Testosterone & dihydrotestosterone changes in male & female athletes relative to training status

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TESTOSTERONE & DIHYDROTESTOSTERONE CHANGES IN MALE & FEMALE ATHLETES RELATIVE TO TRAINING STATUS

ABSTRACT

Purpose:
To establish if training volume was associated with androgen baselines, and androgen responsiveness to acute exercise.

Methods:
During a 'high-volume' training phase, 28 cyclists (14 males, 14 females) undertook VO_2 and maximal work capacity testing. Two days later they completed a repeat sprint protocol, which was repeated three weeks later during a 'low-volume' phase. Blood and saliva samples were collected before and after (+5, +60 minutes) the repeat sprint protocol. Blood was assayed for total testosterone (TT), free testosterone (FT), and dihydrotestosterone (DHT); saliva for testosterone (ST) and DHT (SDHT).

Results:
Pre-trial TT, FT and DHT concentration was greater for males ($p<0.001$, large effect size [ES] differences), and in both genders TT, DHT and SDHT was higher during high-volume loading (moderate to large ES). Area under the curve analysis revealed larger TT, FT and DHT responses to the repeat sprint protocol among females, and high-volume training was linked to larger TT, DHT and SDHT responses (moderate to large ES). Baseline TT and FT correlated with VO_2 and work capacity in both genders ($p<0.05$).

Conclusion:
DHT showed no acute performance correlation but was responsive to volume of training, particularly in females. This work informs on timelines and relationships of androgenic biomarkers in males and females across different training loads, adding to the complexity which should be considered in interpretation thereof. We speculate testosterone may impact acute performance via behavioral mechanisms of motivation and attention; DHT, via training volume induced androgenic promotion, may facilitate long-term adaptive changes especially for females.

KEY WORDS: DHT, gender, training load, androgen, performance

INTRODUCTION

Physical exercise can trigger an acute change in circulating androgens,[1] with the magnitude of responsiveness affected by baseline androgen concentration[2] and, potentially, training status.[3,4] Testosterone, a steroid hormone from the androgen group, has attracted particular interest in scientific literature because the putative functional outcomes following increases after high-intensity exercise includes emotional and behaviour change,[3,5,6] increased work output,[1,7,8] enhanced chronic training adaptation,[9] and enhanced competitive performance.[5,8,9] The mechanisms by which these functional outcomes occur include increased behavioural motivation and cognition,[2,10] activation of signalling pathways which promote mobilization of energy stores,[11] regulation of neuromotor units,[12] and the accumulation of protein for skeletal muscle hypertrophy via mTor.[13] Timing of testosterone measurement is important when considering the pathways by which these functional outcomes occur. In recreational trained males, for example, testosterone changes over the course of a workout show little direct change to hypertrophy,[14] however the addition of testosterone to hypogonadal males, to achieve an eugonadal state, is associated with muscle hypertrophy.[15] Therefore, testosterone, while facilitating chronic training adaptations via pre-workout motivational factors and via permissive support, may be of lower importance across an acute training session.

Interest in dihydrotestosterone (DHT) as part of the androgenic pathway also exists.[1,16] DHT, although an androgenic hormone in its own right because of its production in the prostate,[17] is produced in males and females mostly from the rapid and irreversible reduction of free testosterone (FT) in peripheral tissues of the body by 5α-reductase.[16,18] Although some differences in opinions exists, it is believed that relatively little metabolism of testosterone to DHT occurs in muscle;[1,16] however, blood DHT concentration is often similar to that of FT.[18] DHT has greater androgen receptor binding affinity compared to testosterone[18] and potentially works on similar pathways to testosterone with respect to mobilization of energy stores,[2,19] protein synthesis and modulation of neuromotor units,[1,2] amongst other mechanisms.[16] Given its greater androgenic potential DHT may be more 'potent' than testosterone with respect to human performance.[1]

Similarly to testosterone, DHT is acutely elevated following high intensity exercise in males.[1,20-22] Testosterone responsiveness to high intensity exercise is partly dependent on baseline concentration;[23] it is typically higher in trained versus untrained individuals[23] and, despite typically having lower baseline concentrations than males, testosterone responsiveness (expressed as a percentage change from baseline values) to an exercise or competitive stressor appears similar for females as it is for males.[7] However, it is unclear whether similarities exist for testosterone (total and free components) and DHT responsiveness relative to training status or gender, and whether acute exercise performance is related to the DHT response.[1]

There were several aims to this study; 1) to establish whether training volume (high vs. low) was associated with baseline androgen concentration (testosterone and DHT); 2) to determine if there was a difference between genders on these outcomes; and 3) was there a link to acute performance for DHT similarly to testosterone.

