acute responses of Comprehensive gonadosteroids and corticosteroids to resistance exercise before and after 10 weeks of supervised strength training

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NEW FINDINGS

What is the central question of this study?

While acute responses of the principal gonadosteroid and corticosteroid hormones to resistance exercise is well documented, there is no information regarding how the key smaller-concentration intermediary hormones respond and potentially influence these hormonal pathways.

What is the main finding and its importance?

This study provides evidence for cascading conversions of some gonadosteroids and the data suggest that testosterone concentration increases independent to these hormones. These findings challenge future studies to determine the exact physiological roles of the smaller-concentration gonadosteroid and corticosteroids during and immediately following resistance exercise.

ABSTRACT

Resistance training is a potent stimulus for muscle growth and steroid hormones are known to play a role in this adaptation. However, very little is known about the acute exercise-induced gonadosteroid and corticosteroid hormone responses, including key smaller-concentration intermediate hormones. The present study determined the acute responses of these steroid hormone families using quantitative UHPLC mass spectrometry (MS) following resistance exercise in strength-trained men. Venous and fingertip blood was drawn pre-, mid-, 5 min post- and 15 min post-resistance exercise, both before and after 10 weeks of supervised resistance training. The experimental resistance exercise sessions consisted of 3 sets of 10 repetitions in the bilateral leg press and 3 sets of 10 repetitions in the unilateral knee extension with 2 and 1 min recovery between sets, respectively. Statistically significant ($P<0.05$) increases in the concentration of hormones in the gonadosteroid (including; dehydroepiandrosterone (DHEA), androstenedione, testosterone, estrone) and the corticosteroid (including; cortisol, corticosterone and cortisone) families were demonstrated after both experimental resistance exercise sessions, irrespective of training status. Correlation analyses revealed relationships

between: 1) DHEA and androstenedione, 2) DHEA and cortisol, 3) androstenedione and estrone, and 4) 11-deoxycortisol and cortisol. Testosterone appears to acutely increase independently of other intermediary hormones following resistance exercise. In conclusion, smaller-concentration intermediary gonadosteroids (e.g. estrone) and corticosteroids (e.g. corticosterone) respond robustly to resistance exercise in strength-trained men, although it seems that testosterone concentrations are regulated by factors other than the availability of precursor hormones and changes in plasma volume.

KEYWORDS: testosterone, cortisol, androstenedione, DHEA, 11-deoxycortisol, estrogen, UHPLC mass spectroscopy

INTRODUCTION

Resistance training is a potent stimulus for muscular strength and mass enhancements (Häkkinen et al. 2000; Narici et al. 1989; O'Shea 1966). The resulting adaptations are regulated, in part, by the signaling actions of the steroid hormone families (Bhasin et al. 1996). Multiple families of steroid hormones exist, including the gonadosteroids (androgens, estrogens and progestagens) and corticosteroids (glucocorticoids and mineralocorticoids). Each steroid hormone is generated by multi-step bio-synthetic pathways originating with cholesterol and ending with the principal hormone (e.g. testosterone, cortisol, estradiol etc.). Nonetheless, there is considerable complexity, with multiple hormonal intermediates exerting unique independent actions. Currently, there exists much uncertainty as to the potential acute responses of such intermediary hormones to resistance exercise and any potential role in exerting their function on skeletal muscle.

The principal androgen hormone is testosterone, which stimulates skeletal muscle anabolism through multiple mechanisms (Ferrando et al. 1998; Wu, 1997). Testosterone synthesis requires the conversion of dehydroepiandrosterone (DHEA) to either androstenedione or androstenediol. Each of these intermediaries can then be subsequently converted to testosterone and then 5α-dihydrotestosterone or estrone (which participates in the estrogen pathway) (Baggett et al. 1959). Direct neuronal activation of the testes to release testosterone can occur (Frankel & Ryan 1981), however, some authors suggest that testosterone concentrations increase during exercise in response to both hemoconcentration (Kargotich et al. 1997) and reduced hepatic uptake (Ahtiainen et al. 2015; Cadoux-Hudson et al. 1985) of the molecule, rather than from abundant release by Leydig cells. However, while the effects of acute resistance exercise on DHEA and testosterone have been documented, there is still a lack of data describing the effect of resistance exercise on other potentially significant gonadosteroids, including the precursors, androstenedione and 5α-dihydrotestosterone. Consequently, it has not been possible to infer or interpret the possible role(s), if any, of acute gonadosteroid concentrations on tissue adaptation.

Acute resistance exercise also stimulates the release of pituitary-derived adrenocorticotropic hormone (ACTH), which in turn triggers the secretion of cortisol from the adrenal cortex (McKenna et al. 1979). Cortisol exerts significant tissue specific and pleotropic actions, with a role in protein catabolism, lipolysis and liver gluconeogenesis (Viru and Viru 2004). Although cortisol, along with other hormones, can be stimulated directly by ACTH, cortisol can also be synthesized from other glucocorticoids such as 11-deoxycortisol or cortisone (Jenkins & Sampson 1967). Currently no data exist describing the interconversion and subsequent actions of these corticosteroid hormones in response to resistance exercise.

Given the importance of gonadosteroid and corticosteroid hormones for tissue plasticity and metabolism, surprisingly little is known about the coordinated regulation of the family of hormonal intermediates and principle hormones in response to exercise. To address this lack of understanding, documentation of the temporal concentrations of a comprehensive array of gonadosteroids and corticosteroids is needed. The traditional approach of assessing a small number of selected hormones, using targeted analysis techniques (e.g. chemiluminescent immunoassay or enzyme-linked immunosorbent assays) (Ahtiainen et al. 2015; Cumming et al. 1987; Kraemer et al. 1990; Häkkinen et al. 1998; Häkkinen et al. 2000; Nindl et al. 2001; Smilios et al. 2003) limits the interpretation of potential interactions between hormones. Ultra-high performance liquid chromatography mass spectroscopy (MS), on the other hand, allows for the detection and quantification of families of structurally related intermediates, including members of the gonadosteroid and corticosteroid pathways that are typically present in human circulation in low (micromolar) concentrations.

