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Pharmacology of anabolic steroids

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Abstract

Go to:

Athletes and bodybuilders have recognized for several decades that the use of anabolic steroids can promote muscle growth and strength but it is only relatively recently that these agents are being revisited for clinical purposes. Anabolic steroids are being considered for the treatment of cachexia associated with chronic disease states, and to address loss of muscle mass in the elderly, but nevertheless their efficacy still needs to be demonstrated in terms of improved physical function and quality of life. In sport, these agents are performance enhancers, this being particularly apparent in women, although there is a high risk of virilization despite the favourable myotrophic–androgenic dissociation that many xenobiotic steroids confer. Modulation of androgen receptor expression appears to be key to partial dissociation, with consideration of both intracellular steroid metabolism and the topology of the bound androgen receptor interacting with co-activators. An anticatabolic effect, by interfering with glucocorticoid receptor expression, remains an attractive hypothesis. Behavioural changes by non-genomic and genomic pathways probably help motivate training. Anabolic steroids continue to be the most common adverse finding in sport and, although apparently rare, designer steroids have been synthesized in an attempt to circumvent the dope test. Doping with anabolic steroids can result in damage to health, as recorded meticulously in the former German Democratic Republic. Even so, it is important not to exaggerate the medical risks associated with their administration for sporting or bodybuilding purposes but to emphasize to users that an attitude of personal invulnerability to their adverse effects is certainly misguided.

Keywords: anabolic steroids, clinical, designer, health, mechanism, performance, receptor, SARMs, sport

Introduction

Go to:

Androgens

Androgens exert their effects in many parts of the body, including reproductive tissues, muscle, bone, hair follicles in the skin, the liver and kidneys, and the haematopoietic, immune and central nervous systems (Mooradian *et al*., 1987). The androgenic effects of these hormones can be generally considered as those associated with masculanization and the anabolic effects as those associated with protein building in skeletal muscle and bone.

In the male foetus, androgens stimulate the development of the Wolffian ducts (epididymis, vas deferens, the seminal vesicles and ejaculatory duct) and the male external genitalia (penis, urethra and scrotum) (Wilson *et al*., 1981). During puberty, the androgenic effects resulting from increased testicular steroidogenesis are manifested by growth of the testes, external genitalia and the male accessory reproductive glands (prostate, seminal vesicles and bulbourethral), and secretory activity begins. Further, the secondary sexual characteristics manifested during puberty can be divided into those that are a result of androgenic and anabolic effects. The androgenic effects are the enlargement of the larynx causing a deepening of the voice, the growth of terminal hair (in the pubic, axillary and facial regions; in other regions such growth depends on a number of factors), an increase in sebaceous gland activity (can lead to acne), and CNS effects (libido and increased aggression). Anabolic effects are the growth of skeletal muscle and bone, the stimulation of linear growth eventually ceasing due to the closure of the epiphysis. In men, androgens are essential for sustaining reproductive function, and they play an important role in maintaining skeletal muscle and bone, cognitive function and a sense of well-being.

The most important androgen secreted is testosterone; in the eugonadal man, the Leydig cells in the testes produce ~95% of the testosterone in the body. The ovaries and the adrenal glands (in both sexes) produce very little testosterone but secrete weaker androgens; in particular, dehydroepiandrosterone (DHEA; and its sulpho-conjugate) and androstenedione are of physiological importance in the women, not least because they can undergo peripheral conversion to more potent androgens, for example to testosterone and 5α-dihydrotestosterone (DHT). Another weaker endogenous androgen, androstenediol, also binds to oestrogen receptors.

The effects of androgens are modulated at cellular level by the steroid-converting enzymes within the particular target tissue (Figure 1). In reproductive target tissues, testosterone can be considered to be a prohormone, being readily converted by 5α-reductase to the more potent androgen DHT. In other tissues, such as adipose tissue and parts of the brain, testosterone is converted by aromatase to the oestrogen, oestradiol. In bone, the mechanism of action of the anabolism of androgens has not been entirely elucidated but both a direct effect of testosterone and a mediated effect by aromatization to oestradiol are important (Orwoll, 1996; Zitzmann and Nieschlag, 2004). In the human skeletal muscle (collected less than 12 h post-mortem), 5α-reductase activity (either type 1 or 2) is not detectable (Thigpen *et al*., 1993), so testosterone itself is chiefly binding to the androgen receptor (as supported also by a number of animal studies, mainly in the rat). Aromatase expression and activity is significant in human skeletal muscle (Larionov *et al*., 2003) but whether the conversion of androgens to oestrogens within this tissue is physiologically important for mediating some of the myotrophic effect of androgens is yet to be determined.

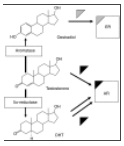

Figure 1

Testosterone can bind directly with the androgen receptor (AR). In target tissues where intracellular enzymes are present, the action of testosterone is mediated by metabolism. Testosterone is irreversibly converted by the enzyme 5α-reductase ...

Modulation of the effects of androgens may also occur at the molecular level due to differences in the distribution of androgen receptor coregulators in various tissues, these coregulators being proteins that affect the transcriptional activity of the androgen receptor (Heinlein and Chang, 2002b; Wolf and Obendorf, 2004). This is a developing field and the comparative importance of many of these coregulators is yet to be established for any particular cell type, let alone their relative *in vivo* importance in examining tissue differences in androgen action. It is envisaged that genetic manipulation of the mouse will assist in elucidating their physiological relevance.

With structural modifications to testosterone, the anabolic effects of androgens can be enhanced but, even so, these cannot be divorced entirely from their androgenic effects. Hence, a more accurate term for anabolic steroids is anabolic–androgenic steroids, but, for simplicity, the shorter term is used within this paper. The disassociation of anabolic from androgenic effects can be at cellular level, depending on the intracellular metabolism of the anabolic steroid in different tissues, with the activity of 5α-reductase being particularly important (see the section 'Intracellular metabolism and the myotrophic–androgenic index'). An appealing hypothesis is that anabolic–androgenic dissociation can also

occur as a result of anabolic steroids inducing specific conformational changes of the androgen receptor complex, which then affects subsequent interaction with various coregulators in different tissues (see the section 'Androgen receptor expression and the importance of coregulators'). There is little data, as yet, to support such a hypothesis, but it is known that the androgen co-activator FHL2 is expressed predominantly in the heart (Muller *et al*., 2000; Wolf and Obendorf, 2004), and it is possible that a number of other androgen receptor coregulators could be tissue specific. How an anabolic steroid may affect androgen receptor conformation and interaction with particular coregulators is of obvious interest, as such knowledge may eventually offer an additional mechanism for anabolic–androgenic dissociation.

The development of nonsteroidal selective androgen receptor modulators (SARMs) may offer better dissociation of biological effects than anabolic steroids and possibly even permit the therapeutic targeting of specific tissues and organs. Potential therapeutic modalities could then be specific agonists for restoration of fat-free muscle mass and strength in those with chronic illnesses such as HIV and specific antagonists for the treatment of prostate cancer in men or hirsutism in women (Wolf and Obendorf, 2004; Bhasin *et al*., 2006). In anticipation of the potential of such agonists for performance enhancement in sport, SARMs have been added to the World Anti-Doping Agency's (WADA's) 2008 list of prohibited substances in sport, despite none yet being available on the market.

Control of anabolic steroids

Anabolic steroids are controlled substances in several countries, including Australia, Argentina, Brazil, Canada, the United Kingdom and the United States. Even so, there is a readily available supply of steroids worldwide for non-medicinal purposes, because, in most countries, anabolic steroids can be sold legally without a prescription (Hermansson, 2002; Cramer, 2005). Thus, many foreign distributors do not violate the laws of their own country when they sell these substances to customers overseas via the Internet and by e-mail orders. The majority of the hormone products in the European market come from countries within the European Union and Russia, but also sometimes from Thailand, Turkey, Egypt, India and Pakistan (Hermansson, 2002). In the United States, significant quantities of anabolic steroids come from Mexico, as well as other countries such as Russia, Romania and Greece (Cramer, 2005).

In the United Kingdom, anabolic steroids are controlled under Schedule IV Part 2 of the Misuse of Drugs Act; the Act includes most of the anabolic steroids, together with clenbuterol (adrenoreceptor stimulant) and human growth hormone. There is no restriction on the possession of these substances when they are part of a medicinal product and are for self-administration. However, prosecutions of intent to supply have been made of individuals found in possession of large quantities of these substances without a prescription for them. A Home Office licence is required for importation and exportation of anabolic steroids, except in cases of small quantities for legitimate purposes.

As to doping control in human sport, the International Olympic Committee (IOC) Medical Commission introduced anabolic steroids as a banned class in 1974 (Kicman and Gower, 2003b). The name of this banned class was amended to anabolic agents in the 1990s to incorporate out-of-competition testing for clenbuterol and other β_2-agonists, which are also considered to have anabolic activity. In 1999, WADA was set up as a foundation under the initiative of the IOC with the support and participation of intergovernmental organizations, governments, public authorities, and other public and private bodies fighting against doping in human sport. Under WADA, the rules and technical documents concerning anabolic steroids (and other drugs) are constantly evolving and for up to date information the reader is strongly advised to access the WADA web site (http://www.wada-ama.org/en/).

Misuse of anabolic steroids in sport and society

The use of anabolic steroids for cosmetic benefits among both adults and adolescents in society may be incorrectly regarded as a comparatively harmless pharmacological manipulation that can aid the development of bulging muscles and a well-toned figure. Surveys of anabolic steroid abuse by gymnasia users found that, overall, around 5% were using such drugs (Korkia and Stimson, 1993), whereas among people attending gyms equipped for competitive bodybuilding, the proportion of current or previous users was around 25–50% (Lenehan *et al*., 1996; Korkia and Stimson, 1997). Nevertheless, it is difficult to estimate the true number of anabolic steroid users in the whole of the United Kingdom but these drugs are used on a nationwide basis, as discussed in depth by the report from the British Medical Association (BMA, 2002). Similar surveys indicate a high prevalence of use in the United States (Yesalis *et al*., 1993, 1997; Yesalis and Bahrke, 2000).

For drug control in sport, anabolic steroids are regarded (correctly) as performance enhancers, as well as harmful to health. Of the 198 143 urine samples analysed in 2006 by 34 WADA-accredited laboratories, 4332 (2%) were found to contain a prohibited substance ('A-sample'), of which 1966 (45% of all the adverse findings) were positive for anabolic steroids. Comparison of the adverse findings for worldwide testing for over a decade show that there has been little change year after year, the most common steroids being testosterone, nandrolone, stanozolol and methandienone. Testosterone has an unfavourable anabolic–androgenic dissociation compared with other anabolic steroids, but it is more difficult to prove its administration, as it is also produced endogenously. Some consider that the WADA statistics do not reflect the real extent of doping with anabolic steroids, particularly within top-level athletics but few would dispute that the urge to succeed and the rewards of success, both financial and otherwise, have provided powerful incentives to some competitors to look for every possible means of improving their performance, despite the risk of denunciation and penalties.

Chemical structures and activity

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Common anabolic steroids

Some of the structural modifications that have been introduced into the testosterone in an attempt to maximize the anabolic effect and minimize the androgenic are shown in Figure 2, and examples of anabolic steroids are given in Figure 3. Many of these steroids have been withdrawn as licensed products in numerous countries worldwide but they continue to be available as pharmaceutical preparations in others, for example, methandienone, methyltestosterone, oxandrolone and stanozolol. The only preparations currently available as licensed products for human use within the United Kingdom are testosterone and its esters, nandrolone (as the decanoate ester), mesterolone and oxymetholone (named patient basis only). Boldenone and trenbolone are restricted to veterinary purposes only in some countries, but, nonetheless, sports competitors and bodybuilders have been known to administer these anabolic steroids.