METHODS

Participants

Twenty-eight trained cyclists (14 males, 14 females) from road and mountain-bike disciplines were recruited to this study. Participant demographics are described in Table 1. All participants had a minimum training history of six years. The level of cycling activity varied for both genders; when questioned whether they considered their activity as competitive or recreational, approximately 50% (in each gender) answered yes on each option. However,

we had no further evidence of this, so to avoid any potential bias arising from differences in competitive level, data were pooled across each gender for analysis. Training volume was self-recorded, and subsequently self-reported, by the cyclists. Each participant provided written informed consent after receiving a full briefing of the study aims, procedures, and benefits. Ethical approval was granted via the National Research Ethics Service, UK (reference number 10/H0808/124).

Experimental protocol

A within-subject design was used to investigate the impact of training hours on the androgen responses of male and female athletes to an acute bout of sprint cycling exercise. Participants were asked to record their training hours and to present when they were in their highest and lowest volume phases (within a 4-week period). Each participant first presented to a laboratory, during a high-volume training phase (termed 'heavy' phase training), where they undertook VO_2 and work capacity testing. Two days later they presented to the laboratory again, this time in a fasted state, for a repeat sprint protocol. Workload for the repeat sprint protocol was prescribed as a proportion of work capacity established from previous testing. Blood and passive drool saliva samples were collected from participants five minutes prior to the sprint protocol (pre), and 5 (5-min post) and 60 (60-min post) minutes after exercise. Participants refrained from consuming any alcohol or caffeine, and were instructed to be as consistent as possible with undertaking any exercise, in the 24 hours prior to testing on both occasions. They were also encouraged to stay well hydrated. On the day of testing, participants were instructed to not consume any food or water for at least 30 min prior to testing, and were permitted to massage their gums to increase saliva flow. Participants were inactive between completion of the repeat sprint protocol and blood and saliva sampling. Blood samples were later tested for total testosterone (TT = FT + protein bound testosterone), FT, and DHT. Saliva samples were tested for testosterone (ST) and DHT (SDHT). The sprint protocol was repeated 3 weeks later when the participants were in their lowest training volume; termed their 'light' training phase.

Incremental exercise trial

Consistent with previous work,[1] to ascertain aerobic capacity and set workloads for subsequent sprint exercise, an incremental test to exhaustion was completed on a cycle ergometer (Schoberer Rad Messtechnik; SRM, Jülich, Germany). Starting load was self-selected based on warm up intensity, and the exercise protocol consisted of 30-W increments every 3 min for 15 min followed by 20-W increments per minute until exhaustion. Power output during the final minute of exercise was averaged to represent work capacity (W_{max}). Expired gases were collected (in a Douglas bag) in the final minute of exercise and analysed, as per previous methodology.[1] These measurements were used to determine the maximal rate of oxygen consumption ($\dot{V}O_{2PEAK}$). Prior to testing, each analyzer was calibrated with gases of known composition and volume within the physiological range, as certified by prior gravimetric analysis (British Oxygen, Guildford, UK).

Repeat sprint exercise trial

On subsequent visits to the laboratory (>48 h after initial performance testing), participants completed a single bout of repeated sprint exercise on a cycle ergometer (SRM, Germany). Similarly to Smith et al.,[1] this consisted of 10 × 30 s sprints, at a target load of 150% of the W_{max} determined from the incremental test (see above), interspersed with 90 s of recovery cycling at a low intensity of less than 100 W. To account for circadian variation in circulating hormones,[24] all testing was conducted in the morning between two and four hours after waking. Workload was self-paced, and participants were given real-time numerical and

graphical feedback on their current power output, cycling cadence, and time elapsed. If a participant was unable to sustain the target workload, they were encouraged to perform maximally during the remaining sprints.

