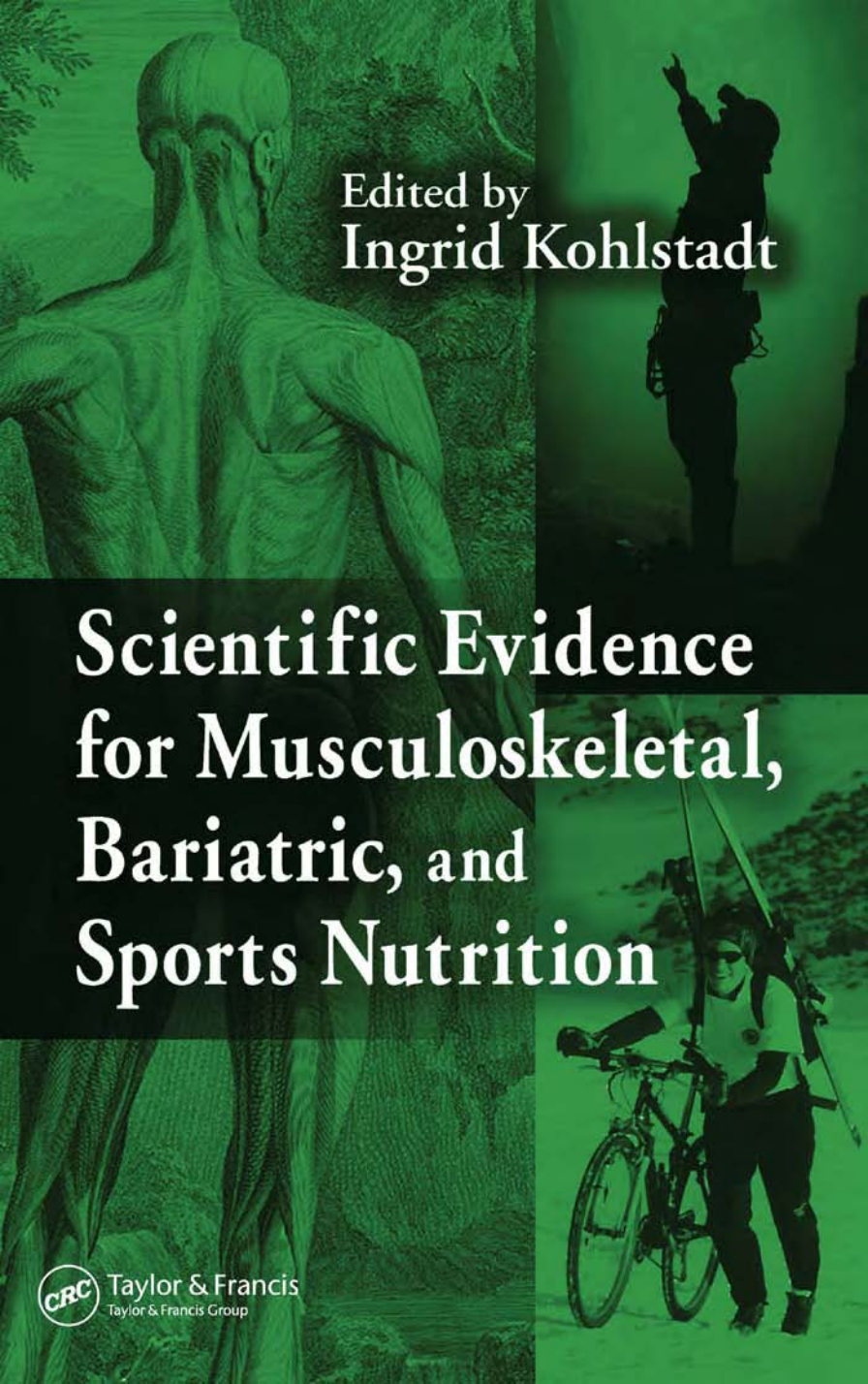

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Scientific Evidence for Musculoskeletal, Bariatric, and Sports Nutrition

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20 Muscle Hypertrophy

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CONTENTS

MUSCLE GROWTH

Introduction

Skeletal muscle hypertrophy can develop in response to overload or resistance training exercise. Muscle hypertrophy occurs as a result of an increase in proteins, leading to elevations in muscle cross-sectional area, length, or muscle mass. Advances in cellular and molecular research over the past two decades have provided a basis for understanding some of the mechanisms that contribute to these responses leading to muscle hypertrophy.

Muscle Hypertrophy Results from Increased Protein Accumulation

If muscle hypertrophy is to occur, there must be a net increase in protein accumulation. This can occur through an increase in protein synthesis, a decrease in protein degradation, or both. It is clear that exercise and various types of overload have a profound effect on muscle protein metabolism. If the stimulus is sufficient, net protein synthesis will increase, resulting in muscle hypertrophy. Recent improvements in the methods used to study muscle hypertrophy including the use of isotopic tracers and muscle biopsies to identify muscle protein synthesis and the use of magnetic resonance imaging or computerized tomographic scanning to carefully document changes in muscle cross-sectional area or muscle volume have advanced our understanding of mechanisms leading to muscle hypertrophy.

Exercise as a Stimulus for Muscle Growth

Acute resistance exercise stimulates increased protein synthesis,[1] but aging may increase the need for available amino acids to induce muscle growth.[2] The

summation of acute responses of protein synthesis to exercise or overload results in chronic increases in net protein accumulation or muscle hypertrophy.[3] Long-term adaptations to resistance exercise might result in protein synthesis rates not significantly different from those of untrained muscles as a plateauing effect.[4] It is possible that increasing the intensity of the training or improving the amino acids or other nutrients required for protein synthesis could improve muscle hypertrophy[5] once this plateau has been reached. However, in a study by Chesley et al.,[6] muscle protein was not increased in spite of an intensive training stimulus, and this suggests that nutritional or other elements are at least as important as the training stimulus for inducing muscle hypertrophy.

Muscle Hypertrophy Requires Adding New Nuclei

Because myonuclei are postmitotic,[7] satellite cells, which are also called muscle precursor cells (MPCs), provide the only important source for adding new nuclei to initiate muscle regeneration, muscle hypertrophy, and postnatal muscle growth in muscles of both young and aged humans.[7] MPCs are critical for muscle growth because muscle hypertrophy is markedly reduced or eliminated completely after irradiation to prevent MPC activation.[8] An example of activated MPCs is shown in Figure 20.1. Growth of adult skeletal muscle requires activation and differentiation of MPCs and increased protein synthesis and accumulation of proteins.

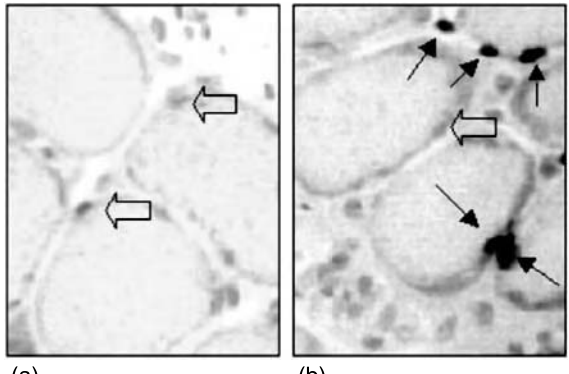

FIGURE 20.1 Satellite cells, also called muscle precursor cells, proliferate to add new nuclei during muscle hypertrophy. a. Control muscle has myonuclei that are not capable of proliferating. Myonuclei examples are shown with open arrows. b. Satellite cells or MPCs are activated and proliferate in loaded muscles. Closed arrows show examples of activated satellite cells that have a marker incorporated for DNA synthesis. The open arrow shows an example of a nonproliferating myonucleus.