Figure 2
Structural modifications to the A- and B-rings of testosterone that increase anabolic activity; substitution at C-17 confers oral or depot activity (i.m.). Figure from Kicman and Gower (2003b), a commissioned article by the Analytical Investigations Standing **...**

Figure 3
Structures of anabolic–androgenic steroids with corresponding diagnostic metabolites and examples of registered trade names. Superscripts ($^{1-6}$) refer to 17β-hydroxyl-esterified preparations: ^{1}undecylenoate; ^{2}acetate; ^{3}propionate; **...**

Oral activity can be conferred by substitution of the 17α-H on the steroid nucleus with a methyl or ethyl group to make the 17α-alkylated anabolic steroids. Alkyl substitution prevents deactivation of the steroid by first-pass metabolism by sterically hindering oxidation of the

17β-hydroxyl group. A methyl group attached to C-1 can also confer oral activity, as in methenolone or mesterolone, but these two anabolic steroids are considered to be relatively weak in pharmacological activity.

Parenteral preparations do not require a 17α-alkyl group but usually the 17β-hydroxyl group is esterified with an acid moiety (van der Vies, 1993) to prevent rapid absorption from the oily vehicle, usually arachis oil plus a small amount of benzyl alcohol. Once in the circulation, hydrolysis rapidly occurs by the action of blood esterases to yield the active compound. The esters include cyclohexylpropionate, decanoate, laurate and phenylpropionate for nandrolone; acetate, cypionate, decanoate, enanthate, isocaproate, phenylpropionate, propionate and undecanoate for testosterone, undecylenate for boldenone and acetate for trenbolone. The mechanism of action of the nandrolone esters and other anabolic steroids, and the effect of drug delivery systems on their biological activity have been studied by van der Vies (1993). The duration of action of the esters depends upon the rate of absorption from the site of administration. This is dependent on the chain length of the acid moiety and also the formulation, being related to the partition coefficient of the derivatives between the oil used in the formulation and plasma. In general, the longer the chain length, the more slowly the preparation is released into circulation, thus prolonging the duration of action. Furthermore, testosterone undecanoate is also orally active, the 11 carbon chain ester making the molecule so lipophilic that its route of absorption is partially shifted from the hepatic portal vein to the lymph system, thus bypassing first-pass metabolism to some extent, it being released into the circulation via the thoracic duct (Coert *et al.*, 1975; Horst *et al.*, 1976).

Non-pharmaceutical water-based testosterone suspensions for injection are advertised on bodybuilding web sites and cheats in sport may find these attractive as, in theory, these should be relatively short acting. Non-pharmaceutical-based preparations, whether oil or water based, may be a particular hazard to health as the contents may not have been prepared under sterile conditions.

Transdermal formulations are invariably testosterone based, legitimately designed for replacement therapy, and include the 'patch' and hydroalcoholic gels, to be applied on a daily basis. Other short-acting testosterone preparations include those that are designed to be administered by the sublingual or buccal route. Such short-acting formulations are of particular concern in sport, as the exogenous source of testosterone is rapidly eliminated following cessation of treatment. Increased out-of-competition testing helps to combat the cheat who is using short-acting preparations and ceasing administration prior to competition in anticipation of testing. It is of interest that an illicit preparation called 'The Cream' was designed for transdermal application (see the section 'Designer steroids').

Steroid dietary supplements

A current cause for concern is the recent manufacture of analogues of established anabolic steroids to tap into the bodybuilding market. To avoid the statutory controls of countries regarding the manufacture and supply of drugs, these compounds are often widely marketed as nutritional/dietary supplements, examples being DHEA, androstenedione, androstenediol, and their 19-nor equivalents (these steroids are prohormones), and analogues of testosterone and stanozolol called 1-testosterone and prostanozolol, respectively (Figure 4). It is a consequence of their widespread availability that a minority of athletes will also use these steroids in an attempt to improve sporting performance, and because they are structurally related to mainstream anabolic steroids, sports antidoping laboratories are made to incorporate such compounds into their drug screens under the WADA rules. These steroids are supplied for oral administration, and are therefore subject to first-pass metabolism, a very important factor as to the extent the steroid is deactivated or converted to a more active form. Some of the putative metabolites of dietary supplements have been identified by mass spectrometry, but not by other analytical techniques such as nuclear magnetic resonance spectroscopy to confirm configuration of the structure; the interested reader is referred to the extensive review by Van Eenoo and Delbeke (2006).

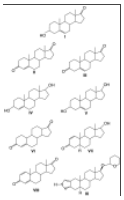

Figure 4
The 'supplements' (I) dehydroepiandrosterone (DHEA), (II) and (III) androstenedione (Δ4 and 5, versions respectively), (IV) and (V) androstenediol (Δ4 and 5 versions, respectively), (VI) 19-norandrostenedione (only Δ4 version ...

With respect to prohormone supplements of testosterone, as recently reviewed by Brown *et al.* (2006), these are modelled on steroids that are endogenously produced, that is, androstenedione, androstenediol and DHEA. However, supplements of the weaker androgens DHEA or androstenedione may be of little or no benefit to healthy young men who wish to improve their strength and sporting performance if, as would be expected, any anabolic effect is primarily mitigated through peripheral conversion to testosterone. Ingestion of DHEA can result in an increase in circulating DHEA and androstenedione, but it is not resolved as to whether there is an increase in plasma testosterone, see for example Brown *et al.* (1999). This is not surprising because in the adult men the overall peripheral contribution of these precursor steroids to circulating testosterone is small. Any contribution from exogenous DHEA or androstenedione will be largely moderated by the large amount of testosterone contributed by the testis. In women, an increase in performance may be possible following ingestion of these supplements, as circulating testosterone would be expected to increase. The plasma concentration of endogenous testosterone is approximately 1/10th that found in men and the relative proportion arising from peripheral conversion of weaker androgens is much greater. Even though only 12–14% of androstenedione is converted peripherally to testosterone (Horton and Tait, 1966; Bardin and Lipsett, 1967), this amount accounts for about half the circulating testosterone in the women. As the peripheral contribution to blood testosterone is far greater in the young adult women than the men, ingestion of modest amounts of androstenedione, DHEA or androstenediol (the natural steroid or the Δ_4 analogue) is likely to significantly raise circulating testosterone. There are modest-to-large increases in circulating testosterone following androstenedione administration to women (Leder *et al.*, 2002; Kicman *et al.*, 2003a; Bassindale *et al.*, 2004; Brown *et al.*, 2004). Women who chronically administer large doses of weaker androgens that can be converted to more potent steroids would be expected to suffer from virilizing effects. In 2004, the FDA (Food and Drug Administration), as part of its public health mission, sent warning letters to 23 companies in the United States requesting them to cease distributing androstenedione as dietary supplements (FDA, 2004).

Designer steroids

Designer anabolic steroids are considered as ones that are manufactured specifically to circumvent doping tests in human sport, and, therefore, for obvious reasons, they are supplied in a clandestine fashion. There are few examples to draw on. Classified documents (Franke and Berendonk, 1997) saved after the collapse of the German Democratic Republic revealed that, since 1983, a pharmaceutical company had produced preparations of epitestosterone propionate exclusively for the governmental doping programme. Epitestosterone, an epimer of testosterone, is a steroid with no anabolic activity but its administration with testosterone simultaneously or sequentially enables an athlete to manipulate the test for testosterone administration if the test is based solely on determination of the urinary testosterone/epitestosterone (T/E) ratio. Recently, a company in California called BALCO (Bay Area Laboratory Co-operative; Burlingame, CA, USA) attracted much media attention due to the high profile of the

athletes involved, not least because of the supply of a transdermal preparation coded as 'The Cream' containing testosterone and epitestosterone, as well as a sublingual preparation of a new anabolic steroid coded as 'The Clear', which was identified from the contents of a spent syringe as tetrahydrogestrinone (THG) by the WADA-accredited laboratory within the University of California, Los Angeles (UCLA) (Catlin *et al*., 2004).

Tetrahydrogestrinone can be easily manufactured by the catalytic hydrogenation of the ethynyl group of the progestogen gestrinone (Figure 5). This relatively simple synthetic step hides the thinking that probably lay behind the design of THG. Given the close homology of their receptors, there is an overlap between the activity of progestogens and androgens, especially those xenobiotic steroids that lack the C-19 methyl group, but which activity predominates depends on whether the alkyl substituent at carbon-17 is ethynyl or ethyl. Substitution of the 17α-H with an ethynyl group on nandrolone, a 19-nor anabolic steroid with some progestational activity, will result in a potent orally active progestogen, this being called norethisterone (norethindrone), a steroid that is still used in some contraceptives today. The synthetic route is described in a seminal paper by Djerassi *et al*. (1954). However, substitution with an ethyl group on nandrolone rather than ethynyl group results in another anabolic steroid known as norethandrolone, which also has oral activity. Gestrinone, is a pharmaceutically available progestogen that lacks the C-19 angular methyl group but has a 17α-ethynyl group, and it follows that reduction of this ethynyl group to the tetrahydro product should make THG a 'potent' androgen. This is indeed the case, as subsequently THG was found to be a highly potent androgen (and progestogen) in an *in vitro* bioassay system expressing human steroid receptors (Death *et al*., 2004), and it promotes muscle accretion in orchidectomized male rats (Jasuja *et al*., 2005). Despite the presence of the 17α-alkyl function, which should make the steroid resistant to first-pass metabolism, it is of interest that the instructions from BALCO Laboratories were to place a few drops of the liquid preparation under the tongue, that is, a sublingual route of administration. THG was invisible on the routine gas chromatography–mass spectrometry screen employed by the WADA-accredited laboratories and necessitated the development of a liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) screen for its detection; for a current and detailed review on the analysis of anabolic steroids see Kicman *et al*. (2008).

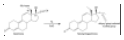

Figure 5
Catalytic hydrogenation of gestrinone to form tetrahydrogestrinone (THG). An example of a catalyst is palladium on carbon (Pd/C), as described in a procedure employed by Catlin *et al*. (2004).

Underground chemists appear also to be accessing information concerning other steroids that were synthesized several decades ago by pharmaceutical companies but were never marketed. Such steroids that have been detected until recently are norbolethone (Catlin *et al*., 2002), which was reputed to have been the active ingredient of 'The Clear' before being replaced by THG, and madol (Sekera *et al*., 2005), which is also referred to as desoxymethyltestosterone (by the WADA-accredited laboratory in Montreal, who identified this steroid around the same time as the accredited laboratory at UCLA). Although the extent of this activity appears to be limited, as screening procedures rely on targeting selecting ions for monitoring by mass spectrometry, unknown steroids may escape detection. To demonstrate how this problem may be addressed, Thevis *et al*. (2005) developed an LC–MS/MS screening method based on common fragmentation pathways and Nielen *et al*. (2006) used a combination of androgen bioassay detection and electrospray quadrupole time-of-flight mass spectometric identification.

Mechanisms of action

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Anabolic steroids are thought to exert their actions by several different mechanisms. These mechanisms include modulating androgen receptor expression as a consequence of (i) intracellular metabolism and by (ii) directly affecting the topology of the androgen receptor and thus subsequent interaction with co-activators and transcriptional activity. Other mechanisms include (iii) an anticatabolic effect by interfering with glucocorticoid receptor expression; and (iv) by non-genomic, as well as by genomic pathways, in the CNS resulting in behavioural changes. These mechanisms are discussed herein.