Blood and saliva sampling

Participants arrived at the laboratory in a fasted state. Next, a phlebotomist collected 5 ml of venous blood (i.e., pre-exercise sample) from a superficial antecubital vein without stasis. Two post-exercise samples were collected, the first ~5 min after exercise and the second after 60 mins. Blood samples were suspended in serum tubes (Sarstedt, Germany) for 15 min before being centrifuged for 15 min at 1,500 *g*. The supernatant was immediately transferred to microfuge tubes and frozen at −20°C until analysis. Saliva was self-collected (~1 mL) into 5 mL polypropylene tubes (Sarstedt, Germany) using a passive drool technique without stimulation. Similarly to blood, saliva samples were centrifuged to separate cellular pellet from supernatant. The samples were then stored at −20°C before analysis. Sampling of saliva commenced at the same time as blood sampling.

Hormonal analysis

Serum samples were analysed for TT, FT, and DHT concentrations using enzyme-linked immunoassay (ELISA) kits (IBL, Hamburg, Germany), as per the manufacturers' instructions. Saliva samples were assayed for ST and SDHT concentration by ELISA kits from the same manufacturer. All participants' serum and saliva samples were assayed on the same plate to eliminate inter-assay variation in measured hormones (all < 9.0%).

To quantify androgen responsivity across the sprint exercise session, hormonal output was determined by area under the curve (AUC) analysis. Typically hormones peak 5 – 30 min after exercise.[1,10] The AUC was calculated using a linear trapezoidal method with respect to change from baseline concentration[25] using the 5-min post and 60-min post data collections. Prior to calculation, data were log (natural) transformed to yield a normal distribution of AUCs and correct for non-uniformity bias arising from gender differences in absolute hormone concentration.[7] Subsequently, the AUC results are more interpretable as a ratio rather than a concentration measure per unit of time.

Statistical Analysis

First, paired or unpaired T-tests were used to assess for gender differences in age, body size, physical performance, and training volume (heavy and light), as well as differences in training volume (heavy vs. light) within male and female cyclists. To determine the impact of gender and training hours on baseline androgen levels and androgen responsivity to exercise, we applied a two-factor (Gender [2 level] × Training condition [2 level]) analysis of variance (ANOVA) with training condition as the within-subjects factor. The ANOVA tests were conducted within a linear mixed-model framework using the *lmerTest* package (version 3.1-0)[26] in the R programming environment (version 4.0.2).[27] Each model was specified with a random intercept for each participant. For model parsimony, we applied a backwards elimination procedure, using the *lmerTest* step function, to exclude all non-significant factors. Significant main effects or interactions were explored using Tukey contrasts. Cohen's *d* was calculated as an effect size (ES) statistic and results classified, as follows: small (0.20), medium (0.50), and large (0.80), respectively.

To identify individual performance regulators of androgen baselines or reactivity to exercise, we tested for between-person relationships between each performance (training hours, VO_2, W_{max}) and hormonal (pre-exercise concentration, AUC) measure using Pearson product-moment correlation coefficients (*r*). Consistent with other work, correlations were broadly interpretable as being either weak ($r \geq 0.30$), moderate ($r \geq 0.50$), strong ($r \geq 0.70$) or

perfect (r = 1.00)[28]. Statistical significance was set at an alpha level of p<0.05 for all analyses.

RESULTS

Participant demographics and performance

The male cyclists were significantly older, taller, and heavier (including a larger body mass index) than the female cyclists with large ES differences (Table 1). The male cyclists also produced superior outcomes (VO_2, W_{max}) during initial performance testing (p<0.001), again with large ES differences. Although self-reported training hrs during the heavy phase was similar for both genders, it differed significantly during the light phase (males > females) with a large ES. Phase differences were verified by lower (p<0.001) reported training hrs during a light (vs. heavy) training phase for both males (ES= -6.1) and females (ES= -4.7).

Insert Table 1 here.