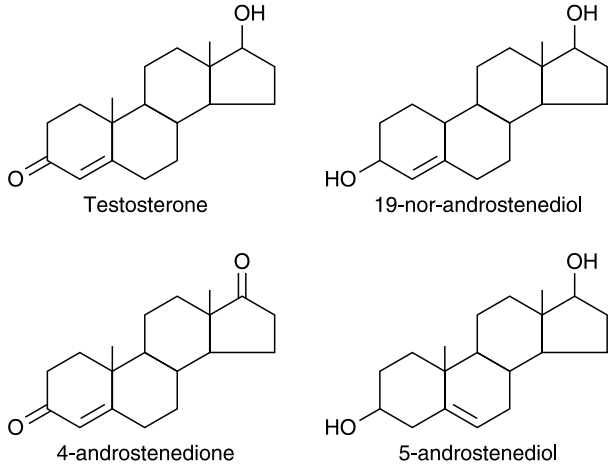

FIGURE 20.2 Chemical structures of anabolic hormones.

A proposed model of how MPC activation may lead to greater muscle hypertrophy after anabolic steroid treatement is presented in Figure 20.2.

Editor's Note

Exogenous anabolic steroids, which excede physiologic levels, cause nutrients to be used for muscle growth instead of other, perhaps more crucial, functions. Laboratory and clinical data provide a rationale for treating anabolic steroid-induced nutrient imbalances.

THE STIMULUS FOR MUSCLE GROWTH MAY ALSO INCREASE MUSCLE BREAKDOWN

Acute Exercise May Increase Muscle Protein Breakdown

The net effects of muscle growth in response to an exercise or overload stimulus depend upon an elevation in protein synthesis and any changes in net protein degradation. Studies conducted over the past two decades have shown divergent results. Indirect evaluations of muscle protein breakdown via urea and 3-methylhistididine excretion suggested that protein degradation increases,[9] decreases,[10] or remains unchanged.[11] More recent methods have examined muscle degradation using isotopic enrichment of the intracellular protein pool and have shown increases in

muscle protein degradation after resistance training,[1] although not as much as the increase in protein synthesis. The availability of required nutrients may be a determining factor.

THE STIMULUS FOR MUSCLE GROWTH CAN EXCEED THE BODY'S ABILITY TO ADEQUATELY NOURISH THE MUSCLE

The stimulus for muscle growth (e.g., resistance exercise) includes increases in both muscle protein synthesis and degradation. For a net increase in protein accumulation, the cellular conditions must favor increasing protein synthesis more than protein degradation. This requires a much greater metabolic demand than if only protein synthesis was increased with resistance training. Although the stimulus for hypertrophy is important, dietary composition may be a critical factor regulating the degree of muscle hypertrophy[12] because inadequate nutrients will not position the muscle's environmental milieu to favor net protein synthesis and accumulation.

What Limits Muscle Hypertrophy?

We can learn some clues from what limits hypertrophy by studying aging muscles. Both young adults and aged humans can achieve some degree of muscle hypertrophy as long as muscles are exposed to resistance exercise or overload of adequate intensity and duration.[13] Aging appears to reduce the relative change in muscle mass to loading.[14] Assuming that the stimulus is adequate and the intensity is sufficient to fully activate the mechanical and molecular signaling pathways, the limitations to muscle hypertrophy may occur at several levels. First, the inability to fully recruit adequate MPCs will limit hypertrophy. Secondly, inadequate hormonal environments for activation and differentiation of MPCs will limit hypertrophy. Finally, increases in apoptosis (programmed cell death) that remove activated MPCs will limit or reduce the potential for hypertrophy. Thus, anything that limits activation, proliferation, and differentiation of MPCs will limit muscle hypertrophy.

1. *A low activation of MPCs will limit hypertrophy.* Without sufficient new nuclei, muscle specific gene transcription will be limited, and this will result in less total protein synthesis and muscle hypertrophy even in the face of adequate muscle stimulus for growth.[15]
2. *Apoptosis in response to overload may limit hypertrophy.* MPC proliferation is a critical event that provides additional nuclei, enabling muscle to increase its mass. However, even if MPCs are activated normally during repetitive loading, but they either do not differentiate or do not survive to participate in increased protein synthesis, then muscle hypertrophy cannot occur. One possibility that could explain

the lower differentiation (largely lower survival) of activated MPCs in aging muscles is the result of the elevation of apoptosis protein levels in muscles from aged animals. We have recently shown that the most recently activated MPCs during loading are also the most susceptible to apoptosis.[16] On the basis of these data, we hypothesize that satellite cell activation is lower in muscle of old animals and that apoptosis is higher in these cells, activated during repetitive loading, so fewer MPCs survive and contribute to muscle hypertrophy. Apoptosis might also limit hypertrophy in young athletes, especially in conditions of overtraining.

3. *Inadequate or inappropriate hormonal milieu will limit hypertrophy.* Skeletal muscle tissue constitutes ~40 to 45% of total body weight in humans and is among the body's most metabolically active tissues. The muscle's hormonal environment (i.e., milieu) has a major impact upon protein synthesis and therefore on muscle growth and hypertrophy. Human skeletal muscle undergoes protein turnover even under nonexercised conditions, and protein metabolism increases during hypertrophy. Hormones such as testosterone, growth hormone (GH), insulin, insulin-like growth factor-I (IGF-I), and glucocorticoids are important regulators of muscle remodeling including determining whether muscle growth or atrophy will occur. Furthermore, skeletal muscles act as a reserve for free amino acids, which provide precursors for glucose via gluconeogenesis during caloric restriction, but clearly such conditions are not favorable for muscle hypertrophy.

Some of the hormonal responses occurring in muscle hypertrophy are difficult to characterize *in vivo* because one hormone or metabolite can interact with other hormones or metabolites. A complete review of the endocrine effects on muscle protein turnover is beyond the scope of this chapter; however, the responses of major hormones that affect protein synthesis and degradation will be summarized here. The effects of testosterone and exogenous testosterone will be examined in detail.