As an adjunct, much of the physiological importance of non-genomic actions of androgens is still to be elucidated, not least with respect to androgen-induced cell-cycle progression. The induction of second messenger signal transduction cascades by steroids commonly occurs within seconds to a few minutes, in contrast to genomic activity of the classic steroid receptors that takes 30–60 min. Regarding androgens, several non-genomic mechanisms appear to be involved, including mediation by the membrane-bound sex hormone-binding globulin receptor and also a putative G-protein-coupled receptor that androgens directly bind with, as well as through stimulation of nonreceptor tyrosine kinase c-SRC. The complexity of these mechanisms is described in detail elsewhere (Cato *et al*., 2002; Heinlein and Chang, 2002a; Losel *et al*., 2003). Ultimately, gene transcription may be modulated by these 'non-genomic' pathways but a well-recognized exception is the rapid elevation of calcium ion influx by a pathway that is confined to the cytoplasm. It is not currently known whether non-genomic actions of androgens at physiological concentrations are important in skeletal muscle growth, let alone what the non-genomic effects may be evoked by the administration of anabolic steroids. For the sake of brevity, this review will only very briefly touch again on non-genomic pathways under 'Behavioural Effects' (see the section 'Behavioural mechanisms').

Intracellular metabolism and the myotrophic–androgenic index

The structural changes to testosterone by medicinal chemists were designed to enhance the protein anabolic effect relative to the androgenic effect. Unfortunately, the anabolic effects could not be divorced entirely from the androgenic effects, although some synthetic steroids present a remarkable dissociation, at least based on the myotrophic–androgenic index. It may be argued that by today's standards this *in vivo* approach, which was developed over 50 years ago, is unsophisticated given the huge developments in molecular biology since that time. Despite the criticism that this approach has attracted, it is of note that anabolic steroids with high myotrophic activity and favourable index values, for example, nandrolone (esterified), oxymetholone, methandienone and stanozolol are still available as medicines in many countries. These steroids remain desirable as a doping agent to enhance sporting performance (as evident by the statistics collated by WADA) and for bodybuilding purposes. For this reason, it is logical to summarize this approach, based on growth of a particular skeletal muscle called the levator ani relative to that of androgenic target tissue, usually the prostate gland, and attempt to explain the underlying mechanism of dissociation of the growth of the two tissues (compared with controls).

Eisenberg and Gordan (1950) proposed the use of the rat levator ani muscle as a bioassay of protein anabolic activity; the anatomical drawings from the dissection of the male rat, displaying the location of this muscle, the prostate and seminal vesicles are displayed in this paper. The rat levator ani muscle is part of the perineal complex of striated muscles that envelope the rectum. This muscle was chosen because previous workers had reported that testosterone propionate stimulated the growth of the perineal complex in infantile rats, and, additionally, this complex was easily separated from other tissues. Eisenberg *et al*. demonstrated that the levator ani muscle in castrated, immature rats responded well to the administration of various steroids such as testosterone propionate, 17α-methyltestosterone and pituitary growth hormone (extracted from the anterior lobes of ox pituitaries). In contrast, there was a much smaller unparalleled increase in the weight of the seminal vesicles.

The foundation of the commonly used procedure of the myotrophic–androgenic index was based on a modification of the Eisenberg and Gordan method by Hershberger *et al.* (1953). Hershberger and co-workers preferred the use of the ventral part of the prostate rather than the seminal vesicles as a measure of tissue androgenic response in immature gonadectomized rats. They proposed a measure of hormonal myotrophic-to-androgenic activity using the following ratio:

Index ratio=(experimental levator ani weight–control levator ani weight)/(experimental ventral prostate weight–control ventral prostate weight) =increase in levator ani weight/increase in ventral prostate weight

Many investigators employed the approach proposed by Hershberger *et al.* (1953), but some made their own modifications to it, and others still used the seminal vesicles as a bioassay of androgenicity.

Like the index value, the myotrophic or androgenic effects were themselves expressed as ratios to other reference steroids, for example, 17α-methyl testosterone or testosterone for oral routes and testosterone propionate for parenteral routes. A comprehensive comparison of the anabolic and androgenic activities of many anabolic steroids and their dissociation index is given elsewhere (Potts *et al.*, 1976) but some examples are displayed in Table 1.

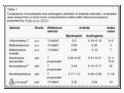

Table 1

Comparison of myotrophic and androgenic activities of anabolic steroids —examples were drawn from a much more comprehensive table (with referenced papers) presented by Potts *et al.* (1976)

Kruskemper (1968) discusses the many failings of the procedures used for determining the myotrophic–androgenic index, for example, the seminal vesicles react more slowly to certain androgens, so that with short test administration, distortions can arise in favour of the myotrophic effect. The harshest criticism of this index was given by Nimni and Geiger (1957), Scow and Hagan (1957) and Hayes (1965). Testosterone administration for 56 days to young gonadectomized rats (castrated at 20–23 days of age) had no effect on the growth of the thigh muscle compared with controls, yet there was considerable growth in the perineal musculature (Scow, 1952; Scow and Hagan, 1957). Testosterone propionate or norethandrolone (17α-ethyl-19-nortestosterone; also an anabolic steroid) administration promoted the growth of the levator ani muscle even in young normal or castrated rats on a protein-free diet, that is, a local anabolic effect proceeding at the expense of catabolic processes in other organs. Hayes (1965) stated that the rat levator ani muscle is not homologous to this muscle in other species, that is, it is not a typical sphincter muscle and does not lift the anus in rodents but is part of the male reproductive system. Thus, Hayes renamed the levator ani muscle, calling it the dorsal bulbocavernosus. All three groups of workers showed that the levator ani muscle reflects a general genitomyotrophic response rather than an overall response to androgens. Later, Hervey (1982) claimed that the male rat's characteristics are determined shortly after birth (due to a brief secretion of testosterone), and, thereafter, any increase in body mass is not affected by androgens.

Contrary to the opinions described above, there is nonetheless biochemical evidence that suggests that the genitomyotrophic response of the levator ani muscle may serve as an indicator of the general myotrophic responses in the developing rat for the following reasons. The same classic androgen receptor can be characterized in the prostate, the bulbocavernosus/levator ani muscle and typical skeletal muscles of the rat (Krieg and Voigt, 1977). Nandrolone (19-nortestosterone) and 5α-DHT have a higher binding affinity than testosterone with the receptor. The prostate has 7 times the concentration of androgen receptors than the bulbocavernosus/levator ani muscles which in turn has 10 times more than other skeletal muscle. *In vitro* studies by Gloyna and Wilson (1969) and Massa and Martini (1974) have shown that 5α-reductase activity is very high in rat sexual tissue such as the prostate and seminal vesicles but negligible, if at all, in skeletal muscle such as the levator ani and thigh muscle. Intracellular DHT is, therefore, low in skeletal muscle, and it is worth emphasizing that its presence is further diminished because of the high activity of the enzyme 3α-hydroxysteroid-dehydrogenase in this tissue (and cardiac tissue as well), the enzyme that converts DHT irreversibly to 3α-androstanediol (Massa and Martini, 1974; Smith *et al.*, 1980). The very low activity of 5α-reductase in skeletal (and cardiac) muscle was subsequently confirmed by other investigators (Krieg *et al.*, 1976; Bartsch *et al.*, 1980), and although the enzymatic activity within the levator ani appears to be significantly higher, it still represents only 5% of that within the prostate. The rat levator ani may be a somewhat atypical striated muscle because of its greater concentration of androgen receptors, but, due to its very low 5α-reductase activity, it can also be argued that it is not a typical part of target tissues associated with the reproductive system. Celotti and Cesi (1992), in their review of possible mechanisms of action of anabolic steroids, discuss that the peculiar androgen sensitivity of this muscle is intermediate between that present in the skeletal muscles and that of the prostate. The myotrophic effect of anabolic steroids may be reflected by the amplified response of the levator ani muscle due to its higher concentration of androgen receptors, an effect that is not apparently sufficient in other (typical) rat skeletal muscles to be observed using differences in weight (compared with controls) as the measurand.

A possible basis for increasing the myotrophic-to-androgenic ratio may be by exploiting the fundamental difference between the 5α-reductase concentrations in skeletal muscle and androgenic tissue. One way of increasing the anabolic–androgenic dissociation is to administer a steroid that has a greater binding affinity for the androgen receptor but upon reduction to a 5α-metabolite has a lesser affinity. Among the anabolic steroids, 19-nortestosterone (nandrolone) was one of the first synthesized, the most used and probably the best studied. Although DHT has a greater binding affinity for the androgen receptor than its parent steroid testosterone, by contrast the 5α-reduced form of 19-nortestosterone, 5α-dihydro-19-nortestosterone, has a lesser binding affinity than its parent steroid 19-nortestosterone (Toth and Zakar, 1982). Hence, in androgenic tissue, testosterone is converted to a more potent metabolite, whereas 19-nortestosterone is converted to a less potent one. As 5α-reduction occurs readily in androgenic tissue but is negligible in skeletal muscle, this explains why 19-nortestosterone has a greater myotrophic-to-androgenic ratio when compared with testosterone (Figure 6). If the model is correct, such a diminishment in androgenic activity should not be confined to the accessory reproductive tissues in the human such as the prostate, but also in non-genital target tissues where clear roles for the metabolism to DHT have been defined such as the male patterns of facial and body hair growth, thus allowing more muscle per whisker. Moreover, even where testosterone rather than DHT appears to stimulate other secondary sexual characteristics, such as voice deepening, with the discovery of two isozymes of 5α-reductase (for review see Russell and Wilson, 1994), it cannot be ruled out that some of these actions attributed to testosterone need to be re-evaluated, the results of which may have relevance to the applicability of anabolic steroids with a high myotrophic–androgenic index. Much of the knowledge of the separate roles of testosterone and DHT came from 5α-reductase deficiency syndrome, but these effects are all ascribed to mutations in the type 2 isoenzyme (Randall, 1994) and the biological role of the 5α-reductase type 1 is harder to ascertain as there is no recognized type 1 deficiency. For example, type 2 5α-reductase appears not to be necessary for the sebaceous gland response to androgens and the development of acne, but it is now known that the principal isoenzyme in this gland is the type 1 form (Thiboutot *et al.*, 1995; Sato *et al.*, 1998). As an adjunct, dutasteride (Avodart; manufactured by GlaxoSmithKline), which inhibits both type 1 and type 2 5α-reductases

and is used in the treatment of benign prostatic hyperplasia (Clark *et al.*, 2004) and male pattern hair loss (Olsen *et al.*, 2006), appears not to be helpful in the treatment for acne vulgaris (Leyden *et al.*, 2004). This suggests that further work at the molecular level is required to better understand the action of androgens on sebaceous gland function.

Figure 6
In androgenic tissues, nandrolone (19-nortestosterone) is readily converted by the enzyme 5α-reductase into 5α-dihydro-19-nortestosterone, i.e., the double bond between C4 and C5 is reduced. This metabolite binds with weaker affinity to **...**

This mechanism of myotrophic–androgenic dissociation does not explain why other anabolic steroids that do not undergo 5α-reduction, for example, those with an extra double bond in the A-ring, such as chlorodehydromethyltestosterone and methandienone (Schanzer, 1996), have a favourable mytotrophic–androgenic index. Even so, it is possible that that myotrophic–androgenic dissociation may occur, simply because the effect of the particular steroid cannot be amplified by 5α-reduction in androgenic target tissues, in common with the hypothesis proposed for the differential action of a steroidal SARM (see the section 'Selective androgen receptor modulators' for an explanation of the term) called MENT (7α-methyl-19-nortestosterone; trestolone) (Agarwal and Monder, 1988; Kumar *et al.*, 1992), as reviewed by Sundaram and Kumar (2000).

Recently, as part of investigations to assess whether the designer steroid THG had anabolic and androgenic properties (see also next section), three papers report the effects of its administration on the growth of the levator ani, prostate and seminal vesicles compared with control steroids (Jasuja *et al.*, 2005; Labrie *et al.*, 2005; Friedel *et al.*, 2006a). Notwithstanding the possible differences in pharmacokinetics and bioavailability between THG and the control steroids administered, there appeared to be little myotrophic–androgenic dissociation, but, nonetheless, the bioassays clearly demonstrated that THG had anabolic and androgenic activity *in vivo*, and, therefore, belonged within the banned doping class of anabolic agents in sport, as defined by WADA.