Baseline androgen concentration

In the first instance, a preliminary analysis of baseline (pre-trial) hormones was conducted using a two-way (Gender, Training, Gender × Training) ANOVA followed by the stepwise procedure. As can be seen in Figure 1, pre-trial TT (1A), FT (1C), DHT (1E), ST (1G), and SDHT (1I) concentration for males was significantly higher than for females (all large ES differences). Also, we identified significantly higher concentrations for the TT, DHT, ST, and SDHT measures with heavy training load status compared to light training load status (all small to large ES differences). A gender × training status interaction also emerged for ST (p=0.009), but post-hoc results paralleled the above gender and training status effects (p<0.001, large ES differences).

Insert Figure 1 here.

Androgen (AUC) response to sprint exercise

The AUC data are presented (see Figure 1) as estimated marginal means (EMM) with a 95% CI. There was a significant gender difference in the serum TT (1B), FT (1D) and DHT (1F) AUC results (all p<0.01, medium to large ES differences), with greater responsivity noted among females than males, but no significant gender differences were observed for ST and SDHT AUC. Significant training-related differences were also seen for serum TT (1B), DHT (1F), and SDHT (1J) AUC, being higher during a heavy training phase relative to a light phase (all large ES differences); and a significant gender × training status interaction emerged for serum TT AUC. Post-hoc contrasts revealed the highest TT AUC among females during a heavy training phase (EMM = 19.4, 95% CI 12.2, 26.6), which differed (p<0.01, large ES) from their light phase (EMM = 6.4, 95% CI -0.8, 13.6) and both training phases among males (heavy EMM 4.3, 95% CI -2.9, 11.5; light EMM = 4.0, 95% CI -3.2, 11.2).

Individual correlates of hormone activity and reactivity to sprint exercise

Several moderate to strong correlations were seen in this study (p<0.05, see Table 2), most noticeably for baseline TT concentration for males and females regardless of training status; however, the relationship was stronger for males. We saw similar relationships for baseline FT and training status when training was heavy for both males and females, and for FT when compared with VO_2 and maximum work capacity. Fewer (significant) correlations, and of weaker strength, emerged between exercise performance and the androgen AUC results.

Insert Table 2 here.

DISCUSSION

This study aimed to establish whether a difference in androgen concentrations and responsiveness exists among trained cyclists during high (heavy) volume versus low (light) volume training periods. An additional aim was to explore whether male versus female differences exist on these outcomes; and, finally, we also sought to establish whether the androgens testosterone and DHT were correlated to acute exercise performance.

Perhaps not surprisingly, baseline testosterone and DHT concentrations were typically higher with heavy volume than the light volume, and males were higher than females in both loading phases, but the change from heavy to light volume was more marked in females. Androgen responsiveness to exercise appeared greater during a heavy volume, and for females. This helps to explain the gender × training status interaction observed for baseline ST, and for serum TT responsiveness to exercise. There were several important correlations to note between baseline serum testosterone and ST (with both TT and FT often linked to self-assessed state and $\dot{V}O_2$ particularly for females) and for heavy training load. While testosterone markers correlated to acute performance, there was no significant DHT correlations with these particular exercise performance indices, despite clear correlations of DHT to load. Consequently, and building on results from Smith et al.,[1] our data suggests that, similarly to testosterone, DHT increases in response to acute exercise; and DHT responsiveness to exercise appears greater than testosterone. However, the novelty in our results was that androgen responsivity to exercise (i.e. both testosterone and DHT when expressed relative to baseline) appears greater for females compared to males, and for high volume versus low volume states as indicated by effect sizes. Further, somewhat contrary to the hypothesis presented by Smith et al.,[1] increases in DHT in this study did not associate with acute exercise performance; testosterone did however. Testosterone has been linked to emotional and behaviour change related to training,[3,5,6] and enhanced competitive performance,[5,8,9] suggesting that a major linkage between testosterone and training could be motivational in nature.