Therefore, the present study aimed to profile the acute responses of the gonadosteroid and corticosteroid hormonal families during and immediately after a heavy resistance exercise session. Experienced strength-trained (men) volunteered for the study, and experimental exercise sessions were completed both before and after a 10-week supervised resistance-training period in order to reduce inherent variability due to training status and check for consistency in acute responses.

METHODS

Ethical Approval

The subjects were thoroughly informed (both written and verbally) about the study's objectives, methods, use of results, and possible risks/harms from participation, and were given the opportunity to ask questions of the researchers regarding the information provided. Thereafter, all subjects provided written informed consent. The study was approved by the ethics committee of Edith Cowan University (No. 9223) and conducted according to the Declaration of Helsinki (2008), except for registration in a database.

Subjects

Twenty strength-trained men volunteered for the study and 18 men completed all study requirements (two men completed only one of the two experimental sessions and were removed from the analyses). The remaining 18 subjects' baseline characteristics were; age 21 ± 2 y, height 180 ± 7 cm, weight 78 ± 12 kg, BMI 24 ± 3, lean body mass 59 ± 7 kg. The subjects were currently performing regular resistance training at least twice per week (duration = 1–6 years) and were free from illness, injury, disease and use of performance enhancing substances. Initial 10- RM inclined leg press load was 2.0 ± 0.6 kg:BM. The present study reports on additional data collected during completion of a previously published study (Walker et al. 2017).

Experimental resistance exercise sessions

Each subject was allocated a specific test start time to arrive at the lab for both experimental exercise sessions, and the sessions were performed from 9:00 until 16:00, which matched the subject's own training time over the 10 weeks. Ingestion of caffeine and alcohol were prohibited prior to the test for 6 and 48 hours, respectively. Also, habitual resistance training was not allowed for 48 hours prior to

the experimental exercise sessions. Dietary intake was recorded over 12 hours prior to the test and the same diet was replicated during the second experimental exercise session after 10 weeks of strength training (described below). Subjects performed a high-intensity resistance exercise protocol consisting of 3 sets of bilateral inclined leg press (45° leg press, Cybex international Inc, Medway, USA) and then 3 sets of unilateral knee extensions (VR3 leg extension, Cybex International Inc, Medway, USA) to failure using 10-repetition maximum (RM) loads with 2-min and 1-min inter-set rests, respectively. Concentric and eccentric phases of the lifts were completed with a 2:2 s tempo (i.e., 4 s in total), which was monitored by the investigator. All exercises were performed from a knee angle of ~85° to a maximum extension of 175° (180° = full extension). Light assistance was provided by the investigator in order to ensure that 10 repetitions were completed in each set, and the load was reduced for the following set if a subject could not voluntarily complete 10 repetitions. Antecubital and fingertip blood samples were taken before exercise after 5 min of quiet sitting (pre-exercise), 2 min after completion of leg press exercise (mid-exercise), and then 5 and 15 min after completion of the leg extension exercise (5 min post-exercise and 15 min post-exercise). Only a small amount of water (<200 mL) was allowed *ad libitum* during the experimental session. After the final blood draw, subjects were provided with a mixed protein (23 g), carbohydrate (3 g), and fat (1.6 g) drink (Total+ Vital Strength, Power Foods International Plc, Marrickville, Australia). The experimental exercise sessions took place before and after a 10-week period of supervised resistance training. This intervention consisted of training twice per week with a session of 3 × 6-RM once per week and a session of 3 × 10-RM once per week using bilateral leg press, unilateral knee extension and bilateral knee flexion exercises (see Walker et al. 2017 for further details). As noted in our previous publication, maximum isometric strength increased 14.3 ± 10.7%, lower-limb lean mass increased 3.2 ± 2.4%, and volume load during the experimental resistance exercise sessions increased 31.4 ± 29.3% as a consequence of training.

Blood sampling and analyses

Venous blood samples were collected into heparinized serum separator tubes (8.5 mL Venosafe SST 2 advance, Becton Dickinson and Co. vacutainer, Plymouth, UK), which stood at room temperature for 15 min before being centrifuged (5702R centrifuge, Eppendorf AG, Hamburg, Germany) for 10 min at 1849 x *g*. The serum was pipetted into 1.5 mL tubes and stored at -80°C until further analysis.

Fingertip blood samples were collected onto LactatePro strips (Arkray Inc, Kyoto, Japan), into HemoCue microcruvettes (HemoCue 201, HemoCue AB, Ängelholm, Sweden) and into non-heparinized capillary tubes (32 µL micro-capillary tubes, Selzer GmbH, Waghäusel, Germany) and used to analyze blood lactate concentration, hemoglobin and hematocrit, respectively. Blood lactate and hemoglobin analyses were performed immediately, according to the manufacturer's instructions. To measure hematocrit, the capillary tubes were plugged with plasticine, centrifuged at 14,328*g* for 5 min (MPW-212 centrifuge, MPW medical instruments, Poland) and the serum:red blood cell ratio was determined. Hemoglobin analysis was performed in duplicate and hematocrit in triplicate, with the mean value used for analysis (CV% = 3.2% and 1.9%, respectively). Hemoglobin and hematocrit values were then used to assess plasma volume changes using the Dill and Costill (1974) equation.