As a final and very important point, it is of note that complete dissociation has not been achieved with any anabolic steroid synthesized, and, therefore, the chronic administration of these drugs, even those with a very high myotrophic–androgenic index value, such as found with nandrolone (19-nortestosterone), will result in hirsutism and, eventually, virilization of women and children.

Androgen receptor expression and the importance of coregulators

The androgen receptor belongs to the family of nuclear receptor superfamily (Mangelsdorf *et al.*, 1995), these intracellular receptors eliciting so-called 'classical' or genomic, actions by interacting with DNA and modulating transcription. A DNA-binding domain, a ligand-binding domain and at least two transcriptional activation domains, characterize these receptors. Apart from binding with the steroid, the ligand-binding domain also functions in dimer formation and mediates transcriptional activation. The DNA-binding domain targets the receptor to specific DNA sequences known as steroid (or hormone) response elements. On the receptor, the DNA-binding domain consists of two subdomains called 'zinc-fingers'; each subdomain contains four cysteine residues that coordinate with a zinc atom, thus, stabilizing the 'finger' structure. The zinc-fingers are inserted between specific grooves of the DNA helix, thus, allowing maintenance of DNA-binding activity.

A general model of steroid receptor action is displayed in Figure 7. In the absence of hormone, it is by and large accepted that steroid receptors exist as an inactive oligomeric complex, being sequestered by the heat-shock protein (Hsp), Hsp90, which acts as a molecular chaperone. Hsps are so-called because they were discovered to accumulate under stress conditions including within heat-traumatized cells, but many are present and functionally important under normal conditions; they are named according to their molecular weight in kilodaltons. Another chaperone called p23 stabilizes the aporeceptor complex by blocking Hsp90 in the ATP-bound substrate conformation. Co-chaperones utilizing tetratricopeptide repeat motifs are necessary for docking of the Hsp90. As an adjunct, other chaperones, called Hsp40 and Hsp70 and an organizing protein called Hop (heat-shock organizing protein) are important in the assembly of the steroid receptor–Hsp90 complex. Picard (2006) gives a clear overview of molecular chaperones and cofactors that are relevant to steroid receptor action. Phosphorylation of the receptors is also important in regulation of receptor function (Weigel and Moore, 2007).

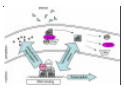

Figure 7
In the absence of hormone, the steroid receptor exists as an inactive oligomeric complex with the molecular chaperone heat-shock protein, Hsp90, and p23, and co-chaperones utilizing tetratricopeptide repeat (TPR) motifs. After hormone binding, the receptor–Hsp90 **...**

The steroid receptor–Hsp90 complex appears to be necessary for the receptor to stabilize in a conformation for binding to the ligand with high affinity and also to maintain its solubility in the cell. It is generally accepted that, although the receptor is held in this complex, it is inactive as a transcription factor, that is, the Hsp90 complex acts as a repressor of transcriptional activity by preventing one or several of the following: nuclear localization, dimerization, DNA binding and interaction with transcriptional co-activators.

Steroids are relatively small molecules, for example, testosterone has a molecular weight of 288, and they can passively diffuse into cells. In target tissues, that is, the cells that contain steroid receptors, the hormone binds to the receptor ligand-binding domain, causing dissociation of the receptor–Hsp90 complex, the resultant conformational (allosteric) change making the receptor active. In the case of the androgen (and glucocorticoid) receptor, the chaperone complex resides in the cytoplasm, and following dissociation from the chaperone the activated receptor is translocated into the nucleus. Activated receptors interact as homodimers with the steroid response element on the chromatin, the effect of two receptors binding being cooperative (greater affinity and stability). This attachment to the DNA, in turn, triggers the formation of a transcription complex, a cluster of coregulators (also called comodulators) that fit around the receptors like 'pieces in a jigsaw puzzle'. Coregulators can be either positive or negative regulatory proteins, referred to as co-activators or corepressors, respectively (Perissi and Rosenfeld, 2005). Co-activator and corepressor complexes are required for nuclear receptor-mediated transcriptional regulation, generally liganded receptors recruiting co-activators resulting in gene activation, transcription of the gene, translation and a resultant alteration in cell function, growth or differentiation.

The function of the transcriptional activation domains on the receptor is to mediate the binding of the receptor to

the comodulators. The receptor has an N-terminal activation function-1 (AF-1) and a second activation function-2 (AF-2) in the C-terminal ligand-binding domain. The mechanisms of AF-1 and AF-2 gene activation, with emphasis on AF-1 and AF-2 conformation and co-activator binding, have been reviewed by Warnmark *et al.* (2003). The mechanism of AF-1 gene activation is not well understood due to the lack of conformational information but, by contrast, many crystal structures of the ligand-binding domain of different nuclear receptors have been achieved, allowing a fuller understanding of AF-2-mediated transcriptional activation. AF-2 is dependent on ligand binding to the receptor for its activity, which causes the folding of a C-terminal helix (helix-12), acting as a lid over the ligand pocket upon ligand binding. This folding creates the activation surface/AF-2 domain, allowing the docking of AF-2 co-activators and the formation of a charge clamp that stabilizes co-activator interaction, these co-activators having the leucine-X-X-leucine-leucine (LXXLL) motif necessary for such interaction (X is any amino acid).

The molecular biology of the androgen receptor has been reviewed by Klocker *et al.* (2004). In contrast to other steroid receptors, most of its transcriptional activity is mediated through the N-terminal AF-1 domain, there being a reduced capacity of AF-2 in the androgen receptor to recruit LXXLL-containing co-activators. Instead, it has been suggested that the AF-2 of the androgen receptor acts primarily as an interaction platform for the recruitment of co-activators to the N-terminal region, this regulation of gene expression through the intradomain interaction and communication being unique to this receptor.

To date, several families of co-activator proteins have been identified but only two direct inhibitors of androgen receptor function have been identified *in vivo*, SHP and DAX-1, these being atypical orphan receptors that lack DNA-binding domains. Using X-ray crystallography, the interaction between peptide segments of SHP containing LXXLL-like motifs and the ligand-binding domain on the androgen receptor was investigated, and it was found that the LKKIL motif formed a complex, binding with a hydrophobic groove on the androgen receptor (Jouravel *et al.*, 2007). It was suggested that this transcriptional activity of androgen receptors might be inhibited by SHP competing for binding to androgen receptor co-activators. The binding motif to the androgen receptor by DAX-1 is still to be elucidated. Another corepressor, FoxG1, appears to be a likely candidate for interaction with the androgen receptor *in vivo* but studies are necessary to prove whether this is the case (Obendorf *et al.*, 2007).

Anabolic steroids bind to the androgen receptor with different affinities. Saartok *et al.* (1984) compared the relative binding affinity of the anabolic steroids ethylestrenol, fluoxymesterone, mesterolone, methandienone, methenolone, 17α-methyltestosterone, nandrolone and oxymetholone to that of tritiated methyltrienolone (17β-hydroxy-17α-methyl-4,9,11-estratrien-3-one; a steroid resembling trenbolone but with a 17α-alkyl substituent) to androgen receptors in cytosol isolated from skeletal muscle and the prostate gland of the rat and skeletal muscle of the rabbit. The order of relative binding affinities in comparison with methyltrienolone, which had the strongest affinity, was nandrolone>methenolone>testosterone>mesterolone; a group which had relatively high and generally similar affinity for the androgen receptor in all three tissues. These investigators did not rank 17α-methyltestosterone, but it had a relative binding affinity of 0.1, which made it an efficient competitor. The relative binding with fluoxymesterone, methandienone and stanozolol was much weaker and that with oxymetholone and ethylestrenol was too low to be determined. There is a large discrepancy as to what is known about the *in vivo* activities of these steroids compared with their *in vitro* activity, even taking into account possible differences in the bioavailability and clearance of these steroids (not least determined by the affinity to sex hormone-binding globulin in the blood circulation). For example, oxymetholone and stanozolol have low relative binding affinity compared with 17α-methyltestosterone in the *in vitro* study, but, conversely, these steroids have a relatively high myotrophic activity compared with the same steroid when administered to the castrated rat (see Table 1). Furthermore, Feldkoren and Andersson (2005) found that stanozolol and methandienone have significantly lower binding affinities compared with testosterone but all three steroids were potent activators in a cell-based androgen receptor-dependent transactivation assay. Clearly, the degree of physical binding to the androgen receptor, as measured by ligand-binding assays, does not fully explain the biological activity of anabolic steroids. Distinct target gene expression profiles due to androgen receptor activation by structurally different androgens has also been reported (Holterhus *et al.*, 2002), the study including three anabolic steroids nandrolone, oxandrolone and stanozolol, together with what the investigators term three 'virilizing androgens' (testosterone, DHT and methyltrienolone) and two testosterone precursors (DHEA and androstenedione). The model used was three structurally different androgen promoter constructs in co-transfected Chinese hamster ovary cells. All the steroids proved to be potent activators of the androgen receptor, but the anabolic steroids and the testosterone precursors showed characteristic promoter activation profiles distinct from the virilizing androgens. The assumption is that the specific ligand-induced conformation determines how the hormone receptor complex can specifically interact with coregulators and neighbouring transcription factors and also that the transactivation capability depends on the structure of the response element. Even though many co-activators have been identified as enhancing the ligand-induced transcriptional activity of the androgen receptor, their relative importance with respect to particular cell types and tissues is unclear (Heinlein and Chang, 2002b). The emerging knowledge concerning androgen receptor interaction with its coregulators in different tissues clearly has relevance to understanding how anabolic steroids exert their actions and will give further insight into how favourable anabolic–androgenic dissociation may be achieved.

Most recently, *in vitro* bioassays have been employed to determine that the designer anabolic steroid THG is indeed a potent androgen. Death *et al.* (2004) demonstrated that THG was about one order of magnitude more potent than nandrolone, testosterone and trenbolone in yeast cells expressing human androgen receptors. Friedel *et al.* (2006b) also used a reporter gene assay based in a yeast strain containing transfected androgen receptor constructs and found that THG was about 10 times lower than the EC_{50} of the reference substance DHT. (Jasuja *et al.* (2005) found that THG upregulated androgen receptor expression in mesenchymal multipotent cells by measuring the translocation of the receptor to the nucleus using immunohistochemical and analyses, but this was not significantly different from DHT. The authors make the important point that it is not known whether yeast-based systems express the repertoire of coregulators that is present in mammalian androgen-responsive tissues. Labrie *et al.* (2005) studied the genomic signature of THG and compared it with the effects of DHT on gene expression in mouse tissues by extracting RNA, converting it to cDNA and then transcribing it *in vitro* to produce biotinylated cRNA for analysis. These investigators found that THG and DHT modulated in a similar fashion 671 genes in the mouse levator ani muscle, 95 genes in the gastrocnemius muscle and 939 genes in the prostate.

The use of *in vitro* assays based on androgen receptor expression, as described above, can help to assess whether future designer steroids have anabolic–androgenic activity, and can help to minimize *in vivo* experiments. These approaches can provide useful evidence to government agencies involved in the regulation of drugs to protect public health. Moreover, the employment of such assays should be of particular benefit to sporting authorities to help stifle legal challenges based on the premise that new designer steroids have unproven anabolic activity and thus should not be subject to doping control and the penalties associated with their administration.

