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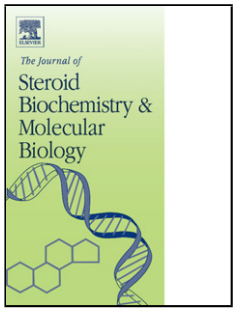

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Comparing metabolic profiles between endurance athlete and non-athlete females reveals differences in androgenic and corticosteroids levels

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Abstract

Endurance training is associated with physiological changes in elite athletes, but little is known about female-specific effects of endurance training. Despite the significant rise in female sports participation, findings from studies performed on male athletes are largely extrapolated to females without taking into consideration sex-specific differences in metabolism. Subsequently, this study aimed to investigate the steroid hormone profiles of elite female endurance athletes in comparison with their non-athletic counterparts. Untargeted metabolomics-based mass spectroscopy combined with ultra-high-performance liquid chromatography was performed on serum samples from 51 elite female endurance athletes and 197 non-athletic females. The

results showed that, compared to non-athletic females, certain androgenic, pregnenolone, and progestin steroids were reduced in elite female endurance athletes, while corticosteroids were elevated. The most significantly altered steroid hormones were 5alpha-androstan-3alpha,17alpha-diol monosulfate (FDR = 1.90×10^{-05}), androstenediol (3alpha, 17alpha) monosulfate (FDR = 2.93×10^{-04}), and cortisol (FDR = 2.93×10^{-04}). Conclusively, the present study suggests that elite female endurance athletes have a unique steroid hormone profile with implications on their general health and performance.

Keywords: steroids; endurance training; metabolomics; female; athletes; sports.

1. Introduction

Endurance training is a type of exercise that seeks to progressively improve the anaerobic threshold, i.e., the point when anaerobic metabolism is initiated, and results in complex alterations to the muscle metabolism [1]. Skeletal muscle cells respond to regular bouts of endurance exercise via mitochondrial adaptation, including increase in mitochondrial numbers, energetic demand, and fatty acid oxidation [2]. In fact, fatty acid oxidation is improved by endurance training, which also contributes to a decreased utilization of carbohydrates in elite endurance athletes [3]. Endurance training also increases the capacity for oxygen transport, resulting in an enhanced performance and greater resistance to fatigue [4]. Like other types of exercise, endurance training is generally beneficial to one's health, but, at the elite level, it can cause damage to the airways as well as an increased susceptibility to asthma development [1,5].

Despite the dramatic increase in female sports participation in the past decades, studies that are specific to elite female athletes lag behind those on males, and the physiological findings from male-specific research are simply extrapolated to their female counterparts [3,6]. The lack of female-specific data is especially problematic considering that elite female endurance athletes are commonly affected with impaired bone health, low energy availability, and menstrual dysfunction, a trio of conditions that is referred to as the female athlete triad [7]. Disordered eating is also prevalent among elite female endurance athletes, a condition that further exacerbates the female athlete triad and contributes to a deficiency of steroid hormones [8–10].

Steroid hormones can be grouped into two major categories: the corticosteroids and the sex steroids. Corticosteroids, which are further divided into glucocorticoids and mineralocorticoids, mediate stress responses, inflammation, and carbohydrate metabolism, while the sex steroids, which are classified into androgens, estrogens, and progestogens, are involved in the development of sexual characteristics [11]. In a sports context, different concentrations of

circulating steroid hormones, namely testosterone and estrogen, can significantly impact the athletic performance of female athletes.

Circulating levels of testosterone, the primary androgen in males, are generally accepted as the hormonal basis for sex-based differences in athletic performance, with men having circulating testosterone levels that are 15- to 20-fold higher than those in women [12]. Female athletes with circulating testosterone levels above the normal range, either due to doping or hyperandrogenism, exhibited a significantly enhanced performance compared to their normal counterparts [13]. In contrast, estrogen contributes to reduced athletic performance and a higher risk of catastrophic ligament injury in female athletes, and circulating levels of estradiol, the primary estrogen in females, fluctuate through the month due to the menstrual cycle [13,14].

Premenopausal females are thought to be better equipped than males to handle endurance sports due to their superior utilization of fatty acids [15–17]. During low- to moderate-intensity exercise, women typically derived a larger share of their exercise energy expenditure from fatty acids compared to their male counterparts [18]. This sex disparity in substrate utilization is thought to be related to the higher levels of circulating estrogen in women as well as differences in body fat composition and distribution [19–22]. It was suggested that the beta oxidation cycle, which degrades fatty acids (acyl-CoA) into two-carbon (acetyl-CoA) units, may be regulated by estrogen due to the fact that several beta-oxidation enzymes, such as medium-chain acyl-CoA dehydrogenase (MCAD) and short-chain hydroxyacyl-CoA dehydrogenase (SCHAD), have upstream estrogen response elements [20].

