Circulating Testosterone as the Hormonal Basis of Sex Differences in Athletic Performance

Short title: Hyperandrogenism and Female Athletics

David J. Handelsman[1], Angelica Lindén Hirschberg[2], Stephane Bermon[3]

[1]ANZAC Research Institute, University of Sydney and Department of Andrology, Concord Hospital, Sydney, New South Wales, Australia,

[2] Department of Women´s and Children´s Health, Karolinska Institutet and Department of Gynecology and Reproductive Medicine, Karolinska University Hospital, Stockholm, Sweden

[3] Université Côte d'Azur, LAMHESS Nice, France and International Association of Athletics Federations, Health and Science Department, Monaco

Keywords: testosterone, sex difference, athletic performance, muscle, hemoglobin, disorders of sex differentiation

Words: 13,172 **References:** 161 **Tables:** 5 **Figures:** 8

Correspondence:
Prof DJ Handelsman
ANZAC Research Institute
Sydney NSW 2139
Australia
E: djh@anzac.edu.au

Disclaimer:

DJH is a medical and scientific consultant for International Association of Athletics Federations (IAAF) and member of the International Olympic Committee (IOC) working group on hyperandrogenic female and transgender athletes and to the Australian Sports Anti-Doping Agency and is also a member of World Anti-Doping Agency (WADA)'s Health, Medicine and Research Committee. He has received institutional grant support from Besins Healthcare and Lawley for investigator-initiated clinical studies in testosterone pharmacology and has provided expert testimony in testosterone litigation.

ALH is a medical and scientific consultant for the Swedish Olympic Committee and a member of the IAAF and IOC working groups on hyperandrogenic female athletes and transgender athletes. She has received grant support from IAAF for a study on testosterone and physical performance in women.

SB is a medical and scientific consultant for the IAAF and a member of the IAAF and IOC working groups on hyperandrogenic female athletes and transgender athletes

The authors have no other involvement with any entity having a financial interest in the material discussed in the manuscript. Opinions expressed in this paper are the personal view of the authors and do not represent those of the IAAF, IOC, WADA or Swedish Olympic Committee.

Acknowledgements: The authors are grateful for helpful insights and comments from Alan Vernec and Osquel Barroso (WADA), Peter Harcourt (AFL, FIBA) and Richard Budgett (IOC).

Abstract

Elite athletic competitions have separate male and female events due to men's physical advantages in strength, speed and endurance so that a protected female category with objective entry criteria is required. Prior to puberty, there is no sex difference in circulating testosterone concentrations or athletic performance but from puberty onwards sex difference in athletic performance emerges as circulating testosterone concentrations rise in men because testes produce 30 times more testosterone than before puberty with circulating testosterone exceeding 15-fold those of women at any age. There is a wide sex difference in circulating testosterone concentrations and reproducible dose-response relationship between circulating testosterone and muscle mass and strength as well as circulating hemoglobin in both men and women. These dichotomies largely accounts for the sex differences in muscle mass and strength and circulating hemoglobin levels resulting in at least an 8-12% ergogenic advantage in men. Suppression of elevated circulating testosterone of hyperandrogenic athletes results in negative effects on performance, which are reversed when suppression ceases. Based on the non-overlapping, bimodal distribution of circulating testosterone concentration (measured by liquid chromatography-mass spectrometry) and making allowance for women with mild hyperandrogenism including that of polycystic ovarian syndrome, who are over-represented in elite athletics, the appropriate eligibility criterion for female athletic events should be a circulating testosterone of less than 5.0 nmol/L. This would include all women other than those with untreated hyperandrogenic disorders of sexual development (DSD), testosterone-treated female-to-male (F2M) transgender, noncompliant male-to-female (M2F) transgender or androgen doping.

1. Background

Virtually all elite sports are segregated into male and female competitions. The main justification is to allow women a chance to win, as women have major disadvantages against men who are, on average, taller, stronger, faster and have greater endurance due to their larger, stronger muscles and bones as well as a higher circulating hemoglobin. Hence, elite female competition forms a protected category with entry that must be restricted by an objective eligibility criterion related by necessity to the relevant sex-specific physical advantages. The practical need to establish an eligibility criterion for elite female athletic competition led the International Association of Athletic Federations (IAAF) to establish a rule in 2011, endorsed by the International Olympic Federation (IOC) in 2012, for hyperandrogenic women. The first IAAF regulation stated that for athletes to be eligible to complete in female events, the athlete must be legally recognised as a female and, unless she has complete androgen insensitivity, maintain serum testosterone less than 10 nmol/L. That IAAF eligibility rule was challenged by an athlete to the Court for Arbitration in Sports (CAS) which ruled in 2015 that, although an eligibility criterion was justified, the scientific grounds for the original IAAF rule was considered insufficient, notably in the extent of the competitive advantage enjoyed by hyperandrogenic athletes who had circulating testosterone greater than 10 nmo/L. The CAS suspended the hyperandrogenism eligibility rule pending receipt of such evidence. In that context, the present paper reviews the available evidence on the hormonal basis for sex differences in athletic performance. It concludes that the evidence justified a revised eligibility criterion of a threshold circulating testosterone concentration of 5 nmol/L (measured by a mass spectrometry method).

2. Sex, Fairness and Segregation in Sport

If sport is defined as the organized playing of competitive games according to rules (1), fixed rules are fundamental in representing the boundaries of fair sporting competition. Rule breaking, whether by breaching eligibility or competition rules, such as use of banned drugs, illegal equipment or match fixing, creates unfair competitive advantages that violates fair play. Cheating constitutes a fraud against not just competitors but also spectators, sponsors, the sport and the public. In the absence of genuine fair competition, elite sport would lose its wide popular appeal and ability to captivate and inspire with the authentic attraction of genuine contest between highly trained athletes.

Nevertheless, fairness is an elusive, subjective concept with malleable boundaries that may change over time as social concepts of fairness evolve. For example, until the late 19th century when organized sports trainers emerged, training itself was considered a breach of fairness since competition was envisaged at that time as a contest based solely on natural endowments. Similarly, sports once distinguished between amateurs and professionals. The concept of fairness has deep and complex philosophical roots mainly

focused on notions of distributive justice. These considerations impact on sport through the universal application of anti-discrimination and human rights legislation. Less attention is given to the philosophical basis of fair competition in elite sport where the objectives are not egalitarian but aim to discover a hierarchy of achievement derived from a mixture of unequal natural talent and individual training effort. Excellent, insightful discussion of the legal and moral complexities of sex and fair competition in elite sports from a legal scholar and former elite female athlete is available (2).

The terms sex and gender are often confused and used as if interchangeable. Sex is an objective, specific biological term with distinct, fixed facets, notably genetic, chromosomal, gonadal, hormonal and phenotypic (including genital) sex, each of which has a characteristic defined binary form. While all facets of biological sex are almost always aligned so that assignment of sex at birth is straightforward, rare instances where any two or more facets of biological sex conflict constitute an intersex state, now referred to as Disorders (or Differences) of Sex Development (DSD)(3). By contrast, gender is a subjective, malleable, self-identified social construct which defines a person's individual gender role and orientation. Prompted by biological, personal and societal factors, volitional expression of gender can take on virtually any form limited only by the imagination with some individuals asserting they have not just a single natal gender but two genders, none, a distinct third gender or gender that varies (fluidly) from time to time. Hence, while gender is usually consistent with biological sex as assigned at birth, in a few it can differ during life. For example, if gender were the basis for eligibility for female sports, an athlete could conceivably be eligible to compete at the same Olympics in both female and male events. These features render the unassailable personal assertion of gender identity incapable of forming a fair, consistent sex classification in elite sport.

The strongest justification for sex classification in elite sport is that after puberty men produce 20 times more testosterone than women (4-7) resulting in circulating testosterone concentrations 15 times higher than in children or women of any age. Age-grade competitive sporting records show no sex-related advantages prior to puberty onwards, whereas from the age of male puberty onwards there is a strong and ongoing male advantage (8). The striking male post-pubertal increase in circulating testosterone provides a major, ongoing, cumulative and durable physical advantage in sporting contests by creating larger and stronger bones, greater muscle mass and strength, and higher circulating hemoglobin as well as possible psychological (behavioural) differences. In concert, these render women, on average, unable to compete effectively against men in power-based or endurance-based sports.

Sex classification in sport therefore requires proof of eligibility as only women should compete in the protected (female) category. This deceptively simple requirement for fairness is taken for granted by peer female competitors who regard participation by males, or athletes with physical features closely resembling males, as unfair. This makes policing of eligibility inescapable for sports to avoid unfair male

participation in female events. Yet, such policing inevitably intrudes into highly personal matters so that it must be achieved with respect for dignity and privacy demanding use of the least invasive, scientifically reliable means. Unsurprisingly this dilemma has always been highly contentious since it first entered international elite sports in the early 20th century and it has become increasingly prominent and contentious in recent decades; nevertheless, the requirement to maintain fair play in female events will not disappear as long as separate female competitions exist. Over recent decades there has been progressively better understanding of the complex biology of genetic sex determination and the impact of pubertal sexual maturation in establishing phenotypic sexual dichotomy in physical capabilities. These sex dichotomous physical features form the basis of, but remain quite distinct from, adult gender roles and identity. Over the last century as knowledge grew the attempts to formalize a scientific basis for the unavoidable necessity of policing eligibility for the female category have been continually challenged. Most recently, the increasing assertion of gender self-identification as a social criterion has further challenged the hegemony of biology for determining "sports sex", Coleman's apt term (2). Allowing subjective gender self-identification to become the sole criterion of sports sex would allow for gaming and perceptions of systematic unfairness to grow. The case for women's sports being defined by sex rather than gender, including the consequences of acceding to gender-based classification have been outlined (9) in arguing the importance of proper medical management of athletes intending to compete in female events.

Separate male and female events in sport is a dominant form of classification that is superimposed on other graduated age group and weight (e.g. weightlifting, power lifting, wrestling, boxing, rowing) classifications, which reflect differences in strength, power, speed to ensure fairness in terms of opportunity to win and, additionally, safety in contact sports. Age and weight classifications rely on objective criteria (birth date, weigh-in weight) for eligibility as necessarily should sex classification. Nevertheless, some power sports dependent on explosive strength and power (eg throwing events, sprinting) do not segregate weight classes, while other sports where height is an advantage (eg basketball, jockeys) do not have height classifications. These sports disproportionately attract athletes with greater weight and/or power-to-weight ratio or advantageous stature, respectively. If sex classification were eliminated such open or mixed competitions would be dominated almost exclusively by men. It therefore seems highly unlikely that sex classification would ever be discarded despite calls on philosophical or sociological grounds to end "gender" classification in sport (10).

3. Sex difference in circulating testosterone levels

3.1 Testosterone biosynthesis, secretion and regulation in men and women

An androgen is a hormone capable of developing and maintaining masculine characteristics in reproductive tissues (notably the genital tract, and other tissues and organs associated with secondary sexual

characteristics and fertility) and contributing to the anabolic status of non-reproductive body tissues (11). The two dominant bioactive androgens circulating in mature mammals, including humans -- testosterone and its more potent metabolite, dihydrotestosterone (DHT) -- account for the development and maintenance of all androgen-dependent characteristics, and their circulating levels in men and non-pregnant women arise from steroids synthesized *de novo* in the testes, ovary or adrenals (12).

The sexually undifferentiated gonads in the embryo develop into either ovaries or testes according to whether a y chromosome (or at least the *sry* gene) is present. After birth and until puberty commences, circulating testosterone concentrations are essentially the same in boys and girls, other than briefly in the neonatal period of boys when higher levels prevail. The onset of male puberty, a brain-driven process triggered by a still mysterious hypothalamic or higher cerebral mechanism (13), initiates the hormonal cascade of puberty. In males this leads to enhanced pituitary luteinizing hormone (LH) secretion that stimulates the 500 million Leydig cells in the testes to secrete 3-10 mg (mean 7 mg) of testosterone daily (4, 6, 7, 14, 15). This creates a very high local concentration of testosterone within the testis as well as a steep downhill concentration gradient into the bloodstream that maintains circulating testosterone levels at adult male levels, which are tightly regulated by strong negative hypothalamic feedback of circulating testosterone. However, in the absence of testes these mechanisms do not occur in females. In girls, serum testosterone increases during puberty (16), peaking at age 20-25 years before declining gradually with age (17, 18) but it remains less than 2 nmol/L at all ages, as determined by a reliable method (see below). In adult women, circulating testosterone is derived from three roughly equal sources – direct secretion from the adrenal gland or the ovary as well as indirectly from extra-glandular conversion (in liver, kidney, muscle, fat, skin) from testosterone precursors secreted by the adrenal and ovary. However, in combination these different sources produce about 0.25 mg of testosterone daily so that throughout life women maintain circulating testosterone levels of less than 2 nmol/L. Circulating testosterone concentrations in women are subject to little dynamic physiological regulation. As a result, circulating testosterone concentrations in healthy pre-menopausal women are stable (non-fluctuating) and not subject to strong negative feedback by exogenous testosterone like men. Even the small rise (50%) at the time of the mid-cycle LH surge triggering ovulation (19), remains within the physiological range for pre-menopausal females. In summary, only when circulating testosterone concentrations in male adolescents rises above the circulating pre-pubertal concentrations does the virilisation characteristic of men commence, progress and remain throughout adult life at least until old age (18).

3.2 Male and female reference ranges for circulating testosterone

A reliable threshold for circulating testosterone must be set using measurement by the reference method of liquid (or gas) chromatography-mass spectrometry (LC-MS) rather than using one of the various available commercial testosterone immunoassays. The necessary reliance on steroid mass spectrometry for clinical

applications in endocrinology, reproductive medicine and sports medicine is widely recognized. It has been standard for decades in anti-doping science (20) and the growing consensus is that it is required for high quality clinical research and practice recognized by cognate professional societies (21, 22) and editorials in leading clinical endocrinology (23) and reproductive medicine (24) journals. The inherently limited specificity of testosterone immunoassays arises from antibody cross-reactivity with structurally related steroids (such as precursors and metabolites) other than the intended target. As a result, all steroid immunoassays including for testosterone display method-specific bias whereby, for example, the lower limit of a testosterone reference range in healthy young men varies from 7.3 to 12.6 nmol/L according to the immunoassay used, so that no consensus definition of a lower limit could be obtained independent of the commercial immunoassay method used (25). Further, testosterone immunoassays are optimized for circulating levels in men but display increasing inaccuracy at the lower, by an order of magnitude, circulating testosterone concentrations in women or children. In contrast to immunoassays, LC-MS based methods are highly specific and do not depend on proprietary antibodies. Using LC-MS-based measurements, method-specific bias can be avoided and a fixed consensus lower reference limit defined (see table 1). Hence, for the precision required in sports medicine, whether for eligibility criteria or anti-doping applications, testosterone in serum must be measured by LC-MS methods.

Prior to puberty, levels of circulating testosterone as determined by LC-MS are the same in boys and girls (16) as well as remaining lower than 2 nmol/L in women of all ages. However, from the onset of male puberty the testes secrete 20 times more testosterone resulting in circulating testosterone levels that are 15 times greater in healthy young men than age similar women. Using LC-MS measurement, circulating testosterone in adults has a strikingly, non-overlapping bimodal distribution with wide and complete separation between men and women. Table 1 summarises data from appropriate reported studies using MS-based methods to measure serum testosterone in healthy men and women. Based on a number-weighted pooling with conventional 95% two-sided confidence limits of the eight available studies using LC-MS measurements of serum testosterone, the limits of the reference range for healthy young men (18 to 40 years) is 7.7 nmol/L to 29.4 nmol/L. Similarly, summarising the nine available studies for healthy menstruating women under 40 years, the 95% (two sided) reference range is 0 to 1.7 nmol/L. These reference limits neglect factors such as oral contraceptive use (26, 27), menstrual phase (19), SHBG (28, 29), overweight (30, 31), fasting and smoking (32), as well as diet (31) and physical activity (33, 34) in women and men, all of which have small effects on circulating testosterone but without materially influencing the divergence between the non-overlapping bimodal distribution of male and female reference ranges of circulating testosterone.

In creating a threshold for eligibility for female events it is also necessary to make allowance for hyperandrogenic women including women with polycystic ovary syndrome (PCOS) and non-classical

adrenal hyperplasia. PCOS is a relatively common disorder among women of reproductive ages with a prevalence of 6-10%, depending on the diagnostic criteria used (35), in which mild hyperandrogenism is a key clinical feature and has higher than expected prevalence among elite female athletes (26, 36-38). Non-classical adrenal hyperplasia is a milder and later (adult) onset variant of classical congenital adrenal hyperplasia (39) with a much higher but still rare population prevalence (1:1000 vs 1:16,000 for the classical variant (40). Table 2 summarises clinical studies (n=16, ≥40 women) reporting serum testosterone concentrations measured by LC-MS in samples from women with PCOS. The pooled data reveals that the upper limit of serum testosterone in women with PCOS is 3.1 nmol/L (95% confidence interval, one sided) or 4.8 nmol/L (using a 99.99% confidence interval, one sided) (table 3). Hence a conservative threshold for circulating testosterone of 5 nmol/L measured by LC-MS would identify fewer than 1:10,000 women with PCOS as false positives, based on circulating testosterone measurement alone. Circulating testosterone higher than this threshold is likely to be due to testosterone-secreting adrenal or ovarian tumors, intersex/DSD, badly controlled or non-compliant M2F transgender athletes or testosterone doping.

3.3 The physiological effects of testosterone depend on the circulating testosterone, not its source (endogenous or exogenous)

Testosterone, whether of natural endogenous or manufactured exogenous source, has an identical chemical structure and biological effects, aside from minor differences in isotopic composition which are biologically insignificant. Regardless of its source, at equivalent doses and circulating levels, exogenous testosterone exerts the same biological and clinical effects on every known androgen-responsive tissue or organ, apart from effects on spermatogenesis, which as discussed below is only a matter of degree. Consequently, exogenous testosterone is a fully effective substitute for endogenous testosterone in therapeutic use, countering the effects of testosterone deficiency due to hypogonadism (reproductive system disorders). Any purported differences between endogenous and exogenous testosterone are, like the differences between men and women, due to corresponding differences in the endogenous production rate or exogenous dose. Such differences in effective exposure lead to corresponding differences in circulating testosterone levels and its effects according to the dose-response curves for testosterone.