- *Insulin.* Shortly after the discovery of insulin, it was found that exogenous insulin reduced muscle wasting, which is often associated with diabetes. Anecdotal and case study evidence also indicates that insulin therapy improves muscle hypertrophy in athletes (especially those who have diabetes). Nevertheless, the literature has conflicting data evaluating the efficacy of insulin on *in vivo* protein synthesis. Part of the confusion may be the manner in which insulin is studied. For example, infusion of insulin to the venous systems causes a significant reduction in blood amino acid levels. This reduces the available amino acids that can be provided to a muscle for protein synthesis. Thus, when amino acid levels are not increased along with insulin, there is no increase in protein synthesis,[17,18] but if amino

acids are elevated during insulin therapy, protein synthesis can be increased[19] even in atrophic muscle.[20] Insulin has also been shown to reduce protein catabolism, thereby improving net protein accumulation and muscle mass in healthy subjects.[21]

- *Growth hormone and insulin-like growth factor-I.* It is thought that the anabolic properties of growth hormone (GH) largely stem from its actions through insulin-like growth factor-I (IGF-I), which is produced in the liver in response to GH. However, both GH and IGF-I have been shown to increase muscle protein synthesis in resting and inactive muscles[22,23] and in atrophic muscles[24] or after resistance exercise,[25] but this may be dependent upon adequate nutrition.[26,27] Nevertheless, other studies have failed to find any increase in muscle protein synthesis by IGF-I or GH in resting or exercised muscles.[28]

It is now understood that tissues other than the liver express IGF-I and that there are local as well as systemic forms of IGF-I that have different functions. Two alternatively spliced variants of IGF-I have been identified so far, and these are both expressed in skeletal muscle. One splice variant is expressed in response to physical activity, which has now been called 'mechano-growth factor' (MGF).[29] The other is similar to the systemic or liver type (IGF-IEa) and is important because it supplies the mature IGF-I required for upregulating protein synthesis in skeletal muscle and other tissues. MGF differs from systemic IGF-IEa in that it has a different peptide sequence, which is responsible for replenishing the MPCs in skeletal muscle. The ability to produce MGF declines with age,[29] and this occurs concomitantly with the decline in circulating GH levels. GH treatment upregulates the level of IGF-I gene expression in older people, and when combined with resistance exercise, more is spliced toward MGF and hence should improve the ability of muscle to respond to physical activity.[29,30]

- *Cortisol.* High levels of cortisol can reduce muscle mass during inactivity or injury and can limit the extent of muscle hypertrophy even given an adequate stimulus for muscle growth.[4,31] The time of day when exercise is done may also affect the level to which cortisol is expressed, with greater levels found during evening exercise sessions.[32] Presumably, reduced cortisol levels should reduce protein catabolism and improve protein accumulation, and this can at least be partially accomplished by providing higher levels of amino acids and other nutrients.[33]
- *Testosterone.* Exogenous testosterone and testosterone derivatives have received considerable political and press attention and have been the focus of numerous studies. Most studies examining androgens and testosterone has been aimed at hypogonadal men,[34] in persons having muscle-wasting diseases,[35] or aging-associated (sarcopenia) muscle loss.[36] Compounds such as nandrolone decanoate have been used to improve tendons and rotator cuff tendon healing.[37] However, there have been long debates in the scientific literature regarding whether

testosterone and related compounds improve muscle protein synthesis and muscle hypertrophy and whether any anecdotal changes in muscle mass and performance are caused by placebo effects or water retention by subjects. However, Bhasin and colleagues[38] have shown quite clearly that testosterone injections improve muscle mass in nonexercised subjects as compared with placebo injections. The increase in muscle mass occurs from an increase in protein synthesis[39] and may also be in part because of a decrease in protein degradation.[33]

PHYSIOLOGIC EFFECTS OF EXOGENOUS TESTOSTERONE AND NUTRIENTS AIMED AT STIMULATING ENDOGENOUS TESTOSTERONE PRODUCTION

Effects of Exogenous Testosterone

Testosterone is a 19-carbon steroid with a 4,5 double bond and a keto-group at position 3 and a hydroxyl group at position 17 of its sterane ring (see Figure 20.3). Androgenic–anabolic steroid testosterone derivatives are synthetically derived versions of testosterone. Early attempts to generate anabolic steroids produced synthetic androgens that retained both androgenic and anabolic properties. The androgenic functions include developing male secondary sexual characteristics including lowering of the tone of the voice, male pattern hair growth, and hair loss. Anabolic effects include increasing muscle mass and strength. The most important anabolic function of testosterone and synthetic derivatives of this hormone is to increase protein synthesis and inhibit protein breakdown.[40]

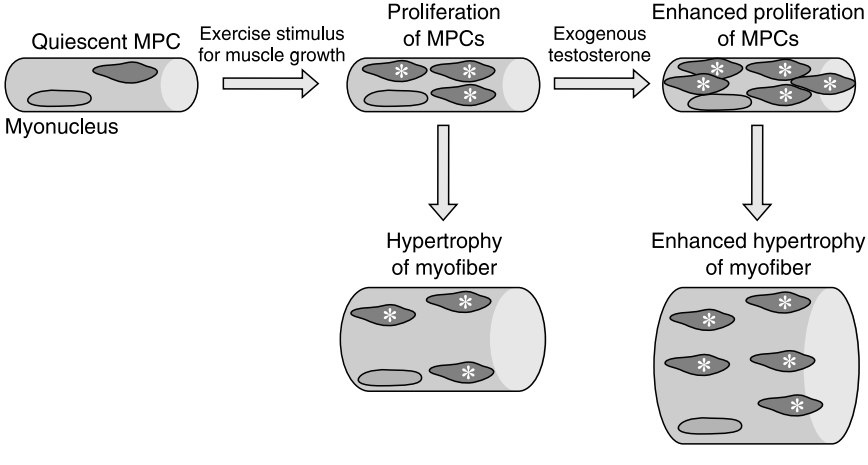

FIGURE 20.3 Proposed model of enhanced satellite cell or muscle precursor cell activation leading to improved muscle hypertrophy with exogenous testosterone.

Testosterone or anabolic steroids administered orally or by intramuscular injection are rapidly metabolized, so they cannot exert any significant effects on the human body. However, pharmacological manipulations of testosterone have been developed to reduce this problem. Three major modifications have been made. These include alkylation at the 17-α position with an ethyl or methyl group, esterification at the 17-β position, and alterations of testosterone's ring structure (see Figure 20.3).

Alkylation is important for creating oral active compounds that slow degradation by the liver. Esterification of testosterone and nortestosterone at the 17-β position slows metabolism and prolongs the effects of the compounds. Injectable oil soluble compounds can remain in the body (and detectable) for several months. Finally, alterations of the testosterone ring structure have been made for both oral and parenteral agents, which increase the activity of these compounds.[40]

Medical Use of Exogenous Testosterone

Medical use of anabolic steroids has been prescribed to counteract testicular dysfunction (i.e., low testosterone levels) and improve the hypothalamus–pituitary–gonadal axis (i.e., male hypogonadism). Androgen and anabolic steroid therapy is primarily used in hypogonadal men to improve or maintain muscle strength and function.[41] Anabolic steroids have also been used to improve nitrogen balance to favor muscular anabolism in several muscle wasting conditions[42] including sarcopenia, which is the age-associated loss of muscle mass,[42] and some forms of anemia,[43] hereditary angioneurotic edema,[44] and osteoporosis.[45] Anabolic steroid use is also beneficial for improving respiratory muscle mass and strength in individuals who have tetraplegia and ventilatory insufficiency.[46]

Small amounts of anabolic steroids improve nitrogen balance after trauma and in patients after abdominal surgery and after burn injury,[47] and this should reduce muscle loss in these patients. In addition, patients with chronic obstructive pulmonary disease and HIV have been shown to have improved lean body mass after treatment with anabolic steroids; however, the effect of the improvement also appears to be related to the nutritional status of the patients.[48] Exogenous testosterone and anabolic steroids have been used to treat muscular dystrophies for many years because they improve muscle mass and reduce muscular dysfunction in muscular dystrophy.[49] However, the number of clinical trials has not been extensive, and the degree to which anabolic steroid treatment can improve various wasting diseases has not been widely studied in humans.

