormone and its binding protein, although it is also important to know that results may vary depending on the equation used.[103,104]

Regarding continuous effect-size modifiers, greater increases on testosterone after 30 and 60 min were noted in those individuals with higher BMI. Taking into account that most of the studies were conducted on trained individuals and on professional or semi-professional athletes, it seems

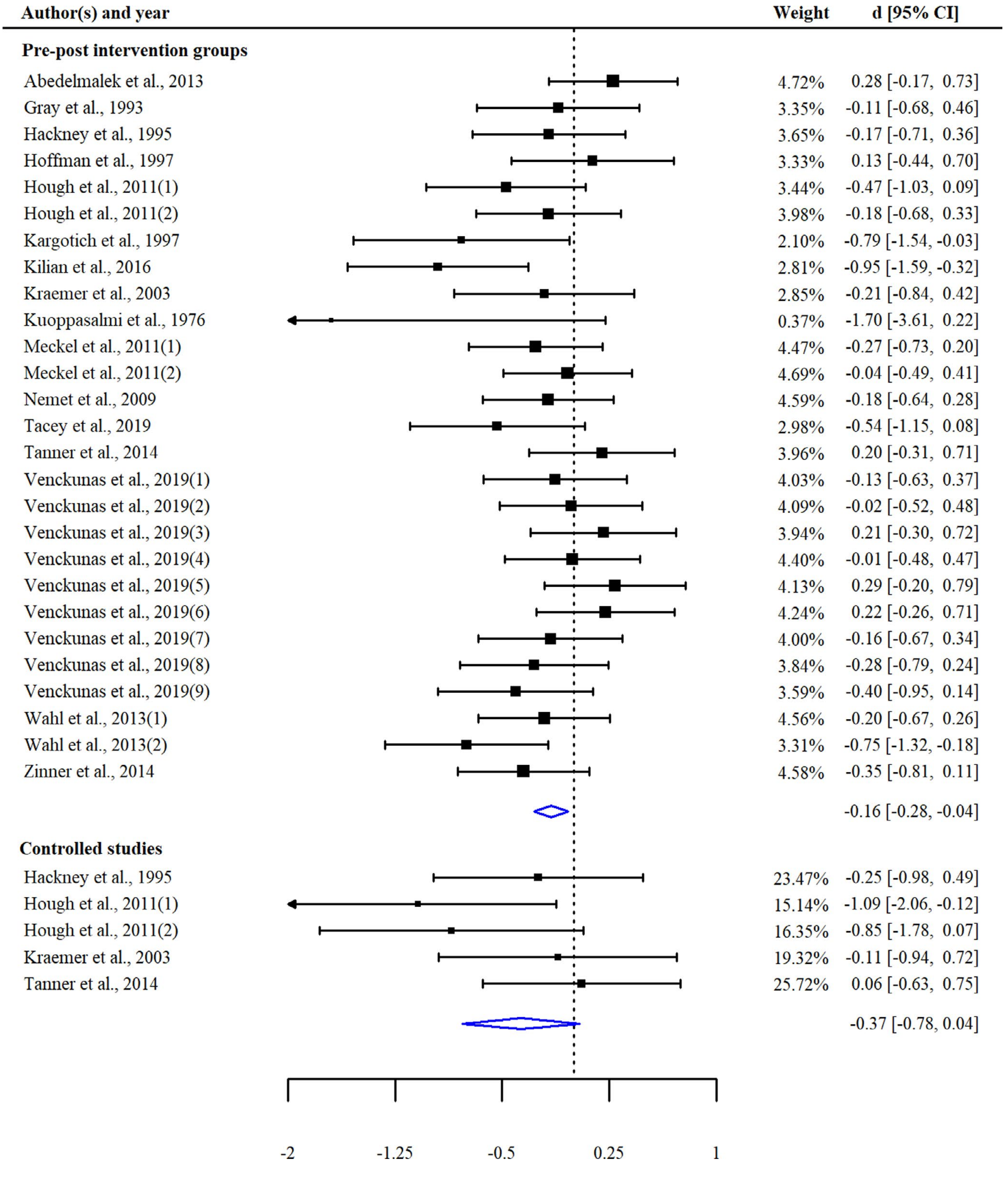


FIGURE 4 Forest plot of the standardized mean differences (d) for testosterone at 60 min, grouped by pre-post intervention groups and controlled trials. A negative value means a reduction of the outcome after high-intensity interval training, whereas a positive value means an increase of the outcome after high-intensity interval training. Abbreviations: CI, confidence interval

plausible that those individuals with higher muscle mass were those individuals with higher BMI. In the same vein, adolescent weightlifters with more than two years of training experience have been shown to produce a greater increase in testosterone levels in response to exercise than those with less than 2 years of experience.[105]