Like all hormones and drugs, over their effective range of biological activity the dose-response relationship for testosterone is usually a sigmoidal curve with lower and upper plateaus joined by a monotonically rising middle region, which may be linear in the natural scale but more often log-linear (linear on the log or similar transformed scale). In the middle portion of the typical sigmoidal dose-response curve for the same increase in testosterone dose (or concentration), the response would be increased in simple proportional (ie linear) but more often on a logarithmic scale. By contrast, at the lower and upper plateaus of dose or concentrations, changes in testosterone exposure may evoke minimal or no response on the endpoint. For example, in women of any age circulating testosterone concentrations are along the lower plateau of the dose-response

curve, so that increases in circulating testosterone concentrations within that lower plateau may have minimal or no effect. In female athletes with the mild hyperandrogenism of PCOS, higher performance has been shown (38) with their muscle mass and power performance correlating with androgen levels (26). However, beyond these effects where endogenous testosterone concentrations are in the high-normal adult female range, it is only when the increases in circulating testosterone concentrations substantially and consistently exceed those prevailing in childhood (<2 nmol/L) and among women including those with PCOS (<5 nmol/L) that the effects would replicate rising testosterone concentrations of boy's in mid- to late puberty (typically >8 nmol/l) which cause the masculinizing effects of increased muscle, bone and hemoglobin characteristics of men. As shown above, the circulating testosterone of most women never reaches consistently above 5 nmol/L, a level which boys must sustain for some time to exhibit the masculinizing effects of male puberty.

Secondarily, the effects of testosterone are modulated in a form of fine tuning by the patterns of exposure, such as whether the circulating testosterone is delivered in the un-physiological steady-state format (e.g. quasi-steady state delivery by implant or transdermal products) or by the peak-and-trough delivery of injections as opposed to the natural state of endogenous fluctuations in serum testosterone around the average adult male levels. However, these latter pattern effects are subtle and the dominant effect remains that of dose and average testosterone concentrations in blood, however they arise. Furthermore, there is evidence that the androgen sensitivity of responsive tissues differ and may be optimal at different circulating testosterone concentrations (41).

Male sexual function is maintained by endogenous testosterone at adult male circulating concentrations. These effects can be replicated by exogenous testosterone if and only if it achieves comparable circulating testosterone concentrations. For example, in a well-controlled prospective study of older men with prostate cancer (42), androgen deprivation achieving castrate levels of circulating testosterone sustained over 12 months markedly suppressed sexual desire and function, whereas those effects did not occur in age-matched men having non-hormonal treatment for prostate cancer or those without prostate cancer. In healthy younger men whose endogenous testosterone is fully suppressed, their sexual function completely recovers when circulating testosterone was restored to the physiological male range by administration of exogenous testosterone (43). Similar effects were also observed in healthy, middle-aged men in whom male sexual function was fully maintained (compared with placebo) during 2 years of treatment with an exogenous androgen (DHT) despite it causing sustained, complete suppression of endogenous testosterone (44). This further supports the key interpretation that the biological effects of exogenous or endogenous testosterone are the same at comparable circulating levels.

Clinically, exogenous testosterone replicates fully all effects of endogenous testosterone on every reproductive and non-reproductive organ or tissue, with the sole exception of the testis. Sperm production

in the testis requires a very high concentration of testosterone (typically 100 times greater than in the general bloodstream), which is produced in nature only by the action of the pituitary hormone LH. LH stimulates the Leydig cells in the interstitial space of the testis between seminiferous tubules to produce high intra-testicular concentrations of testosterone, which are necessary and sufficient to initiate and maintain sperm production in the adjacent seminiferous tubules. This high concentration of testosterone also provides a downhill gradient to supply the rest of the body, where circulating testosterone acts on androgen-responsive tissues to maintain masculine patterns of androgenization. When exogenous testosterone (or any other androgen) is administered to men, pituitary LH is suppressed by negative feedback and the sperm production halts for as long as exogenous testosterone or androgen exposure continues, after which it recovers (45). However, even the reduction in spermatogenesis and testis size when men are treated with exogenous testosterone is only a matter of degree. It is well established in rodents (46, 47) that spermatogenesis is induced by exogenous testosterone if the testosterone concentrations in the testis are high enough to replicate what occurs naturally via LH stimulation (48). However, direct replication that high dose testosterone also initiates and maintains spermatogenesis in humans is not feasible as these testosterone doses are 10-100 times higher than could be safely given to humans. Nevertheless, confirmatory evidence in humans is available from rare cases of men with an activating mutation of the CG/LH receptor (49, 50). This mutation causes autonomous testicular testosterone secretion leading to precocious puberty arising from the premature adult male circulating testosterone concentrations which lead to complete suppression of circulating gonadotropin (LH, FSH) secretion. In this illustrative case the testis was exposed to non-physiologically high testosterone concentrations (but without any gonadotropin stimulation) which induced sperm production and allowed for natural paternity (49). This indicates that even for spermatogenesis, exogenous testosterone can replicate all biological effects of endogenous testosterone in accordance with the relevant dose-response characteristics.

The most realistic view is that increasing circulating testosterone from the childhood or female range to the adult male range will have the same physiological effects whether the source of the additional testosterone is endogenous or exogenous. This is strongly supported by well-established knowledge about the relationship of circulating testosterone concentrations with the timing and manifestations of male puberty. The characteristic clinical features of masculinisation (muscle growth, increased height, increased hemoglobin, body hair distribution, voice change etc) appear only if and when circulating testosterone concentrations rise into the range of males at mid-puberty which are higher than in women at any age even after the rise in circulating testosterone in female puberty. If and only if the pubertal rise in circulating testosterone fails, the males affected are clinically considered hypogonadal. Such a failure of male puberty may occur for genetic reasons (arising from mutations that inactivate any of the cascade of proteins whose activity is critical in the hypothalamus to trigger male puberty) or as a result of acquired conditions, caused by pathological disorders of the hypothalamus or pituitary or functional defects arising from severe deficits of energy or

nutrition (eg extreme overtraining, undernutrition), the latter being comparable with hypothalamic amenorrhea or anorexia nervosa in female athletes/ballet dancers. If male puberty fails, testosterone replacement therapy is fully effective in replicating the all the distinctive masculine features apart from spermatogenesis.

3.4 Elevated circulating testosterone concentration caused by DSDs

Rare genetic intersex conditions known as DSDs can lead to markedly increased circulating testosterone in women and, when coupled with ambiguous genitalia at birth, appearing as undervirilized male, or virilized females. This can cause athletes who were raised and identify as women to have circulating testosterone levels comparable with men and much exceeding that of non-DSD (and non-doped) women, including those with PCOS. Key congenital disorders in this category are 46 XY DSDs namely 5α reductase deficiency (51), 17β-hydroxysteroid dehydrogenase type 3 deficiency (52), androgen insensitivity (53, 54) as well as congenital adrenal hyperplasia (55), which is a 46 XX DSD. There is evidence that the first three conditions, components of 46 XY DSDs, are 140 times more prevalent among elite female athletes than expected in the general population (56).

Genetic 5α reductase deficiency is due to an inactivating mutation in the 5α reductase type II enzyme (51). This leads to a deficit of DHT during fetal life when DHT is required for converting the sex-undifferentiated embryonic and fetal tissue to form the sex-differentiated masculine form external genitalia. Although genetic males (46 XY) with 5α reductase deficiency will develop testes, they usually remain undescended and labial fusion to form a scrotum and phallic growth does not occur. Hence at birth the external genitalia may appear feminine, leading to a female assigned natal sex. Thus, individuals with 5α reductase deficiency may have male chromosomal sex (46 XY), gonadal sex (testes), and hormonal sex (adult male testosterone concentrations), but such severely under-virilized genitalia that affected individuals may be raised from birth as females rather than as under-virilized males. However, from the onset of male puberty, testicular Leydig cells start producing large amounts of testosterone, and the steep rise in circulating testosterone to adult male levels (with the permissive role of 5α reductase activity) leads to masculine virilisation, including male patterns of muscle and bone growth, hemoglobin levels and other masculine body habitus features (hair growth pattern, voice change), as well as phallic growth (56). Such changes of male puberty prompt around half affected individuals who had female sex assigned at birth and developed as girls prior to puberty to adopt a male gender identity and role (57). Sperm are formed in the testes so that, using in vitro fertilization, these individuals may father children (58).

Seventeen β-hydroxysteroid dehydrogenase type 3 deficiency (52) has a similar natural history to 5α reductase deficiency. This disorder is due to inactivating mutations in a steroidogenic enzyme expressed only in the testis and which is essential for testosterone formation in the fetus. In the absence of a functional

enzyme, the testis makes little testosterone but instead secretes large amounts of androstenedione, the steroid immediately prior to the enzymatic block. In the circulation, the excess of androstenedione is converted to testosterone (mainly by the enzyme AKR1C3(12)). Although the circulating testosterone is then converted to circulating DHT, insufficient DHT is formed locally within the urogenital sinus to virilise genitalia at birth. This causes the same severe under-virilisation of the external genitalia of genetically male individuals, leading to ambiguous genitalia at birth despite male chromosomal, gonadal and hormonal sex. When puberty arrives, the testes start producing the adult male testosterone output this leads to marked virilisation and subsequent assumption of a male gender identity by some affected individuals, conflicting with a female assigned natal sex and childhood upbringing.

Androgen insensitivity, which arises from mutation in the androgen receptor (AR), poses different but complex challenges for eligibility for female athletic events. As the AR is located on the X chromosome, genetic males (46 XY) are hemizygous, so that an inactivating mutation in the AR can be partially or fully insensitive to androgen action. Affected individuals have male internal genitalia (testes in the inguinal canal or abdomen with Wolffian ducts) and consequently adult male circulating testosterone concentrations after puberty. These non-lethal mutations have a wide spectrum of functional effects, ranging from full resistance to all androgen action in complete androgen insensitivity syndrome (CAIS) where individuals have a full female phenotype with normal female external genitalia, to partial androgen insensitivity syndrome (PAIS) where some androgen action is still exerted leading to various degrees of ambiguous genitalia, or to mild androgen insensitivity which produces a very mild, under-virilised male phenotype (normal male genital and somatic development but with little body hair and no male pattern balding) (53). Testosterone (and dihydrotestosterone) have no consistent effect of inducing normal nitrogen retention (anabolic) responses in patients with CAIS (59-62) although some reduced androgen responsiveness is retained by patients with PAIS (60, 63-66). Athletes with CAIS can fairly compete as females because the circulating testosterone, although at adult male levels, has no physiological effect so that, in terms of androgen action and the ensuing physical somatic advantages of male sex, affected individuals are indistinguishable from females and gain no benefits of the sex difference arising from unimpeded testosterone action. A more complex issue arises with athletes having PAIS reflecting the degree of incomplete impairment of AR function. Residual androgen action in such AR mutations is harder to characterise quantitatively as there is no standardized, objective *in vitro* test to quantify AR functionality. Hence, although individuals with PAIS may have adult male circulating testosterone concentrations but variable androgen sensitivity, at present this requires a case-by-case evaluation, primarily based on the degree of virilisation. The current best available clinical approach to determining the functional impact (degree of functionality/sensitivity) of an AR mutation is based on the degree of somatic, primarily genital, virilisation assessed according to the Quigley classification of grade of androgen sensitivity (67).

Congenital adrenal hyperplasia (CAH) is a relatively common defect in adrenal steroidogenesis in the

enzymatic pathway leading to synthesis of cortisol, aldosterone and sex steroid precursors. The disease varies in severity from life-threatening (adrenal failure) to mild (hirsutism and menstrual irregularity), or even asymptomatic and undiagnosed. The most common mutations causing CAH occur in the 21 hydroxylase enzyme, accounting for 95% of cases (55). The defect leads to a bottleneck, creating a major backing up of precursor steroids which then overflow into other steroid pathways, leading to diagnostic high levels of 17 hydroxyprogesterone and, in female patients, excessive circulating testosterone or other adrenal-source androgen precursors (eg androstenedione, DHEA) which may be converted to testosterone in tissues. A common clinical problem with management of CAH is that glucocorticoid/mineralocorticoid treatment is not always fully effective partly due to variable compliance, which may leave high circulating testosterone, including well into or even above the normal male range (68). It is unlikely that mild non-classical congenital adrenal hyperplasia is a major contributor to the mild hyperandrogenism prevalent among elite female athletes. The prevalence of PCOS (6-16%) is about 100 times higher than mild non-classical congenital adrenal hyperplasia (0.1%, (40)) while a disproportionately high number of elite female athletes (especially in power sports) have PCOS (36). In one study of hyperandrogenic female athletes, even mild NCAH was ruled out by normal 17 hydroxyprogesterone (26) and in another (38) reported serum androstenedione and cortisol did not differ from controls, ruling out significant congenital adrenal hyperplasia..

4. Sex difference in muscle, hemoglobin, bone and athletic performance relating to adult circulating testosterone concentrations

Following puberty, testosterone production increases (16) but remains below 2 nmol/L in women whereas in men testosterone production increases 20-fold (from 0.3 mg a day to 7 mg a day) leading to a 15-fold higher circulating testosterone concentrations (15 vs 1 nmol/L). The greater magnitude of sex difference in testosterone production (20 fold) compared with circulating levels (15 fold) is due to women's higher circulating SHBG, which retards testosterone clearance creating a slower circulating half-time of testosterone. This order of magnitude difference in circulating testosterone concentrations is the key factor to men's superior athletic performance due to androgen effects principally on muscle, bone and hemoglobin.

4.1 Muscle

4.1.1 Biology:

It has been known since ancient times that castration influences muscle function. Modern knowledge of the molecular and cellular basis for androgen effects on skeletal muscle involves effects due to androgen (testosterone, DHT) binding to the androgen receptor which then releases chaperone proteins, dimerizes and translocates into the nucleus to bind to androgen response elements in the promoter DNA of androgen sensitive genes. This leads to increases in (a) muscle fibre numbers and size, (b) muscle satellite cell numbers,

(c) numbers of myonuclei, and (d) size of motor neurons (69). Additionally there is experimental evidence that testosterone increases skeletal muscle myostatin expression (70), mitochondrial biogenesis (71), myoglobin expression (72) and insulin-like growth factor (IGF-I) content (73) which may augment energetic and power generation of skeletal muscular activity.

Customized genetic mouse models can provide unique physiological insight in targeting specific molecules or their receptors to provide experimental insight into mammalian physiology which is unobtainable by human experimentation. The tight evolutionary conservation of the mammalian reproductive system explains why genetic mouse models have provided consistent, high fidelity replication of the human reproductive system (74, 75). Genetic males (46XY) with androgen insensitivity displaying similar features occur through spontaneously occurring inactivating AR mutations in all mammalian species studied including human, where they are known as women with CAIS. The converse, genetic females (46XX) resistant to all androgen action, cannot occur naturally in humans or other mammals. This is because fully androgen resistant females must have both X chromosomes carrying an inactivated AR. In turn this requires acquiring one X chromosome from their father. However, the potential fathers are sterile as hemizygous males bearing a single copy an X chromosome with an inactive AR produce no sperm, as a functional AR is biologically indispensable for making sperm in any mammal. However, androgen resistant females can be bred by genetic engineering using the Cre-Lox system (76). An important finding from such studies is that androgen-resistant female mice have essentially the same muscle mass and function compared with wild-type androgen sensitive females bearing normal AR whereas androgen-resistant male mice have smaller and weaker muscle mass and function than wild-type males but are comparable instead with the muscle of wild-type females (77). This indicates that androgen action, represented by circulating testosterone, is the key determinant of the higher muscle mass and strength characteristic of males compared with females. Furthermore, endogenous circulating testosterone has minimal effects on skeletal muscle mass and strength in female mice. Although these experiments cannot be replicated in humans, their key insight is that the higher circulating testosterone in males is the determinant of the male's greater muscle mass and function compared with females. Nevertheless, there is also evidence that hyperandrogenic women, mostly with PCOS, have increased muscle mass and strength that correlates with mildly increased circulating testosterone in the high-normal female range (26, 38).

4.1.2 Observational data:

There is a clear sex difference in both muscle mass and strength (78-80) even adjusting for sex differences in height and weight (80, 81). On average, women have 50-60% of men's upper arm muscle cross-sectional area (CSA) and 65-70% of men's thigh muscle CSA; and women have 50-60% of men's upper limb strength and 60-80% of men's leg strength (82). Young men have on average a skeletal muscle mass of over 12kg greater than age-matched women at any given body weight (80, 81). While numerous genes and

environmental factors (including genetics, physical activity and diet) may contribute to muscle mass, the major cause of the sex difference in muscle mass and strength is the sex difference in circulating testosterone.

Age-grade competitive sports records show minimal or no female disadvantage prior to puberty, whereas from the age of male puberty onwards there is a strong and ongoing male advantage. Corresponding to the endogenous circulating testosterone increasing in males after puberty to 15-20 nmol/L (sharply diverging from the circulating levels that remain <2 nmol/L in females), male athletic performances go from being equal on average to those of age-matched females to 10-12% better in running and swimming events, and 20% better in jumping events (8) (figure 1). Corroborative findings are provided by a Norwegian study that examined performance of adolescents in certain athletic events but without reference to contemporaneous circulating testosterone concentrations (83). The striking post-pubertal increase in male circulating testosterone provides a major, ongoing, cumulative and durable advantage in sporting contests by creating at least greater muscle mass and strength such that these sex differences render women unable to compete effectively against men, especially (but not only) in power sports.

These findings are supported by studies of non-athletic women showing that muscle mass is increased in proportion to circulating testosterone in women with mildly elevated testosterone levels due to PCOS (84, 85), a condition which is more prevalent among elite female athletes who exhibit these features (26, 36, 38), often undiagnosed (37), but which may provide an ergogenic advantage (38), consistent with the graded effects of circulating testosterone on explosive performance in men and women (86).