Testosterone Therapy in Women

Like men, women have a loss in muscle mass and strength with age.[50] Likewise, there is a decrease in circulating blood levels of testosterone with increasing age

TABLE 20.1
Examples of anabolic androgenic steroids used by athletes

Clostebol	Dianabol
Drostanolone	Ethylestrenol
Fluoxymesterone	Metandren
Mesterolone	Methenolone
Norethandrolone	Nortestosterone
Oxandrolone	Oxymesterone
Oxymetholone	Stanozolol
Stenbolone	Testosterone
Tetrahydrogestrinone	Turinabol

in women, with ~50% of the decrease occurring by the age of 40.[51,52] Although levels of serum estradiol and 25-hydroxy vitamin D have a higher correlation with muscle mass than serum testosterone levels in women over 65 years of age,[52] there have been an insufficient number of studies to fully evaluate the effects of testosterone therapy combined with resistance exercise on muscle mass and strength in postmenopausal women.

Testosterone Therapy and Anabolic Steroids to Improve Muscle Hypertrophy

It is well known that exogenous testosterone in the form of androgenic–anabolic steroids increases the rate of muscle protein synthesis, promotes muscle growth, and reduces fat deposition.[39] Examples of anabolic–androgenic steroids available to athletes are given in Table 20.1.

Several studies have shown that muscle mass increases more than does strength, although both increase with testosterone therapy in men.[53,54] Although muscle hypertrophy occurs with anabolic steroid treatment, the gains are markedly greater when resistance training (to provide a growth stimulus) is combined with anabolic steroid therapy.[38] Women who use anabolic steroids in conjunction with resistance training have increases in both type I (slow twitch) and type II (fast twitch) fiber cross-sectional area,[55] whereas men who use exogenous testosterone and anabolic steroids while resistance training appear to induce preferential type II fiber hypertrophy.[13] The improvement in muscle mass after anabolic steroid treatment appears to be attributable to increased lean tissue rather than hydration.[56] Androgen receptors are preferentially expressed on mature muscle cells and myonuclei[57] and MPCs.[58] A model whereby exogenous testosterone increases satellite cell proliferation to support enhanced muscle hypertrophy is shown in Figure 20.2.

ANABOLIC PROHORMONES — THEIR MISUSE AMONG ATHLETES AND NUTRITIONAL PRODUCT MANUFACTURERS

Introduction

The prohormone, androstenedione first became available for public consumption over-the-counter in 1996. It is frequently marketed with nutrition products. Subsequently, several other prohormones including androstenediol, norandrostenedione, and norandrostenediol became available to the public. The manufactures of these compounds claim that peripheral enzymatic conversion of prohormones to testosterone or nortestosterone (via ingestion of androstenedione and androstenediol or 19-nor-androstenedione and 19-nor-androstenediol, respectively) leads to anabolic or ergogenic effects including improvements in muscle strength and mass. It has also been suggested that prohormones might help to offset the age-associated reduction in endogenous testosterone, DHEA, and sexual interest.[59]

The effects of oral 4-androstenedione administrations have received the most investigation in humans. Although androstenedione is structurally a "steroid," the anabolic effects of this steroid have not been clearly shown in healthy humans. Structurally similar prohormones such as 19-nor-4-androstenedione, 19-nor-4-androstenediol, 4-androstenediol, 5-androstenedione, and 5-androstenediol are also available to the public. There are subtle differences between the structures of prohormones and testosterone. For example, 19-norsteroids contain one less carbon atom in their sterane ring structure than testosterone. 4-androstenedione contains keto groups at positions 3 and 17, instead of one keto and one hydroxyl group, whereas 4-androstenediol contains two hydroxyl groups at these positions. 5-androstenedione and 5-androstenediol have a 5,6 double bond instead of the 4,5 double bond like testosterone (Figure 20.2).

The ubiquitous enzyme 17 regulates the synthesis of testosterone from the -dione, and synthesis of testosterone from -diol prohormones is regulated by 3-β-hydroxysteroid dehydrogenase. The isozymes in this enzyme family have specific metabolic activities and substrate specificity, which makes it likely that prohormone consumption could affect the synthesis of androgenic steroids other than testosterone such as dehydroepiandrosterone, estradiol, or luteinizing hormone either directly or via indirect pathways. In women, the activation of androgens appears to occur in the abdominal visceral and omental adipose tissue,[60] which suggests that adiposity may influence metabolic inactivation of testosterone or prohormone-induced testosterone.

Androstenedione and Dehydroepiandrosterone

Androstenedione (4-androstenedione) is the immediate precursor of testosterone. Androstenedione is produced by the testes and by the adrenal glands from dehydroepiandrosterone (DHEA).[61] Androstenedione supplementation has been

speculated to increase plasma testosterone levels and improve muscle hypertrophy.[62] Thus, it is not surprising that androstenedione supplements have become popular for improving performance in athletes. Androstenedione is marketed to increase blood testosterone levels, with the idea that this will directly improve muscle strength and mass. Manufacturer-suggested daily doses for oral use of this prohormone range from 100 to 1200 mg/d. It is also available as patches, percutaneous gels, and sublingual sprays.[63] So far, clinical applications of androstenedione have not been reported.

Although androstenedione has been studied previously in rodents,[64] its use was first described in 1962 as a case study in two women, who were given 100 mg of oral androstenedione.[65] This study reported that the oral androstenedione increased plasma testosterone by fourfold to sevenfold in these two subjects, with peak testosterone levels found within 60 min of ingestion of the prohormone. In spite of these large changes in plasma testosterone, plasma levels of androstenedione that had been undetectable before supplementation only had very small increases 60 to 90 min after androstenedione ingestion.[65] DHEA given at a similar dose was shown to have similar effects with ~4-fold increases in plasma testosterone within 90 min of ingestion. These data suggest that oral supplementation with androstenedione or DHEA improves blood levels of testosterone in women.[65]

Blaquier and coworkers[66,67] reported that 4-androstenediol (androstenediol) exhibited ~2.8-fold greater conversion to testosterone than did 4-androstenedione. This introduces the possibility that 4-androstenediol might provide greater anabolic/androgenic results than 4-androstenedione. This higher efficacy may be because androstenedione is converted to androstenediol before becoming testosterone.[67] It is therefore unclear why most research and earlier marketing focused on androstenedione instead of androstenediol in spite of the greater likelihood for androstenediol to elevate testosterone.

Effects of Androstenedione on Blood Hormonal Levels and Muscle Hypertrophy

The extent by which elevated blood levels of prohormones increase muscle growth is unclear from current studies. A critical review of key studies allows clinicians to decide if the information can be generalized to their patients and if additional nutritional recommendations are needed to increase effectiveness.