Another potential modifier of the effect sizes could be the nutritional status of participants, including energy and

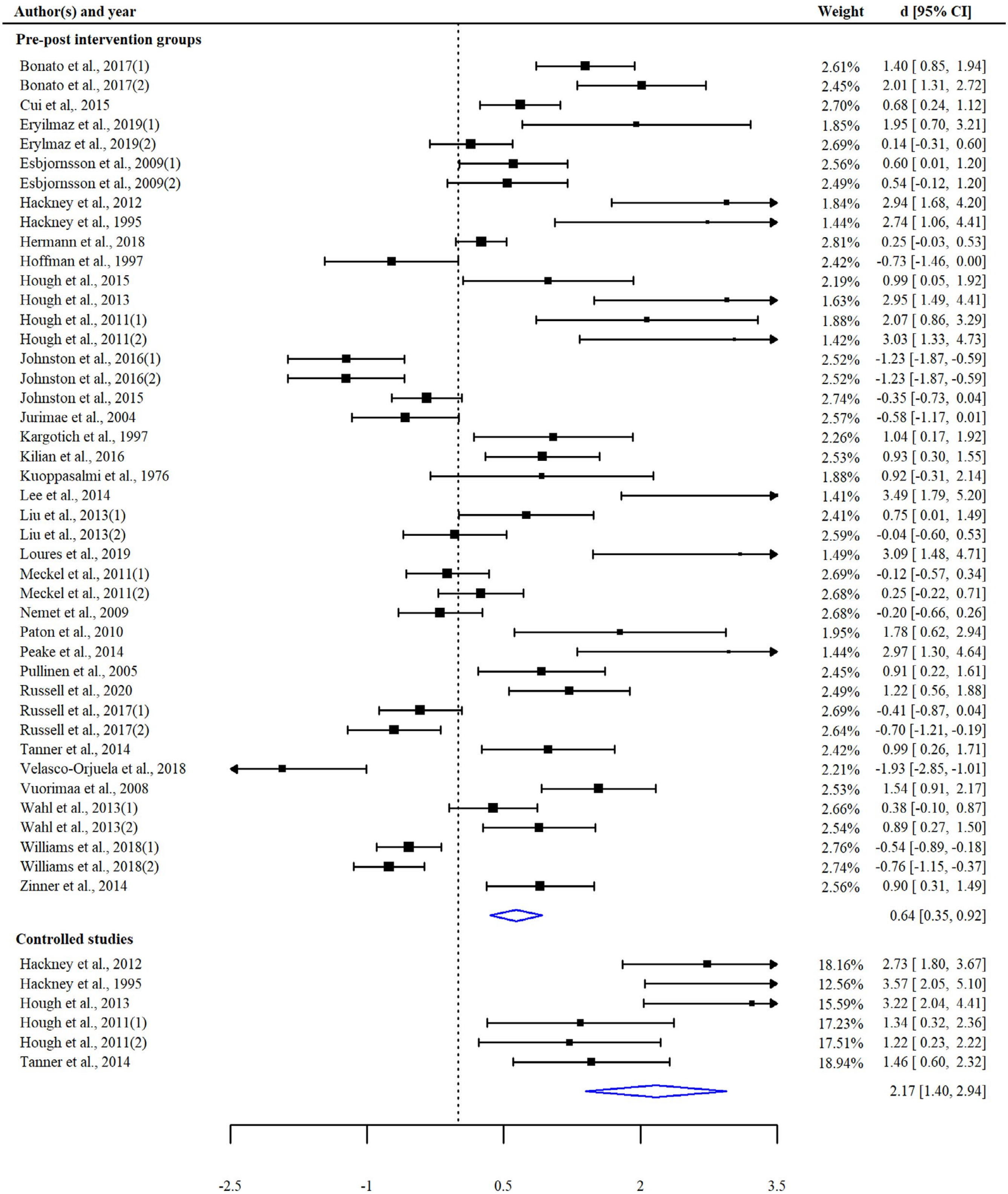


FIGURE 5 Forest plot of the standardized mean differences (d) for cortisol at 0 min, grouped by pre-post intervention groups and controlled trials. A negative value means a reduction of the outcome after high-intensity interval training, whereas a positive value means an increase of the outcome after high-intensity interval training. Abbreviations: CI, confidence interval

macronutrient intake, and meal timing prior to and following exercise, since these factors greatly impact testosterone and cortisol levels.[106] Diet control implemented in the included studies in this meta-analysis varies considerably, partly due to study designs, thus we were not able to investigate the moderator effect. In future research, it is necessary