Studies of elite female athletes further corroborate these findings. One study demonstrates dose-response effects of better performance in some (400m, 400m hurdles, 800 m running, hammer throw, pole vault) but not all athletic events correlated with significantly higher endogenous testosterone in female, but not male, athletes. Even within the low circulating testosterone levels prevailing within the normal female range, in these events there was a significant advantage of 1.8% to 4.5% among those in the highest compared with the lowest tertile of endogenous testosterone (27). A further study of elite female athletes corroborates and extends these observations in that endogenous androgens are associated with a more anabolic body composition as well as enhanced muscular performance (26). In this study 106 Swedish Olympic female athletes were compared with 117 age- and weight (BMI)-matched sedentary control women for their muscle and bone mass (by dual energy X-ray absorptiometry, DEXA), their muscular strength (squat and countermovement jumps), and testosterone and DHT, as well as androgen precursors (DHEA, androstenedione) and urinary androgen glucuronide metabolites (androsterone, etiocholanolone, 3 and 17 3α-diols) measured by liquid chromatography-mass spectrometry (26). The athletes displayed higher muscle (and bone) mass than the sedentary control women, with strength tests correlating strongly with muscle mass whether in total or just in the legs. In turn, muscle mass and strength were correlated with androgens and androgen precursors. Considering that such studies may be confounded by factors such as menstrual

phase and dysfunction, and heterogeneous sports disciplines, which weaken the power of the study, these findings can be regarded as quite robust.

4.1.3 Interventional data:

Dose-response studies show that, in men whose endogenous testosterone is fully suppressed, add-back administration of increasing doses of testosterone that produce graded increases in circulating testosterone, causes a dose-dependent (whether expressed according to testosterone dose or circulating levels) increase in muscle mass (measured as lean body mass) and strength (41, 87). Taken together, these studies prove that testosterone doses leading to circulating concentrations from well below to well above the normal male range have unequivocal dose-dependent effects on muscle mass and strength. These data strongly and consistently suggest that the sex difference in lean body mass (muscle) is largely, if not exclusively, due to the differences in circulating testosterone between men and women. These findings have strong implications for power-dependent sport performance and largely explain the potent efficacy of androgen doping in sport.

The key findings providing conclusive evidence that testosterone has prominent dose-response effects in men are reported in studies by Bhasin et al that proved a monotonic dose-response, extending from sub- to supra-physiological range for men for testosterone effects on muscle mass, size and strength in healthy young men, findings that have been replicated and confirmed by an independent group (41). Both sets of studies used a common design of fully suppressing all endogenous testosterone (to castrate levels) for the full duration of the experiment by administering a GnRH analog. In the Bhasin studies, participants were then randomized to five groups who received weekly injections of 25 mg, 50 mg, 125 mg, 300 mg or 600 mg of testosterone enanthate for 20 weeks. In effect this was two sub- and two supra-physiological testosterone doses. In these studies, the lowest testosterone dose produced a mean serum testosterone of 253 ng/dl (8.8 nmol/L) in younger men and 176 ng/dl (6.1 nmol/L) in older men. The studies showed a consistent dose-response for muscle mass and strength that was clearly related to testosterone dose and consequential blood testosterone concentrations (upper panel, figure 2).

The study of Finkelstein et al involved the same design and involved 400 healthy men aged 20 to 50 years of age who had complete suppression of endogenous testosterone for the 16 weeks of the study with testosterone added back using daily doses of 0, 1.25 g, 2.5 g, 5 g or 10 g of a topical 1% testosterone gel (41). This again created a graded dose-response curve for serum testosterone and for muscle mass and strength. The inclusion of a zero (placebo) dose allowed differentiation between the zero and lowest testosterone dose. The placebo (zero) dose produced a serum testosterone of 0.7 nmol/L, the typical mean for castrated men, childhood, and women of any age. Meanwhile the lowest testosterone dose (1.25 g gel per day) produced a serum testosterone of 6.9 nmol/L, which is equivalent to that of a male in early to mid-puberty. A key finding for this review is that, from this study of men, the increase in serum testosterone from mean of normal female concentration (0.9 nmol/L) to supra-physiological female concentrations (6.9 nmol/L) produced significant increases of 2.3% for total body lean (muscle) mass, 3.0% for thigh muscle area, and

5.5% increase in leg press strength (digitised data pooling both cohorts from lower panel, figure 2).

Studies of the ergogenic effects of supra-physiological concentrations of circulating testosterone require studies administering graded doses of exogenous testosterone for months. Due to ethical concerns regarding risks of unwanted virilisation and hormone-dependent cancers, however, few studies have administered supra-physiological testosterone doses to healthy women. One well designed, randomized placebo-controlled study of postmenopausal women investigated the effects of different testosterone doses on muscle mass and performance and physical function (88). Sixty-two women (mean age 53) all had a standard estrogen-replacement dose administered during a 12 week run-in period (to eliminate any hypothetical confounding effects of estrogen deficiency), after which they were randomized to one of five groups receiving weekly injections of testosterone enanthate (doses: 0, 3 mg, 6.25 mg, 12.5 mg, and 25 mg respectively) for 24 weeks. The increasing doses of testosterone produced an expected dose-response in serum testosterone concentrations (by LC-MS) with the highest testosterone dose (25 mg/week) produced a mean nadir concentration of 7.3 nmol/L. The women whose testosterone concentrations were increased to 7.3 nmol/L achieved significant increases in muscle mass and strength (table 4), ranging from 4.4% for muscle (lean) mass to between 12% and 26% for measures of muscle strength (chest and leg press, loaded stair climb). As muscle strength measurement is effort-dependent, the placebo-controlled design of the Huang study support the further interpretation that the highest dose of testosterone also had prominent mental motivational effects in the effort-dependent tests of muscle strength. These findings provide salient direct evidence of the ergogenic effects of hyperandrogenism in female athletes confirming that at least up to average circulating testosterone concentrations of 7.3 nmol/L, women display a similar dose-response relationship as do men for supra-physiological testosterone with significant gains in muscle mass and power.

These effects of testosterone administration on circulating testosterone concentrations in females may be compared with the effects in males from the Finkelstein and Bhasin studies. In men, the lowest testosterone dose (1.25 g/day) increased mean serum testosterone to 6.9 nmol/L equivalent to early to mid-male puberty resulting in significant increases of total body lean (muscle) mass (2.3%), thigh muscle area (3.0%), and leg press strength (5.5%) compared with the placebo dose which resulted in a serum testosterone of 0.7 nmol/L. In the Huang study (figure 3), muscle mass and strength in postmenopausal women displayed a flat response at the 3 lower doses, when circulating testosterone concentrations remain below 5 nmol/L, and displayed a significant increase only when the mean circulating testosterone concentration produced by the highest testosterone dose first increased circulating testosterone concentrations above 5 nmol/L. This pattern, flat at lower doses and rising at highest dose, represents the lower plateau and the earliest rising portion, respectively, of the sigmoidal dose-response curve of testosterone for muscle.

Data corroborating the Huang study results comes from another well-controlled study in which post-menopausal women who were administered methyl testosterone following a run-in period of estrogen

replacement displayed a significant increase in lean (muscle) mass as well as upper and lower limb power during a 16-week double-blind, parallel group study (89).

Similarly, two prospective studies of the first 12 months treatment of transmen (F2M transgender) shows a consistent major increase in muscle mass and strength due to testosterone administration. In one study testosterone treatment of 17 transmen achieving adult male circulating testosterone levels (mean 31 nmol/L) increased muscle mass by 19.2% (90) whereas, conversely, testosterone suppression (using an estrogen-based treatment regimen) in 20 transwomen reduced circulating testosterone levels from adult male range to adult female range led to a 9.4% reduction in muscle mass (measured as cross-sectional area). In a second study, 23 transmen administered adult male testosterone doses also produced striking increases in total body muscle size and limb muscle size (by 6.5-16.6%) and grip strength (by 18%) compared with age-matched untreated control women (91).

4.1.4 Effects on athletic performance:

In summary, muscle growth, and the increase in strength and power it brings, has an obvious performance-enhancing effect, in particular in sports that depend on strength and (explosive) power, such as track and field events (83, 86). There is convincing evidence that the sex differences in muscle mass are sufficient to account for the increased strength and aerobic performance of men compared with women and are in keeping with the differences in world records between the sexes (92). The basis for the sex difference in muscle mass and strength is the sex difference in circulating testosterone as clearly shown (for example) by (a) the enhanced athletic performance of men compared with pre-pubertal boys and women (8); (b) the close correspondence of muscle growth (muscle size) with muscle strength in ascending dose studies in men by Bhasin et al (87, 93-95) and Finkelstein et al (41) and in postmenopausal women by Huang et al (88) (c) the effect of male castration in reducing muscle size and strength, effects which are fully rectified by testosterone replacement; and (d) the striking efficacy of androgen doping on the sports performances of GDR female athletes (96).

4.2 Hemoglobin

4.2.1 Biology

It is well known that circulating hemoglobin is androgen-dependent and consequently higher in men than in women; however, the physiological mechanism by which androgens like testosterone boosts circulating hemoglobin is not fully understood (97). Testosterone increases secretion of and sensitivity to erythropoietin, the main trophic hormone for erythrocyte production and thereby hemoglobin synthesis as well as suppressing hepcidin (98), a crucial iron regulatory protein that governs the body's iron economy. Hepcidin has to balance the need for iron absorption from foods (the only source of iron required for body's iron-

containing proteins) against the risk that the body has no mechanism to shed excess iron which can be toxic. Adequate iron availability is essential for normal erythropoiesis and synthesis of key heme, iron-containing oxygen-transporting proteins such as hemoglobin and myoglobin (99) as well as other iron-dependent proteins such as cytochromes and DNA synthesis and repair enzymes. Experimental evidence in mice shows that testosterone increases myoglobin content of muscle with potential for augmenting aerobic exercise performance (72), but this has not been evaluated in humans.

Increasing the amount of hemoglobin in the blood has the biological effect of increasing oxygen transport from lungs to tissues, where the increasing availability of oxygen enhances aerobic energy expenditure. This is exploited to its greatest effect in endurance sports (1). The experiments of Ekblom in 1972 (see redrawn figure 4) demonstrated strong linear relationships between changes in hemoglobin (due to withdrawal or re-transfusion of 1, 2 or 3 units (400 mL) of blood) and aerobic capacity, established by repeated testing of maximal exercise-induced oxygen consumption before and after each procedure (100). As already noted, circulating hemoglobin levels are on average 12% higher in men than women (101). It may be estimated that as a result the average maximal oxygen transfer will be about 10% greater in men than in women, which has a direct impact on their respective athletic capacities.

4.2.2 Observational data:

Circulating hemoglobin levels are on average 12% higher in men than in women (101), likely to be due to the sex difference in average circulating testosterone concentrations. This interpretation is supported by the fact that male castration (eg for advanced prostate cancer) (102) and androgen deficiency due to reproductive system disorders (103) reduce circulating hemoglobin in men, eliminates the sex difference whereas testosterone replacement therapy restores circulating hemoglobin to adult male levels (97, 103, 104).

Women with CAH require glucocorticoid replacement therapy but exhibit widely varying levels of hormonal control (55). An unusually informative observational study provides unique insight into testosterone effects on circulating hemoglobin in otherwise healthy women (68). The degree of poor control is associated with increasing levels of circulating testosterone ranging from normal female concentrations up to 36 nmol/L and these correlates closely (r=0.56) with circulating hemoglobin (figure 5). Interpolating from the dose-response regression, increases in circulating testosterone measured by LC-MS from 0.9 nmol/L to 5 nmol/L, 7 nmol/L, 10 nmol/L and 19 nmol/L were associated with a strong dose-response relationship of increased circulating hemoglobin by 6.5%, 7.8%, 8.9% and 11%, respectively. An 11% increase in circulating hemoglobin translates to a 10% difference in maximal oxygen transfer (100), which may account for virtually all the 12% sex difference in male and female circulating hemoglobin (101). To put this into context, any drug that achieved such increases in hemoglobin would be prohibited in sport for blood doping, as this difference is sufficient to have ergogenic effects. That is even regardless of any testosterone effects on muscle mass or strength for

which data were not available in that study. Conversely, among elite female athletes with circulating testosterone in the healthy pre-menopausal female range, circulating hemoglobin does not correlate with athletic performance (27). In women with the mild hyperandrogenism of PCOS circulating hemoglobin and hematocrit are reported as not (105) or marginally increased (106), findings which may be influenced by the fact that PCOS is associated with reduced or absent menstruation, thereby reducing the iron loss of regular menstruation.

4.2.3 Interventional data:

In the Bhasin studies, in both young and older men the highest testosterone dose produced a 12% increase in blood hemoglobin compared with the lowest dose reflecting a strong dose-response relationship (figure 6) (107). Analogous findings were reported for testosterone treatment effects in postmenopausal women where the highest dose (25 mg weekly) of testosterone, which increased mean serum testosterone to 7.3 nmol/L, had the largest increase (3%) in blood hemoglobin and hematocrit (88).

Corroborative findings are available from studies of transmen (F2M transgender), natal females who receive testosterone treatment at replacement doses to create adult male circulating testosterone concentrations, who exhibit increases in circulating hemoglobin to male levels (reviewed (108-110)). One prospective 12 month study of transgender (non-athlete) individuals reported that testosterone suppression (by an estrogen-based regimen) to normal female levels in 20 (M2F) transwomen reduced hemoglobin by 14%, whereas conversely testosterone treatment in 17 (F2M) transmen which created mean circulating testosterone levels of 31 nmol/L increased hemoglobin levels by 15% (90).

If such an increase in hemoglobin were produced by any chemical substance, it would be considered doping, according to the World Anti-Doping Code.

4.3 Bone

4.3.1 Biology:

There is extensive experimental evidence from genetic mouse models showing that the sex difference in bone size, mass and function are due to the sex difference in circulating testosterone. These effects have been reported from studies of global and tissue or cell-selective inactivation of AR or estrogen receptors (ER) which show that androgen effects are mediated by both direct effects on the AR as well as indirect effects mediated via aromatisation of testosterone to estradiol to act on ER (reviewed in (111)). Bone grows in length due to epiphyseal chondral growth plates which provide cartilage forming the matrix for lengthening of long bone which is terminated by estrogen-dependent mechanism that depends on aromatisation of testosterone to estradiol. Similarly, bone width and density are increased through appositional growth from periosteal and endosteal expansion which depend on bone loading and androgen exposure together with other factors.

An important difference between androgen effects on bone compared with effects on muscle or hemoglobin is that developmental bone effects of androgens are likely to be irreversible.

4.3.2 Observational data:

Men have distinctively greater bone size, strength and density than women of the same age. As with muscle, sex differences are absent prior to puberty but then accrue progressively from the onset of male puberty due to the sex difference in exposure to adult male circulating testosterone concentrations (reviewed in (111)). Girl's earlier onset of puberty and its growth spurt as well as earlier estrogen-dependent epiphyseal fusion explains their shorter stature than boys. As a result, on average men are 7-8% taller with longer, denser and stronger bones whereas women have shorter humerus and femur cross-sectional area being 65-75% and 85%, respectively, compared to men (82). These changes create an advantage of greater bone strength and stronger fulcrum power from longer bones. In addition, whereas passing through puberty enhances boy's physical performance, the widening of the female pelvis during puberty, balancing the evolutionary demands of obstetrics and locomotion (112, 113), retards the improvement in girl's physical performance, possibly driven by ovarian hormones rather than absence of testosterone (114, 115).

Sex differences in height have been the most thoroughly investigated measure of bone size as adult height is a stable, easily quantified measure in large population samples. Extensive twin studies show that adult height is highly heritable with predominantly additive genetic effects (116) which diverge in sex-specific manner from the age of puberty onwards (117, 118), which effects are likely to be due to sex differences in adult circulating testosterone concentrations.

Bone density (total and medullary cross-sectional area) is increased in women with CAH with variably elevated serum testosterone (including into the male range) when it is only partially suppressed by glucocorticoid treatment (119) although more effective glucocorticoid suppression lowers bone density (120).

4.3.3 Interventional data:

Well designed, placebo-controlled direct interventional studies of supra-physiological androgen effects on bone in females are few, rarely feasible and unlikely to be performed for ethical and practical reasons. Unlike muscle which responds relatively rapidly to androgen effects so that muscle studies in humans can be completed within 3-4 months (41, 87, 88, 95, 121), comparable bone studies would typically take a year or more to reach plateau effects. Hence such direct investigational studies in otherwise healthy women would risk side-effects of virilisation which may be only slowly and partly, if at all, reversible as well as potential promotion of hormone-dependent cancers making such studies ethically and practically not feasible.

4.3.4 Effects on athletic performance:

The major effects of men's larger and stronger bones would be manifest via their taller stature as well as the larger fulcrum with greater leverage for muscular limb power exerted in jumping, throwing or other explosive power activities. The greater cortical bone density and thereby resistance to long bone fractures is unlikely to be relevant to the athletic performance of young athletes in whom fractures during competition are extremely rare and not expected to be linked to sex. On the other hand, stress fractures in athletes, mostly involving the legs, are more frequent in females with the male protection attributable to their larger and thicker bones (122).

4.4 Other androgen-sensitive sex dichotomous effects:

4.4.1 Biology and observational data:

Many if not most other aspects of physiology exhibit sex difference so that they may enhance the impact of the male advantage in sports performance of the dominant determinants (muscle, hemoglobin). Examples include sex differences in exercise-induced cardiac (123, 124) and lung (125) function and mitochondrial biogenesis and energetics (71). However, the limited knowledge of the magnitude and hormonal mechanisms involved, specifically the degree of androgen dependence of these mechanisms, means that it is difficult to estimate their contribution, if any, towards the sex difference in athletic performance. The sex difference in pulmonary function may be largely explained by the androgen-sensitive sex difference in height, which is a strong predictor of lung capacity and function (125). Further physiological studies of the androgen dependence of other physiological sex differences are awaited with interest.

Psychological differences between men and women on mental function (eg rotational orientation (126)) as well as mood, motivation and behavioural effects may involve androgen sensitive effects during pre- and perinatal as well as post-pubertal effects (127, 128).