Steroid hormones were measured using mass spectrometry. The internal standards were cortisol-d4 for cortisol and cortisone, corticosterone-d8 for corticosterone, 11-deoxycortisol, DHEA, estrone and andrastenedione and testosterone-d3 for testosterone. 100 µL of internal standard (60, 60 and 20 $ng \cdot mL^{-1}$, respectively, in water) was added to 200 µL of plasma. Steroid hormones were extracted using 1 mL of ethyl acetate (Merck KGaA Darnstadt, Germany). The organic layer was removed to a new tube and dried. The residue was re-suspended in 80 µL of 60% methanol (Merck) and 40% water, and transferred to HPLC injector vials. A volume of 12 µL was injected onto a UHPLC mass spectrometer system consisting of an Accela MS pump and autosampler followed by an Ion Max APCI source on a Finnigan TSQ Quantum Ultra AM triple quadrapole mass spectrometer, all controlled by Finnigan Xcalibur software (Thermo Electron Corporation, San Jose, CA, USA). Steroid hormone separation was achieved during a 10-min run using a gradient of increasing methanol from 60 to 80% (balance water) flowing at 400 $\mu L.min^{-1}$ through a Kinetex PFP 2.6µm, C18, 100A, 100 × 3.0-mm column at 40°C (Phenomenex, Auckland, New Zealand). Retention times were: 2.4

min for cortisol, cortisol-d4 and cortisone; 3.2 min for corticosterone and corticosterone d8; 3.4 min for 11-deoxycortisol; 4.2 min for androstenedione; and 4.6 min for testosterone, testosterone-d3, DHEA and estrone. Ionization was in positive mode, and Q2 had 1.2 mTorr of argon for all steroid hormones. The mass transitions followed were: cortisol-d4 367.2→121.2, cortisol 363.2→122.2, cortisone 361.1→163.1, corticosterone 347.1→121.0, corticosterone-d8 355.2→125.2, 11-deoxycortisol 347.1→97.1, androstenedione 287.1→97.05, testosterone 289.1→97.1, testosterone-d3 292.1→97.1, DHEA 271.1→197.0, and estrone 271.1→132.0 all between 22 and 28 V. Analysis was carried out using Xcalibur software (ThermoFisher scientific, Waltham, USA). Steroid hormone concentrations were calculated from the peak area ratio steroid/internal standard compared with standard curves generated from pure compounds. The limits of detection were: DHEA = 0.02 nmol·L^{-1}, Androstenedione = 0.02 nmol·L^{-1}, Testosterone = 0.035 nmol·L^{-1}, Estrone = 0.28 nmol·L^{-1}, 11-deoxycortisol = 0.14 nmol·L^{-1}, Corticosterone = 0.02 nmol·L^{-1}, Cortisone = 0.001 nmol·L^{-1}, Cortisol = 0.28 nmol·L^{-1}. Intra- and inter-assay coefficient of variation % for reported hormones were between 10–25%, as previously reported (Baume et al. 2008).

Statistical analyses

All statistics were performed using IBM SPSS software (version 24, IBM, USA). Standard procedures were used to obtain means and standard deviations, which are used to describe the data. The data were explored and then log transformed if normal distribution was not observed. Repeated measures ANOVA (2 exercise sessions × 4 time points) was used to assess main effects. Bonferroni post-hoc tests were applied to determine the source of any differences. Correlations between the exercise-induced changes (% of pre-exercise) in hormone concentrations were performed using Pearson's Product Moment Correlation. The results of the statistical analyses were the same whether correcting for plasma volume change or assessing uncorrected concentrations (Table 1). Criterion alpha for statistical significance was set at $P \leq 0.05$.

RESULTS

Gonadosteroids

Pre-exercise concentrations of gonadosteroid hormones remained similar (n.s.) before vs. after the 10-week training period and within the typical ranges for men. Pre-exercise concentrations of DHEA were 7.74 ± 9.20 and 5.38 ± 5.34, androstenedione were 1.92 ± 0.80 and 2.06 ± 0.84, testosterone were 13.70 ± 8.57 and 11.90 ± 4.96, and estrone were 1.36 ± 0.55 and 1.74 ± 0.74 $nmol \cdot L^{-1}$ before and after the training period, respectively (Table 1).

Significant main effects for Time were observed for DHEA ($F = 3.9$, $P = 0.018$), testosterone ($F = 9.9$, $P < 0.001$), androstenedione ($F = 8.0$, $P < 0.001$), and estrone ($F = 25.2$, $P < 0.001$). A significant main effect for Exercise Session was also observed for DHEA ($F = 4.8$, $P = 0.037$).

In response to the experimental resistance exercise sessions, statistically significant acute increases in androstenedione concentration were observed before the training period and in DHEA and testosterone after 10-weeks of training, while estrone increased before and after the training period (Table 1). There were no statistically significant differences in the magnitude of the response when comparing exercise sessions before versus after the training period (i.e. all responses were similar during both experimental exercise sessions, Figure 1).

Corticosteroids

Pre-exercise concentrations of 11-deoxycortisol were 1.96 ± 4.73 and 0.63 ± 0.78, corticosterone were 8.84 ± 15.75 and 6.13 ± 4.02, cortisone were 0.512 ± 0.186 and 0.506 ± 0.137, and cortisol were 275 ± 131 and 276 ± 76 $nmol \cdot L^{-1}$ before and after the 10-week training period, respectively (Table 1). There were no statistically significant differences in pre-exercise concentrations before vs. after the 10-week training period.

Significant main effects for Time were observed for cortisol ($F = 14.2$, $P < 0.001$), cortisone (before: $F = 5.9$, $P = 0.003$), corticosterone ($F = 21.7$, $P < 0.001$), and 11-deoxycortisol ($F = 3.6$, $P = 0.035$). A significant main effect for Exercise Session was also observed for 11-deoxycortisol ($F = 4.4$, $P = 0.044$).