In a sports context, analysis of steroid metabolites is largely limited to anti-doping surveillance, and metabolomics profiling is not yet a routine analytical activity of the World Anti-Doping Agency (WADA) [23]. However, emerging lines of scientific inquiry suggests that epigenome-induced changes in metabolism influence elite athletic performance [24]. Only a few studies have investigated the differences in steroid metabolite profiles between elite endurance athletes and non-athletes, especially for females. To help fill this gap, this study aims to explore whether elite female endurance athletes exhibit a distinct steroid signature that distinguishes them from non-athletic females.

2. Material and methods

2.1 Study design

Serum samples from 51 consenting elite female endurance athletes from different endurance sports backgrounds were collected from the anti-doping laboratory in Italy. Briefly, blood samples were collected by doping officers in serum separator tubes, then delivered to the anti-doping laboratory within 36 hours under cooling conditions. Once received, samples were immediately centrifuged to separate the serum and then stored at − 20 °C until analysis. **Figure 1** classifies the sports background of each participant based on the intensity level of the dynamic component, i.e., the estimated percent of maximal oxygen uptake, and the static component, i.e., the estimated percent of maximal voluntary contraction [25]. Elite female endurance athletes were included only if they had competed at the national and/or international levels and had tested negative for prohibited substances. . To act as non-elite athlete controls, metabolomics data from 197 healthy females from a Qatar Biobank cohort was extracted [26]. All sample collection was carried out in accordance with the Declaration of Helsinki, and all protocols carried out in this study were approved by the Institutional Review Boards of Qatar University (QU-IRB 1277-E/20).

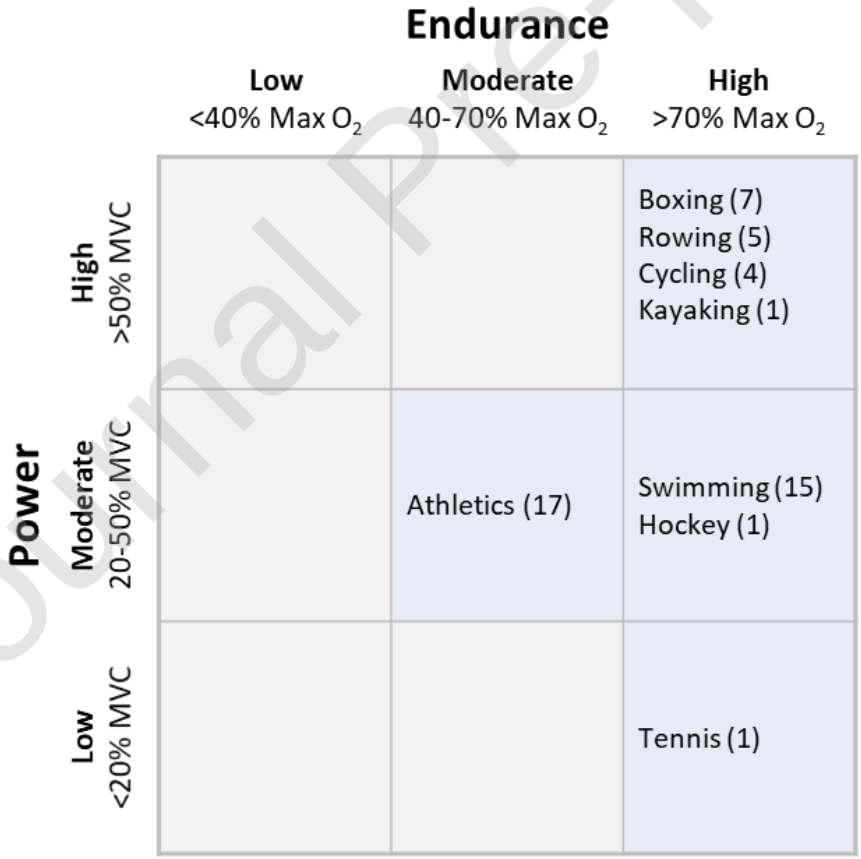

Figure 1. Classification of elite female athletes' sports based on peak dynamic (endurance) and static (power) components achieved while competing.

2.2 Untargeted metabolomics

Serum samples were shipped on dry ice to Metabolon, Inc. (Durham, North Carolina) for untargeted metabolomic profiling in line with established protocol [27]. Briefly, metabolite measurement was carried out using Waters ACQUITY ultra-high-performance liquid chromatography as well as a Thermo Scientific Q-Exactive high-resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. Detected chromatographic peaks were identified by comparing their fragmentation spectra (MS/MS), mass-to-charge (m/z) ratios, and retention time indices to those of authenticated standards in Metabolon's known chemical reference library [28].

2.3 Statistical Analysis

2.3.1 Multivariate Analysis

Analysis was performed using R version 4.0.3 (www.r-project.org/). Raw data from both cohorts were pooled together, quantile normalized to correct for batch effects, and log transformed. Principal component analysis (PCA) was conducted using SIMCA® version 16.0.2 (Sartorius AG, Göttingen, Germany) with the metabolite missingness cut-off set at 50%. Next, orthogonal partial least squares discriminant analysis (OPLS-DA) was performed using SIMCA® version 16.0.2 to examine the discriminatory effect of the metabolites on the phenotype of interest. For this study, our focus was on 29 metabolites related to steroid biosynthesis.