4.4.2 Interventional data:

There is some limited direct evidence from well-designed, placebo-controlled trials that administration of testosterone or other androgens at supra-physiological doses directly affect mood and behaviour, notably inducing hypomania (129). In a randomized placebo-controlled study of testosterone administration in postmenopausal women (88) with the highest dose (the only one causing circulating testosterone levels to exceed female range), there was not only an increase in muscle mass (4.4%) but a strikingly greater increase in muscle strength (12-26%) suggesting an enhanced mental motivational effect of testosterone on the effort-dependent tests of muscle strength.

5. Alternative mechanisms proposed to explain sex differences

Alternative explanations for the sex difference in muscle mass and strength, other than it being due to the sex difference in post-pubertal circulating testosterone have been proposed. These include that sex differences in athletic performance might instead be due to (a) sex differences in height because height is a predictor of muscle mass (92), (b) genetic sex differences due to influence of unspecified Y chromosome genes (130) and (c) sex differences in growth hormone (GH) secretion (92),

5.1 Effects of height

One proposal has been that, as men are taller than women, height differences may explain the sex differences in muscle mass and function which explains some athletic success (92). Numerous factors contributes to the regulation of adult muscle mass including genetics, race, adiposity, hormones, physical activity (exercise/training), diet, birth order and bone size (including height) (reviewed in (131)). Among the non-hormonal factors, genetics explains a large proportion (about 50-60% from pooled twin studies (132)) of the variability in muscle mass and strength (133, 134) and may be explained in turn by the equally high genetic contributions to circulating testosterone (28, 29). Some factors influencing muscle mass and strength such as physical activity, adiposity and bone size are also partly androgen dependent. Prior to puberty there is no sex difference in skeletal features including height (135, 136). However, with the onset of puberty, girls aged 11 and 12 years old are transiently taller than peer-aged boys due to their earlier onset of the female pubertal growth spurt but from age of 14 years onward the taller stature in males emerges and stabilises (117). Hence, like muscle mass, sex differences in bone size (including length, density and height) arise after male puberty establishes the marked dichotomy between men and women in adult circulating testosterone concentrations. Taller height is advantageous in some sports (basketball, some football codes, combat sports) but in others (jockeys, cycling, gymnastics, weightlifting, bodybuilding) short stature provides a greater power/strength-to-weight ratio as well as superior rotational balance, speed and agility. Yet the male advantages in speed, strength and endurance apply regardless of whether height is advantageous or not. Hence the sex difference in height, where they exist, are largely dependent on post-pubertal differences in circulating testosterone when sex differences in height are first expressed.

5.2 Genetic effects of Y chromosome

It has also been proposed that the sex difference in athletic performance may be due to genetic effects of an unspecified Y chromosome gene(s) that may dictate taller stature (130) as height is correlated with men's greater muscle mass. The small human Y chromosome has few functional genes and none with a known effect on height other than the short stature homeobox gene (SHOX) gene, located in the pseudoautosomal regions of the tip of the short arms of X and Y chromosomes (137). Adult height displays an apparent dose dependency on SHOX gene copy number that is a major factor contributing to explaining both the short

stature of 45XO females (Turner's syndrome), who have a single copy of the SHOX gene, as well as the tall stature of 47XXY males (Klinefelter's syndrome), who have three copies (137). However, when SHOX copy number is the same, men with additional supernumerary Y chromosomes (eg 47 XYY) are the same height as 47 XXY men (138). Hence there is no evidence supporting a dosage-dependent Y chromosomal gene effects on height independent of SHOX gene copy number, and nor does men's possession of a Y chromosome explain the height difference between adult men and women. On the contrary, the tall stature of 47 XXY men is at least partly due to the concomitant androgen deficiency leading to pubertal delay. Pubertal delay prolongs long bone growth due to delayed epiphyseal closure, an estrogen-dependent effect that requires adequate production of testosterone as a substrate for aromatisation to estradiol, resulting in tall stature. Similar eunuchoidal features and taller stature are evident in 46 XY men with congenital hypogonadotropic hypogonadism (Kallmann's syndrome and its variants) with comparable congenital onset of androgen deficiency, also manifest as pubertal delay and long bone overgrowth. Hence, taller height is better explained by impaired testicular function with delayed puberty and epiphyseal closure rather than unspecified Y chromosome dosage effects. In any case, rare aneuploidies in themselves do not explain the sex difference in height in the general population of individuals with normal sex chromosomes.

5.3 Growth hormone.

The proposal that the sex difference in muscle mass and function might be due to sex differences in endogenous GH secretion (92) is refuted by the extensive and conclusive clinical evidence that endogenous GH secretion in young women is consistently higher (typically twice as high) as in young men of similar age (139-146). Those findings cannot explain the male advantage in muscle mass and strength unless GH retards muscle growth/function, for which there is no evidence. Furthermore estrogens inhibit GH-dependent, hepatic IGF-I production, the major pathway of GH action (147, 148). The weak observational association between low circulating IGF-I and some, but not other, measures of weak muscle strength and limited mobility among older women may reflect general age-associated debility rather than any specific hormonal effects (149). Finally, the evidence that endogenous GH plays no role in sex differences in muscle mass and function is supported by evidence from the most extensive interventional study of GH treatment to non-GH deficient adults, daily GH administration for 8 weeks to healthy recreational athletes produced only marginally significant improvement in exercise performance of men, and none in women (150). These findings are consistent with the speculation that GH (or IGF-I) may be an amplifier of testosterone effects and therefore be a consequence of the sex difference in circulating testosterone rather than its cause.

6. The impact of adult male circulating testosterone concentrations on sports performance

Plausible estimates of the magnitude of the ergogenic advantage of adult male circulating testosterone

concentrations are feasible from the limited available observational and interventional studies.

Population data on the ontogeny of puberty shows that prior to puberty boys and girls have comparable athletic performance whereas sex differences in athletic performance emerge coinciding with the rise in circulating testosterone from the onset of male puberty. Male puberty results in circulating testosterone concentrations rising from the prepubertal and female post-pubertal range (<2 nmol/L) to adult male circulating testosterone concentrations (18). This is associated with a 10-12% better performance in running and swimming events and 20% enhancement in jumping events (8).

A minimal estimate of the impact of adult male testosterone concentrations on muscle size and strength on females is provided by the Huang study in postmenopausal women (88). In this study the highest testosterone dose (weekly injections of 25 mg testosterone enanthate) increased mean circulating testosterone from 0.9 nmol/L to 7.3 nmol/L, which is equivalent to the circulating testosterone of boys in early to mid-puberty. After 24 weeks of testosterone treatment, the increase in circulating testosterone concentrations led to significant increases in muscle size of 4.4% and in muscle strength of 12 to 26%. Given the limited testosterone dose (and concentration) as well as study duration, it is likely these findings underestimate the magnitude of the impact that sex difference in circulating testosterone has on muscle mass and strength, and therefore on athletic performance.

Converse effects of reduced athletic performance in athletes who undergo suppression of circulating testosterone concentrations from those in the male into the female range have been reported. Among recreational (non-elite) athletes, an observational study show a consistent deterioration in athletic performance of transwomen (M2F transgender) athletes corresponding closely to the suppression of circulating testosterone concentrations (151). Similarly, among elite athletes with circulating testosterone in the male range due to DSDs, comparable findings of athletic performance reduced by an average of 5.7% when circulating testosterone was suppressed from the male range to below 10 nmol/L (152). Subsequently when the IAAF hyperandrogenism rule was suspended in 2015, and so these elite athletes could train and compete with unsuppressed serum testosterone levels, their athletic performances increased by a similar amount. Additionally, circulating hemoglobin levels in these untreated DSD athletes were comparable with male athletes or else female athletes doping with erythropoietin (figure 7). However, when circulating testosterone was suppressed to below 10 nmol/L their hemoglobin were 12% lower and again comparable with non-doped, non-DSD females, corresponding to the 12% magnitude of the sex difference in hemoglobin between men and women (101).

Congruent findings are also known for an elite female athlete whose serial athletic performance based on publicly available best annual times between 2008 and 2016 for the 800m running event are depicted in

relation to the original 2011 IAAF hypernadrogenism regulation (figure 8).

Based on the established dose-response relationships, suppression of circulating testosterone to <10 nmol/L would not eliminate all ergogenic benefits of testosterone for athletes competing in female events. For example, according to the Huang study (88), reducing circulating testosterone to a mean of 7.3 nmol/L would still deliver a 4.4% increase in muscle size and a 12-26% increase in muscle strength compared with circulating testosterone at the normal female mean value of 0.9 nmol/L. Similarly, according to the Karunasena study (68), reducing circulating testosterone concentration to 7 nmol/L would still deliver 7.8% more circulating hemoglobin than the normal female mean value. Hence the magnitude of the athletic performance advantage in DSD athletes, which depends on the magnitude of elevated circulating testosterone concentrations, is considerably greater than the 5-9% difference observed in reducing levels below 10 nmol/L.

The physiological mechanism underlying these observations is further strengthened by prospective controlled studies of initiation of cross-sex hormone treatment in transgender individuals (90, 153). These show that, over the first 12 months muscle mass (area) was decreased by 9.4% and hemoglobin by 14% in twenty transwomen (M2F transgender) treated with an estrogen-based regimen that reduced circulating testosterone concentrations from the male range to female levels. Conversely, in seventeen transmen (F2M transgender) treated for the first time with testosterone for 12 months (which increased circulating testosterone levels to a mean of 31 nmol/L), muscle mass increased by 19.2% and hemoglobin by 15% (90). The muscle mass findings remained stable between 1 and 3 years of initiation of treatment although fat mass continued to change between 1 and 3 years of testosterone treatment (153). These studies did not report muscle strength but other studies of testosterone dose-response relationships for muscle mass and strength show consistently positively correlation (41, 69, 93, 95) although with disproportionately greater effect on muscle strength than on muscle mass. Hence the muscle mass estimates in these prospective treatment initiation studies in transgender individuals likely underestimate the muscle strength gains from elevated testosterone levels where the circulating testosterone markedly exceeds female range to be within the male range as occurs in severe hyperandrogenism of DSD females or transwomen (M2F transgender). These effects are also the biological basis of the ergogenic efficacy of androgen doping in women.

Finally, to put these competitive advantages into context, the winning margin (the difference in performance by which a competitor misses a gold medal, any medal or making the final) in elite athletic or swimming events over the last 3 Olympics is <1% equally for both male and female events (table 5).

7. Gaps in knowledge and research limitations

The major limitations on scientific knowledge of the impact of adult male circulating testosterone

concentrations on the sex differences in athletic performance is the lack of well-designed studies. Ideally, these would need to replicate adult male circulating testosterone concentrations for sufficient time in women to investigate the effects on muscle, hemoglobin, bone and other androgen-sensitive measures that display consistent sex dichotomy in the population. However, the ethical and safety concerns preventing such studies hitherto are likely to remain formidable obstacles due to the risk of unacceptable and potentially irreversible virilization as well as of promoting hormone-dependent cancers in women.

With the exception of one interventional study using a relatively low testosterone dose (ie low for males), all available data comprises observational studies that can only examine the effects of serum testosterone within physiological female limits or sparse and mostly uncontrolled data from intersex/DSD athletes. While the available observational findings in healthy females are informative, the key question is the magnitude and dose-response of effects at still higher circulating testosterone concentrations on the performances of women. While a testosterone dose-response relationship has been established in women at relatively low (for men) testosterone dose and circulating concentrations, it remains unproven even if clearly plausible that the testosterone dose-response relationships established in men for muscle, hemoglobin and bone can be extrapolated to women when they are exposed to higher (ie comparable with males) circulating testosterone concentrations. It is theoretically possible there could be differences between men and women in muscle responses to testosterone, as muscle cell populations might express genetic differences in androgen sensitivity (for which there are no data), or alternatively the long-term prior pattern of testosterone exposure from conception to adulthood might lead to differences in testosterone dose-responsiveness after maturity. Although the dose-response relationship may be similar in women as in men, there is also anecdotal evidence that the dose-response curves may be left-shifted so that testosterone has greater potency in women than in men at comparable doses and circulating levels. The prediction is supported by the anecdotal evidence from the surreptitious East German national doping program in which the supervising doctors asserted from their experience of illicit cheating that androgens had more potent ergogenic effects in women than in men (96), a speculative opinion shared by many experienced sports medicine physicians.

There is no known means of increasing endogenous testosterone in women to anything like the requisite degree to attempt to answer these questions. In healthy men, circulating testosterone originates almost exclusively from a single source (testicular Leydig cell) and is subject to tight hypothalamic negative feedback control, so that either direct stimulation (by hCG) or indirect reflex effects (eg from estrogen blockers operating via negative feedback) to enhance Leydig cells testosterone secretion are feasible. However, similar mechanisms do not operate in women in whom circulating testosterone originates from three different sources (adrenal, ovary, extra-glandular conversion of androgen precursors), none of which is subject to tight testosterone negative feedback control. As a result, it is not feasible to produce a sufficient increase in circulating testosterone in women either by direct ovarian stimulation or indirect reflex effects to test this hypothesis even if were deemed ethical and safe. On the other hand, carefully controlled, graded-

dose studies in F2M transgender individuals might be informative but are largely lacking at this time.

Hence the only feasible design of such studies would be testosterone (or another androgen) administration to healthy young women. The only well-designed, placebo-controlled study of testosterone in otherwise healthy postmenopausal women was restricted to relatively low testosterone doses which, while clearly supra-physiological for women, were only 20-25% of male testosterone replacement doses (88). We are currently performing a double-blind, randomized, placebo-controlled study on the effects of moderately increased testosterone concentration on physical performance and behaviour in young healthy women (ClinicalTrials.gov ID: NCT03210558). However, obtaining ethical approval (and practical difficulties in recruitment) to administer supra-physiological testosterone doses that maintain circulating testosterone in the male range for sufficiently prolonged periods are likely to remain an obstacle to definitive resolution of this question.

In men, analogous ethical concerns over short and long-term adverse effects delayed the definitive studies of supra-physiological testosterone doses to healthy young and older men but were eventually overcome. This was despite the fact that, uniquely among hormones, there is no known disease state in men due to pathologically excessive testosterone secretion. By contrast, in women, supra-physiological testosterone effects are known to produce virilization side-effects which may be only slowly and partially, if at all, reversible. Yet maintaining clearly supra-physiological testosterone concentrations would require treatment for months (muscle) or years (bone) and would replicate not only a known hyperandrogenic disease state (PCOS) but also potentially increasing risk of hormone-dependent cancers. In these circumstances, it could only be justifiable to replicate in women the salient testosterone dose-response studies available from men if the available evidence of dose-response relationship in men was not sufficiently convincing or and/or there was reason to believe that these dose-response characteristics would be substantially different in women. Overall, the unequivocal dose-response evidence in men together with the available overlap evidence in women appears sufficiently persuasive, so that it is doubtful that women would respond differently from men if their circulating testosterone were raised to levels in the male range. More broadly, there is no more reason to require separate studies in women vs men any more than there is for every different ethnic subgroup of people. An aesthetic preference for splitting categories is not a sound reason to require the virtually impossible standard of establishing fresh and comprehensive empirical evidence in women of testosterone dose-response effects ranging into male circulating testosterone concentrations.

An analogy can be drawn to WADA's practice of accepting salient surrogate evidence for banning drugs where it is not feasible or ethical to require direct proof of the ergogenic effects of the plethora of existing and new drugs with potential but individually unproven ergogenic effects. In that context, firmly established ergogenic efficacy of androgens (on muscle mass and strength) and increased hemoglobin (on endurance) (evidence reviewed in (1)) mean that chemical substances or methods which increase endogenous

testosterone, erythropoietin or hemoglobin are also considered ergogenic (154). By parity of reasoning, if a condition causes a female athlete's circulating testosterone levels to be in the male range, well exceeding female ranges, with consequential increases in muscle, hemoglobin and bone effects (at least), an ergogenic effect may be reasonably be assumed.

8. Conclusions

The available albeit incomplete evidence makes it highly likely that the sex difference in circulating testosterone of adults explains most if not all the sex differences in sporting performance. This is based on the dose-response effects of circulating testosterone to increase muscle mass and strength, bone size and strength (density), and circulating hemoglobin, each of which alone increases athletic capacity, as well as other possible sex dichotomous, androgen-sensitive contributors such as mental effects (mood, motivation, aggression) and muscle myoglobin content. These facts explain the clear sex difference in athletic performance in most sports, on which basis it is commonly accepted that at least in those sports competition has to be divided into male and female categories.

The first IAAF hyperandrogenism regulation specified a hormonal eligibility criterion of a serum testosterone of less than 10 nmol/L for participation in the protected category of female athletic events in an athlete with normal androgen sensitivity. This threshold was based on serum testosterone measurements by immunoassays. However no reliable method-independent consensus threshold could be established using commercial testosterone immunoassays, as these assays differ systematically due to method-specific bias arising unavoidably from the specificity of the different proprietary antibodies employed (25). Therefore, if the objective is to require female athletes with congenital conditions that cause them to have serum testosterone concentrations in the normal male range to bring those levels down to the same range as other female athletes, then (allowing for PCOS athletes) the threshold used should be no more than 5.0 nmol/L. This represents a conservative criterion that includes all healthy young (<40 yr) women, including those with PCOS. Conversely, this criterion is generous to hyperandrogenic females and transwomen in allowing them to maintain a higher serum testosterone than most non-PCOS competitors in female events even though increases in muscle mass and strength and hemoglobin would be expected in this range. This is so even though the range remains below the circulating testosterone levels of mid-male puberty when the major biological effects of men's higher circulating testosterone begin to be fully expressed. Ongoing compliance with the eligibility criterion is also an important variable since the estrogen-based suppression of circulating testosterone, typically using daily administered estrogen products, has a rapid onset and offset. Adequate monitoring to prevent gaming of eligibility criteria would require regular random rather than announced blood sampling.