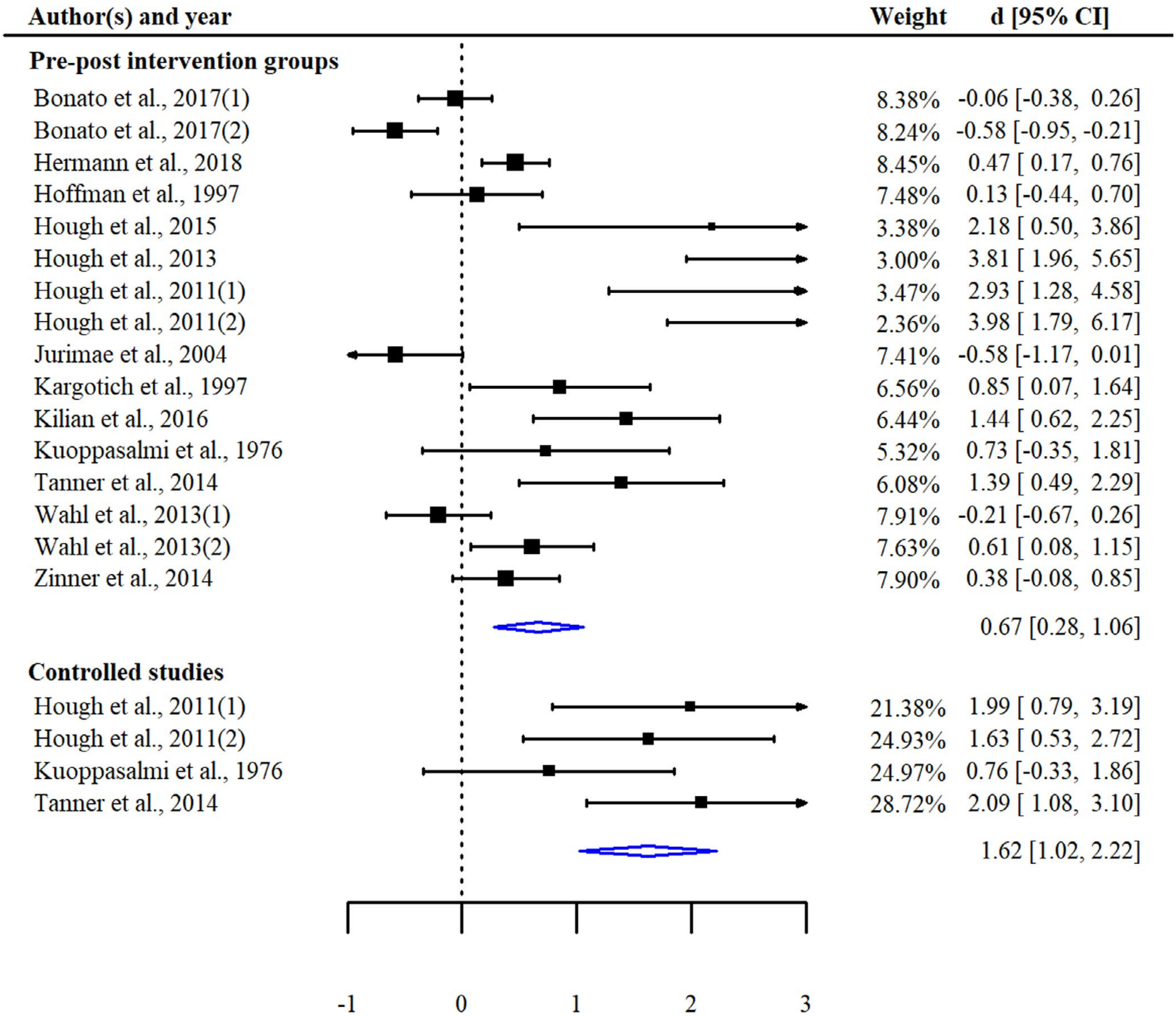


FIGURE 6 Forest plot of the standardized mean differences (d) for cortisol at 30 min, grouped by pre-post intervention groups and controlled trials. A negative value means a reduction of the outcome after high-intensity interval training, whereas a positive value means an increase of the outcome after high-intensity interval training. Abbreviations: CI, confidence interval

to standardize diet as much as possible to mitigate the influence of different nutritional status between and within individuals.[106]