2.3.2 Univariate Regression

A general linear model was run by regressing the dependent variable on independent confounders and phenotype of interest, i.e., athletes and non-athletic females incorporated as categorical variables. The linear model consisted of PC1 as a confounder and was used to assess the difference in steroid synthesis in athletes versus non-athletic females. PC2 was not included in the model as it showed association with the phenotype in the study. The false discovery rate (FDR) was used to correct for multiple testing, and the significance of association was determined to be FDR < 0.05.

3. Results

3.1 Principal component analysis (PCA)

Non-targeted metabolomics was applied to compare the metabolic signatures of 51 female elite athletes and age-matched non-athletic female controls. PCA was performed based on 29 steroid metabolites to capture the global view of the data. Whereas PC1 showed no clear separation between the two groups, PC2 explained 6.53% of the total variance in the data (**Figure 2).** We removed PC2 from the linear regression analysis due to its role as a possible confounder. No other batch effects were seen in our data.

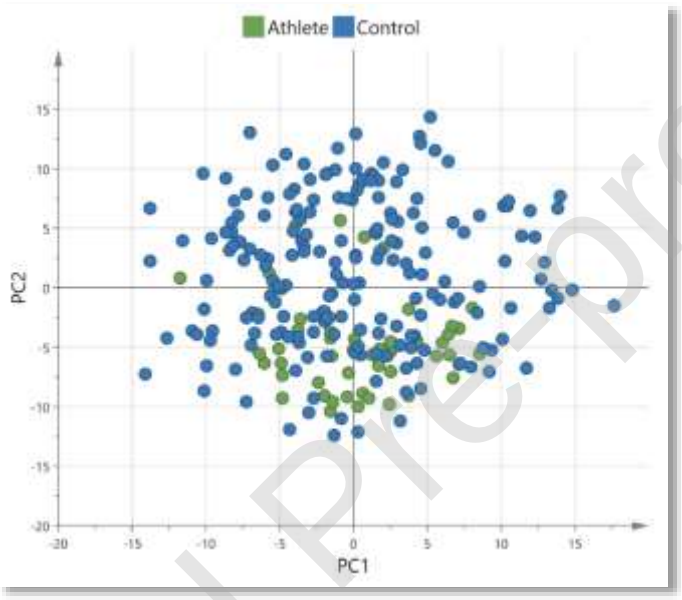

Figure 2. Principal component analysis (PCA) of steroid metabolite levels in elite female endurance athletes and non-athletic females.

3.2 Orthogonal partial least squares discriminant analysis (OPLS-DA)

Based on 29 steroid metabolites, OPLS-DA was performed to identify components that best differentiate between the study groups (elite female endurance athletes and non-athletic females), while dissecting orthogonal components which do not differentiate between these groups. The OPLS-DA score plot showed a remarkable separation of the two phenotypes, suggesting that steroid metabolites are significantly different between the two groups (**Figure 3**). The discriminatory component accounted for 96% of the variation between elite female endurance athletes and non-athletes (R2Y = 0.96, Q2Y=0.90).

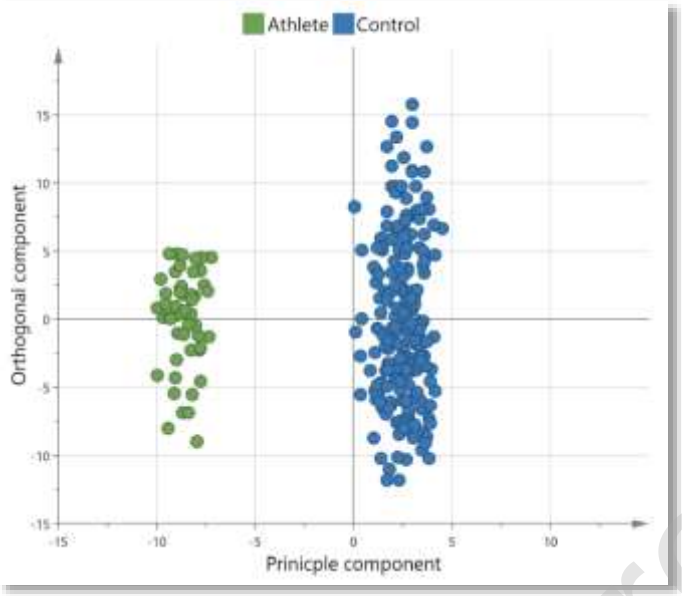

Figure 3. Orthogonal partial least squares discriminant analysis (OPLS-DA) model comparing steroid metabolite levels between elite female endurance athletes and non-athletes.

The corresponding loading scores revealed that androgenic, progestin, and pregnenolone steroids were increased in non-athletic females, while corticosteroids were increased in elite female endurance athletes (**Figure 4**).