A related matter is how long such a threshold of circulating testosterone should be maintained. In both intersex/DSD and transgender individuals, the developmental effects of adult male circulating testosterone concentrations will have established the sex difference in muscle, hemoglobin and bone, some of which is fixed and irreversible (bone size) and some of which is maintained by the male circulating testosterone concentrations (muscle, hemoglobin). The limited available prospective evidence from initiation of transgender cross-sex hormone treatment suggests that the advantageous increases in muscle and hemoglobin due to male circulating testosterone concentrations are induced or reversed over the first 12 months and the androgenic effects may plateau after time. This time course is much faster than the somatic effects of male puberty, which evolve over years and for some variables (eg peak bone mass) are not complete for up to a decade after the start of puberty. However, the abrupt hormonal changes induced by medical treatment in intersex/DSD or transgender individuals may be telescoped compared with male puberty where circulating testosterone concentrations increase irregularly and incompletely for some years. Additional data is available from the unique investigative model of men undergoing castration for prostate cancer. Just as androgen sensitivity to testosterone may differ between tissues (41), the time-course of offset of androgen effects following withdrawal of male testosterone concentrations may also differ between the major androgen-responsive tissues. For example, circulating hemoglobin shows a progressive fall for 6 months reaching a nadir and plateau at 12-16 months in 6 studies involving 534 men undergoing medical castration for prostate cancer (155-160). Although these studies of older men with prostate cancer must be extrapolated with caution, age, stage of disease, race and baseline circulating testosterone concentration did not affect the rate or extent of decline in hemoglobin (155, 157). Comparable longitudinal studies of muscle loss, strength and performance following castration for prostate cancer are well summarised (161) showing progressive loss for 24 months (see figure 4). Further clinical studies to define the time-course of changes, mainly offset, in testosterone-dependent effects, notably on muscle and hemoglobin, are badly needed to determine the optimal duration for cross-sex hormone effects in sport.

Table 1: Serum testosterone measurements by LC-MS methods in studies of healthy men and women

Author (year)	Sample (age 18-40 yr)	N	Lower 95% CL	Upper 95% CL
Men			**nmol/L**	**nmol/L**
Sikaris (2005)	Elite, eugonadal	124	10.4	30.1
Turpeinen (2008)	Convenience	30	10.1	31.2
Kushnir (2010)	Convenience	132	7.2	24.2
Salameh (2010)	Convenience	264	7.1	39.0
Neale (2013)	Convenience	67	10.6	31.9
Kelsey (2014)	Secondary pooled analysis	1058	7.2	25.3
Hart (2015)	Birth cohort	423	7.4	28.0
Travison (2017)	Pooled two cohorts	1656	7.9	31.1
	Number-weighted mean		**7.7**	**29.4**
Women				
Turpeinen (2008)	Convenience	32	0.8	2.8
Kushnir (2010)	Convenience	104	0.3	2.0
Salameh (2010)	Convenience	235	0.03	1.5
Haring (2012)	Population-based	263	0.04	2.0
Neale (2013)	Convenience	90	0	1.7
Bui (2013)	Convenience	25	0.30	1.69
Rothman (2013)	Convenience	31	0.4	0.92
Bermon (2017)	Elite athletes	1652	0	1.62
Eklund (2017)	Elite athletes and controls	223	0.26	1.73
	Number-weighted mean		**0.06**	**1.68**

Sikaris et al. 2005 Reproductive hormone reference intervals for healthy fertile young men: evaluation of automated platform assays. J Clin Endocrinol Metab. 2005 Nov;90(11):5928-36; **Turpeinen et al** 2008 Determination of testosterone in serum by liquid chromatography-tandem mass spectrometry. Scand. J. Clin. Lab. Invest. 68:50-57; **Kushnir et al**. 2010 Liquid chromatography-tandem mass spectrometry assay for androstenedione, dehydroepiandrosterone, and testosterone with pediatric and adult reference intervals. Clin Chem. 2010 Jul;56(7):1138-47; **Salameh et al** 2010 Validation of a total testosterone assay using high-turbulence liquid chromatography tandem mass spectrometry: total and free testosterone reference ranges. Steroids 75:169-175; **Haring et al** 2012 Age-specific reference ranges for serum testosterone and androstenedione concentrations in women measured by liquid chromatography-tandem mass spectrometry. J. Clin. Endocrinol. Metab. 97:408-415; **Neale et al**. 2013 Adult testosterone and calculated free testosterone reference ranges by tandem mass spectrometry. Ann Clin Biochem. 2013 Mar;50(Pt 2):159-61; **Bui et al**. 2013 Dynamics of serum testosterone during the menstrual cycle evaluated by daily measurements with an ID-LC-MS/MS method and a 2nd generation automated immunoassay. Steroids. 2013 Jan;78(1):96-101; **Rothman et al**. 2013 Reexamination of testosterone, dihydrotestosterone, estradiol and estrone levels across the menstrual cycle and in postmenopausal women measured by liquid chromatography-tandem mass spectrometry. Steroids. 2011 Jan;76(1-2):177-82**; Hart et al.** Testicular function in a birth cohort of young men. Hum Reprod. 2015 Dec;30(12):2713-24; **Travison et al** 2017 Harmonized Reference Ranges for Circulating Testosterone Levels in Men of Four Cohort Studies in the United States and Europe. J. Clin. Endocrinol. Metab. 102:1161-1173; **Bermon et al** 2014 Serum androgen levels in elite female athletes. J. Clin. Endocrinol. Metab. 99:4328-4335; **Bermon S, Garnier PY**. 2017 Serum androgen levels and their relation to performance in track and field: mass spectrometry results from 2127 observations in male and female elite athletes. Br J Sports Med 51:1309-1314; **Eklund et al.** 2017 Serum androgen profile and physical performance in women Olympic athletes. Br J Sports Med 51:1301-1308

Table 2: Summary of serum testosterone (nmol/L) by LC-MS in women with PCOS from 16 studies

Study	N	Mean	SD
Moran 2017	92	*0.24*	*0.08*
Munzker 2017	274	*0.93*	*0.19*
O'Reilly 2017	114	*0.55*	*0.19*
Handelsman 2017	152	0.38	0.25
Paquali 2016	156	1.17	0.47
Yang 2016	1159	2.2	1.44
Tosi 2016	116	1.33	0.55
Daan 2015	170	*1.64*	*0.53*
Bui 2015	44	*0.85*	*0.3*
Keefe 2014	52	*1.7*	*0.97*
Yasmin 2013	165	1.99	1.02
Janse 2011	200	*1.12*	*0.47*
Jedel 2011	72	*0.23*	*0.08*
Legro 2010 (Mayo)	596	*2.12*	*0.89*
Legro 2010 (Quest)	596	*1.98*	*0.97*
Stener-Victorin 2010	74	1.53	0.62
Sum	4032		1072
Number-weighted mean		1.69	0.87

Data taken directly from paper or interpolated from other data (eg median, quartiles, ranges, sample size) supplied as described by Wan et al 2014 (Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. BMC Med Res Methodol 14:135) shown in *red italics*.

Stener-Victorin et al 2010 Are there any sensitive and specific sex steroid markers for polycystic ovary syndrome? J. Clin. Endocrinol. Metab. 95:810-819; **Legro et al** 2010 Total testosterone assays in women with polycystic ovary syndrome: precision and correlation with hirsutism. J. Clin. Endocrinol. Metab. 95:5305-5313; **Jedel et al** 2011 Sex steroids, insulin sensitivity and sympathetic nerve activity in relation to affective symptoms in women with polycystic ovary syndrome. Psychoneuroendocrinology 36:1470-1479; **Janse et al** 2011 Assessment of androgen concentration in women: liquid chromatography-tandem mass spectrometry and extraction RIA show comparable results. Eur. J. Endocrinol. 165:925-933; **Yasmin et al** 2013 The association of body mass index and biochemical hyperandrogenaemia in women with and without polycystic ovary syndrome. Eur. J. Obstet. Gynecol. Reprod. Biol. 166:173-177; **Bui et al** 2015 Testosterone, free testosterone, and free androgen index in women: Reference intervals, biological variation, and diagnostic value in polycystic ovary syndrome. Clin. Chim. Acta 450:227-232; **Daan et al** 2015 Androgen levels in women with various forms of ovarian dysfunction: associations with cardiometabolic features. Hum. Reprod. 30:2376-2386; **Tosi et al** 2016 Implications of Androgen Assay Accuracy in the Phenotyping of Women With Polycystic Ovary Syndrome. J. Clin. Endocrinol. Metab. 101:610-618; **Yang et al** 2016 Assessing new terminal body and facial hair growth during pregnancy: toward developing a simplified visual scoring system for hirsutism. Fertil. Steril. 105:494-500; **Pasquali et al** 2016 Defining Hyperandrogenism in Women With Polycystic Ovary Syndrome: A Challenging Perspective. J. Clin. Endocrinol. Metab. 101:2013-2022; **Handelsman et al** 2017 Performance of mass spectrometry steroid profiling for diagnosis of polycystic ovary syndrome. Hum. Reprod. 32:418-422; **O'Reilly et al** 2017 11-Oxygenated C19 Steroids Are the Predominant Androgens in Polycystic Ovary Syndrome. J. Clin. Endocrinol. Metab. 102:840-848; **Munzker et al** 2017 High salivary testosterone-to-androstenedione ratio and adverse metabolic phenotypes in women with polycystic ovary syndrome. Clin. Endocrinol. (Oxf). 86:567-575; **Moran et al** 2017 The association of the lipidomic profile with features of polycystic ovary syndrome. J. Mol. Endocrinol. 59:93-104.

Table 3: Upper confidence limits on serum testosterone in women with PCOS

Confidence interval	Likelihood*	SDs#	One-sided¶	Two-sided¶
95%	1:20	1.96	3.13	3.39
99%	1:100	2.35	3.47	3.73
99.9%	1:1000	3.10	4.21	4.39
99.99%	1:10,000	3.72	4.77	4.95

* indicates the likelihood that a woman with PCOS would exceed that limit by chance
# indicates the number of standard deviations for each confidence limit
¶ Two-sided confidence intervals are conventional for a result that could exceed or fall below confidence limits, but here as we focus only on values exceeding the upper limit, so that one-sided confidence limits are appropriate.

Table 4 (from Huang et al, 2014 (88)): Effects of testosterone on muscle mass and strength in women

Androgen sensitive variable	Baseline	Increase	% increase
Lean muscle mass (kg)	43 ± 6	1.9 ± 0.5	4.4
Chest press (Watts)	100 ± 26	26 ± 7	26
Leg press (Newtons)	744 ± 172	90 ± 30	12
Loaded stair climb power (Watts)	406 ± 77	56 ± 13	14

Data shown as mean and standard error of the mean derived from table 1 and digitized from figure 4 from Huang et al showing the effects of testosterone (mean circulating concentration 7.3 nmol/L) on muscle mass and strength in women treated with the highest testosterone dose (n=11; 25 mg testosterone enanthate per week).

Table 5 The winning margin in elite athletic or swimming events over the last three Olympics

Median margin (%)[1]	n	Win gold	Win medal	Make final
Athletics[2]				
Running	81	0.62	0.31	0.22
Jumping	24	0.92	0.42	0.92
Throwing	24	1.93	0.70	0.75
Swimming[3]				
Backstroke	12	0.56	0.28	0.16
Breaststroke	12	0.84	0.14	0.17
Butterfly	12	0.52	0.48	0.12
Freestyle	30	0.49	0.23	0.14
Relay	18	0.37	0.35	0.12

1. Winning margin is defined as the difference (expressed as a percentage of the faster time) between 1st and 2nd place (Win gold), between 3rd and 4th place (Win medal) and between the last into the final and the first that missed out (Make final). Years (2008, 2012, 2016) and sexes were combined as there was no significant differences in winning margin between them.

2. Running includes 100m, 200m, 400m, 800m, 1500m, 5000m, 10,000m, marathon and 3,000m steeplechase, 110m(male)/100m(female) and 400m hurdles, 4 x 100m and 4 x 400m relays, 20km and 50km walk events, Jumping includes high jump, long jump, triple jump and pole vault events and Throwing includes javelin, shot put, discus and hammer events. Heptathlon and decathlon were not included as their final results are in points, not times.

3. Events comprise 100m and 200m for the form strokes and 50m, 100m, 200m, 400m, 800m(female)/1500 m (male) and marathon 10km with the relays being the 4x100m medley, 4x100m and 4 x200m freestyle relays.

Figure Legends

Figure 1: Sex differences in performance (in percentage) according to age (in years) in running events including 50 m to 2 miles (upper left panel) and in jumping events including high jump, pole vault, triple jump, long jump and standing long jump (upper right panel), for details see (8). The lower panel is a fitted sigmoidal curve plot of sex differences in performance (in percentage) according to age (in years) in running, jumping and swimming events, as well as the rising serum testosterone concentrations from a large dataset of serum testosterone of males. Note that in the same dataset female serum testosterone concentrations did not change over those ages, remaining the same as in pre-pubertal boys and girls. Data shown as mean and standard error of the mean of the pooled sex differences by age.

Figure 2 Strong dose-response relationship between testosterone dose and circulating concentration with muscle mass and strength in men:

Upper panel (from Bhasin et al (87)) shows the strong dose-response relationships of muscle mass shown as "lean" or "fat-free" mass (A) or volume of thigh (D) and quadriceps (E) muscle and of leg muscle strength (C) with increasing testosterone dose (upper row) or circulating concentration (lower row). Serum testosterone concentrations are in US units (ng/dl), divide by 28.8 to get nmol/L.

Lower panel (from Finkelstein et al (41)) shows the strong dose-response relationships of whole body muscle mass (B), thigh muscle mass (E) and leg press strength (F) with increasing testosterone dose. Cohorts 1 and 2 were treated with the same increasing doses of testosterone but either without (blue fill, cohort 1) or with (red fill, cohort 2) an aromatase inhibitor (anastrozole), which prevents conversion of testosterone to estradiol. The differences between cohorts (ie use of anastrozole) was not significant for muscle mass and strength so can be ignored with results of the two cohorts pooled.

Figure 3 (from Huang et al (88)): Dose-response effects on lean (muscle) mass and three measures of muscle strength as a result of increasing doses of weekly testosterone enanthate injections in women. Note significant effects on all four parameters of the highest testosterone dose, the only one that produced circulating testosterone concentrations exceeding normal female range.

Figure 4: Redrawn results from Ekblom et al 1972 (100). Results from the transfusion of additional blood are shown in dark red circles and those after blood withdrawal in light red circles.

Figure 5: Plot of circulating hemoglobin against the natural logarithm of serum testosterone in women with congenital adrenal hyperplasia (from Karunasena et al (68)). The filled circles represent a cohort where serum testosterone was measured by immunoassay. The open triangles denote a second cohort, where serum testosterone was measured by LC-MS. Note the systematic overestimation of testosterone by the immunoassay used in cohort 1 vs LC-MS measurement in cohort 2. Despite that over-estimation, however, the correlations were similar in both cohorts.

Figure 6 (from Coviello et al, 2000 (107)): Depicts the strong dose-response relationship between increasing testosterone dose with resulting change in blood hemoglobin in young and older men

1172 Figure 7: Mean hemoglobin concentrations (g/dl) of 12 elite athletes in four groups of three XY or XX middle
1173 distance runners. The hemoglobin concentrations were collected as a part of the Athlete Biological Passport
1174 and analysed according to the WADA standard methods. Each bar (athlete) is the mean of a minimum of three
1175 blood samples. In the 46 XY DSD group, blood was collected in a period when the athlete was not undergoing
1176 hormonal suppressive treatment

1177

1178 Figure 8: Best annual 800m times of an elite female athlete between 2008 and 2016

1179
1180

Figure 1

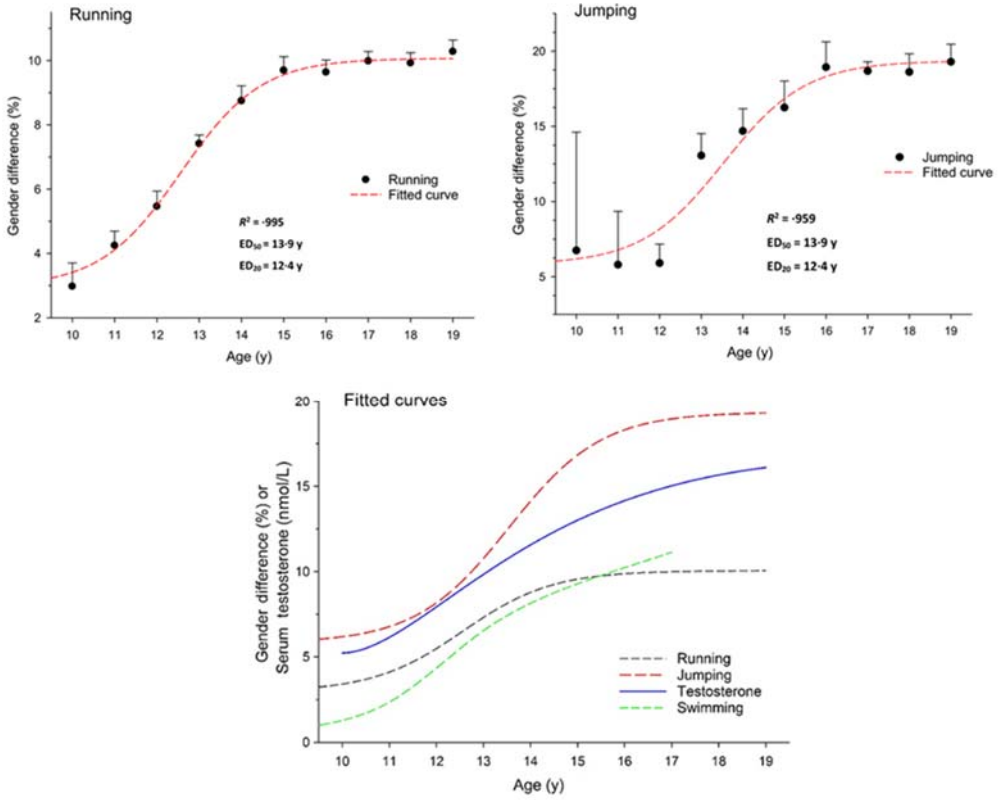

Running
Gender difference (%)
Age (y)
Running
Fitted curve
R^2 = -995
ED_{50} = 13·9 y
ED_{20} = 12·4 y
Jumping
Gender difference (%)
Age (y)
Jumping
Fitted curve
R^2 = -959
ED_{50} = 13·9 y
ED_{20} = 12·4 y
Fitted curves
Gender difference (%) or
Serum testosterone (nmol/L)
Age (y)
Running
Jumping
Testosterone
Swimming