In response to the experimental resistance exercise sessions, statistically significant acute increases were observed in cortisol before the training period, in corticosterone both before and after training, and in cortisone after the training period (Table 1). The magnitude of acute responses before versus after the training period did not change (Figure 2).

Plasma volume

There were minor changes in plasma volume during each experimental exercise session, although the changes did reach statistical significance ($P < 0.05$) at mid-exercise and 5 min post-exercise both before and after the training period. Before the training period, plasma volume changed by -5.5 ± 0.04% mid-exercise, by -2.7 ± 0.04% 5 min post-exercise and by -0.5 ± 0.05% 15 min post-exercise. After the training period, plasma volume changed by -2.8 ± 0.08% mid-exercise, by -0.2 ± 0.08% 5 min post-exercise and by 0.1 ± 0.06% 15 min post-exercise.

Blood lactate

Significant main effects for Time ($F = 379.3$, $P < 0.001$), Exercise Session ($F = 10.1$, $P = 0.003$) and Interaction ($F = 3.8$, $P = 0.012$) were observed for blood lactate.

After both experimental exercise sessions, statistically significant increases in blood lactate concentration were observed at all time points (Table 1). The baseline corrected area-under-the-curve concentration during/after exercise was greater after 10 weeks of training versus before the training period (17.1 ± 3.3 vs. 14.1 ± 4.2 $mmol \cdot L^{-1} \cdot 30min^{-1}$, $P = 0.008$, Figure 3).

Bivariate correlations

The full correlation matrix of exercise-induced changes (from pre-exercise to 15 min post-exercise) in concentration between the studied hormones is presented in Table 2. The overall number of statistically significant relationships observed increased after the training period. Some notable relationships are mentioned in the following text. The resistance exercise-induced change (% from pre-exercise to 15 min post-exercise) in androstenedione concentration was related to the change in estrone concentration before ($r = 0.828$, $P = 0.002$, $n = 11$) and after ($r = 0.882$, $P < 0.001$, $n = 16$) the 10-week training period. Also, the exercise-induced change in DHEA was significantly related to the change in androstenedione after ($r = 0.880$, $P < 0.001$, $n = 16$) the training period. However, there were no relationships between the change in androstenedione and testosterone.

The change in cortisol concentration was positively and significantly related to the change in DHEA concentration at all times points (e.g. 15 min post-exercise before training $r = 0.702$, $P = 0.002$, $n = 17$; 15 min post-exercise after training $r = 0.859$, $P < 0.001$, $n = 17$, Figure 4A). Also, the change in 11-deoxycortisol was positively and significantly related to the change in cortisol at mid- and 15 min post-exercise both before and after training (e.g. 15 min post-exercise before training $r = 0.507$, $P = 0.038$, $n = 18$; 15 min post-exercise after training $r = 0.797$, $P < 0.001$, $n = 18$, Figure 4B).

DISCUSSION

The present study investigated the acute responses in gonadosteroid and corticosteroid hormonal families following resistance exercise sessions performed before and after a 10-week supervised (heavy strength training) training program in already-trained men. Analysis was performed using a targeted mass spectroscopy technology enabling quantification of (smaller-concentration) intermediary hormonal metabolites of each pathway that are typically not qualified in most clinical studies. Statistically significant increases in DHEA, androstenedione and estrone were observed in

response to the present study's resistance exercise protocol. The correlations between the acute increases in these hormones suggest a direct conversion cascade during/following acute resistance exercise. Although serum testosterone also increased in response to resistance exercise, this response appears to occur independent of the other measured gonadosteroids. Of the corticosteroids, corticosterone concentration increased significantly both before and after the training period, suggesting a role of mineralocorticoid action during acute resistance exercise sessions. Cortisol concentrations increased significantly before but not after the 10-week training program, perhaps indicating that the same resistance exercise protocol was not as physiologically/psychologically stressful.

Acute gonadosteroid responses to resistance exercise

Previously it has been proposed that plasma volume changes explain the measured increase in testosterone concentration after acute (albeit high-intensity swimming) exercise (Kargotich et al. 1997). However, correction for plasma volume shift did not influence the results in the present study, thus, other mechanisms may be responsible for the observed changes. Androstenedione is an intermediary hormone that can be converted to testosterone. Acute increases in androstenedione (~60%) occurred before the training period while acute increases in testosterone (~5%) only occurred after the training period (Table 1). Furthermore, no relationships between the acute increases in androstenedione and testosterone were observed in the present study. Androstenediol, having been converted from DHEA, can also be converted to testosterone, but again a discordance was observed in the present study between acute increases in DHEA and testosterone concentrations. In a study by Cumming and colleagues (1987) it was concluded that it *"seems unlikely that the late exercise testosterone increment is gonadotropin-mediated"* (p.237) and a discourse in luteinizing hormone and testosterone pulsatility has clearly been demonstrated in rats (Robaire & Bayly 1989). Therefore, there are likely other reasons for the increase in testosterone during acute exercise than the simple top-down steroidogenesis pathway.

One possible candidate for acute increases in testosterone during resistance exercise is reduced uptake by the liver (Ahtiainen et al. 2015), perhaps due to lower hepatic blood flow during exercise. Another is that direct neural stimulation of the testes during stressful conditions leads to testosterone release (Frankel & Ryan 1981), which would by-pass upstream androgens. Further evidence that acute exercise may modify the assumed gonadosteroid conversion pathway (i.e. DHEA–androstenedione–testosterone–5α-dihydrotestosterone) was observed by Sato and colleagues (2016). Here, low-intensity endurance exercise increased DHEA and free testosterone concentrations in untrained subjects but 5α-dihydrotestosterone increased only during high-intensity endurance exercise. On the other hand, all examined gonadosteroid concentrations increased following high-intensity endurance exercise in athletes. Ultimately, identifying the mechanisms mediating such observations is still unresolved.