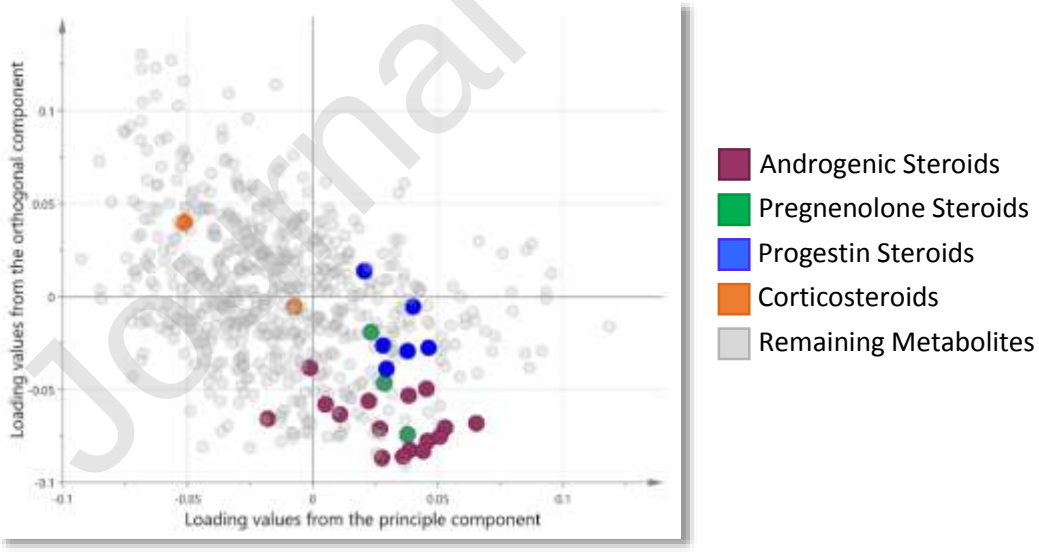

Figure 4. Loading plot of OPLS-DA showing the principal and orthogonal components after quantile normalization.

OPLS-DA also yielded a variable importance projection (VIP) list that reflected the overall importance of the steroid metabolites differentiating elite female endurance athletes and non-athletic females (**Table S1**).

3.3 Linear regression

In order to identify steroid metabolites that significantly change between the two studied groups, the steroid metabolites were regressed against elite female endurance athletes versus non-athletic females after correcting for PC1. Out of 29, 15 steroid metabolites were found to be significantly changed between the two groups (FDR<0.05) (**Table 1**). Androgenic and progestin steroids were lower in elite female endurance athletes, while the corticosteroid cortisol was higher compared to controls. The results of the univariate linear regression model shown in **Table 1** corresponded with the top metabolites shown in the VIP list (**Table S1**) from the multivariate OPLS-DA model.

Table 1. Steroid metabolites that most significantly differentiate between elite female endurance athletes and non-athletic females.

Metabolites	Sub-pathway	Count	Estimate	Std Error	p-value	FDR
5alpha-androstan-3alpha,17alpha-diol monosulfate	Androgenic Steroids	197	-0.45	0.09	6.78×10^{-07}	1.90×10^{-05}
Androstenediol (3alpha, 17alpha) monosulfate (2)	Androgenic Steroids	244	-0.40	0.09	3.13×10^{-05}	2.93×10^{-04}
Cortisol	Corticosteroids	246	0.41	0.09	2.65×10^{-05}	2.93×10^{-04}
Androstenediol (3beta,17beta) monosulfate (1)	Androgenic Steroids	247	-0.38	0.10	1.74×10^{-04}	1.22×10^{-03}
Andro steroid monosulfate C19H28O6S (1)*	Androgenic Steroids	245	-0.47	0.13	2.86×10^{-04}	1.60×10^{-03}
5alpha-androstan-3alpha,17beta-diol monosulfate (2)	Androgenic Steroids	223	-0.43	0.12	6.38×10^{-04}	2.98×10^{-03}
Androsterone sulfate	Androgenic Steroids	248	-0.36	0.11	1.86×10^{-03}	6.52×10^{-03}
5alpha-pregnan-3beta,20beta-diol monosulfate (1)	Progestin Steroids	247	-0.62	0.19	1.75×10^{-03}	6.52×10^{-03}
16a-hydroxy DHEA 3-sulfate	Androgenic Steroids	248	-0.39	0.13	2.67×10^{-03}	8.30×10^{-03}
Androstenediol (3alpha, 17alpha) monosulfate (3)	Androgenic Steroids	248	-0.30	0.10	4.83×10^{-03}	1.13×10^{-02}
21-hydroxypregnenolone disulfate	Pregnenolone Steroids	248	-0.26	0.09	4.66×10^{-03}	1.13×10^{-02}
5alpha-pregnan-3beta-ol,20-one sulfate	Progestin Steroids	185	-0.47	0.16	4.45×10^{-03}	1.13×10^{-02}
5alpha-pregnan-3beta,20alpha-diol monosulfate (2)	Progestin Steroids	247	-0.62	0.23	7.73×10^{-03}	1.66×10^{-02}
Epiandrosterone sulfate	Androgenic Steroids	248	-0.28	0.11	1.27×10^{-02}	2.53×10^{-02}
5alpha-pregnan-3beta,20alpha-diol disulfate	Progestin Steroids	248	-0.41	0.19	2.96×10^{-02}	5.53×10^{-02}

[1] *Count*: number of included samples; *Estimate*: estimated beta value (effect size); *Std error*: standard error; *p-value*: nominal p-value; *FDR*: false discovery rate.