Figure 2

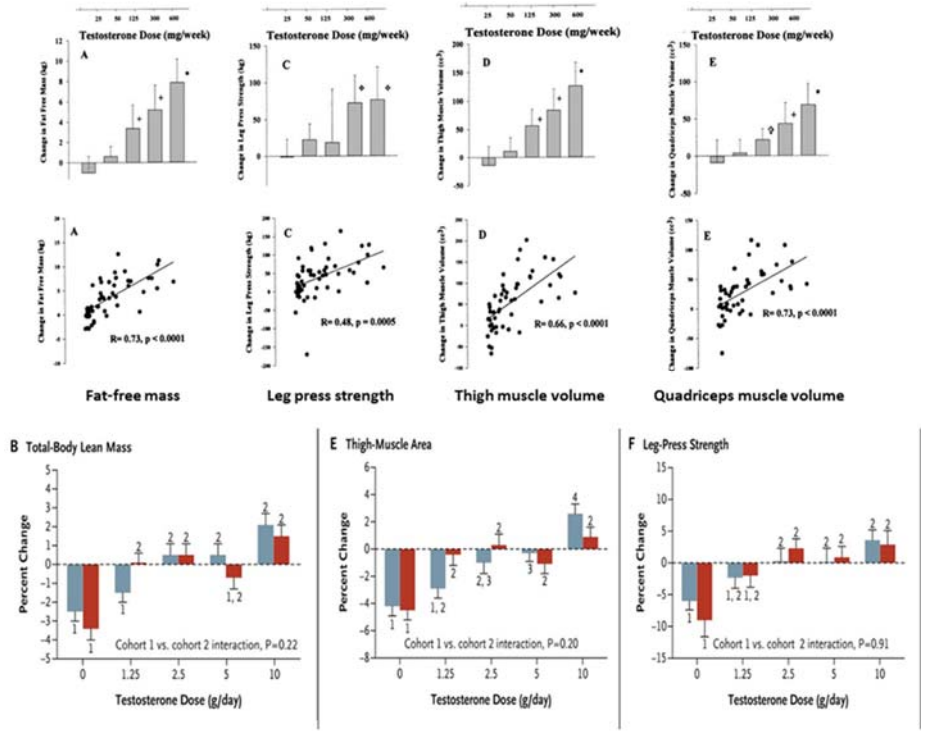

Testosterone Dose (mg/week)
A
C
D
E
R= 0.73, p < 0.0001
R= 0.48, p = 0.0005
R= 0.66, p < 0.0001
R= 0.73, p < 0.0001
Fat-free mass
Leg press strength
Thigh muscle volume
Quadriceps muscle volume
B Total-Body Lean Mass
E Thigh-Muscle Area
F Leg-Press Strength
Percent Change
Cohort 1 vs. cohort 2 interaction, P=0.22
Cohort 1 vs. cohort 2 interaction, P=0.20
Cohort 1 vs. cohort 2 interaction, P=0.91
Testosterone Dose (g/day)

Figure 3

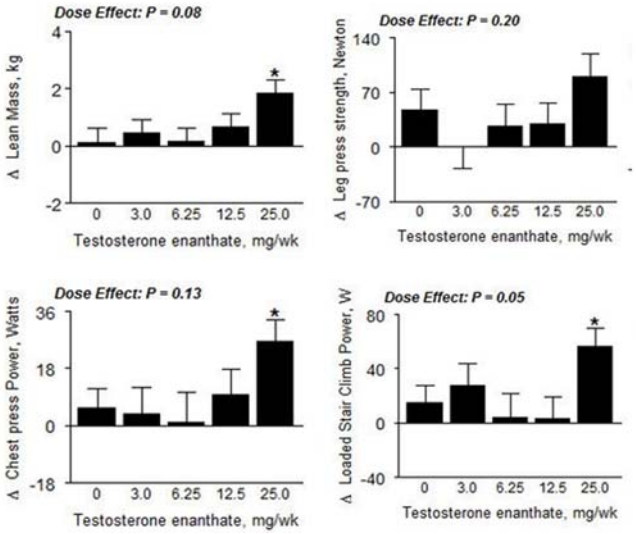


Figure 4

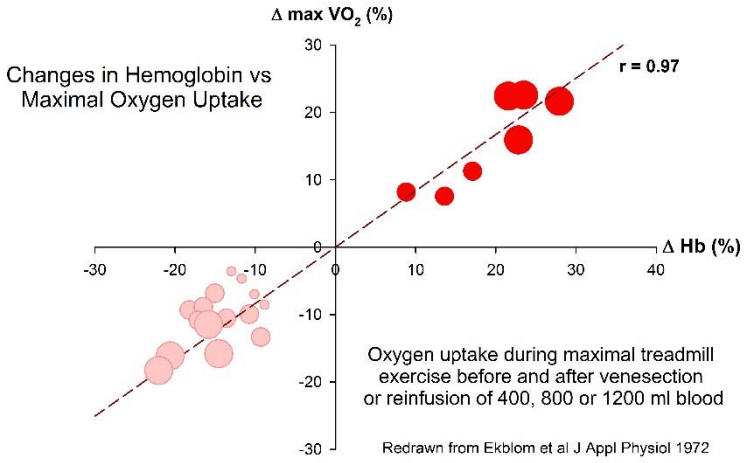


Figure 5

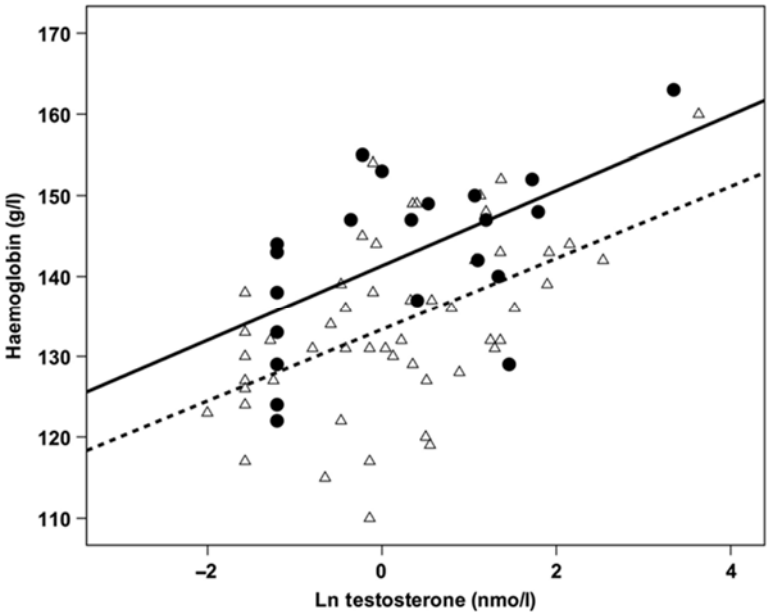


Figure 6

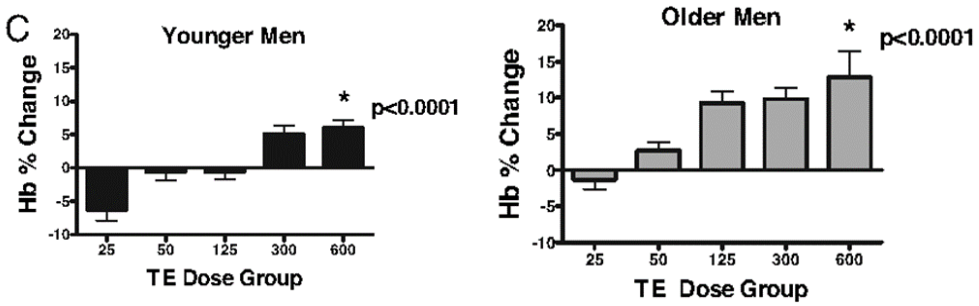


Figure 7

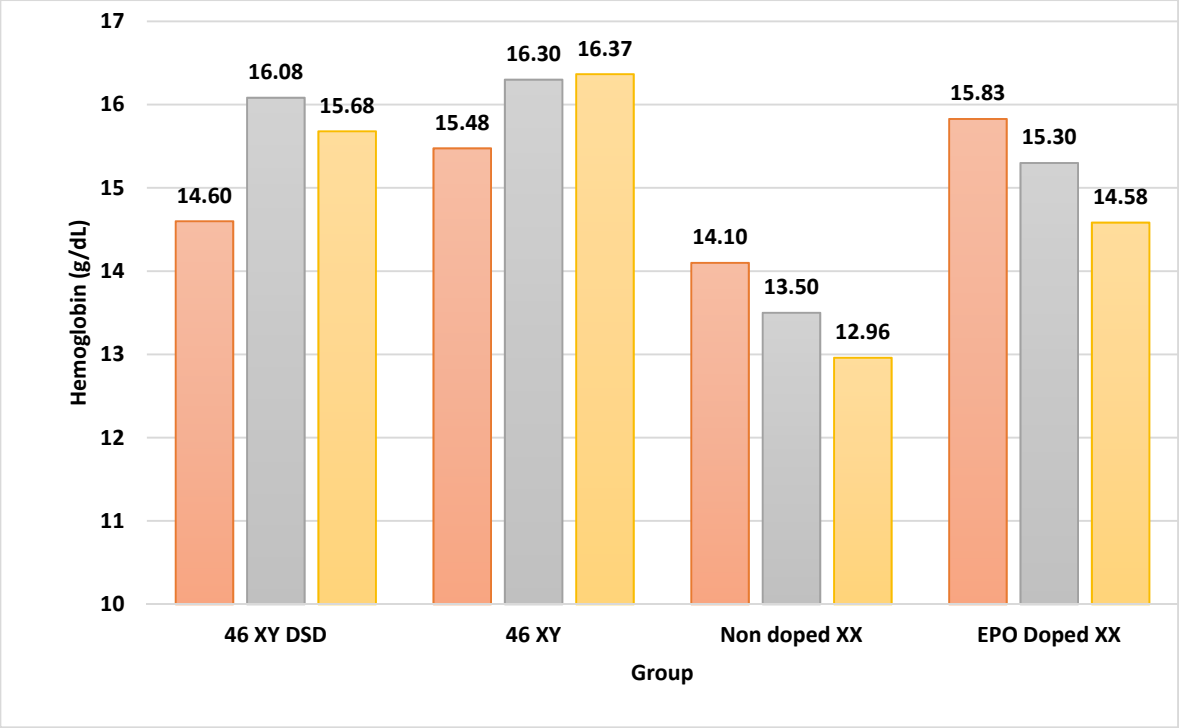
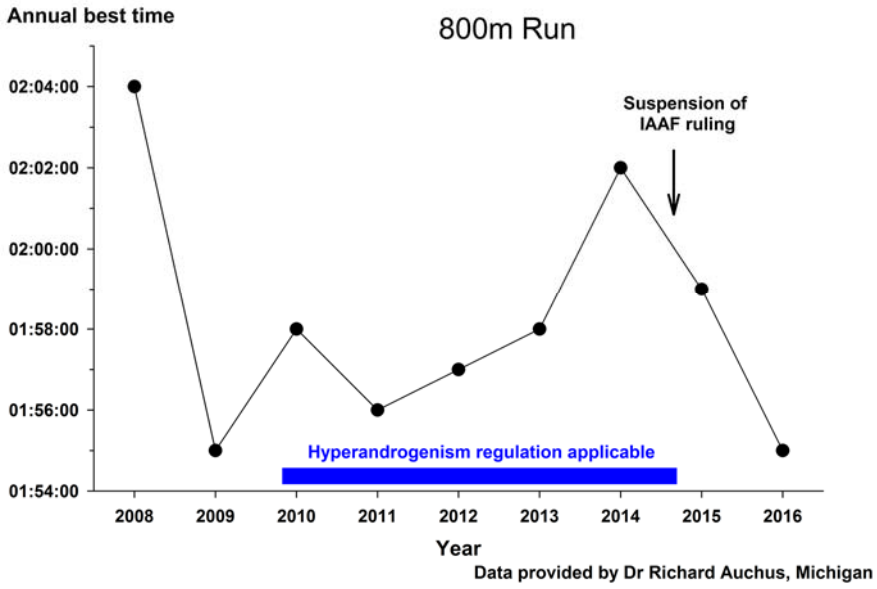


Figure 8



References

1. **Handelsman DJ** 2015 Performance Enhancing Hormones in Sports Doping. Chap 24.In: DeGroot LJ, Jameson JL eds. Endocrinology. 7th ed.: Elsevier Saunders, Philadelphia; pp.441-454
2. **Coleman DL** 2017 Sex in Sport. Available at https://ssrn.com/abstract=2928106, Accessed 22 October 2017
3. **Lee PA, Nordenstrom A, Houk CP, Ahmed SF, Auchus R, Baratz A, Baratz Dalke K, Liao LM, Lin-Su K, Looijenga LH, 3rd, Mazur T, Meyer-Bahlburg HF, Mouriquand P, Quigley CA, Sandberg DE, Vilain E, Witchel S, Global DSDUC** 2016 Global Disorders of Sex Development Update since 2006: Perceptions, Approach and Care. Hormone research in paediatrics 85:158-180
4. **Southren AL, Tochimoto S, Carmody NC, Isurugi K** 1965 Plasma production rates of testosterone in normal adult men and women and in patients with the syndrome of feminizing testes. J Clin Endocrinol Metab 25:1441-1450
5. **Horton R, Tait JF** 1966 Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of its conversion to testosterone. J Clin Invest 45:301-313
6. **Southren AL, Gordon GG, Tochimoto S** 1968 Further studies of factors affecting metabolic clearance rate of testosterone in man. Journal of Clinical Endocrinology and Metabolism 28:1105-1112
7. **Saez JM, Forest MG, Morera AM, Bertrand J** 1972 Metabolic clearance rate and blood production rate of testosterone and dihydrotestosterone in normal subjects, during pregnancy, and in hyperthyroidism. J Clin Invest 51:1226-1234
8. **Handelsman DJ** 2017 Sex differences in athletic performance emerge coinciding with the onset of male puberty. Clin Endocrinol (Oxf) 87:68-72
9. **Auchus RJ** 2017 Endocrinology and women's sports: the diagnosis matters. Duke Law: Law and Contemporary Problems 80:127-138
10. **Foddy B, Savulescu J** 2011 Time to re-evaluate gender segregation in athletics? Br J Sports Med 45:1184-1188
11. **Handelsman DJ** 2015 Androgen Physiology, Pharmacology and Abuse. Chap 138.In: DeGroot LJ, Jameson JL eds. Endocrinology. 7th ed.: Elsevier Saunders, Philadelphia; pp.2368-2393
12. **Miller WL, Auchus RJ** 2011 The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr Rev 32:81-151
13. **Abreu AP, Kaiser UB** 2016 Pubertal development and regulation. Lancet Diabetes Endocrinol 4:254-264
14. **Horton R, Shinsako J, Forsham PH** 1965 TESTOSTERONE PRODUCTION AND METABOLIC CLEARANCE RATES WITH VOLUMES OF DISTRIBUTION IN NORMAL ADULT MEN AND WOMEN. Acta Endocrinol (Copenh) 48:446-458
15. **Rivarola MA, Saez JM, Meyer WJ, Jenkins ME, Migeon CJ** 1966 Metabolic clearance rate and blood production rate of testosterone and androst-4-ene-3,17-dione under basal conditions, ACTH and HCG stimulation. Comparison with urinary production rate of testosterone. J Clin Endocrinol Metab 26:1208-1218
16. **Courant F, Aksglaede L, Antignac JP, Monteau F, Sorensen K, Andersson AM, Skakkebaek NE, Juul A, Bizec BL** 2010 Assessment of circulating sex steroid levels in prepubertal and pubertal boys and girls by a novel ultrasensitive gas chromatography-tandem mass spectrometry method. J Clin Endocrinol Metab 95:82-92
17. **Davison SL, Bell R, Donath S, Montalto JG, Davis SR** 2005 Androgen levels in adult females: changes with age, menopause, and oophorectomy. J Clin Endocrinol Metab 90:3847-3853
18. **Handelsman DJ, Sikaris K, Ly LP** 2016 Estimating age-specific trends in circulating testosterone and sex hormone-binding globulin in males and females across the lifespan. Ann Clin Biochem 53:377-384
19. **Rothman MS, Carlson NE, Xu M, Wang C, Swerdloff R, Lee P, Goh VH, Ridgway EC, Wierman ME** 2011 Reexamination of testosterone, dihydrotestosterone, estradiol and estrone levels across the menstrual cycle and in postmenopausal women measured by liquid chromatography-tandem mass spectrometry. Steroids 76:177-182
20. **Muller RK** 2010 History of doping and doping control. Handb Exp Pharmacol:1-23
21. **Rosner W, Hankinson SE, Sluss PM, Vesper HW, Wierman ME** 2013 Challenges to the Measurement of Estradiol: An Endocrine Society Position Statement. J Clin Endocrinol Metab 98:1376-1387.