4.1 | Strengths and limitations

Our findings should be interpreted with caution because they are limited to the data obtained from the included studies. Firstly, there were a reduced number of controlled studies available; several outcomes did not have the required number of studies for desirable statistical power. Therefore, we included uncontrolled pre-post intervention groups, although these could influence the effect sizes of HIIT due to the effects of uncontrolled variables. Secondly, high heterogeneity was found across included studies in respect to some HIIT and participant characteristics and diet control. Lastly, due to the fact that only 9.1% of the total sample was composed of women, the results are representative of healthy youth and adult men; they might not, therefore, be extra polatable to women or individuals with acute or chronic diseases. Despite the limitations, several strengths also need to be mentioned. This study provides the first comprehensive picture of the effects of HIIT on testosterone and cortisol levels. Moreover, we investigated those HIIT components and participant's characteristics, which may drive the greatest responses in testosterone and cortisol. This data may be valuable for both physicians and trainers in exercise prescription.

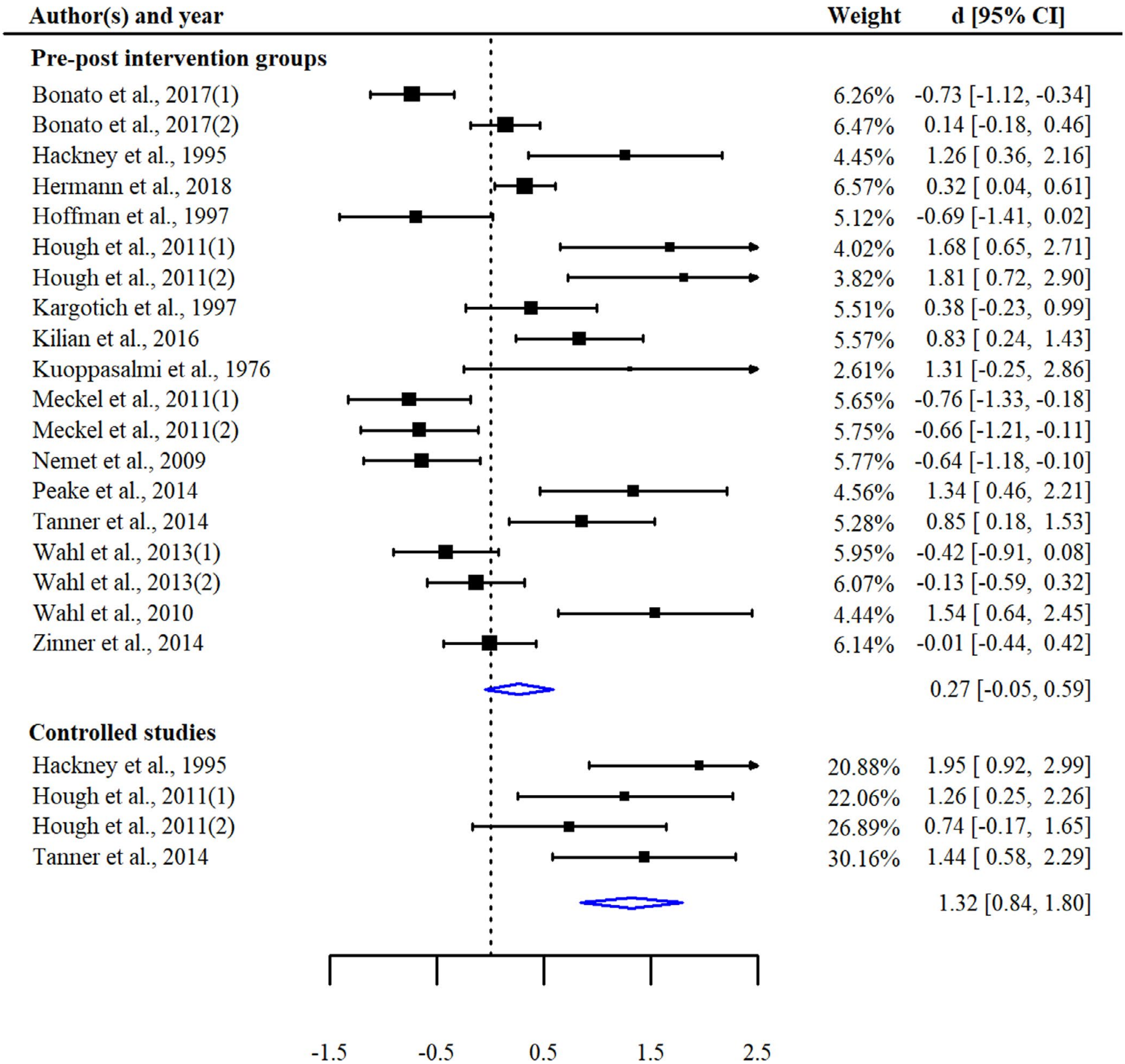


FIGURE 7 Forest plot of the standardized mean differences (d) for cortisol at 60 min, grouped by pre-post intervention groups and controlled trials. A negative value means a reduction of the outcome after high-intensity interval training, whereas a positive value means an increase of the outcome after high-intensity interval training. Abbreviations: CI, confidence interval