The most significantly associated steroid metabolites in elite female endurance athletes were revealed to be 5alpha-androstan-3alpha,17alpha-diol monosulfate (FDR = 1.90 x 10^{-05}), androstenediol (3alpha, 17alpha) monosulfate (2) (FDR = 2.93 x 10^{-04}), cortisol (FDR = 2.93 x 10^{-04}), androstenediol (3beta,17beta) monosulfate (1) (FDR = 1.22 x 10^{-03}), 16a-hydroxy DHEA 3-sulfate (FDR = 8.30 x 10^{-03}), androsterone sulfate (FDR = 6.52 x 10^{-03}), and 5alpha-pregnan-3beta-ol,20-one sulfate (FDR = 1.13 x 10^{-02}) (**Figure 5**).

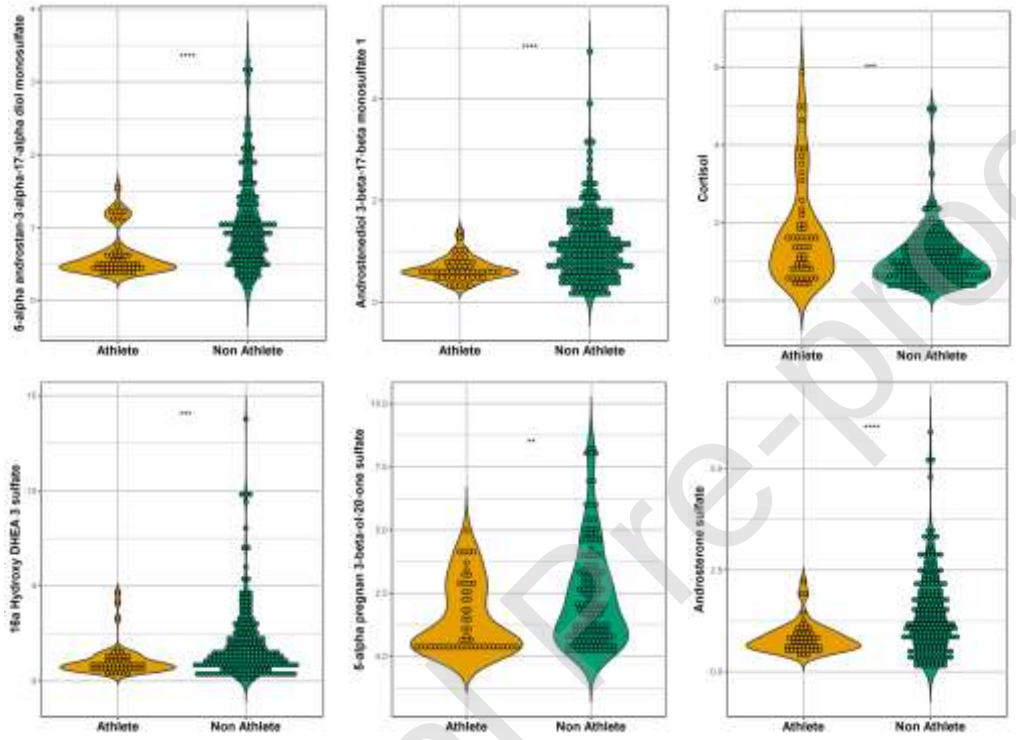

Figure 5. Boxplots of the steroid metabolites that are significantly associated with elite female endurance athletes as revealed by linear regression (FDR < 0.05). ***/***/**** indicate a nominal p-value < 0.001/0.0001/0.00001.*

The biochemical relationships of the 15 significantly associated steroid metabolites (FDR<0.05) were ascertained from the Kyoto Encyclopedia of Genes and Genomes (KEGG) PATHWAY database (https://www.genome.jp/pathway/) (steroid biosynthesis reference pathway map00140) (**Figure 6**).