King et al.[62] studied 30 untrained normotestoterogenic men who performed 8 weeks of whole-body resistance training. Acute androstenedione (100 mg) had no effect on total testosterone, free testosterone, luteinizing hormone (LH), or follicle stimulating hormone (FSH) but increased serum androstenedione concentrations by 175 to 350%. Serum estradiol concentrations were higher after 2, 5, and 8 weeks of resistance training compared with the androstenedione group. Total testosterone was unaffected by androstenedione ingestion, but the free testosterone concentration was significantly higher at 0 and 8 weeks of the study. Despite the elevated blood levels, androstenedione did not appear to alter

body composition, fat free mass, cross-sectional areas of type I and type II fibers from the vastus lateralis, and muscle strength relative to placebo consumption in the trained subjects. The generalizability of the study to competitive athletes may be limited because the men in this study had body fat in excess of 20%. Adipose is likely to minimize the effectiveness because adiposity increases conversion of androstenedione to estrogens because of peripheral aromatization in adipose tissue.[68]

Wallace et al.[69] studied the effects 12 weeks of supplementation with 50 mg two times daily of either androstenedione or DHEA in a group of middle-aged men. No significant improvements in testosterone levels, lean body mass, or strength were observed in either supplement group compared with the placebo group. This lack of effect was the result of the low dose provided to the subjects. The dose is comparable with that used in the treatment of elderly subjects before surgery to preserve muscle and bone. It is not surprising that the positive results of muscle preservation before stress in frail elderly men and women is not generalizable to muscle enhancement in middle-aged men.

Ballantyne et al.[70] examined oral androstenedione supplementation in male subjects over a 24 h period and then gave the subjects 200 mg of oral androstenedione (in two divided doses). Venous blood samples were obtained every 3 h over the following 12 h and then 24 h after ingestion of the final capsule. Heavy resistance training increased serum testosterone, and androstenedione supplementation increased serum androstenedione by ~200% and luteinizing hormone by ~100%, without concomitant increases in serum testosterone, free testosterone, or estradiol. Because most androstenedione is converted first to estrone in blood, bone, skeletal muscle, and adipose tissue rather than to estradiol, it is possible that estrone rather than estradiol levels would have been a more sensitive measure of the effect of androstenedione on serum estrogens.[71] However, exercise in the androstenedione group significantly elevated plasma estradiol by approximately 83% for 90 min. On the basis of the lack of change in testosterone levels, these data suggest that androstenedione may not improve testosterone release or synthesis and therefore may not provide male athletes a marked anabolic improvement above that provided by heavy resistance exercise.[70]

Leder et al.[71] found that healthy men aged 20 to 40 years given 300 mg of oral androstenedione had serum testosterone concentrations increased by 34%; however, baseline testosterone levels, obtained 24 h after androstenedione administration, did not change.[71] Serum estradiol significantly increased by 42 and 128% in subjects consuming 100 and 300 mg/d of androstenedione, respectively. These data suggest that oral androstenedione, when given in dosages of 300 mg/d, increases serum testosterone and estradiol concentrations in some healthy men, but the effects are variable between subjects.[71]

Recent studies by Brown and colleagues[72,73] have evaluated the effectiveness of a nutritional supplement containing androstenedione and herbs that were designed to enhance serum testosterone concentrations and prevent the formation of dehydrotestosterone and estrogens. Healthy 30 to 59 year old men consumed a combination of 300 mg androstenedione, 150 mg dehydroepiandrosterone,

540 mg saw palmetto, 300 mg indole-3-carbinol, 625 mg chrysin, and 750 mg tribulus terrestris per day or a placebo for 28 days. Whereas the ingestion of androstenedione combined with herbal products increased serum-free testosterone concentrations in older men, these herbal products did not prevent the conversion of ingested androstenedione to estradiol and dehydrotestosterone in the short duration of this study.

Broeder and colleagues[74] studied the effects of oral androstenedione and androstenediol supplementation during 12 weeks of intense resistance training in the so called Andro Study. Fifty healthy men (age 35 to 65 years) were randomly assigned to a placebo, androstenedione (200 mg/d taken in two divided doses), or androstenediol (200 mg/d taken in two divided doses) group. Each subject participated 3 days per week in a heavy resistance training program that included an average of three sets of nine exercises (i.e., a total of 81 sets per week) using 60 to 95% of their pre-training 1 repetition maximum. Total and free testosterone levels increased in the androstenedione group over 1 to 2 months but returned to basal concentrations by the third month of the study. Compared with week 0, concentrations of estrone, estradiol, and DHEA-sulfate increased significantly in the androstenedione and androstenediol groups at week 12. Neither androstenedione nor androstenediol supplementation improved the resistance training improvements in muscle strength compared with the placebo group and may have a negative impact on blood lipids and cholesterol.[75] However, the conclusion regarding the impact on muscle mass should be viewed carefully because the subjects were probably hypocaloric because the average energy intake was only ~120 kJ/kg/d or 29 kcal/kg/d, whereas current nutrition recommendations for individuals undergoing intense exercise training suggest that these subjects needed ~3 kJ/kg/d or 13 kcal/kg/d more than they received in this study.[76] Thus, the training program in this study[74] may have been too strenuous or the nutritional intake inadequate, considering that it failed to positively alter improve muscle mass even in the placebo group. This underscores the importance of maintaining adequate or superior nutrition during exercise training such as resistance training and that supplementation per se may be inadequate to invoke changes in muscle mass even given adequate muscle stimulus to hypertrophy and having a muscle milieu that includes potential anabolic compounds.

Gender Differences with Androstenedione

Horton and Tait[77] examined the interconversions of androstenedione and testosterone in women. These researchers showed that with intravenous administration of androstenedione and testosterone, ~60% of plasma testosterone is derived from androstenedione, whereas less than 3% of the plasma androstenedione is derived from testosterone. Therefore, the conversion of androstenedione to testosterone contributes largely to the circulating testosterone concentrations in women. In contrast, in men only ~0.3% of plasma testosterone is derived from androstenedione, whereas ~37% of plasma androstenedione is derived from plasma testosterone.

Therefore, men appear to be capable of preferentially converting testosterone into androstenedione, and the contribution of androstenedione to blood testosterone levels is likely to be minimal. From further investigation of gender differences in hormone metabolism, Horton and Tait concluded that men can convert testosterone to androstenedione more easily than women, but women appear to convert androstenedione to testosterone more readily than men. These data provide a rationale for using androstenedione for increasing androgen levels and reducing sarcopenia and osteoporosis in postmenopausal women and hypogonadal men.[78]

PHYSICAL EXAM AND LABORATORY SCREENING — FALSE POSITIVES AND FALSE NEGATIVES

Clinical and Laboratory Characteristics of Exogenous Testosterone Use

Exogenous steroid use alters clinical and laboratory findings. Much of the information relating to clinical and laboratory effects of exogenous testosterone is obtained from case reports, which even include fatalities.[79] The long-term clinical health effects are dependent upon the type of exogenous testosterone compound, dose, frequency of use, and age of the athlete. Assessment of health consequences and evaluation of the physical examination are complicated in that some athletes combine one or two anabolic steroids or other hormones and several prohormones or just take prohormones. Some athletes use the compounds in tandem for long periods of time, and some use them more periodically.[80] Comprehensive scientific studies are complicated by the ethical implications of giving volunteers the supramaximal doses typically used by athletes. The final problem is that some of the compounds obtained by athletes are obtained on the "black market," and the quality, amount, and composition of the compounds cannot be known.[81]

There are several physical attributes that might together provide some evidence of exogenous testosterone use, but these must be followed up with more objective data before drawing conclusions on use or misuse. Many of the clinical and laboratory characteristics are summarized in Table 20.2.