5 | CONCLUSIONS

In conclusion, the present results reveal that following a HIIT bout, testosterone increases immediately after, returns to baseline levels between 15–30 min, and drops below baseline levels between 60–180 min. HIIT-induced cortisol acute elevations may last longer, since cortisol increases between 0–60 min and drops below baseline levels between 120–180 min. Both hormones return to baseline levels after 24h, indicating that it may be the enough time to recover from a single session of HIIT. Furthermore, HIIT long intervals (≥60 s) may be the type of HIIT producing a greater increase in cortisol.

6 | PERSPECTIVE

Recently, HIIT has become a focal point on research and clinical practice, being applied worldwide; yet, its acute effect on testosterone and cortisol levels was still unclear. The beneficial chronic adaptations triggered by HIIT are the result of several acute bouts of HIIT; hence investigating the effects of a single HIIT session is essential for understanding not only its acute physiological responses, but also the long-term effects of HIIT. Aimed at closing this knowledge gap, this systematic review and meta-analysis provides a better understanding of the endocrine response to a single HIIT session,

TABLE 2 Pooled standardized mean differences (d) and heterogeneity for secondary testosterone and cortisol outcomes in pre-post intervention groups

Outcome	k	Participants (% women)	d (95% CI)[a]	Z	P	Q	P	I^2
Testosterone after 15 min	7	137 (0%)	0.14 (−0.34 to 0.62)	0.58	0.559	28.63	**<0.001**	79.05%
Testosterone after 120 min	8	99 (0%)	−0.48 (−0.70 to −0.27)	−4.36	**<0.001**	9.82	0.199	28.76%
Testosterone after 180 min	6	59 (0%)	−0.29 (−0.51 to −0.06)	−2.53	**0.011**	4.46	0.484	0.00%
Testosterone after 1440 min	8	107 (0%)	−0.15 (−0.42 to 0.12)	−1.11	0.267	14.57	**0.041**	51.97%
Free testosterone at 0 min	6	93 (0%)	0.43 (−0.17 to 1.03)	1.40	0.160	25.96	**<0.001**	80.74%
Cortisol after 15 min	13	281 (16.7%)	1.63 (0.97 to 2.29)	4.86	**<0.001**	181.76	**<0.001**	93.40%
Cortisol after 120 min	9	109 (0%)	−0.95 (−1.45 to −0.45)	−3.71	**<0.001**	42.17	**<0.001**	81.03%
Cortisol after 180 min	5	50 (0%)	−1.08 (−1.90 to −0.26)	−2.60	**0.009**	13.87	**0.007**	71.16%
Cortisol after 1440 min	10	131 (4.6%)	−0.02 (−0.29 to 0.25)	−0.14	0.890	24.38	**0.004**	63.09%

Bold values indicates statistically significant.

Abbreviations: CI, confidence interval.

[a]Negative value means a reduction of the outcome after acute high-intensity interval training, whereas a positive value means an increase of the outcome after high-intensity interval training.

which could be useful for both clinicians and coaches in the prescription of exercise programs to enhance health and performance. For instance, testosterone and cortisol may be used as sensitive biomarkers to monitor the anabolic and catabolic response to HIIT in order to detect potential disorders before observing clinical symptoms (e.g., overtraining, anxiety, depression).[12,13] Future well-designed randomized controlled trials with larger sample size, diet control, adjusting for plasma volume changes after HIIT, as well as including women as participants, controlling for the menstrual cycle phase, are necessary to confirm these findings.

CONFLICT OF INTEREST

None of the authors have any conflict of interests.

AUTHORS' CONTRIBUTIONS

Manuel Dote-Montero: Conceptualization, Methodology, Formal analysis, Writing - Original Draft. Almudena Carneiro-Barrera: Methodology, Formal analysis, Writing - Review & Editing. Vicente Martinez-Vizcaino: Writing - Review & Editing. Jonatan R. Ruiz: Writing - Review & Editing. Francisco J. Amaro-Gahete: Conceptualization, Methodology, Writing - Review & Editing.

DATA AVAILABILITY STATEMENT

The authors declare that data supporting the findings of this study are available within the article and its supplementary information files.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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