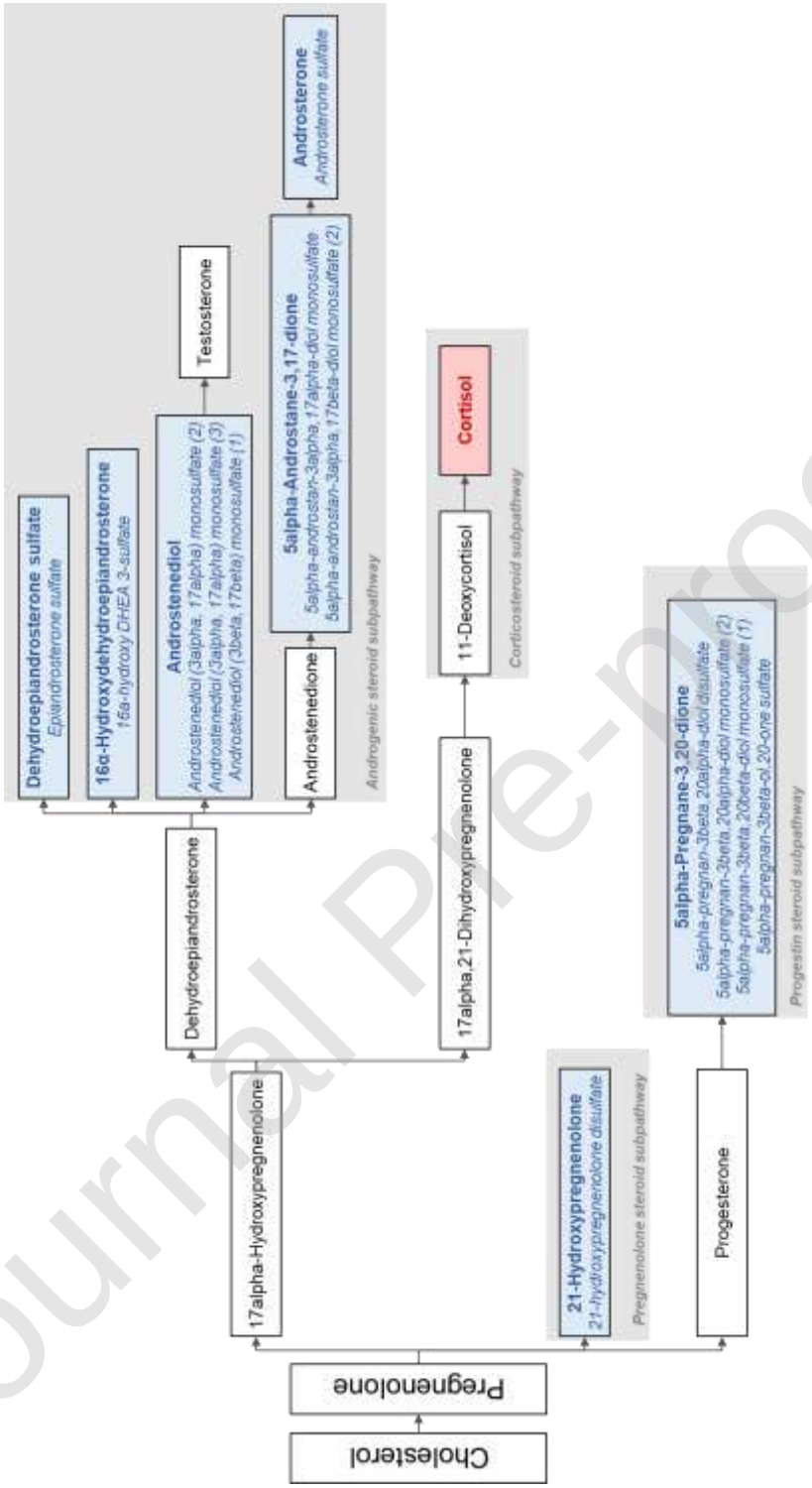

Figure 6. A summary of the biochemical relationships between the steroid metabolites that were significantly associated with elite female endurance athletes. This summary is based on the steroid biosynthesis reference pathway (map00140) from the Kyoto Encyclopedia of Genes and Genomes (KEGG). *Blue shading indicates down-regulated steroid metabolites, while red shading represents up-regulated steroid metabolites. Steroid metabolites are italicized when no exact match is found in KEGG.*

Discussion

Elite athletic performance is dependent on genetics as well physiological adaptation to years of training [29–32]. In particular, endurance-based training remodels the skeletal muscle metabolome by enhancing its oxidative enzyme activity and increasing its mitochondrial content, resulting in a distinct metabolic profile that sets high-endurance athletes apart from their high-power counterparts [27,33]. Previous findings have shown that exercise-induced changes in steroid hormone concentrations are modulated by both the exercise intensity as well as the level of physical fitness [27,34,35]. Taking it further, this study aimed to investigate the differences in steroid metabolism between elite female endurance athletes and non-athletic females.

Our findings revealed that 15 steroid metabolites were significantly altered between elite female endurance athletes and non-athletic females. Except for cortisol, all the significantly altered steroid metabolites were in a sulfated form, causing them to remain inactive until desulfation [36]. Sulfation is an integral part of phase II metabolism, the latter of which encompasses various conjugation reactions that allow metabolites to be excreted from the body [11]. Steroids from four metabolic sub-pathways, i.e., androgens, corticosteroids, progestin, and pregnenolone, were identified as being significantly altered between the two groups.