Anticatabolic activity

It is accepted that the administration of anabolic steroids to healthy women and children has an anabolic effect, and that with the virilizing effects, there is a gain in muscle mass and strength. However, for many years, it was difficult to prove conclusively that the administration of these steroids had a myotrophic effect in healthy young sportsmen, as discussed by Ryan (1976) (see the section 'Anabolic steroids as performance enhancers in sport'). Around that period, an interesting but speculative biochemical explanation for this difference in response between the sexes was that due to the exposure to testosterone during puberty in men, there is a downregulation of receptors (decrease in responsiveness of receptors often followed by decrease in numbers) in the skeletal muscle and that the androgen receptor population is then saturated with testosterone in the adult, so that no further response can be induced by pharmacological doses of androgens (Wilson, 1988). It was therefore reasoned that any possible myotrophic effect from administration of anabolic steroids to eugonadal men could be via an anticatabolic mechanism rather than a direct anabolic effect. However, the proposed downregulation of androgen receptors in skeletal muscle because of increased androgen exposure was based on a few animal studies at that time (Dahlberg *et al*., 1981; Rance and Max, 1984) and conflicting evidence was presented by Michel and Baulieu (1980) and more recently by others, for example, Antonio *et al*. (1999). Indeed, Antonio *et al*. speculate that upregulation may occur with the administration of pharmacological amounts of androgens, converting muscles that normally have a minor, or no response, to muscles with enhanced androgen responsiveness. Androgen receptor regulation in different groups of skeletal muscle in response to physiological and supraphysiological exposure to testosterone is intricate, let alone what may occur following administration of xenobiotic anabolic steroids, and the interested reader is referred to the detailed review by Dr F Kadi in the same issue of this journal.

Indirect evidence of an antiglucocorticoid effect comes from a case report concerning partial androgen insensitivity syndrome (Tincello *et al*., 1997). A patient with a single amino-acid mutation in the androgen receptor DNA-binding domain (Arg-608 to Lys), which explained his lack of overall response to high-dose androgen treatment at different times in his life, nonetheless, could be induced into a positive nitrogen balance with testosterone administration. An appealing explanation for this finding is that anabolic steroids act as glucocorticoid receptor antagonists. Most binding studies, however, indicate that anabolic steroids have very low binding affinity for the glucocorticoid receptor (Hickson *et al*., 1990), a notable exception being THG, which binds with high affinity (Friedel *et al*., 2006b). An alternative hypothesis, therefore, is that anabolic steroids may interfere with glucocorticoid receptor expression at the gene level.

Over the years, it has become apparent that the endocrinology of skeletal muscle is highly complex, and there is a delicate balance between synthesis and breakdown during growth, health, disease and ageing, as considered by Sheffield-Moore and Urban (2004). It is this complexity that makes it challenging to resolve the significance of anabolic steroids as anticatabolic (and anabolic) agents across the spectrum, from the healthy athlete who desires faster recovery from arduous training schedules where cortisol may be somewhat raised (Hervey, 1982) to the patient with severe physical trauma, such as from a burn injury, where there is extreme hypercortisolaemia and hypoandrogenaemia (Sheffield-Moore and Urban, 2004).

Behavioural mechanisms

The behavioural effects of androgens/anabolic steroids in men and women, including those concerning sexual behaviour, cognitive abilities, aggression and mood, have been reviewed by Lukas (1996), Christiansen (2001, 2004) and Kuhn (2002) and are also discussed in the National Institute on Drug Abuse (NIDA) Research Monographs (Katz and Pope, 1990; Svare, 1990; Yesalis *et al*., 1990). Androgens are critical to the human male sexual behaviour and they can also enhance female sexual desire and arousal. Testosterone appears to play an important role in cognitive functioning, such as attention and alertness, memory and spatial skills, although based on the conclusions of a limited number of studies. With respect to mood, there are significantly positive correlations of endogenous androgen concentrations with a sense of well-being and joyfulness, and negative correlations with depression and anxiety. Major mood syndromes can arise with anabolic steroid use, including mania or hypomania (mania of a mild type) during exposure and depressive symptoms during steroid withdrawal (Pope and Katz, 1994). Anabolic steroid administration is also associated with increased aggression, especially in high-dose users, but this is not a foregone certainty given that the interaction between androgens and behaviour in men and women is complex. It is an entirely reasonable hypothesis that the athlete may learn to recognize and harness the increase in aggression that can arise with steroid use to help drive their training and increase their competitiveness (Brooks, 1978). Furthermore, male athletes who administer anabolic steroids and then withdraw just before competition in anticipation of a drug test may then experience (in the author's opinion) a lack of motivation and possibly depression, because they will be in a state of androgen deficiency, taking time for testicular steroidogenesis to recover. In an effort to avoid this problem, it is possible that some athletes may switch to using fairly small doses of short half-life formulations of testosterone for replacement purposes in the hope that, at the time of collection of their sample for drug testing, the urinary testosterone/epitestosterone ratio will be below the WADA reporting threshold of 4.

Clark and Henderson (2003) have summarized the literature with respect to the effects of anabolic steroids on the neural circuits that underlie behavioural effects; their review focusing on animal models and steroid exposure that mimic human abuse regimes. Androgen receptors mediate the effects of anabolic steroids in the mammalian brain; the expression of progestogen and oestrogen receptors may also be affected. Non-genomic pathways are important too, the best-characterized example being the

allosteric modulation of $GABA_A$ receptor function by anabolic steroids, possibly through a putative binding site for anabolic steroids residing within the transmembrain domain of the receptor. Induction of aggression by anabolic steroids appears to overlap with neural circuits underlying the regulation of aggression by endogenous androgens, these being systems utilizing GABA, serotonin and arginine vasopressin.

Selective androgen receptor modulators

Go to:

Many anabolic steroids were synthesized and their biological activity characterized (myotrophic–androgenic index, metabolic studies in animal and man) over 40 years ago, at a time when molecular endocrinology was in its infancy. With the knowledge gained in the 1980s and 1990s as to how selective oestrogen receptor modulators, such as tamoxifen and raloxifene, may work at molecular level (Jordan, 1998), perhaps it is not surprising that there is currently an interest in the possibility of modulating the androgen receptor in a similar manner. The development of SARMs, including their ligand interactions with the androgen receptor, is reviewed by Bhasin *et al.* (2006). Tissue selectivity may be achieved by synthesizing ligands that modulate the expression of the androgen receptor by inducing specific conformational changes that affect its interaction with coregulators. Androgens, steroidal or nonsteroidal, that offer tissue selectivity based on a divergence in intracellular metabolism are also included under the term SARM, such as the steroid MENT, which cannot undergo 5α-reduction (Kumar *et al.*, 1992) but is almost certainly aromatized to an active oestrogen (Lamorte *et al.*, 1994; de Gooyer *et al.*, 2003). Indeed, the role of 5α-reductase appears to play a critical part in determining the tissue-specific expression of SARMs (Gao and Dalton, 2007a). Notwithstanding, the clinical applications of steroidal androgens are generally limited by poor tissue selectivity, pharmacokinetics and toxicity, and it is hoped that the amenability to structural modifications of nonsteroidal ligands will overcome these limitations. The current nonsteroidal SARM pharmacophores are analogues of aryl propionamide, bicyclic hydantoin, quinoline and tetrahydroquinoline (Gao and Dalton, 2007b) (Table 2).

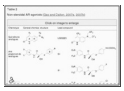

Table 2

Non-steroidal AR agonists (Gao and Dalton, 2007a, 2007b)

Clinically, SARMs may offer unique therapeutic potential to androgen therapy (Negro-Vilar, 1999; Roy *et al.*, 2001; Wolf and Obendorf, 2004) and ultimately those that maintain the anabolic actions of androgens without causing virilization would greatly expand the therapeutic options for women (Gao and Dalton, 2007b). Negro-Vilar (1999) gives a wish list of the desired profile of activity of SARMs, these being tailored to a number of male and female applications. Generally, all include an anabolic effect in muscle and bone, but the androgenic effects are modified to varying degrees from stimulatory, to weak or neutral, depending on the disease state. For example, for the treatment of hypogonadism in elderly men, it is important to minimize induction of growth of the prostate gland to avoid increasing the risk of developing benign prostatic hypertrophy or cancer of the prostate, and, thus, an SARM could be administered with weaker to no activity in this gland. SARMs also could be useful, but not merely confined to, the treatment of osteopaenia, osteoporosis and sarcopaenia in elderly men and postmenopausal women (assuming sufficient anabolic–androgenic dissociation can be achieved for the latter), glucocorticoid-induced osteoporis, HIV wasting, cancer cachexia and different types of muscular dystrophies.

Clinical applications

Go to:

The clinical applications of anabolic steroids has been reviewed recently by Basaria *et al.* (2001) and Shahidi (2001). Historically, the usefulness of anabolic steroids in reversing the catabolic state of patients had not proved convincing and, by the end of the 1980s, many anabolic steroids had been withdrawn as licensed products and those remaining were limited for the purpose of hormone replacement therapy and the treatment of specific diseases (see next paragraph). A detailed analysis of the plethora of clinical reports, including uncontrolled trials and case studies, together with consideration of the risks versus benefits of various anabolic steroids for protein-building purposes is beyond this review. What is especially of note, however, is that lately the potential of anabolic steroids as therapeutic agents to increase weight, lean body mass and strength is being currently revisited. Anabolic steroids, such as testosterone esters, and the 17α-alkylated steroids oxymetholone and oxandrolone, may play a significant role in the treatment of cachexia associated with AIDS, severe burns and renal failure, where nutrition and standard care have been ineffective, as reviewed by Basaria *et al.* (2001). Further, nandrolone decanoate has been demonstrated to be effective in countering sarcopaenia in patients receiving dialysis (Johansen *et al.*, 1999, 2006) and trestolone (MENT) could be a promising new androgen therapy for sarcopaenia (loss of muscle and strength in senescence). In the older woman, oxandrolone administration stimulates muscle protein anabolism (Sheffield-Moore *et al.*, 2006), but the role of anabolic steroid therapy in women with wasting syndromes very much needs to be evaluated (Basaria *et al.*, 2001). Notwithstanding the above, a number of regulatory and conceptual issues are hindering progress in deciding which clinical conditions may benefit from intervention with anabolic steroids (Bhasin *et al.*, 2006), not least what outcomes should constitute evidence of efficacy in clinical trials. For example, although, theoretically, an increase in lean body mass and weight in HIV-infected individuals suffering from weight loss should lead to improved physical functioning and quality of life, and ultimately to increased survival, this has not been demonstrated (Johns *et al.*, 2005). Carefully designed randomized trials may eventually give the definitive answers as to the clinical usefulness of therapy with anabolic steroids, and whether xenobiotic anabolic steroids offer any advantage over supraphysiological doses of testosterone to men. In designing trials involving women, to help reduce unwanted androgenic effects, the administration of a xenobiotic steroid with a favourable myotrophic–androgenic index should be considered. In the interim, at the very least it seems sensible to consider hormone replacement therapy to men in a catabolic state where there is a significant decrease in circulating testosterone associated with the chronic disease, for example, those with severe burn injuries or HIV-associated wasting.

For hormone replacement therapy, testosterone preparations are used in male hypogonadism and male hormonal contraception (where progestogens are administered to inhibit gonadotropin secretion). Mesterolone is also available for the treatment of male hypogonadism but it is seldom used, if at all. Oxymetholone and stanozolol, which induce the production of a C-1 esterase inhibitor, were used in the prevention and control of attacks of hereditary angio-oedema (except in pregnant women and prepubertal patients due to the risk of virilization) but the latter steroid has been recently withdrawn in the United Kingdom. Anabolic steroids also stimulate erythrocyte synthesis, which can be useful in the treatment of hypoplastic anaemias but their use in wealthy countries is likely to be limited with the relative recent availability of recombinant human erythropoietin and its analogues. In postmenopausal women, the treatment of osteoporosis with anabolic steroids, such as nandrolone decanoate, is not advocated given the success of oestrogen replacement and, more recently, with the introduction of the biphosphonates.