Estrone concentrations increased significantly in response to resistance exercise both before and after the 10-week training period. Furthermore, the magnitude of the estrone response strongly matched the androstenedione response ($r > 0.8$). Estrone is a weak estrogen and a precursor for the other estrogens, estradiol and estriol. Little is known about the effect of estrogens on muscle and tendon adaptation in men. There may of course be different mechanisms of adaptation to resistance training between the sexes, but at least in women circulating estrogen levels appear to be highly important (Hansen and Kjaer 2014). Future studies may focus on the potential role of acute circulating estrogen response to resistance exercise and their role in muscular adaptation also in (trained) men.

Acute corticosteroid responses to resistance exercise

Cortisol responds robustly to resistance exercise with concentrations peaking at around 30–60 min after high-volume resistance exercise (Ahtiainen et al. 2003; Geisler et al. 2019; Häkkinen and Pakarinen 1993; Nindl et al. 2001; Smilios et al. 2003; Walker et al. 2011). Similarly, and temporally aligned, significant increases in corticosterone concentration were observed following both resistance

exercise sessions. This may be indicative of direct splanchnic nerve stimulation of the adrenal gland (Holzwarth et al. 1987).

Izquierdo et al. (2009) reported no change in cortisol concentrations immediately after performance of 5 sets of 10 repetitions in the leg press, but a significant increase 15 minutes after completion of the exercise session, suggesting a time-lag in the response. This was also observed in the present study, with no response observed immediately following the 3 × 10 leg press sets (i.e. mid-exercise) and a significant increase 5 min after completion of the 3 × 10 unilateral knee extension sets – approximately 15 min after the completion of the leg press sets. This delayed response suggests that upstream factors play a role in cortisol secretion.

A similar delay was observed in the present study regarding DHEA, and it is plausible that secretion of cortisol and DHEA has been stimulated by adrenocorticotrophic hormone (ACTH) simultaneously due to a neurohumoral link (Kjaer 1992). This notion is supported by the observed relationship between the exercise-induced changes in these hormones (Figure 4A). Cortisol is thought to play an important glucocorticoid role during (prolonged) exercise by supplying substrate for metabolism, but in the case of short-duration resistance exercise, cortisol may play more of a role in providing amino acids for muscle remodeling (Viru and Viru 2004). This may be one reason for some observations of a relationship between exercise-induced increases in cortisol and training-induced muscle hypertrophy (West and Phillips 2012).

Although supposedly involved in production of cortisol, 11-deoxycortisol has also been shown to be synthesized into androstenedione (Auzeby et al. 1991). Nevertheless, the present study did not reveal relationships between responses of these two hormones and so this may not play a role within the exercise setting. The present analysis did identify significant relationships between exercise-induced increases in 11-deoxycortisol and cortisol. There are several possibilities for these relationships, but perhaps likeliest is that: 1) a global glucocorticoid response to exercise has occurred and/or 2) 11-deoxycortisol (being a less potent glucocorticoid) is released as a control system to limit excessive

tissue breakdown- as was hypothesized regarding possible DHEA influence on the liver and kidneys. Once again, however, these hypotheses are speculative and require specific investigation.

General considerations and study limitations

From previous resistance exercise sessions using multi-joint, multi-set, moderate-load and moderate-repetition protocols, average increases in testosterone of ~10–30% tend to be observed while the average increases in cortisol tends to be ~50–200% (e.g. Häkkinen and Pakarinen 1993; Kraemer et al. 1990; Smilios et al. 2003). The observed increases in these hormones before and after 10 weeks of training in the present study were ~11% and ~5% for testosterone and ~75% and ~50% for cortisol, respectively. In this regard, the testosterone and cortisol responses after the training period may be considered to be (qualitatively) at the lower end of expected response magnitude.

This might be explained by the exercise protocol *only* consisting of 3 sets of bilateral leg press and 3 sets of unilateral knee extension. In order to maximize acute increases in serum testosterone and cortisol concentrations, higher total training volumes and exercises targeting large muscle groups or whole-body workouts are needed (Gotshalk et al. 1997; Häkkinen and Pakarinen 1993; Häkkinen et al. 1998; Kraemer et al. 1990; Smilios et al. 2003). For example, 5 sets of 10 repetitions in the back squat exercise led to significant increases in both testosterone and cortisol up to 45 min post-exercise, whereas 5 sets of 10 repetitions in bench press (an exercise using smaller muscle mass) resulted in no change in salivary concentrations (Geisler et al. 2019). Indeed, in the present study, the largest testosterone responses were observed following the bilateral leg press sets and a gradual return to pre-exercise values occurred thereafter despite the subjects subsequently performing the unilateral knee extension sets. Consequently, the ability to detect differences in acute responses from before versus after training might have been limited.

In contrast to the typically reduced acute hormonal responses when the same resistance-exercise protocol is repeated in strength-trained athletes (Ahtiainen et al. 2003), beginners tend to demonstrate

enhanced acute increases in serum hormones after a short-term period of training (Ahtiainen et al. 2003; Izquierdo et al. 2009; Walker et al. 2014). Given that the subjects in the present study were already well-trained, a lowered response reported in the present study would not be surprising. However, statistically this was not the case for any hormone investigated (Figures 1 and 2), and so the present results are not in-line with this hypothesis. Nevertheless, the current training period was 10 weeks while 21 weeks were performed in the study of Ahtiainen and colleagues (2003). Hence, there may be a training period duration after which the same resistance exercise stimulus evokes a lesser acute response.