Androgens can directly enhance sports performance via their effects on behavioral patterns, bone mass, lean body mass, erythropoietin, and visuospatial abilities [13]. In fact, female athletes with high androgen levels are estimated to have a 2-5% greater competitive benefit compared to those with levels within the normal female range [37]. Our findings revealed that among the top changed androgenic metabolites were 5alpha-androstan-3alpha,17alpha-diol monosulfate (FDR = 1.90×10^{-05}), androstenediol (3alpha, 17alpha) monosulfate (FDR = 2.93×10^{-04}), androsterone sulfate (FDR = 6.52×10^{-03}), 16a-hydroxy DHEA 3-sulfate (FDR = 8.30×10^{-03}), and epiandrosterone sulfate (FDR = 2.53×10^{-02}). The precursor to the aforementioned metabolites, dehydroepiandrosterone (DHEA), is classified as a prohibited substance by WADA, but the extent of its impact on athletic performance is still subject to debate, especially in female athletes [38,39].

5alpha-androstan-3alpha,17alpha-diol monosulfate was the most significantly impacted steroid metabolite in elite female endurance athletes (FDR = 1.90×10^{-05}). Interestingly, a previous study found that, in healthy male subjects, changes in 5alpha-androstan-3alpha,17alpha-diol monosulfate levels were associated with resistance exercise and not endurance exercise [40]. 5alpha-androstan-3alpha,17alpha-diol monosulfate levels were significantly decreased in women who used hormone contraceptives and suffered from provoked vestibulodynia, a chronic pain disorder that affected 7 to 16% of the female population [41]. In males, this metabolite was correlated with survival outcome and fatigue levels in prostate cancer patients, and it was identified as a commonly enriched metabolite in young and old Japanese men [42–44]. Metabolome analysis has shown that 5alpha-androstan-3alpha,17alpha-diol monosulfate is

not one of the steroid metabolites impacted by acetaminophen (paracetamol) use [45]. It is worthwhile to note that the vast majority of studies investigating the impact of acetaminophen on endurance performance were conducted in males [46]. Likewise, androstenediol (3alpha, 17alpha) monosulfate has been previously linked to the *SULT2A1* gene during quantitative trait locus analysis [47]. *SULT2A1* encodes a sulfotransferase that catalyzes steroid sulfation in the adrenal glands and liver. Certain *SULT2A1* variants modulate levels of dehydroepiandrosterone sulfate (DHEA-S), an adrenal androgen metabolite, in women with polycystic ovary syndrome [48,49]. DHEA-S is the most abundant steroid hormone in humans, and it is notable in that it, along with its precursor DHEA, is responsible for 75% of estrogens in premenopausal women [50]. Via the activity of the 3-beta–hydroxysteroid dehydrogenase and 5alpha-reductase enzymes, DHEA can be synthesized into epiandrosterone, a testosterone precursor with weak androgenic activity [51].

Epiandrosterone sulfate, the 3-sulfate of epiandrosterone, is associated with the *CYP3A7* variant rs11568825, the latter of which exhibited a significant genome-wide association with DHEA-S [47,52]. Epiandrosterone sulfate has also been identified as a potential biomarker of chronic widespread musculoskeletal pain in adult female twins [53]. Musculoskeletal pain accounts for the majority of young athletes' visits to sports medicine clinics, with female athletes being more likely to suffer from sports-related injuries compared to their male counterparts [54,55]. A growing number of studies have reported that hormonal changes related to the menstrual cycle negatively impact sports performance in elite female athletes [56,57].

Similarly, androsterone sulfate, the 3-sulfate of androsterone, is formed as a result of SULT2A1 activity, and it is the most abundant 5-alpha-reduced androgen metabolite in serum [58]. In men, plasma androsterone sulfate levels can be used as a marker of 5alpha-reductase activity, the latter of which participates in androgen and estrogen metabolism [59]. Interestingly, screening for 5alpha-reductase deficiency can identify disorders of sex development that were previously undiagnosed in young elite female athletes [60].

16alpha-hydroxy DHEA 3-sulfate is an estriol precursor that naturally originates from pregnancy but can be pathological in other contexts, as it is strongly associated with increased breast cancer risk in postmenopausal women [61–63]. This metabolite is also significantly downregulated in cases of rheumatoid arthritis, a disease which affects women 4-5 times more frequently than men [64,65]. In male soldiers undergoing military training, 16alpha-hydroxy DHEA 3-sulfate is significantly correlated with changes in body mass index and energy balance [66].

Unlike the androgenic steroids, the impact of corticosteroids on sports performance is much less agreed upon, with no current evidence that short-term use could enhance performance [67]. In the present study, cortisol, a glucocorticosteroid, was among the top three most significantly altered steroid metabolites between elite female endurance athletes and non-athletic females (FDR = 2.93×10^{-04}). Cortisol is the most abundant endogenous

glucocorticosteroid in humans, mediating the inflammatory and stress responses as well as immune function and carbohydrate metabolism [68]. Prolonged bouts of exercise result in elevated cortisol levels, which, in turn, maintain blood glucose by stimulating gluconeogenesis [69]. Moreover, extreme long-term endurance training might result in telomere shortening, the latter of which may be mediated by cortisol responsivity [70,71]. Other than exercise-induced stress, one possible reason that cortisol was upregulated in the current study could be due to an altered carbohydrate utilization pattern in the elite female endurance athletes.