To some extent this study supports the previous theory of a common pathway of androgenic promotion.[1] More importantly, an interesting and novel finding from this study was the difference observed for androgen responses to exercise when undertaking a recent heavy volume versus a lighter volume of training, seemingly irrespective of being male or female or being a recreational or competitive cyclist. Of particular note was the greater serum DHT response with a recent heavy volume training load. The difference in DHT responsiveness may suggest that training volume status can influence the ability to metabolize testosterone in humans. This observation is similar to work conducted in amateur football in the mid 1980's,[29] and the theory is supported by rat studies which have shown upregulation of 5α-reductase in skeletal muscle of chronically trained rats.[30] It may also explain differing opinions on the relative metabolism of testosterone to DHT;[1,16] training status may not have been considered in the manner of our present study. Nevertheless, given the rapid rate at which 5α-reductase breaks down FT, an upregulation of 5α-reductase is likely to result in a rapid increase in DHT concentration, even in the absence of an increase in serum FT. Consequently, these results are suggestive of androgenic promotion capabilities that increase with training and decline with detraining, as reflected by lower volume.

Surprisingly, and despite an increase in responsiveness of DHT to exercise, DHT was not associated with any index of acute exercise performance. Potentially, given increased affinity of androgen receptors for DHT over testosterone, there may be differences in availability and turnover.[16] Dissociation of DHT from androgen receptors is approximately

three times slower than for testosterone.[16] A possible role therefore for DHT may be longer in nature. While circulating testosterone concentration quickly returns to pre-exercise levels, DHT remains active at the cellular level. Given that DHT is also active in mobilization of energy stores[2,19] and plays a role in protein synthesis,[1,2,31] it may be that DHT has more effect in enhancing chronic training adaptations, providing a better permissive environment. This theory is somewhat intertwined with the concept that androgen promotion capability increases with training and decreases with detraining, especially given the potential upregulation of 5α-reductase with a chronic training load.[19] Further support comes from rodent studies, which have shown a relationship between skeletal muscle mass and DHT concentration,[2] and work showing a rise in baseline serum DHT amongst chronicallytrained aged males.[20] As such, it would be informative to monitor changes in DHT concentration across training cycles. Speculatively, while testosterone may be a marker of acute performance behavior, possibly due to behavioral mechanisms, motivation and attention,[5,10] DHT may be a better marker of adaptive ability and change. That is, the evidence we present suggests that DHT, via training volume induced androgenic promotion, may offer greater long term adaptive support than testosterone; and this may be of larger relative magnitude in females.

Although this study holds a great deal of novelty, it is important that the results are evaluated with knowledge of its limitations. Firstly, we saw some inconsistency in results between serum and saliva outcomes; although measures of serum and salivary androgens are closely related, they do not perfectly correlate[24,32] and temporal sampling factors are a confounder. A limit on this study is that we needed, for compliance, to time saliva collections concurrently with blood sampling. It is well recognised that there is a lag between concentrations in blood and those in saliva.[1,10] It is generally accepted that serum measurement is best practice, therefore slight variation in androgen concentration in saliva from the 'gold standard' could also explain, in part, many of the non-significant correlations for ST and SDHT. The sample size (n=14 per gender) is another consideration when interpreting the performance-androgen correlations, as the minimum correlation we can detect at 80% power (α=0.05) is approximately 0.68. Additionally, performance was assessed some time before hormonal data collection. Secondly, we recognise some experimental bias might arise from the AUC calculations with limited, and uneven, blood- and saliva-sampling points. Nevertheless, our methodological approach and statistical methods were robust and results can be interpreted within that confidence. Finally, it is worth noting the significant difference in age between genders. It is unlikely to have affected the outcomes given the robustness of our methodology (including how participants were grouped), however, it might be recommended that genders are age matched in future studies.

PRACTICAL APPLICATIONS

This work supports a considerable body of research which has shown that short bouts of intense exercise will induce a spike in testosterone, and that acute performance itself may relate to individual testosterone concentration. A novel finding is that in chronic training adaptations DHT may be important to maximise training adaptations. This offers further knowledge towards understanding if, where and how biomarkers may help chart training.