Anabolic steroids as performance enhancers in sport

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The action of anabolic steroid in increasing skeletal muscle mass and strength in women is not questioned. Male and female athletes from the German Democratic Republic (GDR), from about 1972 onwards did exceptionally well in international events, being consistently in the top ranking of medal winners. Sporting performance among their female athletes, particularly in strength-dependent events, was spectacular. Following the reunification of Germany in 1990, ground-breaking documental research was made by the former athlete, Mrs Brigitte Berendonk, and her husband Professor Werner Franke, who had succeeded in acquiring a number of highly classified scientific reports that had not been destroyed. These documents deal with the systematic state-sponsored programme of doping of athletes and included scientific reports, doctoral theses and a hand-written protocol book giving the times and dosage of administration of anabolic steroids to athletes. Several thousand athletes were treated with anabolic steroids every year, including adolescents of each sex. Particular emphasis was placed on the administration of anabolic steroids to women and adolescent girls, despite the virilizing effects, because of the rapid gains in sporting performance. It is important to note that the GDR scientists established (to themselves) that 'androgenic initiation' has permanent effects in girls and women, where increases in strength and performance do not return to pretreatment values after the drug is withdrawn. The current emphasis on out-of-competition drug testing is, therefore, of vital importance to help prevent doping during training followed by a period of drug elimination and then competition.

With respect to men, a most comprehensive review in 1976 of previous results concluded that there was little evidence for supraphysiological doses of testosterone or synthetic anabolic steroids having any appreciable effect on muscle size or strength in healthy men (Ryan, 1976). Even so, many of the studies reviewed had a lack of adequate control and standardization. Despite the debate in the scientific community as to the effectiveness of anabolic steroids as performance enhancers in men, male athletes and bodybuilders continued to use them, knowing from their own experimentation that they were effective. Conclusions from more recent reviews suggested that the administration of anabolic steroids could consistently result in significant increases in strength if male athletes satisfied certain criteria including the timing of doses and dietary factors (Wright, 1980; Haupt and Rovere, 1984; Alen and Hakkinen, 1987; Strauss and Yesalis, 1991). Then in 1996, Bhasin *et al.* (1996) in a very carefully designed study, proved beyond doubt that treatment with testosterone in supraphysiological doses (600 mg i.m. of testosterone enanthate for 10 weeks) increases muscle size and strength, and that with exercise these effects are augmented.

Subsequent work showed that increases in fat-free mass, muscle size, strength and power are highly dose-dependent and correlated with serum testosterone concentrations (Bhasin *et al.*, 2001; Woodhouse *et al.*, 2003) (Note: Strength is the maximum amount of force that can be exerted, for example, the heaviest weight that can be pushed away on a leg press, as opposed to power, which is the product of force and velocity, usually measured in watts, for example, the amount of weight that can be pressed away at speed, often repeatedly. Several track and field events demand explosive power, which depends on athletes first developing a solid strength base). The implications of these subsequent findings need to be emphasized to those concerned with antidoping in sport, in that an approximate doubling of the serum total and free concentrations of testosterone from the baseline values in eugonadal men over a 20-week period caused significant increases in strength and power (see the results reported by Bhasin *et al.* (2001), regarding the weekly regimen of 300 mg testosterone enanthate). Although the serum testosterone was measured 7 days after previous injection, which reflect the lowest values after administration, such androgen exposure is relatively small in the context of the regimens often written about in connection with bodybuilding. Even with respect to athletes, this dose is small compared with the amounts that some athletes may have been administering around 30 years ago, as Wright (1980) comments during that period that 'it is not uncommon for the dose level in national calibre athletes to exceed 1 mg/kg of body weight per day with a rather large number of individuals using two to four times that quantity.' Over a decade later, it is worth noting that Rogol and Yesalis (1992) remark that 'Endurance and sprint athletes use doses closer to those used medically for replacement levels', and the connotation is, therefore, that such athletes by that time recognized that modest drug-induced gains in strength and power may be all that is required to secure an advantage in these type of sports events. The use of smaller doses of anabolic steroids, particularly those formulated for daily administration (such as transdermal applications of testosterone as opposed to i.m. administered testosterone enanthate) would allow rapid elimination of a steroid in anticipation of a drug test. Out-of-competition testing should counter this strategy. Regardless of the above, it should be stressed that due to anabolic steroid administration being covert in athletics for obvious reasons, very little recent information has come to light regarding the doses of anabolic steroids used by elite athletes

who choose to cheat.

Adverse effects

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The undesirable effects arising from anabolic steroid administration (Table 3) have been extensively reviewed (Haupt and Rovere, 1984; Di Pasquale, 1990; Graham and Kennedy, 1990; Landry and Primos, 1990; Shahidi, 2001; Kicman and Gower, 2003b; James and Kicman, 2004). Typically, anabolic steroids are taken in cycles of about 6–12 weeks (the 'on period') followed by a variable period off the drugs, from 4 weeks to several months (the 'off period') in an attempt to reduce the likelihood of undesirable effects but some bodybuilders will take them almost continuously.

Table 3

Adverse effects from anabolic steroid administration

For clinical purposes, the administration of these drugs can be of therapeutic benefit and reasonably safe, with the physician making objective decisions based on the benefit/risk ratio in relation to a patient's condition. By contrast, for the purposes of enhancing performance in sport or for cosmetic purposes, usually because it is a clandestine activity, the athletes and bodybuilders are making subjective decisions regarding the effect these steroids are having on their health. Many probably have an attitude of personal invulnerability because they regard themselves as smart steroid users (Perry *et al*., 1990), their knowledge being based on reconnaissance of the considerable amount of popular literature (also in electronic form) written by steroid 'gurus', consultation of colleagues who are steroid users in the gym and their own personal experiences from experimentation. Furthermore, it may be perceived that athletes who fail a test show no obvious signs of ill-health, such as blatant gynaecomastia, severe steroid acne or hirsutism, and this may imply to others that the adverse effects of anabolic steroid use are exaggerated. These athletes could be exercising moderation in the doses they were administering, which should help to keep adverse effects to a minimum (Millar, 1994). Of note, however, is that many of the adverse effects can be difficult to recognize without a thorough medical examination (and patient–doctor confidentiality would have to be maintained) and other damaging effects are insidious where the athletes themselves will be unaware, such as the potential harmful changes to the cardiovascular system. Even so, it is important not to overstate the medical risks associated with anabolic steroid use (Hoffman and Ratamess, 2006) but to emphasize that the hazards to health are dependent on the sex, the dose, the duration of administration, whether hepatoxic 17α-alkylated steroids are being administered and the susceptibility of the individuals themselves to androgen exposure (likely to be dependent on genetic factors, age and lifestyle). The axiom, particularly among bodybuilders who can use excessively large amounts of steroids, that the 'more you take, the more you grow' should be accompanied with 'the more you may damage your health'. It is difficult to gauge the prevalence of severe adverse effects of what is an underground activity, and, moreover, it would be unethical to mimic the large dose regimens in controlled studies over prolonged periods of time to evaluate the risks to health. Notwithstanding, from the records of the doping programme in the former German Democratic Republic, nowhere did the GDR doctors record a damaging effect that was not described in the 'western' literature. These effects included the irreversible effects of virilization (masculanizing effects) in women and female adolescents, and life-threatening liver damage associated with 17α-alkylated steroids (oral-turinabol was commonly administered), which sadly led to the death of the hammer thrower, Mr Detlef Gertsenberg, following postoperative complications.

Abbreviations

Go to:

AF activation function
BALCO Bay Area Laboratory Co-operative
DHEA dehydroepiandrosterone
DHT 5α-dihydrotestosterone
FDA Food and Drug Administration
Hsp heat-shock protein
LC–MS/MS liquid chromatography–mass spectrometry/mass spectrometry
MENT 7α-methyl-19-nortestosterone
SARM selective androgen receptor modulator
THG tetrahydrogestrinone
UCLA, University of California Los Angeles
WADA World Anti-Doping Agency

Notes

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Conflict of interest

The author states no conflict of interest.

References

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Agarwal AK, Monder C. *In vitro* metabolism of 7-alpha-methyl-19-nortestosterone by rat-liver, prostate, and epididymis. Endocrinology. 1988;123:2187–2193. [PubMed]

Alen M, Hakkinen K. Androgenic steroid effects on serum hormones and on maximal force development in strength athletes. J Sports Med Phys Fitness. 1987;27:38–46. [PubMed]

Antonio J, Wilson JD, George FW. Effects of castration and androgen treatment on

androgen-receptor levels in rat skeletal muscles. J Appl Physiol. 1999;87:2016–2019. [PubMed]
Bardin CW, Lipsett MB. Testosterone and androstenedione blood production rates in normal women and women with idiopathic hirsutism or polycystic ovaries. J Clin Invest. 1967;46:891–902. [PMC free article] [PubMed]
Bartsch W, Krieg M, Voigt KD. Quantification of endogenous testosterone, 5-alpha-dihydrotestosterone and 5-alpha-androstane-3-alpha, 17-beta-diol in subcellular fractions of the prostate, bulbocavernosus/levator ani muscle, skeletal muscle and heart muscle of the rat. J Steroid Biochem. 1980;13:259–264. [PubMed]
Basaria S, Wahlstrom JT, Dobs AS. Anabolic–androgenic steroid therapy in the treatment of chronic diseases. J Clin Endocrinol Metab. 2001;86:5108–5117. [PubMed]
Bassindale T, Cowan DA, Dale S, Hutt AJ, Leeds AR, Wheeler MJ, et al. Effects of oral administration of androstenedione on plasma androgens in young women using hormonal contraception. J Clin Endocrinol Metab. 2004;89:6030–6038. [PubMed]
Bhasin S, Calof OM, Storer TW, Lee ML, Mazer NA, Jasuja R, et al. Drug insight: testosterone and selective androgen receptor modulators as anabolic therapies for chronic illness and aging. Nat Clin Pract Endocrinol Metab. 2006;2:146–159. [PMC free article] [PubMed]
Bhasin S, Storer TW, Berman N, Callegari C, Clevenger B, Phillips J, et al. The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. N Engl J Med. 1996;335:1–7. [PubMed]
Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, et al. Testosterone dose–response relationships in healthy young men. Am J Physiol Endocrinol Metab. 2001;281:E1172–E1181. [PubMed]
BMA Anabolic androgenic steroid use in British gymnasiums Drugs in Sport (Report by the Board of Science and Education of the British Medical Association) 2002. BMJ Books: London; 56–75.75In: Conway L, Morgan D (eds).
Brooks RV. Some aspects of the action of anabolic steroids on athletes Physical Activity and Human Well-being 1978. Symposia Specialists Inc.: Quebec; 219–229.229In: Landry F, Oraban WAR (eds).
Brown GA, Dewey JC, Brunkhorst JA, Vukovich MD, King DS. Changes in serum testosterone and estradiol concentrations following acute androstenedione ingestion in young women. Horm Metab Res. 2004;36:62–66. [PubMed]
Brown GA, Vukovich M, King DS. Testosterone prohormone supplements. Med Sci Sports Exerc. 2006;38:1451–1461. [PubMed]
Brown GA, Vukovich MD, Sharp RL, Reifenrath TA, Parsons KA, King DS. Effect of oral DHEA on serum testosterone and adaptations to resistance training in young men. J Appl Physiol. 1999;87:2274–2283. [PubMed]
Catlin DH, Ahrens BD, Kucherova Y. Detection of norbolethone, an anabolic steroid never marketed, in athletes' urine. Rapid Commun Mass Spectrom. 2002;16:1273–1275. [PubMed]
Catlin DH, Sekera MH, Ahrens BD, Starcevic B, Chang YC, Hatton CK. Tetrahydrogestrinone: discovery, synthesis, and detection in urine. Rapid Commun Mass Spectrom. 2004;18:1245–1249. [PubMed]
Cato AC, Nestl A, Mink S. Rapid actions of steroid receptors in cellular signaling pathways. Sci STKE. 2002;2002:RE9. [PubMed]
Celotti F, Cesi PN. Anabolic steroids—a review of their effects on the muscles, of their possible mechanisms of action and of their use in athletics. J Steroid Biochem Mol Biol. 1992;43:469–477. [PubMed]
Christiansen K. Behavioural effects of androgen in men and women. J Endocrinol. 2001;170:39–48. [PubMed]
Christiansen K. Behaviour correlates of testosterone 2004. Cambridge University Press: UK; 233–254.254In: Nieschlag E, Behre HM (eds).
Clark AS, Henderson LP. Behavioral and physiological responses to anabolic–androgenic steroids. Neurosci Biobehav Rev. 2003;27:413–436. [PubMed]
Clark RV, Hermann DJ, Cunningham GR, Wilson TH, Morrill BB, Hobbs S. Marked suppression of dihydrotestosterone in men with benign prostatic hyperplasia by dutasteride, a dual 5 alpha-reductase inhibitor. J Clin Endocrinol Metab. 2004;89:2179–2184. [PubMed]
Coert A, Geelen J, de Visser J, van der Vies J. The pharmacology and metabolism of testosterone undecanoate (TU), a new orally active androgen. Acta Endocrinol (Copenh) 1975;79:789–800. [PubMed]
Cramer RJ. Anabolic steroids are easily purchased without a prescription and present significant challenges to law enforcement officials 2005. Available atdated 3 November 2005.
Dahlberg E, Snochowski M, Gustafsson JA. Regulation of the androgen and glucocorticoid receptors in rat and mouse skeletal muscle cytosol. Endocrinology. 1981;108:1431–1440. [PubMed]
de Gooyer ME, Oppers-Tiemissen HM, Leysen D, Verheul HAM, Kloosterboer HJ. Tibolone is not converted by human aromatase to 7 alpha-methyl-17 alpha-ethynylestradiol (7 alpha-MEE): analyses with sensitive bioassays for estrogens and androgens and with LC–MSMS. Steroids. 2003;68:235–243. [PubMed]
Death AK, McGrath KCY, Kazlauskas R, Handelsman DJ. Tetrahydrogestrinone is a potent androgen and progestin. J Clin Endocrinol Metab. 2004;89:2498–2500. [PubMed]