22. **Rosner W, Auchus RJ, Azziz R, Sluss PM, Raff H** 2007 Position statement: Utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. J Clin Endocrinol Metab 92:405-413
23. **Handelsman DJ, Wartofsky L** 2013 Requirement for mass spectrometry sex steroid assays in the Journal of Clinical Endocrinology and Metabolism. J Clin Endocrinol Metab 98:3971-3973
24. **Handelsman DJ** 2017 Mass spectrometry, immunoassay and valid steroid measurements in reproductive medicine and science. Hum Reprod:1-4
25. **Sikaris K, McLachlan RI, Kazlauskas R, de Kretser D, Holden CA, Handelsman DJ** 2005 Reproductive hormone reference intervals for healthy fertile young men: evaluation of automated platform assays. J Clin Endocrinol Metab 90:5928-5936.
26. **Eklund E, Berglund B, Labrie F, Carlstrom K, Ekstrom L, Hirschberg AL** 2017 Serum androgen profile and physical performance in women Olympic athletes. Br J Sports Med 51:1301-1308
27. **Bermon S, Garnier PY** 2017 Serum androgen levels and their relation to performance in track and field: mass spectrometry results from 2127 observations in male and female elite athletes. Br J Sports Med 51:1309-1314
28. **Travison TG, Zhuang WV, Lunetta KL, Karasik D, Bhasin S, Kiel DP, Coviello AD, Murabito JM** 2014 The heritability of circulating testosterone, oestradiol, oestrone and sex hormone binding globulin concentrations in men: the Framingham Heart Study. Clin Endocrinol (Oxf) 80:277-282
29. **Coviello AD, Zhuang WV, Lunetta KL, Bhasin S, Ulloor J, Zhang A, Karasik D, Kiel DP, Vasan RS, Murabito JM** 2011 Circulating testosterone and SHBG concentrations are heritable in women: the Framingham Heart Study. J Clin Endocrinol Metab 96:E1491-1495
30. **Fui MN, Dupuis P, Grossmann M** 2014 Lowered testosterone in male obesity: mechanisms, morbidity and management. Asian J Androl 16:223-231
31. **Corona G, Rastrelli G, Monami M, Saad F, Luconi M, Lucchese M, Facchiano E, Sforza A, Forti G, Mannucci E, Maggi M** 2013 Body weight loss reverts obesity-associated hypogonadotropic hypogonadism: a systematic review and meta-analysis. Eur J Endocrinol 168:829-843
32. **Sartorius G, Spasevska S, Idan A, Turner L, Forbes E, Zamojska A, Allan C, Ly L, Conway A, McLachlan R, Handelsman D** 2012 Serum testosterone, dihydrotestosterone and estradiol concentrations in older men self-reporting very good health: The Healthy Man Study. Clin Endocrinol (Oxf) 77:755-763
33. **Webb ML, Wallace JP, Hamill C, Hodgson JL, Mashaly MM** 1984 Serum testosterone concentration during two hours of moderate intensity treadmill running in trained men and women. Endocr Res 10:27-38
34. **Cano Sokoloff N, Misra M, Ackerman KE** 2016 Exercise, Training, and the Hypothalamic-Pituitary-Gonadal Axis in Men and Women. Front Horm Res 47:27-43
35. **Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO** 2016 The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod 31:2841-2855
36. **Hagmar M, Berglund B, Brismar K, Hirschberg AL** 2009 Hyperandrogenism may explain reproductive dysfunction in olympic athletes. Med Sci Sports Exerc 41:1241-1248
37. **Eliakim A, Marom N, Galitskaya L, Nemet D** 2010 Hyperandrogenism among elite adolescent female athletes. J Pediatr Endocrinol Metab 23:755-758
38. **Rickenlund A, Carlstrom K, Ekblom B, Brismar TB, von Schoultz B, Hirschberg AL** 2003 Hyperandrogenicity is an alternative mechanism underlying oligomenorrhea or amenorrhea in female athletes and may improve physical performance. Fertil Steril 79:947-955
39. **Falhammar H, Nordenstrom A** 2015 Nonclassic congenital adrenal hyperplasia due to 21-hydroxylase deficiency: clinical presentation, diagnosis, treatment, and outcome. Endocrine 50:32-50
40. **Auchus RJ** 2015 The classic and nonclassic concenital adrenal hyperplasias. Endocr Pract 21:383-389
41. **Finkelstein JS, Lee H, Burnett-Bowie SA, Pallais JC, Yu EW, Borges LF, Jones BF, Barry CV, Wulczyn KE, Thomas BJ, Leder BZ** 2013 Gonadal steroids and body composition, strength, and sexual function in men. N Engl J Med 369:1011-1022
42. **Donovan KA, Gonzalez BD, Nelson AM, Fishman MN, Zachariah B, Jacobsen PB** 2017 Effect of androgen deprivation therapy on sexual function and bother in men with prostate cancer: A controlled comparison. Psychooncology
43. **Buena F, Swerdloff RS, Steiner BS, Lutchmansingh P, Peterson MA, Pandian MR, Galmarini M, Bhasin S** 1993 Sexual function does not change when serum testosterone levels are pharmacologically varied within the normal male range. Fertil Steril 59:1118-1123

44. **Sartorius GA, Ly LP, Handelsman DJ** 2014 Male sexual function can be maintained without aromatization: randomized placebo-controlled trial of dihydrotestosterone (DHT) in healthy, older men for 24 months. J Sex Med 11:2562-2570
45. **Liu PY, Swerdloff RS, Christenson PD, Handelsman DJ, Wang C** 2006 Rate, extent, and modifiers of spermatogenic recovery after hormonal male contraception: an integrated analysis. Lancet 367:1412-1420
46. **Walsh PC, Swerdloff RS** 1973 Biphasic effect of testosterone on spermatogenesis in the rat. Invest Urol 11:190-193
47. **Singh J, O'Neill C, Handelsman DJ** 1995 Induction of spermatogenesis by androgens in gonadotropin-deficient (*hpg*) mice. Endocrinology 136:5311-5321
48. **Handelsman DJ, Spaliviero JA, Simpson JM, Allan CM, Singh J** 1999 Spermatogenesis without gonadotropins: maintenance has a lower testosterone threshold than initiation. Endocrinology 140:3938-3946
49. **Juel Mortensen L, Blomberg Jensen M, Christiansen P, Ronholt AM, Jorgensen A, Frederiksen H, Nielsen JE, Loya AC, Gronkaer Toft B, Skakkebaek NE, Rajpert-De Meyts E, Juul A** 2017 Germ cell neoplasia in situ and preserved fertility despite suppressed gonadotropins in a patient with testotoxicosis. J Clin Endocrinol Metab
50. **Cunha-Silva M, Brito VN, Macedo DB, Bessa DS, Ramos CO, Lima LG, Barroso PS, Arnhold IJP, Segaloff DL, Mendonca BB, Latronico AC** 2018 Spontaneous fertility in a male patient with testotoxicosis despite suppression of FSH levels. Hum Reprod
51. **Mendonca BB, Batista RL, Domenice S, Costa EM, Arnhold IJ, Russell DW, Wilson JD** 2016 Steroid 5alpha-reductase 2 deficiency. J Steroid Biochem Mol Biol 163:206-211
52. **Mendonca BB, Gomes NL, Costa EM, Inacio M, Martin RM, Nishi MY, Carvalho FM, Tibor FD, Domenice S** 2017 46,XY disorder of sex development (DSD) due to 17beta-hydroxysteroid dehydrogenase type 3 deficiency. J Steroid Biochem Mol Biol 165:79-85
53. **Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM, French FS** 1995 Androgen receptor defects: historical, clinical, and molecular perspectives. Endocr Rev 16:271-321
54. **Lucas-Herald A, Bertelloni S, Juul A, Bryce J, Jiang J, Rodie M, Sinnott R, Boroujerdi M, Lindhardt Johansen M, Hiort O, Holterhus PM, Cools M, Guaragna-Filho G, Guerra-Junior G, Weintrob N, Hannema S, Drop S, Guran T, Darendeliler F, Nordenstrom A, Hughes IA, Acerini C, Tadokoro-Cuccaro R, Ahmed SF** 2016 The long-term outcome of boys with partial androgen insensitivity syndrome and a mutation in the androgen receptor gene. J Clin Endocrinol Metab 101:3959-3967
55. **El-Maouche D, Arlt W, Merke DP** 2017 Congenital adrenal hyperplasia. Lancet
56. **Bermon S, Garnier PY, Hirschberg AL, Robinson N, Giraud S, Nicoli R, Baume N, Saugy M, Fenichel P, Bruce SJ, Henry H, Dolle G, Ritzen M** 2014 Serum androgen levels in elite female athletes. J Clin Endocrinol Metab 99:4328-4335
57. **Imperato-McGinley J, Peterson RE, Gautier T, Sturla E** 1979 Androgens and the evolution of male gender identity among male pseudohemaphrodites with 5-a reductase deficiency. New England Journal of Medicine 300:1233-1237
58. **Kang HJ, Imperato-McGinley J, Zhu YS, Rosenwaks Z** 2014 The effect of 5alpha-reductase-2 deficiency on human fertility. Fertil Steril 101:310-316
59. **Strickland AL, French FS** 1969 Absence of response to dihydrotestosterone in the syndrome of testicular feminization. J Clin Endocrinol Metab 29:1284-1286
60. **Rosenfield RL, Lawrence AM, Liao S, Landau RL** 1971 Androgens and androgen responsiveness in the feminizing testis syndrome. Comparison of complete and "incomplete" forms. J Clin Endocrinol Metab 32:625-632
61. **Hamilton CR, Jr., Kliman B** 1971 Anabolic effect of dihydrotestosterone in testicular feminization syndrome. Metabolism 20:870-877
62. **Zachmann M, Zagalak M, Vollmin JA, Gitzelmann RP, Prader A** 1977 Influence of testosterone on urinary 15N-balance in normal subjects and patients with testicular feminization. Clin Chim Acta 77:147-157
63. **Tincello DG, Saunders PT, Hodgins MB, Simpson NB, Edwards CR, Hargreaves TB, Wu FC** 1997 Correlation of clinical, endocrine and molecular abnormalities with in vivo responses to high-dose testosterone in patients with partial androgen insensitivity syndrome. Clin Endocrinol (Oxf) 46:497-506

64. **Grino PB, Isidro-Gutierrez RF, Griffin JE, Wilson JD** 1989 Androgen resistance associated with a qualitative abnormality of the androgen receptor and responsive to high dose androgen therapy. J Clin Endocrinol Metab 68:578-584
65. **Lundberg Giwercman Y, Nikoshkov A, Lindsten K, Bystrom B, Pousette A, Knudtzon J, Alm J, Wedell A** 2000 Response to treatment in patients with partial androgen insensitivity due to mutations in the DNA-binding domain of the androgen receptor. Horm Res 53:83-88
66. **Holterhus PM, Sinnecker GH, Hiort O** 2000 Phenotypic diversity and testosterone-induced normalization of mutant L712F androgen receptor function in a kindred with androgen insensitivity. J Clin Endocrinol Metab 85:3245-3250
67. **Quigley CA** 1998 The androgen receptor: physiology and pathyphysiology. Chap Nieschlag E, Behre HM eds. Testosterone: Action, Deficiency, Substitution. 2nd ed.: Springer-Verlag, Berlin
68. **Karunasena N, Han TS, Mallappa A, Elman M, Merke DP, Ross RJ, Daniel E** 2017 Androgens correlate with increased erythropoiesis in women with congenital adrenal hyperplasia. Clin Endocrinol (Oxf) 86:19-25
69. **Herbst KL, Bhasin S** 2004 Testosterone action on skeletal muscle. Curr Opin Clin Nutr Metab Care 7:271-277
70. **Dubois V, Laurent MR, Sinnesael M, Cielen N, Helsen C, Clinckemalie L, Spans L, Gayan-Ramirez G, Deldicque L, Hespel P, Carmeliet G, Vanderschueren D, Claessens F** 2014 A satellite cell-specific knockout of the androgen receptor reveals myostatin as a direct androgen target in skeletal muscle. FASEB J 28:2979-2994
71. **Usui T, Kajita K, Kajita T, Mori I, Hanamoto T, Ikeda T, Okada H, Taguchi K, Kitada Y, Morita H, Sasaki T, Kitamura T, Sato T, Kojima I, Ishizuka T** 2014 Elevated mitochondrial biogenesis in skeletal muscle is associated with testosterone-induced body weight loss in male mice. FEBS Lett 588:1935-1941
72. **Manttari S, Anttila K, Jarvilehto M** 2008 Testosterone stimulates myoglobin expression in different muscles of the mouse. Journal of comparative physiology. B, Biochemical, systemic, and environmental physiology 178:899-907
73. **Ferrando AA, Sheffield-Moore M, Yeckel CW, Gilkison C, Jiang J, Achacosa A, Lieberman SA, Tipton K, Wolfe RR, Urban RJ** 2002 Testosterone administration to older men improves muscle function: molecular and physiological mechanisms. Am J Physiol Endocrinol Metab 282:E601-607
74. **Matzuk MM, Lamb DJ** 2008 The biology of infertility: research advances and clinical challenges. Nat Med 14:1197-1213
75. **Matzuk MM, Lamb DJ** 2002 Genetic dissection of mammalian fertility pathways. Nat Cell Biol 4 Suppl:s41-49
76. **Walters KA, Simanainen U, Handelsman DJ** 2010 Molecular insights into androgen actions in male and female reproductive function from androgen receptor knockout models. Hum Reprod Update 16:543-558
77. **MacLean HE, Chiu WS, Notini AJ, Axell AM, Davey RA, McManus JF, Ma C, Plant DR, Lynch GS, Zajac JD** 2008 Impaired skeletal muscle development and function in male, but not female, genomic androgen receptor knockout mice. Faseb J 22:2676-2689
78. **Morrow JR, Jr., Hosler WW** 1981 Strength comparisons in untrained men and trained women athletes. Med Sci Sports Exerc 13:194-197
79. **Miller AE, MacDougall JD, Tarnopolsky MA, Sale DG** 1993 Gender differences in strength and muscle fiber characteristics. Eur J Appl Physiol Occup Physiol 66:254-262
80. **Janssen I, Heymsfield SB, Wang ZM, Ross R** 2000 Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. Journal of applied physiology (Bethesda, Md. : 1985) 89:81-88
81. **Hosler WW, Morrow JR, Jr.** 1982 Arm and leg strength compared between young women and men after allowing for differences in body size and composition. Ergonomics 25:309-313
82. **Sale DG** 1999 Neuromuscular function. Chap 4.In: Tarnopolsky M ed. Gender Differences in Metabolism: Practical and Nutritional Implications. CRC Press, Boca Raton, Florida; pp.61-86
83. **Tonnessen E, Svendsen IS, Olsen IC, Guttormsen A, Haugen T** 2015 Performance development in adolescent track and field athletes according to age, sex and sport discipline. PLoS One 10:e0129014
84. **Carmina E, Guastella E, Longo RA, Rini GB, Lobo RA** 2009 Correlates of increased lean muscle mass in women with polycystic ovary syndrome. Eur J Endocrinol 161:583-589
85. **Douchi T, Oki T, Yamasaki H, Kuwahata R, Nakae M, Nagata Y** 2001 Relationship of androgens to muscle size and bone mineral density in women with polycystic ovary syndrome. Obstet Gynecol 98:445-449

86. **Cardinale M, Stone MH** 2006 Is testosterone influencing explosive performance? Journal of strength and conditioning research / National Strength & Conditioning Association 20:103-107
87. **Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Chen X, Yarasheski KE, Magliano L, Dzekov C, Dzekov J, Bross R, Phillips J, Sinha-Hikim I, Shen R, Storer TW** 2001 Testosterone dose-response relationships in healthy young men. Am J Physiol Endocrinol Metab 281:E1172-1181
88. **Huang G, Basaria S, Travison TG, Ho MH, Davda M, Mazer NA, Miciek R, Knapp PE, Zhang A, Collins L, Ursino M, Appleman E, Dzekov C, Stroh H, Ouellette M, Rundell T, Baby M, Bhatia NN, Khorram O, Friedman T, Storer TW, Bhasin S** 2014 Testosterone dose-response relationships in hysterectomized women with or without oophorectomy: effects on sexual function, body composition, muscle performance and physical function in a randomized trial. Menopause 21:612-623
89. **Dobs AS, Nguyen T, Pace C, Roberts CP** 2002 Differential effects of oral estrogen versus oral estrogen-androgen replacement therapy on body composition in postmenopausal women. J Clin Endocrinol Metab 87:1509-1516
90. **Elbers JM, Asscheman H, Seidell JC, Gooren LJ** 1999 Effects of sex steroid hormones on regional fat depots as assessed by magnetic resonance imaging in transsexuals. Am J Physiol 276:E317-325
91. **Van Caenegem E, Wierckx K, Taes Y, Schreiner T, Vandewalle S, Toye K, Lapauw B, Kaufman JM, T'Sjoen G** 2015 Body composition, bone turnover, and bone mass in trans men during testosterone treatment: 1-year follow-up data from a prospective case-controlled study (ENIGI). Eur J Endocrinol 172:163-171
92. **Sonksen P** 2016 Determination and regulation of body composition in elite athletes. Br J Sports Med
93. **Storer TW, Woodhouse L, Magliano L, Singh AB, Dzekov C, Dzekov J, Bhasin S** 2008 Changes in muscle mass, muscle strength, and power but not physical function are related to testosterone dose in healthy older men. J Am Geriatr Soc 56:1991-1999
94. **Bhasin S, Parker RA, Sattler F, Haubrich R, Alston B, Umbleja T, Shikuma CM** 2007 Effects of testosterone supplementation on whole body and regional fat mass and distribution in human immunodeficiency virus-infected men with abdominal obesity. J Clin Endocrinol Metab 92:1049-1057
95. **Bhasin S, Woodhouse L, Casaburi R, Singh AB, Mac RP, Lee M, Yarasheski KE, Sinha-Hikim I, Dzekov C, Dzekov J, Magliano L, Storer TW** 2005 Older men are as responsive as young men to the anabolic effects of graded doses of testosterone on the skeletal muscle. J Clin Endocrinol Metab 90:678-688
96. **Franke WW, Berendonk B** 1997 Hormonal doping and androgenization of athletes: a secret program of the German Democratic Republic government. Clin Chem 43:1262-1279
97. **Shahani S, Braga-Basaria M, Maggio M, Basaria S** 2009 Androgens and erythropoiesis: past and present. J Endocrinol Invest 32:704-716
98. **Bachman E, Travison TG, Basaria S, Davda MN, Guo W, Li M, Connor Westfall J, Bae H, Gordeuk V, Bhasin S** 2014 Testosterone induces erythrocytosis via increased erythropoietin and suppressed hepcidin: evidence for a new erythropoietin/hemoglobin set point. J Gerontol A Biol Sci Med Sci 69:725-735
99. **Ordway GA, Garry DJ** 2004 Myoglobin: an essential hemoprotein in striated muscle. The Journal of experimental biology 207:3441-3446
100. **Ekblom B, Goldbarg AN, Gullbring B** 1972 Response to exercise after blood loss and reinfusion. J Appl Physiol 33:175-180
101. **Murphy WG** 2014 The sex difference in haemoglobin levels in adults - mechanisms, causes, and consequences. Blood Rev 28:41-47
102. **Grossmann M, Zajac JD** 2012 Hematological changes during androgen deprivation therapy. Asian J Androl 14:187-192
103. **Snyder PJ, Peachey H, Berlin JA, Hannoush P, Haddad G, Dlewati A, Santanna J, Loh L, Lenrow DA, Holmes JH, Kapoor SC, Atkinson LE, Strom BL** 2000 Effects of testosterone replacement in hypogonadal men. J Clin Endocrinol Metab 85:2670-2677
104. **Roy CN, Snyder PJ, Stephens-Shields AJ, Artz AS, Bhasin S, Cohen HJ, Farrar JT, Gill TM, Zeldow B, Cella D, Barrett-Connor E, Cauley JA, Crandall JP, Cunningham GR, Ensrud KE, Lewis CE, Matsumoto AM, Molitch ME, Pahor M, Swerdloff RS, Cifelli D, Hou X, Resnick SM, Walston JD, Anton S, Basaria S, Diem SJ, Wang C, Schrier SL, Ellenberg SS** 2017 Association of Testosterone Levels With Anemia in Older Men: A Controlled Clinical Trial. JAMA internal medicine 177:480-490
105. **Berria R, Gastaldelli A, Lucidi S, Belfort R, De Filippis E, Easton C, Brytzki R, Cusi K, Jovanovic L, DeFronzo R** 2006 Reduction in hematocrit level after pioglitazone treatment is correlated with