Short-term training led to a greater exercise-induced increase in blood lactate concentration after training compared to before training, as previously observed (Izquierdo et al. 2009; Walker et al. 2014; Walker et al. 2015). This is not always observed in the literature as some studies show no differences (Ahtiainen et al. 2005), and sometimes the larger response after training does not reach statistical significance (Kraemer et al. 1998). The reason for the greater increase in blood lactate is unclear but may be explained by a greater anaerobic metabolism (due to the higher volume load) or greater ejection from muscle into the blood. Although the type of resistance exercise protocol influences the magnitude of both blood lactate and hormonal response (Kraemer et al. 1990; Walker et al. 2014), these small differences from before to after training may not greatly influence the responses since the literature overall shows conflicting results.

Plasma volume changes of ~14–22% after intense resistance exercise have been reported in the literature (Collins et al. 1986; Ploutz-Snyder et al. 1995), causing hemoconcentration of circulating hormones. One limitation of using fingertip blood to estimate plasma volume change, as done in the present study, is that it may lead to plasma volume change underestimation compared to venous blood sampling. However, even after consideration of an error of 1-3% (Knowlton et al. 1990), the values obtained in the present study (calculated as <5%) are moderate and presumably would not have substantially affected the observed changes in hormone concentration. Therefore, other factors most likely explain the acute increases in serum hormone concentration.

Collecting blood samples only in the period immediately after completion of the experimental exercise sessions does not allow the tracking of possible conversions/interactions of gonadosteroids or corticosteroids over the following days (e.g. 24–48 h). This might have provided important information relating to how acute resistance exercise influences diurnal variations in hormonal interactions and potentially help to explain the previously-observed reduction in basal testosterone concentrations detected 24–48 h after strenuous resistance-exercise (Häkkinen and Pakarinen 1993). Nevertheless, new evidence of intermediary hormone conversions in the gonadosteroid pathway following resistance exercise in strength-trained men is presented. Given that the primary target hormone (i.e. testosterone) response appears to occur independent to conversions of intermediary hormones, exercise-induced conversions may not necessarily follow the expected theoretical cascading pathway (i.e. DHEA–androstenedione–testosterone).

Of final note, the training-induced changes in strength and muscle mass have been reported in a previous article (Walker et al. 2017). These results indicated that, for some of the well-trained subjects, the training program did not improve strength and muscle mass after the 5-week time point, which is not unexpected in already-trained subjects but should be considered when interpreting the acute hormonal responses presented.

In conclusion, acute, intense resistance exercise led to a robust endocrine response also in smaller-concentration intermediary gonadosteroids (e.g. estrone) and corticosteroids (e.g. corticosterone). Evidence is presented for cascading conversions of some gonadosteroids, which suggest that testosterone concentration increases independently of these hormones during exercise and contrasts the theoretical cascading pathway. There remains uncertainty regarding cascading corticosteroid conversions in the exercise setting. The current 10-week resistance training program did not statistically reduce the magnitude of the acute hormone responses to the experimental exercise session in these already trained men. The challenge for future studies is to determine the exact physiological

roles of the smaller-concentration gonadosteroid and corticosteroids during and immediately following resistance exercise.

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COMPETING INTERESTS

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

The samples for this work were collected in the laboratories of Edith Cowan University, Australia and the mass spectroscopy analyses were performed at the University of Auckland, New Zealand. SW, KH, RUN, DCS, AJB conceived and designed the study. SW and GGH acquired the data, SW, JFM, SP and DCS analyzed the data, and SW, KH, RUN, GGH, DCS and AJB interpreted the findings. SW drafted the manuscript and all authors revised the manuscript at various stages throughout the process. All authors have approved the final version of the manuscript, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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FIGURE LEGENDS

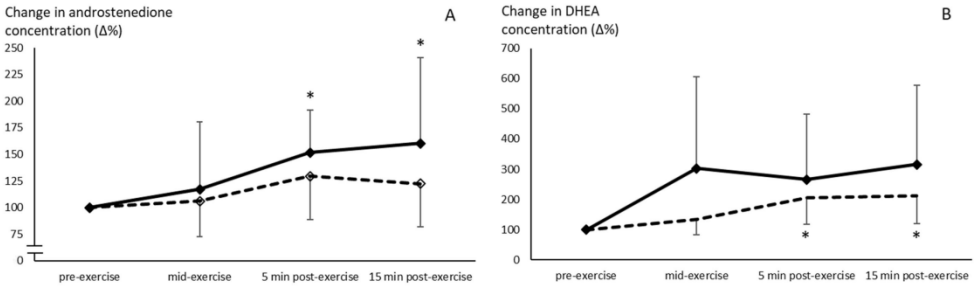

Figure 1. Exercise-induced changes (% of pre-exercise) in androstenedione (A) and DHEA (B) concentrations (mean ± SD). The exercise sessions led to robust increases in smaller-concentration intermediate hormones with a trend towards a blunted response after the 10-week training period. * = $P < 0.05$ versus pre-exercise concentration. Solid line = before training, dashed line = after training.

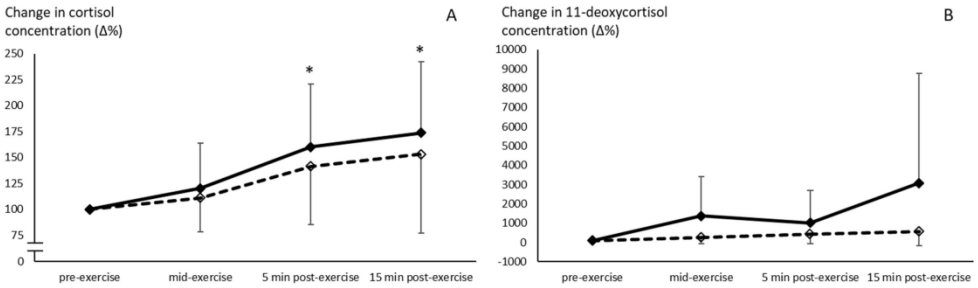

Figure 2. Exercise-induced changes (% of pre-exercise) in cortisol (A) and 11-deoxycortisol (B) concentrations (mean ± SD). Robust increases in cortisol, but highly individual changes in 11-deoxycortisol, were observed before training while no changes were observed in either hormone after the 10-week training period. * = $P < 0.05$ versus pre-exercise concentration. Solid line = before training, dashed line = after training.