Di Pasquale MG. Anabolic Steroid Side Effects—Facts, Fiction and Treatment. MGD Press: Ontario; 1990.
Djerassi C, Miramontes L, Rosenkranz G, Sondheimer F. Steroids .54. Synthesis of 19-nor-17-alpha-ethynyltestosterone and 19-nor-17-alpha-methyltestosterone. J Am Chem Soc. 1954;76:4092–4094.
Eisenberg E, Gordan GS. The levator ani muscle of the rat as an index of myotrophic activity of steroidal hormones. J Pharmacol Exp Ther. 1950;99:38–44. [PubMed]
FDA Health Effects of Androstenedione 2004. Food and Drug Administration: Rockville, MD; White Paper. 11 March 2004.
Feldkoren BI, Andersson S. Anabolic–androgenic steroid interaction with rat androgen receptor *in vivo* and *in vitro*: a comparative study. J Steroid Biochem Mol Biol. 2005;94:481–487. [PubMed]
Franke WW, Berendonk B. Hormonal doping and androgenization of athletes: a secret program of the German Democratic Republic Government. Clin Chem. 1997;43:1262–1279. [PubMed]
Friedel A, Geyer H, Kamber M, Laudenbach-Leschowsky U, Schanzer W, Thevis M, et al. 17 beta-Hydroxy-5alpha-androst-1-en-3-one (1-testosterone) is a potent androgen with anabolic properties. Toxicol Lett. 2006a;165:149–155. [PubMed]
Friedel A, Geyer H, Kamber M, Laudenbach-Leschowsky U, Schanzer W, Thevis M, et al. Tetrahydrogestrinone is a potent but unselective binding steroid and affects glucocorticoid signalling in the liver. Toxicol Lett. 2006b;164:16–23. [PubMed]
Gao W, Dalton JT. Ockham's razor and selective androgen receptor modulators (SARMs): are we overlooking the role of 5alpha-reductase. Mol Interv. 2007a;7:10–13. [PMC free article] [PubMed]
Gao WQ, Dalton JT. Expanding the therapeutic use of androgens via selective androgen receptor modulators (SARMs) Drug Discov Today. 2007b;12:241–248. [PMC free article] [PubMed]
Gloyna RE, Wilson JD. A comparative study of conversion of testosterone to 17beta-hydroxy-5alpha-androstan-3-one (dihydrotestosterone) by prostate and epididymis. J Clin Endocrinol Metab. 1969;29:970–977. [PubMed]
Graham S, Kennedy M. Recent developments in the toxicology of anabolic steroids. Drug Saf. 1990;5:458–476. [PubMed]
Haupt HA, Rovere GD. Anabolic steroids: a review of the literature. Am J Sports Med. 1984;12:469–484. [PubMed]
Hayes KJ. So-called levator ani of rat. Acta Endocrinologica. 1965;48:337–347. [PubMed]
Heinlein CA, Chang C. The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. Mol Endocrinol. 2002a;16:2181–2187. [PubMed]
Heinlein CA, Chang CS. Androgen receptor (AR) coregulators: an overview. Endocrine Rev. 2002b;23:175–200. [PubMed]
Hermansson G. Doping trade: business for the big ones 2002. In: Play the Game, search for author underaccessed on January 2008.
Hershberger LG, Shipley EG, Meyer RK. Myotrophic activity of 19-nortestosterone and other steroids determined by modified levator ani muscle method. Proc Soc Exp Biol Med. 1953;83:175–180. [PubMed]
Hervey GR. What are the effects of anabolic steroids Science and Sporting Performance 1982. Clarendon Press: Oxford; 120–136.136In: Davies B, Thomas G (eds).
Hickson RC, Czerwinski SM, Falduto MT, Young AP. Glucocorticoid antagonism by exercise and androgenic-anabolic steroids. Med Sci Sports Exerc. 1990;22:331–340. [PubMed]
Hoffman JR, Ratamess NA. Medical issues associated with anabolic steroid use: Are they exaggerated. J Sports Sci Med. 2006;5:182–193.
Holterhus PM, Piefke S, Hiort O. Anabolic steroids, testosterone-precursors and virilizing androgens induce distinct activation profiles of androgen responsive promoter constructs. J Steroid Biochem Mol Biol. 2002;82:269–275. [PubMed]
Horst HJ, Holtje WJ, Dennis M, Coert A, Geelen J, Voigt KD. Lymphatic absorption and metabolism of orally administered testosterone undecanoate in man. Klin Wochenschr. 1976;54:875–879. [PubMed]
Horton R, Tait JF. Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of its conversion to testosterone. J Clin Invest. 1966;45:301–313. [PMC free article] [PubMed]
James VHT, Kicman AT. Medical aspects of drug use in the gym. Drug Ther Bull. 2004;42:1–5. [PubMed]
Jasuja R, Catlin DH, Miller A, Chang YC, Herbst KL, Starcevic B, et al. Tetrahydrogestrinone is an androgenic steroid that stimulates androgen receptor-mediated, myogenic differentiation in C3H10T1/2 multipotent mesenchymal cells and promotes muscle accretion in orchidectomized male rats. Endocrinology. 2005;146:4472–4478. [PubMed]
Johansen KL, Mulligan K, Schambelan M. Anabolic effects of nandrolone decanoate in patients receiving dialysis—a randomized controlled trial. JAMA. 1999;281:1275–1281. [PubMed]

Johansen KL, Painter PL, Sakkas GK, Gordon P, Doyle J, Shubert T. Effects of resistance exercise training and nandrolone decanoate on body composition and muscle function among patients who receive hemodialysis: a randomized, controlled trial. J Am Soc Nephrol. 2006;17:2307–2314. [PubMed]
Johns K, Beddall MJ, Corrin RC. Anabolic steroids for the treatment of weight loss in HIV-infected individuals. Cochrane Database Syst Rev. 2005. p. CD005483.
Jordan VC. Designer estrogens. Sci Am. 1998;279:60–67. [PubMed]
Jouravel N, Sablin E, Arnold LA, Guy RK, Fletterick RJ. Interaction between the androgen receptor and a segment of its corepressor SHP. Acta Crystallogr D Biol Crystallogr. 2007;63:1198–1200. [PubMed]
Katz DL, Pope HG., Jr Anabolic–androgenic steroid-induced mental status changes. NIDA Res Monogr. 1990;102:215–223. [PubMed]
Kicman AT, Bassindale T, Cowan DA, Dale S, Hutt AJ, Leeds AR. Effect of androstenedione ingestion on plasma testosterone in young women; a dietary supplement with potential health risks. Clin Chem. 2003a;49:167–169. [PubMed]
Kicman AT, Gower DB. Anabolic steroids in sport: biochemical, clinical and analytical perspectives. Ann Clin Biochem. 2003b;40:321–356. [PubMed]
Kicman AT, Houghton E, Gower DB. Anabolic steroids: metabolism, doping and detection in human and equestrian sports Steroid Analysis 2008. Springer: Dordrecht; In: Makin HLJ, Gower DB (eds).2nd edn.
Klocker H, Gromoll J, Cato ACB. The androgen receptor: molecular biology Testosterone 2004. Cambridge University Press: Cambridge, UK; In: Nieschlag E, Behre HM (eds).3rd edn.
Klotz F, Petersson A, Isacson D, Thiblin I. Violent crime and substance abuse: a medico-legal comparison between deceased users of anabolic androgenic steroids and abusers of illicit drugs. Forensic Sci Int. 2007;173:57–63. [PubMed]
Korkia P, Stimson GV. Anabolic Steroid Use in Great Britain: An Exploratory Investigation 1993. The Centre for Research on Drugs and Health Behaviour: London; (ed)
Korkia P, Stimson GV. Indications of prevalence, practice and effects of anabolic steroid use in Great Britain. Int J Sports Med. 1997;18:557–562. [PubMed]
Krieg M, Dennis M, Voigt KD. Comparison between binding of 19-nortestosterone, 5-alpha-dihydrotestosterone and testosterone in rat prostate and bulbocavernosus/levator ani muscle. J Endocrinol. 1976;70:379–387. [PubMed]
Krieg M, Voigt KD. Biochemical substrate of androgenic actions at a cellular level in prostate, bulbocavernosus/levator ani and in skeletal muscle. Acta Endocrinologica. 1977;85:43–87.
Kruskemper HL. Anabolic Steroids. Academic Press: New York; 1968.
Kuhn CM. Anabolic steroids. Recent Prog Horm Res. 2002;57:411–434. [PubMed]
Kumar N, Didolkar AK, Monder C, Bardin CW, Sundaram K. The biological-activity of 7-alpha-methyl-19-nortestosterone is not amplified in male reproductive-tract as is that of testosterone. Endocrinology. 1992;130:3677–3683. [PubMed]
Labrie F, Luu-The V, Calvo E, Martel C, Cloutier J, Gauthier S, et al. Tetrahydrogestrinone induces a genomic signature typical of a potent anabolic steroid. J Endocrinol. 2005;184:427–433. [PubMed]
Lamorte A, Kumar N, Bardin CW, Sundaram K. Aromatization of 7-alpha-methyl-19-nortestosterone by human placental microsomes *in-vitro*. J Steroid Biochem Mol Biol. 1994;48:297–304. [PubMed]
Landry GL, Primos WA., Jr Anabolic steroid abuse. Adv Pediatr. 1990;37:185–205. [PubMed]
Larionov AA, Vasyliev DA, Mason JI, Howie AF, Berstein LM, Miller WR. Aromatase in skeletal muscle. J Steroid Biochem Mol Biol. 2003;84:485–492. [PubMed]
Leder BZ, Leblanc KM, Longcope C, Lee H, Catlin DH, Finkelstein JS. Effects of oral androstenedione administration on serum testosterone and estradiol levels in postmenopausal women. J Clin Endocrinol Metab. 2002;87:5449–5454. [PubMed]
Lenehan P, Bellis M, McVeigh J. A study of anabolic steroid use in the North West of England. J Perform Enhancing Drugs. 1996;1:57–71.
Leyden J, Bergfeld W, Drake L, Dunlap F, Goldman MP, Gottlieb AB, et al. A systemic type I 5 alpha-reductase inhibitor is ineffective in the treatment of acne vulgaris. J Am Acad Dermatol. 2004;50:443–447. [PubMed]
Losel RM, Falkenstein E, Feuring M, Schultz A, Tillmann HC, Rossol-Haseroth K, et al. Nongenomic steroid action:

controversies, questions, and answers. Physiol Rev. 2003;83:965–1016. [PubMed]
Lukas SE. CNS effects and abuse liability of anabolic–androgenic steroids. Annu Rev Pharmacol Toxicol. 1996;36:333–357. [PubMed]
Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, et al. The nuclear receptor superfamily: the second decade. Cell. 1995;83:835–839. [PubMed]
Massa R, Martini L. Testosterone-metabolism—necessary step for activity. J Steroid Biochem Mol Biol. 1974;5:941–947.
Michel G, Baulieu EE. Androgen receptor in rat skeletal muscle—characterization and physiological variations. Endocrinology. 1980;107:2088–2098. [PubMed]
Millar AP. Licit steroid use—hope for the future. Br J Sports Med. 1994;28:79–83. [PMC free article] [PubMed]
Mooradian AD, Morley JE, Korenman SG. Biological actions of androgens. Endocr Rev. 1987;8:1–28. [PubMed]
Muller JM, Isele U, Metzger E, Rempel A, Moser M, Pscherer A, et al. FHL2, a novel tissue-specific coactivator of the androgen receptor. EMBO J. 2000;19:359–369. [PMC free article] [PubMed]
Negro-Vilar A. Selective androgen receptor modulators (SARMs): a novel approach to androgen therapy for the new millennium. J Clin Endocrinol Metab. 1999;84:3459–3462. [PubMed]
Nielen MWF, Bovee TFH, van Engelen MC, Rutgers P, Hamers ARM, van Rhijn IHA, et al. Urine testing for designer steroids by liquid chromatography with androgen bioassay detection and electrospray quadrupole time-of-flight mass spectrometry identification. Anal Chem. 2006;78:424–431. [PubMed]
Nimni ME, Geiger E. Non-suitability of levator ani method as an index of anabolic effect of steroid. Proc Soc Exp Biol Med. 1957;94:606–610. [PubMed]
Obendorf M, Meyer R, Henning K, Mitev YA, Schroder J, Patchev VK, et al. FoxG1, a member of the forkhead family, is a corepressor of the androgen receptor. J Steroid Biochem Mol Biol. 2007;104:195–207. [PubMed]
Olsen EA, Hordinsky M, Whiting D, Stough D, Hobbs S, Ellis ML, et al. The importance of dual 5 alpha-reductase inhibition in the treatment of male pattern hair loss: results of a randomized placebo-controlled study of dutasteride versus finasteride. J Am Acad Dermatol. 2006;55:1014–1023. [PubMed]
Orwoll ES. Androgens as anabolic agents for bone. Trends Endocrinol Metab. 1996;7:77–84. [PubMed]
Perissi V, Rosenfeld MG. Controlling nuclear receptors: the circular logic of cofactor cycles. Nat Rev Mol Cell Biol. 2005;6:542–554. [PubMed]
Perry PJ, Andersen KH, Yates WR. Illicit anabolic steroid use in athletes. A case series analysis. Am J Sports Med. 1990;18:422–428. [PubMed]
Picard D. Chaperoning steroid hormone action. Trends Endocrinol Metab. 2006;17:229–235. [PubMed]
Pope HG, Jr, Katz DL. Psychiatric and medical effects of anabolic–androgenic steroid use. A controlled study of 160 athletes. Arch Gen Psychiatry. 1994;51:375–382. [PubMed]
Potts GO, Arnold A, Beyler AL. Dissociation of the androgenic and other hormonal activities from the protein anabolic effects of steroids Handbook of Experimental Pharmacology 1976. 43Springer-Verlag: Berlin; 361–401.401In: Kochakian CD (ed).
Rance NE, Max SR. Modulation of the cytosolic androgen receptor in striated-muscle by sex steroids. Endocrinology. 1984;115:862–866. [PubMed]
Randall VA. Role of 5 alpha-reductase in health and disease. Baillieres Clin Endocrinol Metab. 1994;8:405–431. [PubMed]
Rogol AD, Yesalis CE., III Clinical review 31: anabolic–androgenic steroids and athletes: what are the issues. J Clin Endocrinol Metab. 1992;74:465–469. [PubMed]
Roy AK, Tyagi RK, Song CS, Lavrovsky Y, Ahn SC, Oh TS, et al. Androgen receptor: structural domains and functional dynamics after ligand-receptor interaction. Ann NY Acad Sci. 2001;949:44–57.
Russell DW, Wilson JD. Steroid 5-alpha-reductase—2 genes 2 enzymes. Annu Rev Biochem. 1994;63:25–61. [PubMed]
Ryan AJ. Athletics (see also Addendum, page 723) Anabolic–Androgenic Steroids 1976. 43Springer-Verlag: Berlin; 515–534.534In: Kochakian CD (ed).

Saartok T, Dahlberg E, Gustafsson JA. Relative binding-affinity of anabolic–androgenic steroids—comparison of the binding to the androgen receptors in skeletal muscle and in prostate, as well as to sex hormone-binding globulin. Endocrinology. 1984;114:2100–2106. [PubMed]

Sato T, Sonoda T, Itami S, Takayasu S. Predominance of type I 5 alpha-reductase in apocrine sweat glands of patients with excessive or abnormal odour derived from apocrine sweat (osmidrosis) Br J Dermatol. 1998;139:806–810. [PubMed]

Schanzer W. Metabolism of anabolic androgenic steroids (see comments) Clin Chem. 1996;42:1001–1020. [PubMed]

Scow RO. Effect of testosterone on muscle and other tissues and on carcass composition in hypophysectomized, thyroidectomized, and gonadectomized male rats. Endocrinology. 1952;51:42–51. [PubMed]

Scow RO, Hagan SN. Effect of testosterone propionate on myosin, collagen and other protein fractions in striated muscle of gonadectomized rats. Endocrinology. 1957;60:273–278. [PubMed]

Sekera MH, Ahrens BD, Chang YC, Starcevic B, Georgakopoulos C, Catlin DH. Another designer steroid: discovery, synthesis, and detection of 'madol' in urine. Rapid Commun Mass Spectrom. 2005;19:781–784. [PubMed]

Shahidi NT. A review of the chemistry, biological action, and clinical applications of anabolic–androgenic steroids. Clin Ther. 2001;23:1355–1390. [PubMed]

Sheffield-Moore M, Paddon-Jones D, Casperson SL, Gilkison C, Volpi E, Wolf SE, et al. Androgen therapy induces muscle protein anabolism in older women. J Clin Endocrinol Metab. 2006;91:3844–3849. [PubMed]

Sheffield-Moore M, Urban RJ. An overview of the endocrinology of skeletal muscle. Trends Endocrinol Metab. 2004;15:110–115. [PubMed]

Smith K, Krieg M, Schwien S. *In vitro* metabolism of 5 alpha-dihydrotestosterone to 5 alpha-androstane-3 alpha, 17 beta-diol in rat heart, diaphragm, skeletal muscle and bulbocavernosus/levator ani: enzyme characterization and quantification. J Steroid Biochem. 1980;13:917–924. [PubMed]

Strauss RH, Yesalis CE. Anabolic steroids in the athlete. Annu Rev Med. 1991;42:449–457. [PubMed]

Sundaram K, Kumar N. 7 alpha-Methyl-19-nortestosterone (MENT): the optimal androgen for male contraception and replacement therapy. Int J Androl. 2000;23:13–15.

Svare BB. Anabolic steroids and behavior: a preclinical research prospectus. NIDA Res Monogr. 1990;102:224–241. [PubMed]

Thevis M, Geyer H, Mareck U, Schanzer W. Screening for unknown synthetic steroids in human urine by liquid chromatography–tandem mass spectrometry. J Mass Spectrom. 2005;40:955–962. [PubMed]

Thiboutot D, Harris G, Iles V, Cimis G, Gilliland K, Hagari S. Activity of the type-1 5-alpha-reductase exhibits regional differences in isolated sebaceous glands and whole skin. J Invest Dermatol. 1995;105:209–214. [PubMed]

Thigpen AE, Silver RI, Guileyardo JM, Casey ML, McConnell JD, Russell DW. Tissue distribution and ontogeny of steroid 5-alpha-reductase isozyme expression. J Clin Invest. 1993;92:903–910. [PMC free article] [PubMed]

Tincello DG, Saunders PTK, Hodgins MB, Simpson NB, Edwards CRW, Hargreaves TB, et al. 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) 1997;46:497–506. [PubMed]

Toth M, Zakar T. Relative binding affinities of testosterone, 19-nortestosterone and their 5-alpha-reduced derivatives to the androgen receptor and to other androgen-binding proteins—a suggested role of 5- alpha-reductive steroid-metabolism in the dissociation of myotropic and androgenic activities of

19-nortestosterone. J Steroid Biochem Mol Biol. 1982;17:653–660.
van der Vies J. Pharmacokinetics of anabolic steroids. Wien Med Wochenschr. 1993;143:366–368. [PubMed]
Van Eenoo P, Delbeke FT. Metabolism and excretion of anabolic steroids in doping control—new steroids and new insights. J Steroid Biochem Mol Biol. 2006;101:161–178. [PubMed]
Warnmark A, Treuter E, Wright APH, Gustafsson JA. Activation functions 1 and 2 of nuclear receptors: molecular strategies for transcriptional activation. Mol Endocrinol. 2003;17:1901–1909. [PubMed]
Weigel NL, Moore NL. Steroid receptor phosphorylation: a key modulator of multiple receptor functions. Mol Endocrinol. 2007;21:2311–2319. [PubMed]
Wilson JD. Androgen abuse by athletes. Endocr Rev. 1988;9:181–199. [PubMed]
Wilson JD, Griffin JE, George FW, Leshin M. The role of gonadal steroids in sexual differentiation. Recent Prog Horm Res. 1981;37:1–39.
Wolf SS, Obendorf M. Selective androgen receptor modulators Testosterone 2004. Cambridge University Press: UK; 623–640.640In: Nieschlag E, Behre HM (eds).3rd edn.
Woodhouse LJ, Reisz-Porszasz S, Javanbakht M, Storer TW, Lee M, Zerounian H, et al. Development of models to predict anabolic response to testosterone administration in healthy young men. Am J Physiol Endocrinol Metab. 2003;284:E1009–E1017. [PubMed]
Wright JE. Anabolic steroids and athletics. Exerc Sport Sci Rev. 1980;8:149–202. [PubMed]
Yesalis CE, Bahrke MS. Doping among adolescent athletes. Baillieres Best Pract Res Clin Endocrinol Metab. 2000;14:25–35. [PubMed]
Yesalis CE, Barsukiewicz CK, Kopstein AN, Bahrke MS. Trends in anabolic–androgenic steroid use among adolescents. Arch Pediatr Adolesc Med. 1997;151:1197–1206. [PubMed]
Yesalis CE, Kennedy NJ, Kopstein AN, Bahrke MS. Anabolic–androgenic steroid use in the United States. JAMA. 1993;270:1217–1221. [PubMed]
Yesalis CE, Vicary JR, Buckley WE, Streit AL, Katz DL, Wright JE. Indications of psychological dependence among anabolic–androgenic steroid abusers. NIDA Res Monogr. 1990;102:196–214. [PubMed]
Zitzmann M, Nieschlag E. Androgens and bone metabolism Testosterone 2004. Cambridge University Press: UK; 233–254.254In: Nieschlag E, Behre HM (eds).3rd edn.