Sexual and Physical Characteristics

1. *Gynecomastia.* Gynecomastia in male athletes is a common problem and easily recognized in a physical examination. In addition to the pain that usually accompanies gynecomastia, this creates a cosmetic physical problem, especially for bodybuilders, who are judged by the quality of their physiques, and may require corrective surgery.[82] Development of gynecomastia is the result of converting the large amounts of exogenous testosterone to estrogens.[83] Athletes have been reported to use tamoxifen to prevent gynecomastia, and recent findings suggest that this is an effective treatment.[84]

TABLE 20.2
Examples of physical and clinical effects reported for anabolic–androgenic steroid use

Endocrine changes
- Hypothalamic–pituitary dysfunction
- Altered sexual interest
- Altered glucose tolerance
- Hyperinsulinism

Sexual and physical characteristics in men
- Acne
- Gynecomastia
- Lower sperm production
- Lower endogenous testosterone production
- Infertility
- Increased male pattern baldness
- Prostate hypertrophy
- Testicular atrophy
- Voiding alterations

Sexual and physical characteristics in women
- Acne
- Clitoral hypertrophy
- Deepening of the voice
- Male pattern baldness
- Increased facial and body hair
- Infertility
- Menstrual irregularities

Behavioral changes
- Aggressiveness
- Depression
- Irritability
- Irregular "mood" changes

Immune function
- Decreased immunoglobulins
- Depressed immune responses

Cardiovascular changes
- Potential for elevated systolic or diastolic blood pressure
- Altered blood lipid profile
- Increased levels of C-reactive proteins
- Increased hematocrit and red blood cells
- Decreased high-density lipoprotein
- Increased low-density lipoprotein
- Decreased fibrinolytic inhibition
- Increased triglycerides
- Left ventricular hypertrophy

TABLE 20.2
Continued

Adolescent growth
Premature epiphyseal closure
Hepatic function
Altered liver structure
Hepatocellular adenomas
Hematocellular hyperplasia
Cholestatic jaundice
Peliosis hepatis
Altered liver enzymes

2. *Acne.* Obvious indicators upon physical examination include acne around the shoulders, upper and middle back, and face of male and female athletes.[85] The acne will clear upon termination of use of exogenous testosterone.[86] Clearly though, acne by itself is not sufficient for diagnosis and must be considered in combination with other clinical tests.
3. *General characteristics in women.* In women, some irreversible physiological changes associated with anabolic steroid use include male pattern baldness, virilization, and deepening of the voice.[87] There may also be menstrual irregularities.[88] However, such changes could also result from androgen-producing ovarian and adrenal neoplasms, and this possibility should be explored by the practitioner.[87]
4. *Sexual characteristics and reproductive system.* Plenty of anecdotal cases have been cited by the lay media; however, what role anabolic steroids and exogenous testosterone might have in the etiology of various diseases in humans or animals is still not known. Testosterone and steroid use in clinical trials and in controlled laboratory studies has been reported to correlate with a number of deleterious changes in risk factors for infertility. In males, anabolic steroid use could result in lower levels of endogenous testosterone, gonadotrophic hormones, sex hormone-binding globulin levels, sperm motility, sperm count, altered sperm structure, and testicular atrophy.[89] However, termination of anabolic steroid use results in a normalization of sperm production and testes function. Menstrual abnormalities, shrinkage of the breasts, and increased sex drive will usually return to normal after steroid termination in women.[86] However, in women, there are some irreversible physiological changes associated with anabolic steroid use[90] including male pattern baldness, deepening of the voice, increased body hair, and clitoris hypertrophy.[91]

Prostate abnormalities have been reported in humans including an increase in prostatic volume, reduction in urine flow rate, and marked changes in voiding patterns.[75] In contrast, 100 mg of androstenedione does not alter serum levels of prostate-specific antigen,[72] suggesting no overall change in prostate function with this prohormone.

Behavioral Changes

It is reasonable to expect that some of the behavioral signs and symptoms accompanying anabolic steroid use are likely overlooked by healthcare professionals, so it is not possible to determine the number or extent of steroid-related behavioral complications.[79] Female athletes have been reported to display increased aggressiveness when using anabolic steroids.[88] A positive correlation has been established between endogenous testosterone levels and aggressive behavior in males, but some people are much more sensitive to changes in mood and others are much more resistant to steroid-induced changes in mood and behavior.[92] Moderate and low doses of exogenous testosterone have failed to produce changes in male aggressiveness, general outlook or mood, and sexual behavior of subjects.[93] However, the high levels of steroids often used by athletes have been reported to induce psychotic and sometimes violent syndromes and, importantly, psychological dependence on the steroids.[94] This psychological dependence explains the severe withdrawal symptoms.[95] Nevertheless, other reports have not supported a close relationship between steroid use and changes in personality or aggression.[96] Although supraphysiological levels of exogenous testosterone have been found to increase both the subjective and objective assessments of aggression, the interpretation of these data are complicated by the fact that several steroid users in this study had personality disorder profiles including Cluster B personality disorder traits for antisocial, borderline, personality disorder; histrionic personality disorder.[94] Thus it is likely that some athletes, if predisposed as a result of some preexisting personality disorder, may have increased aggression and behavioral changes, but others not prone to personality disorders may not be observed clinically to have any changes in behavior or aggression. It has been estimated that ~300,000 athletes use anabolic steroids yearly in the United States;[97] however, only a small percentage of steroid users experience psychological dependence requiring clinical treatment. Thus, the psychological dependence may be overestimated, or most people using anabolic steroids have not developed symptoms of a significant nature that require interventions.

Immune Function

Other potential areas of concerns studied in animals and humans include reduced T-cell and immune function,[91] which has the potential for the athlete to suffer upper respiratory infections or other more complicated problems.

Cardiovascular Risks

Exogenous steroid use has been linked to increased coronary artery disease, cardiomyopathy, and cerebrovascular accidents.[56,98]

1. *Lipoproteins.* Use of exogenous testosterone compounds has been thought to increase cardiovascular risk factors.[99] The most important are changes in lipoproteins, increased levels of triglycerides, and elevated concentrations of several blood clotting factors. Estimates of anabolic steroid-induced changes in blood lipids include a marked decrease in high-density lipoprotein-2 (HDL-2) levels by ~52% and severe decreases in high-density lipoprotein-b (HDL-b) levels by ~78% along with increases in low-density lipoprotein levels (LDLs) by ~36%.[100,101] However, this observation is not universal and may depend on the steroid used because nandrolone has been reported to show no changes in HDL cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol, triglycerides, the total cholesterol to HDL-C ratio, or the LDL-C/HDL-C ratio from 100 mg/wk of nandrolone in men or women.[102] Nevertheless, athletes frequently use much higher levels of nandrolone and combine this with other steroids, and the effects of the higher doses or combinations of several anabolic steroids and prohormones may alter the lipid profiles in these athletes. The changes in blood lipid profiles provide a negative stimulus that would promote cardiovascular disease. Blood lipids and lipoprotein changes favor an increased atherogenic lipid profile that persists after termination of steroid use.[103] As with the findings of King and colleagues,[62] serum concentrations of HDL-C decreased 10% during the first week of androstenedione supplementation and remained depressed thereafter. However, serum concentrations of total testosterone did not change. These data and others[74,75] support data from earlier studies that indicate an increase in serum estrogen and a decrease in HDL-C in response to oral androstenedione.
2. *Blood profiles and lipids.* Urhausen et al.[104] studied 32 male bodybuilders and powerlifters, comparing the blood profiles of current users with those who had terminated anabolic steroid use ~1 year before the study began. The previous users had taken ~720 mg/wk of combined anabolic–androgenic steroids for 26 weeks per year over ~9 years. The athletes who currently used anabolic–androgenic steroids at the time of the study took ~1030 mg/wk of combined steroids for 33 weeks per year for an average of ~8 years. Blood tests showed that erythrocyte and platelet counts in the former steroid users were normal, but in current steroid users, all hematological cell types were increased. Liver alanine aminotransferase levels were increased in some but not all of the former steroid users. The blood cholesterol

and endogenous testosterone levels of the former steroid users were normal. In general, these data show that lipid blood levels return to normal after cessation of exogenous testosterone use.