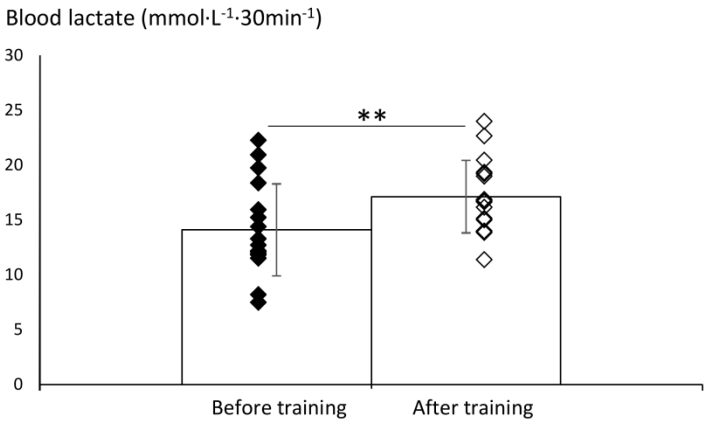

Figure 3. Post-exercise blood lactate area-under-the-curve concentration (mean±SD) after baseline correction (mmol·L^{-1}·30min) from mid-exercise to 15 min post-exercise. Each diamond represents a single subject (filled = before training, open = after training). There was a high anaerobic demand during both exercise sessions with even higher post-exercise blood lactate concentration after the 10-week training period. ** = $P < 0.01$ before versus after training.

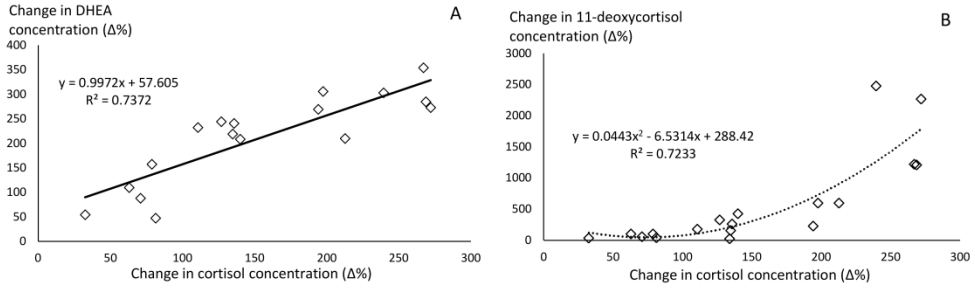

Figure 4. Relationship between the exercise-induced change (% of pre-exercise) in cortisol concentration and both DHEA (A: linear relationship) and 11-deoxycortisol (B: nonlinear relationship). The link between DHEA and cortisol is consistent with the hypothesis that ACTH influenced secretion of these hormones following exercise, and the nonlinear relationship between cortisol and 11-deoxycortisol may indicate the need to block glucocorticoid receptors preventing excessive tissue breakdown. Each diamond represents a single subject.

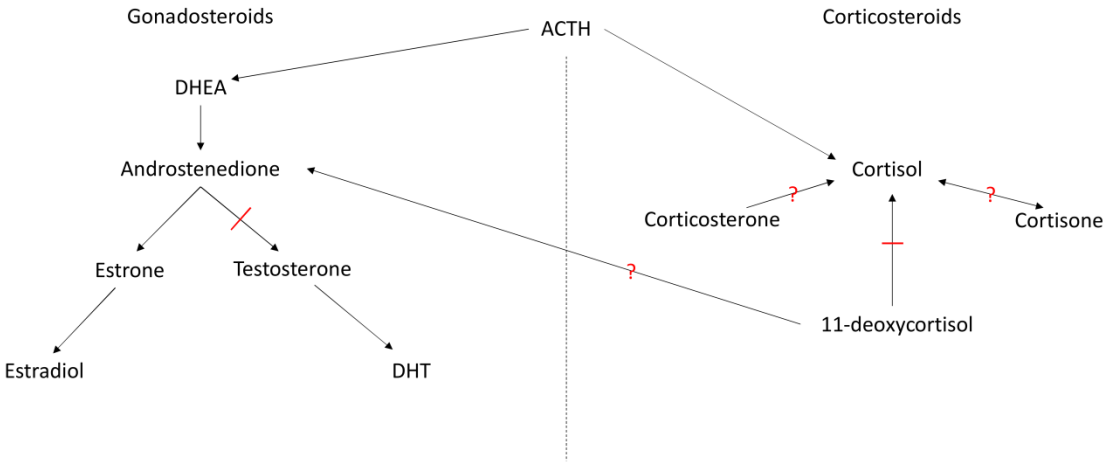

Figure 5. Theoretical hormonal response cascade during resistance exercise in strength-trained men. Clear DHEA secretion from resistance exercise initiates the descending gonadosteroid cascade, but the exercise setting suggests a divergence from the theoretical cascade in that testosterone responds independently to the measured intermediate hormones. It remains uncertain whether intermediate hormones influence cortisol secretion or whether their response is related to other corticosteroid roles or blocking of glucocorticoid receptors.