CONCLUSION

This study demonstrates timelines and relationships of androgenic hormone components to exercise, performance metrics and gender. A novel finding was the difference between heavy and light training volume for androgen responsiveness to exercise irrespective of gender. Testosterone correlated to acute performance, DHT did not. DHT responsiveness was greater with higher volumes, especially in females. We speculate that FT has an impact on acute

performance potentially via the behavioral mechanisms of motivation and attention. Further, we suggest DHT via training load induced androgenic promotion may offer longer term adaptive changes, particularly in females.

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The authors have no conflicts of interests. The results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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FIGURE CAPTIONS

Figure 1. Androgen area under the curve (AUC) responses to a sprint exercise protocol. Sub-figures on the left represent the sampled data, shown as box plots with median line, 25th-75th percentile and 10th-90th percentile error bars. Sub-figures on the right represent the AUC results, presented as estimated marginal means with a 95% CI. TT = serum total testosterone, FT = serum free testosterone, DHT = serum dihydrotestosterone, ST = salivary testosterone, SDHT = salivary dihydrotestosterone. Significant difference between factors *p<0.05.

Table 1. Age, anthropometric, and performance data for trained male and female cyclists. Data are presented as means (±SD).

Variable	Male (n=14)	Female (n=14)	*p* values	Effect size
Age (years)	24.7 (2.1)	21.9 (1.3)	<0.001	1.6
Standing height (m)	1.82 (0.05)	1.70 (0.05)	<0.001	2.3
Body mass (kg)	75.7 (6.3)	58.9 (3.8)	<0.001	3.2
BMI (kg/m^2)	22.9 (1.2)	20.4 (0.9)	<0.001	2.3
Peak VO_2 (mL/kg/min)	59.0 (7.6)	48.5 (6.2)	<0.001	1.5
Maximum power (W)	336 (66.6)	230 (38.5)	<0.001	1.9
Heavy training (hrs)	12.6 (2.7)	11.9 (2.6)	0.477	0.3
Light training (hrs)	2.6 (1.2)	1.4 (1.3)	0.010	1.1

Key: BMI = body mass index.

Table 2. Correlations between individual performance indicators and androgen (pre-test and AUC) concentration measures.

			Pre-test values					AUC values				
Performance	Gender	Training	TT	FT	DHT	ST	SDHT	TT	FT	DHT	ST	SDHT
Training hours	Male	Heavy	**0.93**	**0.79**	0.42	-0.11	0.35	0.18	0.03	0.08	**0.63**	-0.11
		Light	**0.70**	0.48	0.37	0.41	0.46	0.14	0.12	0.15	0.40	0.05
	Female	Heavy	**0.62**	**0.85**	0.35	**0.80**	0.33	0.26	0.34	0.30	0.41	0.21
		Light	**0.57**	0.48	0.26	0.35	0.27	-0.03	-0.20	-0.24	-0.29	-0.12
Peak VO_2	Male	Heavy	0.32	0.18	0.32	-0.46	0.27	-0.06	-0.04	-0.18	0.41	-0.05
		Light	0.09	-0.26	0.38	-0.40	0.20	0.01	0.33	-0.50	0.25	0.16
	Female	Heavy	0.36	**0.78**	0.37	**0.60**	0.39	0.38	0.22	0.24	**0.53**	-0.14
		Light	**0.69**	**0.59**	0.26	0.44	0.33	-0.43	0.08	0.23	0.20	-0.31
Maximum power output	Male	Heavy	0.52	0.46	0.37	-0.21	0.40	0.04	0.03	0.16	0.31	-0.05
		Light	0.48	0.21	0.37	-0.03	0.36	0.00	0.05	-0.31	0.28	0.08
	Female	Heavy	0.33	**0.76**	0.33	0.37	0.26	0.36	0.04	0.09	0.41	-0.19
		Light	**0.58**	0.35	0.08	0.28	0.11	-0.28	0.46	0.22	0.26	-0.17

Key: TT = serum total testosterone, FT = serum free testosterone, DHT = serum dihydrotestosterone, ST = salivary testosterone, SDHT = salivary dihydrotestosterone. Significant correlations (p<0.05) are highlighted in bold