3. *C-reactive proteins.* A recent observation is that anabolic steroids can elevate C-reactive protein concentrations without any relationship to changes in cardiac troponin T. The higher C-reactive protein concentrations may indicate a greater predisposition to developing peripheral arterial disease.[105]
4. *Cardiomyopathy.* The types and extent of clinical changes vary among different types and doses of anabolic steroids and among individuals. The clinical changes appear to be reversible within several months after cessation of anabolic steroid use.[89,104] However, increased left ventricular mass and dilated cardiomyopathy are not reversible upon cessation of anabolic steroid use.[106] Furthermore, several case reports on athletes have linked anabolic steroid use to nonfatal myocardial infarction, atrial fibrillation, and stroke.[98]

Blood and Thrombogenic Characteristics

Testosterone can potentially alter thromogenic activity by increasing thromboxane A^2 receptor activity and platelet aggregability.[107] However, there is no direct evidence that exogenous testosterone or its anabolic steroid compounds are thrombogenic in humans.[108] On the contrary, the reductions of fibrinolytic inhibition and lipoprotein A in men and women who use anabolic steroids[109,110] are considered favorable effects of androgens with regard to the risk of cardiovascular disease. Anabolic steroid use increases whole-body hematocrit and red blood cell volume, and as a result, anabolic steroids have been used to treat patients with renal failure.[111]

Blood Pressure

The literature concerning potential changes in blood pressure during anabolic steroid use is mixed. Some studies have found no change in blood pressure in healthy athletes,[89,112] whereas others have found an increase in systolic or diastolic blood pressure.[91,113] Thus, although it is not clear that anabolic steroids will increase resting blood pressure levels, androgenic compounds may affect the blood pressure more than anabolic substances do, but the exact mechanism of this is unknown.[114,115]

Hepatic Function

Liver structure and function are changed by steroid use.[116] These include peliosis hepatitis, hepatocellular hyperplasia, hepatocellular adenomas, and cholestatic jaundice.[91,117] It has also been shown that high levels of anabolic steroids may lead to hepatocellular carcinomas.[118,119] Liver changes are associated with use of oral 17-α-alkylated anabolic steroids.[91] An interesting case study reported on a

26 year old jaundiced bodybuilder, whose steroid regimen over 5 weeks before hospitalization was 500 mg testosterone injected intramuscularly injection twice weekly and daily consumptions of 40 mg of oral stanazol and 30 mg of methylandrostenediol.[120] This bodybuilder had high aminotransferase and alanine aminotransferase levels but normal bilirubin levels. However, he was found to have hepatocellular necrosis, based upon pathological analysis of his liver biopsy. Clinical signs and laboratory findings for this bodybuilder improved substantially 12 weeks after he discontinued these testosterone derivatives.[120] This type of damage is, however, unusual, and it is in contrast to other case reports, which primarily report a cholestatic-type liver damage with steroid use.[121] Other reports suggest widespread liver changes even with anabolic steroids thought to have low levels of toxicity. For example, acute stanozolol treatment (5 mg/kg/d) was shown to significantly decrease the levels of cytochrome P450 (Cyt. P450) and cytochrome b5 (Cytb5) during the first 48 hr of treatment in rats, whereas subsequently, at 72 and 96 h, the levels of these enzymes significantly increased. In contrast to the acute treatment, both P450 and Cytb5 enzymes decreased with 60 to 90 days of chronic treatment.[122]

Nevertheless, most reports of anabolic steroid-induced hepatotoxicity in humans are primarily based on elevated levels of aminotransferase, aspartate aminotransferase, and creatine kinase levels, but this may not be sufficient assessment of liver function because athletes using steroids do not have changes in hepatic dysfunction, at least based on gamma-glutamyltranspeptidase levels.[123] In general, the data show that blood levels of liver enzymes return to normal,[104] and other data show that liver tumors regress and there is a return to normal liver function after termination of anabolic steroid use.[89]

Endocrine Modifications

1. *Hypothalamic–pituitary dysfunction.* A case report from van Breda et al.[124] described a competitive bodybuilder who initially presented with gynecomastia and widespread acne. However, the physical examination and subsequent laboratory tests showed that this athlete had a hypothalamic–pituitary dysfunction, which was thought to have persisted for several months even after termination of the anabolic steroid use.[124] This is a rather severe problem, and these investigators suggest that to regain normal hypothalamic–pituitary function in athletes with similar conditions, supraphysiological doses of 200 μg LH-releasing hormone (LH-RH) should be considered when the physiological challenge test with LH-RH (50 μg) fails to show an acceptable response and a return toward normal.[124]
2. *Glucose metabolism.* Hyperinsulinimia and reduced glucose tolerance have also been noted in athletes using various steroids.[89] For example, powerlifters who ingested anabolic steroids had a lower glucose tolerance compared with a control group who did not take steroids, in spite of having significantly higher postglucose serum

insulin concentrations.[125] These results suggest that athletes who use anabolic steroids may have diminished glucose tolerance, which is linked to insulin resistance.

Growth in Adolescents

Several reports suggest premature stunting of growth in adolescent anabolic steroid users, presumably by premature closing of the epiphyseal endplates,[126,127] but this has not been carefully and systematically studied.

DRUG TESTING — FALSE POSITIVES AND FALSE NEGATIVES

The use of androstenedione and DHEA is banned by most sports governing bodies including the International Olympic Committee (IOC).[128,129] The prohormones androstenediol and androstenedione are on the "banned list." The USAA limit for the testosterone-to-epitestosterone (T/E) ratio is 4.0 (as compared to the IOC limit of 6.0). The limit for the 19-norandrosterone urine level is greater than 2 ng/ml and the epitestosterone level is above 200 ng/ml. These more stringent standards increase the likelihood of false positive results, but they also reduce the likelihood of having athletes test negative but actually taking a banned hormone or prohormone for competition or training.

Drug testing for some prohormones has been difficult, but newer methods are improving the detection of these compounds in athletes.[130] The other complication of using prohormones is that some preparations have been found to contain anabolic hormones such as testosterone or 19-nortestosterone, without this information available on the manufacturer's label.[131,132] A study funded by the IOC of 634 nonhormonal supplements found that ~15% contained prohormones that were not listed on the label.[133] These findings were found subsequently by another study.[134]

For more than 20 years, urine analysis of the T/E ratio has been used to provide an indirect marker of testosterone or anabolic steroid use in athletes.[135] The body produces endogenous epitestosterone, the 17-α epimer of testosterone, but it has little anabolic activity. The IOC has set a T/E ratio limit of 6 for competitions.[136]

False Negative Tests

Urine analysis is prone to misdiagnosis because some athletes have T/E ratios below 6.0 in spite of using anabolic steroids. Athletes have used epitestosterone to effectively reduce the T/E ratio and therefore become negative for drug use. Because epitestosterone is believed to be a urine-manipulating agent, the IOC has banned it. The epitestosterone that is marketed for normalizing manipulation is synthesized from soybean, which has a low carbon-13 content.[137] These

manufactured commercial compounds appear to have lower levels of carbon-13 than endogenously synthesized steroids, and higher levels of carbon-12.[138] Athletes using anabolic steroids and epitestosterone would be expected to have urinary steroids with higher levels of carbon-12 than those athletes who are not taking these compounds. Not surprisingly, recent improvements in drug testing have focused on analysis of the ratio of carbon-13 to carbon-12, using a gas chromatography–combustion–isotope ratio mass spectrometry method (GC/MS).[138] This method is not influenced by athlete ethnicity[139] and does appear to be sensitive and reliable.[138] Although the carbon-13 to carbon-12 ratio appears to be reliable and valid,[138] it is a new method and therefore should be thoroughly tested to minimize the chances of false positives before being used in international competitions.