Table 1. Uncorrected (black font) and plasma volume-corrected (grey font) serum concentrations (mean±SD) measured pre-, mid-, and post-resistance exercise in gonadosteroid and corticosteroid hormones assessed in the present study before and after the 10-week training period.

	DHEA (nmol·L^{-1})	Androstenedione (nmol·L^{-1})	Testosterone (nmol·L^{-1})	Estrone (nmol·L^{-1})	11-deoxycortisol (nmol·L^{-1})	Corticosterone (nmol·L^{-1})	Cortisone (nmol·L^{-1})	Cortisol (nmol·L^{-1})	Lactate (mmol·L^{-1})
Before									
pre-	7.74±9.20	1.92±0.80	13.70±8.57	1.36±0.55	1.96±4.73	8.84±15.75	0.512±0.186	275±131	1.03±0.16
	8.05±9.40	1.88±0.84	14.46±8.18	1.41±0.55	2.02±4.85	8.67±16.21	0.528±0.178	274±135	1.03±0.16
mid-	14.40±16.38	2.16±1.40	18.14±9.94	2.29±1.33	7.94±12.76	10.64±7.83	0.647±0.217	327±171	**10.09±2.82**
	13.12±15.58	2.02±1.36	18.01±8.25	2.22±1.33	6.46±11.49	9.62±7.25	0.604±0.204	290±142	**9.56±2.77**
post-	17.73±25.09	**2.76±1.08**	15.94±9.73	**3.07±1.37**	13.22±32.35	**30.35±26.82**	0.580±0.188	**406±180**	**8.18±2.12**
	17.63±23.80	**2.65±1.01**	16.34±8.84	**3.11±1.41**	13.16±31.14	**30.00±25.61**	0.574±0.165	**396±170**	**7.94±1.99**
15post-	18.36±20.06	**2.72±0.94**	13.55±6.07	**3.11±1.70**	25.17±49.75	**36.62±38.64**	0.593±0.208	**444±230**	**5.89±2.01**
	19.43±20.68	**2.72±0.98**	14.13±5.11	**3.29±1.74**	26.49±49.87	**38.15±38.01**	0.604±0.128	**451±230**	**5.88±2.07**
After									
pre-	5.38±5.34	2.06±0.84	11.90±4.96	1.74±0.74	0.63±0.78	6.13±4.02	0.506±0.137	276±76	1.69±0.73
	5.48±5.52	2.02±0.87	12.52±4.35	1.78±0.74	0.63±0.81	5.90±4.05	0.520±0.128	274±78	1.69±0.73
mid-	7.70±9.61	2.16±0.63	**15.05±5.49**	2.44±1.52	2.25±6.93	8.99±5.32	0.575±0.157	302±106	**11.60±1.69**
	8.40±11.69	2.16±0.56	**15.11±5.53**	2.44±1.44	2.66±8.46	9.25±5.49	0.576±0.129	305±109	**11.25±1.70**
post-	**8.26±4.0**	2.72±1.05	**13.95±5.**	**3.44±1**	1.04±0.6	**20.17±12.**	**0.582±0.**	358±1	**10.90±2**

	6		**88**	**.74**	1	**25**	**129**	05	**.05**
	8.43±3.92	2.69±1.08	**14.63±5.56**	**3.51±1.66**	1.04±0.61	**20.81±12.05**	**0.587±0.105**	358±105	**10.88±2.24**
15post-	**12.04±15.34**	2.58±1.08	12.24±5.29	**3.51±2.03**	4.53±14.92	**23.78±15.90**	0.571±0.191	388±177	**7.64±1.99**
	13.26±17.49	2.69±1.12	13.19±5.34	**3.74±2.07**	5.19±17.09	**25.35±16.16**	0.595±0.211	405±199	**7.72±1.99**

DHEA = dehydroepiandrosterone. Bolded values signify statistical significance ($P < 0.05$).

Table 2. Correlation matrix for the exercise-induced change (pre-exercise to 15min post-exercise) in hormone concentrations before and after the 10-week training period.

Before training

	DHEA	Androstenedione	Testosterone	Estrone	11-deoxycortisol	Corticosterone	Cortisone	Cortisol	Lactate
DHEA	1								
Androstenedione	-0.080	1							
Testosterone	0.265	-0.178	1						
Estrone	**0.922**	**0.828**	0.185	1					
11-deoxycortisol	**0.834**	-0.369	0.346	**0.664**	1				
Corticosterone	0.366	0.148	-0.049	**0.615**	0.128	1			
Cortisone	0.478	-0.232	**0.664**	0.174	**0.524**	0.117	1		
Cortisol	**0.702**	0.043	0.261	**0.730**	**0.507**	**0.688**	**0.508**	1	
Lactate	0.389	0.461	0.202	0.588	0.107	0.130	0.053	0.220	1

After training

	DHEA	Androstenedione	Testosterone	Estrone	11-deoxycortisol	Corticosterone	Cortisone	Cortisol	Lactate
DHEA	1								
Androstenedione	**0.880**	1							
Testosterone	0.375	0.426	1						

Estrone	**0.947**	**0.882**	0.341	1					
11-deoxycortisol	**0.616**	**0.564**	**0.498**	**0.744**	1				
Corticosterone	**0.734**	**0.612**	0.073	**0.797**	**0.749**	1			
Cortisone	0.472	**0.540**	**0.774**	**0.498**	**0.651**	0.363	1		
Cortisol	**0.859**	**0.766**	0.424	**0.900**	**0.797**	**0.810**	**0.632**	1	
Lactate	**0.555**	**0.605**	0.109	**0.573**	0.318	0.384	0.339	**0.500**	1

Data presented are Pearson's Product Moment Correlation (r) values and bolded values signify statistical significance ($P < 0.05$). DHEA = dehydroepiandrosterone.