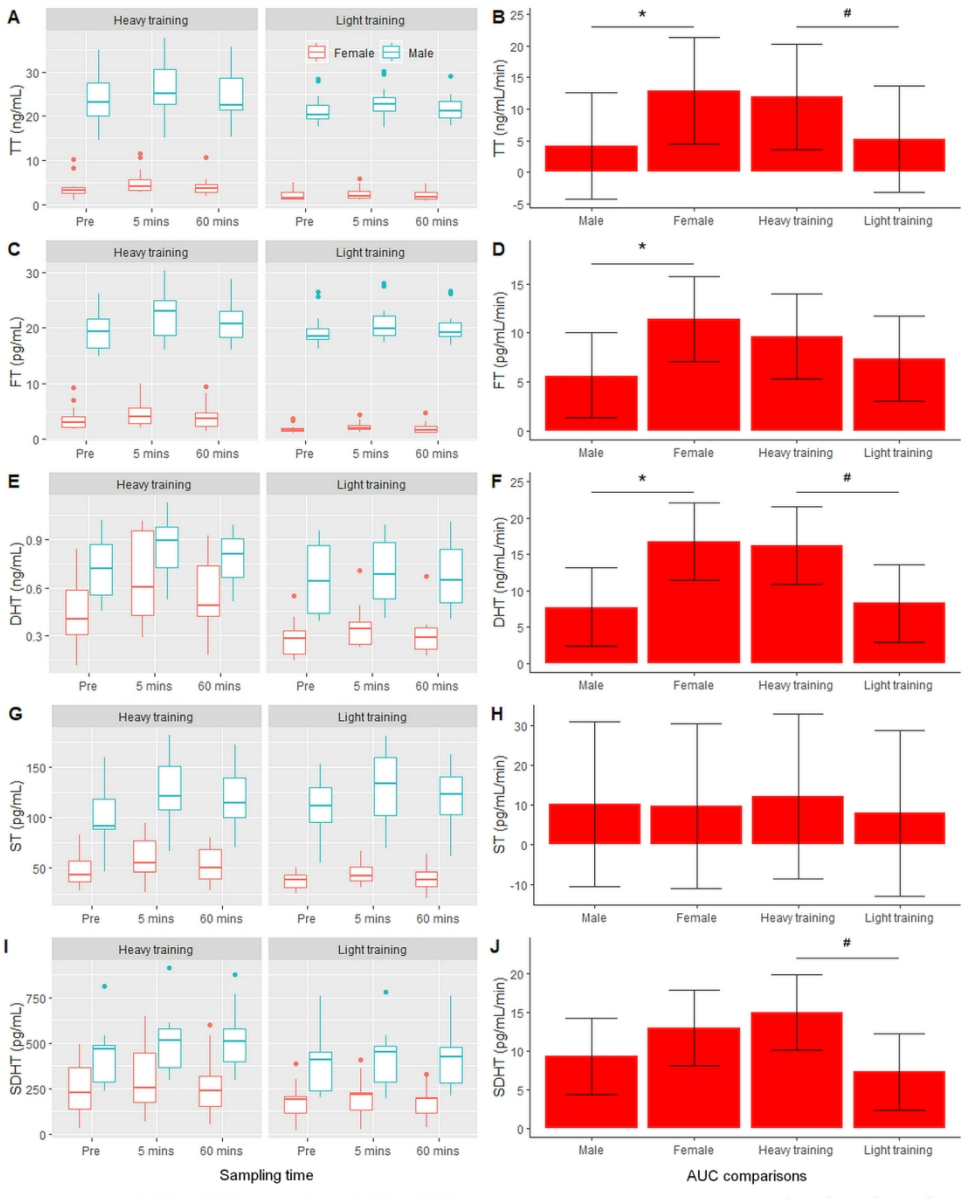

Figure 1. Androgen area under the curve (AUC) responses to a sprint exercise protocol. Sub-figures on the left represent the sampled data, shown as box plots with median line, 25th-75th percentile and 10th-90th percentile error bars. Sub-figures on the right represent the AUC results, presented as estimated marginal means with a 95% CI. TT = serum total testosterone, FT = serum free testosterone, DHT = serum dihydrotestosterone, ST = salivary testosterone, SDHT = salivary dihydrotestosterone. Significant difference between factors *p<0.05.

129x161mm (300 x 300 DPI)