decreased plasma free testosterone level, not hemodilution, in women with polycystic ovary syndrome. Clin Pharmacol Ther 80:105-114
106. **Han Y, Kim HS, Lee HJ, Oh JY, Sung YA** 2015 Metabolic effects of polycystic ovary syndrome in adolescents. Ann Pediatr Endocrinol Metab 20:136-142
107. **Coviello AD, Kaplan B, Lakshman KM, Chen T, Singh AB, Bhasin S** 2008 Effects of graded doses of testosterone on erythropoiesis in healthy young and older men. J Clin Endocrinol Metab 93:914-919
108. **Irwig MS** 2017 Testosterone therapy for transgender men. Lancet Diabetes Endocrinol 5:301-311
109. **Velho I, Fighera TM, Ziegelmann PK, Spritzer PM** 2017 Effects of testosterone therapy on BMI, blood pressure, and laboratory profile of transgender men: a systematic review. Andrology
110. **Jacobeit JW, Gooren LJ, Schulte HM** 2009 Safety aspects of 36 months of administration of long-acting intramuscular testosterone undecanoate for treatment of female-to-male transgender individuals. Eur J Endocrinol 161:795-798
111. **Almeida M, Laurent MR, Dubois V, Claessens F, O'Brien CA, Bouillon R, Vanderschueren D, Manolagas SC** 2017 Estrogens and Androgens in Skeletal Physiology and Pathophysiology. Physiol Rev 97:135-187
112. **Sharma K, Gupta P, Shandilya S** 2016 Age related changes in pelvis size among adolescent and adult females with reference to parturition from Naraingarh, Haryana (India). Homo : internationale Zeitschrift fur die vergleichende Forschung am Menschen 67:273-293
113. **Fischer B, Mitteroecker P** 2017 Allometry and Sexual Dimorphism in the Human Pelvis. Anat Rec (Hoboken) 300:698-705
114. **Riesenfeld A** 1978 Functional and hormonal control of pelvic width in the rat. Acta anatomica 102:427-432
115. **Berdnikovs S, Bernstein M, Metzler A, German RZ** 2007 Pelvic growth: ontogeny of size and shape sexual dimorphism in rat pelves. Journal of morphology 268:12-22
116. **Polderman TJ, Benyamin B, de Leeuw CA, Sullivan PF, van Bochoven A, Visscher PM, Posthuma D** 2015 Meta-analysis of the heritability of human traits based on fifty years of twin studies. Nat Genet 47:702-709
117. **Jelenkovic A, Sund R, Hur YM, Yokoyama Y, Hjelmborg JV, Moller S, Honda C, Magnusson PK, Pedersen NL, Ooki S, Aaltonen S, Stazi MA, Fagnani C, D'Ippolito C, Freitas DL, Maia JA, Ji F, Ning F, Pang Z, Rebato E, Busjahn A, Kandler C, Saudino KJ, Jang KL, Cozen W, Hwang AE, Mack TM, Gao W, Yu C, Li L, Corley RP, Huibregtse BM, Derom CA, Vlietinck RF, Loos RJ, Heikkila K, Wardle J, Llewellyn CH, Fisher A, McAdams TA, Eley TC, Gregory AM, He M, Ding X, Bjerregaard-Andersen M, Beck-Nielsen H, Sodemann M, Tarnoki AD, Tarnoki DL, Knafo-Noam A, Mankuta D, Abramson L, Burt SA, Klump KL, Silberg JL, Eaves LJ, Maes HH, Krueger RF, McGue M, Pahlen S, Gatz M, Butler DA, Bartels M, van Beijsterveldt TC, Craig JM, Saffery R, Dubois L, Boivin M, Brendgen M, Dionne G, Vitaro F, Martin NG, Medland SE, Montgomery GW, Swan GE, Krasnow R, Tynelius P, Lichtenstein P, Haworth CM, Plomin R, Bayasgalan G, Narandalai D, Harden KP, Tucker-Drob EM, Spector T, Mangino M, Lachance G, Baker LA, Tuvblad C, Duncan GE, Buchwald D, Willemsen G, Skytthe A, Kyvik KO, Christensen K, Oncel SY, Aliev F, Rasmussen F, Goldberg JH, Sorensen TI, Boomsma DI, Kaprio J, Silventoinen K** 2016 Genetic and environmental influences on height from infancy to early adulthood: An individual-based pooled analysis of 45 twin cohorts. Scientific reports 6:28496
118. **Jelenkovic A, Hur YM, Sund R, Yokoyama Y, Siribaddana SH, Hotopf M, Sumathipala A, Rijsdijk F, Tan Q, Zhang D, Pang Z, Aaltonen S, Heikkila K, Oncel SY, Aliev F, Rebato E, Tarnoki AD, Tarnoki DL, Christensen K, Skytthe A, Kyvik KO, Silberg JL, Eaves LJ, Maes HH, Cutler TL, Hopper JL, Ordonana JR, Sanchez-Romera JF, Colodro-Conde L, Cozen W, Hwang AE, Mack TM, Sung J, Song YM, Yang S, Lee K, Franz CE, Kremen WS, Lyons MJ, Busjahn A, Nelson TL, Whitfield KE, Kandler C, Jang KL, Gatz M, Butler DA, Stazi MA, Fagnani C, D'Ippolito C, Duncan GE, Buchwald D, Derom CA, Vlietinck RF, Loos RJ, Martin NG, Medland SE, Montgomery GW, Jeong HU, Swan GE, Krasnow R, Magnusson PK, Pedersen NL, Dahl-Aslan AK, McAdams TA, Eley TC, Gregory AM, Tynelius P, Baker LA, Tuvblad C, Bayasgalan G, Narandalai D, Lichtenstein P, Spector TD, Mangino M, Lachance G, Bartels M, van Beijsterveldt TC, Willemsen G, Burt SA, Klump KL, Harris JR, Brandt I, Nilsen TS, Krueger RF, McGue M, Pahlen S, Corley RP, Hjelmborg JV, Goldberg JH, Iwatani Y, Watanabe M, Honda C, Inui F, Rasmussen F, Huibregtse BM, Boomsma DI, Sorensen TI, Kaprio J, Silventoinen K** 2016 Genetic and environmental influences on adult human height across birth cohorts from 1886 to 1994. Elife 5
119. **Bechtold S, Beyerlein A, Bonfig W, Dalla Pozza R, Putzker S, Otto R, Schmidt H, Schwarz HP** 2014 Sexual difference in bone geometry of adult patients with classical congenital adrenal hyperplasia:

data using peripheral quantitative computed tomography. Hormone research in paediatrics 82:171-178

120. **Falhammar H, Filipsson H, Holmdahl G, Janson PO, Nordenskjold A, Hagenfeldt K, Thoren M** 2007 Fractures and bone mineral density in adult women with 21-hydroxylase deficiency. J Clin Endocrinol Metab 92:4643-4649
121. **Bhasin S, Storer TW, Berman N, Callegari C, Clevenger B, Phillips J, Bunnell TJ, Tricker R, Shirazi A, Casaburi R** 1996 The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. N Engl J Med 335:1-7
122. **Moreira CA, Bilezikian JP** 2017 Stress Fractures: Concepts and Therapeutics. J Clin Endocrinol Metab 102:525-534
123. **Foryst-Ludwig A, Kintscher U** 2013 Sex differences in exercise-induced cardiac hypertrophy. Pflugers Arch 465:731-737
124. **Gibala MJ, Gillen JB, Percival ME** 2014 Physiological and health-related adaptations to low-volume interval training: influences of nutrition and sex. Sports Med 44 Suppl 2:S127-137
125. **Townsend EA, Miller VM, Prakash YS** 2012 Sex differences and sex steroids in lung health and disease. Endocr Rev 33:1-47
126. **Levine SC, Foley A, Lourenco S, Ehrlich S, Ratliff K** 2016 Sex differences in spatial cognition: advancing the conversation. Wiley interdisciplinary reviews. Cognitive science 7:127-155
127. **Hines M** 2006 Prenatal testosterone and gender-related behaviour. Eur J Endocrinol 155 Suppl 1:S115-121
128. **Hines M, Spencer D, Kung KT, Browne WV, Constantinescu M, Noorderhaven RM** 2016 The early postnatal period, mini-puberty, provides a window on the role of testosterone in human neurobehavioural development. Current opinion in neurobiology 38:69-73
129. **Pope HG, Jr., Kouri EM, Hudson JI** 2000 Effects of supraphysiologic doses of testosterone on mood and aggression in normal men: a randomized controlled trial. Arch Gen Psychiatry 57:133-140; discussion 155-136
130. **Ferguson-Smith MA, Bavington LD** 2014 Natural selection for genetic variants in sport: the role of Y chromosome genes in elite female athletes with 46,XY DSD. Sports Med 44:1629-1634
131. **Heymsfield SB, Gonzalez MC, Lu J, Jia G, Zheng J** 2015 Skeletal muscle mass and quality: evolution of modern measurement concepts in the context of sarcopenia. The Proceedings of the Nutrition Society 74:355-366
132. **Silventoinen K, Sammalisto S, Perola M, Boomsma DI, Cornes BK, Davis C, Dunkel L, De Lange M, Harris JR, Hjelmborg JV, Luciano M, Martin NG, Mortensen J, Nistico L, Pedersen NL, Skytthe A, Spector TD, Stazi MA, Willemsen G, Kaprio J** 2003 Heritability of adult body height: a comparative study of twin cohorts in eight countries. Twin research : the official journal of the International Society for Twin Studies 6:399-408
133. **Beunen G, Thomis M** 2004 Gene powered? Where to go from heritability (h2) in muscle strength and power? Exercise and sport sciences reviews 32:148-154
134. **Silventoinen K, Magnusson PK, Tynelius P, Kaprio J, Rasmussen F** 2008 Heritability of body size and muscle strength in young adulthood: a study of one million Swedish men. Genet Epidemiol 32:341-349
135. **Seeman E** 2002 Pathogenesis of bone fragility in women and men. Lancet 359:1841-1850
136. **Nishiyama KK, Macdonald HM, Moore SA, Fung T, Boyd SK, McKay HA** 2012 Cortical porosity is higher in boys compared with girls at the distal radius and distal tibia during pubertal growth: an HR-pQCT study. J Bone Miner Res 27:273-282
137. **Oliveira CS, Alves C** 2011 The role of the SHOX gene in the pathophysiology of Turner syndrome. Endocrinol Nutr 58:433-442
138. **Ottesen AM, Aksglaede L, Garn I, Tartaglia N, Tassone F, Gravholt CH, Bojesen A, Sorensen K, Jorgensen N, Rajpert-De Meyts E, Gerdes T, Lind AM, Kjaergaard S, Juul A** 2010 Increased number of sex chromosomes affects height in a nonlinear fashion: a study of 305 patients with sex chromosome aneuploidy. Am J Med Genet A 152A:1206-1212
139. **Wideman L, Weltman JY, Shah N, Story S, Veldhuis JD, Weltman A** 1999 Effects of gender on exercise-induced growth hormone release. Journal of applied physiology (Bethesda, Md. : 1985) 87:1154-1162

140. **Veldhuis JD, Roemmich JN, Rogol AD** 2000 Gender and sexual maturation-dependent contrasts in the neuroregulation of growth hormone secretion in prepubertal and late adolescent males and females--a general clinical research center-based study. J Clin Endocrinol Metab 85:2385-2394
141. **Veldhuis JD** 1996 Gender differences in secretory activity of the human somatotropic (growth hormone) axis. Eur J Endocrinol 134:287-295
142. **Ho KY, Evans WS, Blizzard RM, Veldhuis JD, Merriam GR, Samojlik E, Furlanetto R, Rogol AD, Kaiser DL, Thorner MO** 1987 Effects of sex and age on the 24-hour profile of growth hormone secretion in man: importance of endogenous estradiol concentrations. J Clin Endocrinol Metab 64:51-58
143. **Veldhuis JD, Roelfsema F, Keenan DM, Pincus S** 2011 Gender, age, body mass index, and IGF-I individually and jointly determine distinct GH dynamics: analyses in one hundred healthy adults. J Clin Endocrinol Metab 96:115-121
144. **Veldhuis JD, Patrie JT, Brill KT, Weltman JY, Mueller EE, Bowers CY, Weltman A** 2004 Contributions of gender and systemic estradiol and testosterone concentrations to maximal secretagogue drive of burst-like growth hormone secretion in healthy middle-aged and older adults. J Clin Endocrinol Metab 89:6291-6296
145. **Roelfsema F, Veldhuis JD** 2016 Growth Hormone Dynamics in Healthy Adults Are Related to Age and Sex and Strongly Dependent on Body Mass Index. Neuroendocrinology 103:335-344
146. **Pritzlaff-Roy CJ, Widemen L, Weltman JY, Abbott R, Gutgesell M, Hartman ML, Veldhuis JD, Weltman A** 2002 Gender governs the relationship between exercise intensity and growth hormone release in young adults. Journal of applied physiology (Bethesda, Md. : 1985) 92:2053-2060
147. **Leung KC, Doyle N, Ballesteros M, Sjogren K, Watts CK, Low TH, Leong GM, Ross RJ, Ho KK** 2003 Estrogen inhibits GH signaling by suppressing GH-induced JAK2 phosphorylation, an effect mediated by SOCS-2. Proc Natl Acad Sci U S A 100:1016-1021
148. **Ho KK, O'Sullivan AJ, Wolthers T, Leung KC** 2003 Metabolic effects of oestrogens: impact of the route of administration. Ann Endocrinol (Paris) 64:170-177
149. **Cappola AR, Bandeen-Roche K, Wand GS, Volpato S, Fried LP** 2001 Association of IGF-I levels with muscle strength and mobility in older women. J Clin Endocrinol Metab 86:4139-4146
150. **Meinhardt U, Nelson AE, Hansen JL, Birzniece V, Clifford D, Leung KC, Graham K, Ho KK** 2010 The effects of growth hormone on body composition and physical performance in recreational athletes: a randomized trial. Ann Intern Med 152:568-577
151. **Harper J** 2015 Race times for transgender athletes. Journal of Sporting Cultures and Identities 6:1-9
152. **Bermon S** 2017 Androgens and athletic performance of elite female athletes. Curr Opin Endocrinol Diabetes Obes 24:246-251
153. **Elbers JM, Asscheman H, Seidell JC, Megens JA, Gooren LJ** 1997 Long-term testosterone administration increases visceral fat in female to male transsexuals. J Clin Endocrinol Metab 82:2044-2047
154. **Handelsman DJ** 2006 Clinical review: The rationale for banning human chorionic gonadotropin and estrogen blockers in sport. J Clin Endocrinol Metab 91:1646-1653
155. **Asbell SO, Leon SA, Tester WJ, Brereton HD, Ago CT, Rotman M** 1996 Development of anemia and recovery in prostate cancer patients treated with combined androgen blockade and radiotherapy. Prostate 29:243-248
156. **Strum SB, McDermed JE, Scholz MC, Johnson H, Tisman G** 1997 Anaemia associated with androgen deprivation in patients with prostate cancer receiving combined hormone blockade. Br J Urol 79:933-941
157. **Bogdanos J, Karamanolakis D, Milathianakis C, Repousis P, Tsintavis A, Koutsilieris M** 2003 Combined androgen blockade-induced anemia in prostate cancer patients without bone involvement. Anticancer research 23:1757-1762
158. **Choo R, Chander S, Danjoux C, Morton G, Pearce A, Deboer G, Szumacher E, Loblaw A, Cheung P, Woo T** 2005 How are hemoglobin levels affected by androgen deprivation in non-metastatic prostate cancer patients? Can J Urol 12:2547-2552
159. **Chander S, Choo R, Danjoux C, Morton G, Pearse A, Deboer G, Szumacher E, Loblaw A, Cheung P, Woo T** 2005 Effect of androgen suppression on hemoglobin in prostate cancer patients undergoing salvage radiotherapy plus 2-year buserelin acetate for rising PSA after surgery. Int J Radiat Oncol Biol Phys 62:719-724

160. **Golfam M, Samant R, Eapen L, Malone S** 2012 Effects of radiation and total androgen blockade on serum hemoglobin, testosterone, and erythropoietin in patients with localized prostate cancer. Current oncology (Toronto, Ont.) 19:e258-263
161. **Storer TW, Miciek R, Travison TG** 2012 Muscle function, physical performance and body composition changes in men with prostate cancer undergoing androgen deprivation therapy. Asian J Androl 14:204-221