Although plasma, serum, and salivary cortisol levels are correlated with one another, attempts to compare salivary cortisol levels between athletes and non-athletes have yielded mixed results. One systematic review found that only a few studies reported elevated salivary cortisol levels in female athletes but no such elevation during resting conditions, which indicated a low discriminative capacity between groups [72]. A recent study showed that salivary cortisol levels in elite female athletes were significantly higher in the aftermath of a competition compared to rest or training days [73]. Similarly, another systematic review demonstrated that female athletes did not exhibit a significant anticipatory cortisol response prior to a sport competition [74].

Regarding the remaining subpathways, there was little literature available about the steroid metabolites in the pregnenolone and progestin subpathways. **Table 2** compares the steroid metabolites that were significantly changed in this study with the results of previous serum metabolic profiling studies.

Table 2. Previously reported associations of the 15 significantly changed steroid metabolites between elite female endurance athletes and non-athletic females. *A tick mark indicates that our findings were confirmed in a previous study*.

Cause	Population	Ref.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Acetaminophen use	455 active adults	[45]	✓	✓			✓				✓	✓		✓			
Acute kidney injury	435 cirrhosis patients	[75]									✓						
Depression	99 ART-treated HIV-infected adults	[76]		✓								✓	✓		✓		✓
Early pregnancy	61 young women	[77]	✓				✓	✓	✓						✓	✓	✓
Malaria	199 Gouin children	[78]	✓	✓	✓		✓	✓					✓		✓	✓	
Moderate- and high-endurance sports	191 elite athletes	[27]		✓												✓	
Postmenopausal breast cancer	1,564 female cases and matched controls	[62]	✓			✓		✓			✓			✓			
Provoked vestibulodynia	109 female cases and matched controls	[41]	✓	✓			✓	✓	✓	✓	✓						
Rheumatoid arthritis	236 Arab cases and controls	[64]	✓	✓									✓	✓	✓	✓	✓
Strenuous military training	25 male Norwegian army soldiers	[66]	✓	✓		✓	✓							✓			
T2, T3, and T4 prostate cancers	137 Caucasian male smokers	[79]					✓										
World Trade Center lung injury	223 male firefighters	[80]	✓	✓			✓	✓	✓		✓				✓	✓	✓

1. 16a-hydroxy DHEA 3-sulfate; **2**. 21-hydroxypregnenolone disulfate; **3**. 5alpha-androstan-3alpha,17alpha-diol monosulfate; **4**. 5alpha-androstan-3alpha,17beta-diol monosulfate (2); **5**. 5alpha-pregnan-3beta,20alpha-diol disulfate; **6**. 5alpha-pregnan-3beta,20alpha-diol monosulfate (2); **7**. 5alpha-pregnan-3beta,20beta-diol monosulfate (1); **8**. 5alpha-pregnan-3beta-ol,20-one sulfate; **9**. Andro steroid monosulfate C19H28O6S (1)*; **10**. Androstenediol (3alpha, 17alpha) monosulfate (2); **11**. Androstenediol (3alpha, 17alpha) monosulfate (3); **12**. Androstenediol (3beta,17beta) monosulfate (1); **13**. Androsterone sulfate; **14**. Cortisol; **15**. Epiandrosterone sulfate.

A major limitation of this study is the lack of detailed background information about the study participants. Usage of hormonal contraception and different stages of the menstrual cycle introduce hormonal variations between participants and may act as confounding factors that have not been accounted for in this study [13]. However, the effects of these potential confounders were diluted out when considering the mean differences among the elite female athletes who participated in this study. Other limitations to the present study include the relatively small sample size and the different sports backgrounds of the participants, which is an issue because athletes from different sports disciplines exhibit distinct oxidative, inflammatory, and xenobiotic profiles, all of which may have an effect on steroid metabolism [81–84].

Conclusions

Endurance training on a consistent basis results in physiological and metabolic changes. The findings of the present study suggest that elite female endurance athletes have a distinct steroid hormone profile that sets them apart from non-athletic females. Despite limited information about the participants and possible confounding factors influencing their metabolic profiling, the emerging data revealed significant differences in the levels of various steroid metabolites between the two studied groups. It is worthwhile to note that there is a major dearth of studies in the sports sciences focusing on female athletes. The present study should be viewed as a preliminary exploratory study that will need to be followed by controlled replication studies to confirm our findings and investigate if steroid hormone profiles are linked to demographic factors, health, and/or sports performance in elite female athletes.

Declarations

Supplementary Materials: Table S1 – Variable importance projection (VIP) list of steroid metabolites between elite female endurance athletes and non-athletic females.

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