Four men took up to 150 mg of DHEA for up to 4 days, and urine samples were analyzed using gas chromatography-mass spectrometry (GC-MS) on day 3. Only one of the four subjects had an increase in the T/E ratio above 6.0.[140] However, the doses of DHEA were also lower than suggested by supplement manufacturers, and it is not known if the T/E ratio would exceed the IOC standards to therefore reach a "positive" detection if much higher levels of DHEA are consumed by athletes.

False Positives

On 6 September 1994 a female Olympic 400 m athlete was suspended by the British Athletic Federation Limited for testing positive for testosterone in her urine. Although she was initially suspended, she was reinstated on the basis of the argument that urinary microbes produced testosterone and caused her false positive urine tests for testosterone. The basis of the argument was that unlike prokaryotes (e.g., *Escherichia coli*), many eukaryotic microorganisms can synthesize steroids and steroid substrates including testosterone.[141] Investigation of urine samples of 134 women showed that when normal urine was inoculated with 10,000 or 100,000 CFU/mlof *Candida albicans*, a microbe that is typically found in vaginal flora, the testosterone levels in the urine rose significantly although the absolute increases were small. Although it is potentially possible that a false positive drug evaluation could be made if testosterone alone were examined, this is unlikely because there was a small increase in the absolute concentration of testosterone and a corresponding small increase in the T/E ratios, which are maintained well below 6. The increase in urinary testosterone appears to be caused by conversion of urinary androstenedione rather than by synthesis of testosterone per se. This leaves open the possibility that false positives could arise at least in women with some type of urinary microbe infection.

De la Torre and co-workers[142] investigated the increases in urine testosterone after contamination with 15 different organisms (bacteria, fungi, and molds). In the future, improved handling of urine samples such as freeze-drying will reduce the potential for contamination[143] and false positives. In addition, false positives will be likely reduced by evaluating the isotope ratio of carbon-13 to carbon-12 of

urinary steroids to determine whether the testosterone is of exogenous or endogenous origin.[138] An additional reduction in false positives might occur if we compare the testosterone liberated by glucuronidase hydrolysis or that in the free steroid fraction with the total testosterone as a routine test.

Some nutritional supplements have also been found to contain anabolic or androgenic steroids and banned prohormones, which result in positive drug tests, even though the athletes have not knowingly consumed the drugs.[133,144] Athletes can fail a drug test up to 120 h after consuming food supplements,[131] so this could also be viewed as a "false" positive test[145] although it is really a true positive by the IOC regulations. Nandrolone is indirectly detected by identification of its two main metabolites, 19-norandrosterone (19-NA) and 19-noretiocholanolone (19-NE), in the urine. The IOC has set a limit for these metabolites of 2 ng/ml in men and 5 ng/ml in women[146] because endogenous production is known to occur in humans, especially pregnant women.[147]

Athletes who have tested positive for nandrolone have contested positive drug tests on a variety of grounds, including the fact that physical effort alone can produce increased levels of nandrolone.[145] However, Schmitt et al. showed that exhaustive exercise in trained athletes does not significantly increase endogenous nandrolone production and produces nandrolone metabolites in urine levels that are far below the IOC threshold of 2 ng/ml urine in males.[148] However, it is possible to obtain a false positive for drug screen for urinary 19-norandrosterone in a woman athlete who is pregnant.[147]

The potential for false positive drug tests can also arise from testing for specific markers that are naturally occurring in the body. In the case of DHEA or other banned prohormone supplements, urine markers are commonly measured using gas chromatography-mass spectrometry (GC-MS) in methods such as gas chromatography–combustion–isotope ratio mass spectrometry (GC-C-IRMS). A urinary product of DHEA administration is detectable in samples from subjects given multiple doses of DHEA, and was identified in GC-MS experiments to be 3α,5-cyclo-5α-androstan-6β-ol-17-one (3α,5-cyclo). This metabolite occurs naturally, found in urine samples collected from elite athletes; yet, concentrations are markedly elevated after both single and multiple DHEA administrations.[130] Coupling screening of GC-MS and GC-C-IRMS may become the standard method of detecting androstenedione, testosterone, and dehydrotestosterone use, but it is still possible that some false positives may arise.

Van Thuyne and Delbeke[134] report that GC-MS identified dehydrotestosterone in food supplements even though its presence was not listed on the manufacturer's label. However, follow-up studies with GC-MS were unable to confirm its presence in the food supplement. This emphasizes the point that athletes might indeed test positive for prohormone use in some tests but not in others.

Designer Drugs and Getting Around Drug Tests

Some substances are currently undetectable. Tests have been developed for the known drugs, but resourceful entrepreneurs have manufactured new "designer"

drugs, and without a "footprint" they cannot be detected using routine methods. The so-called tetrahydrogestrinone designer steroid (THG) was only discovered because a syringe reported to contain an "undetectable steroid" was sent to the U.S. Anti-Doping Agency (USADA) by a track coach. The syringe contents were analyzed using liquid chromatograph and mass spectrometry. The compound resembled somewhat the structures of gestrinone, used to treat endometriosis, and trenbolone, an agriculture anabolic steroid used primarily in cattle and by some athletes because it is believed to be minimally affected by aromatase or 5α-reductase. The investigators synthesized the new drug as a combination of gestrinone and trenbolone and made a new compound called THG. THG was given to an animal, and the urine tested positive for THG, and this was the same signature as the original compound in the syringe sent to the USADA.[149]

CONCLUSION

Although some studies suggest that prohormones may change testosterone blood levels, there is currently no compelling evidence that clearly demonstrates that prohormones are effective in enhancing muscle mass or strength even when combined with heavy resistance training. These prohormones may result in negative health consequences and may also in some cases test positive in routine drug tests.[144] Despite the negative studies, recreational use of anabolic steroids is increasing among fitness club members and other amateur athletes.

Clinical changes resulting from exogenous testosterone administration include increased circulating estrogen changes in blood lipids, which increases the risk factors associated with cardiovascular disease, altered liver function, and cardiac hypertrophy. All except the cardiomyopathic changes appear to be reversible once steroid use is discontinued. As with anabolic steroids, supplementation of prohormones including oral androstenedione elevates circulating estrogens and is associated with small but statistically significant decreases in HDL-C. It is not yet known if the small transient negative changes in blood lipids (i.e., 3 to 6 mg/dl reductions in HDL-C) induced by androstenedione will have a major impact on the risk for cardiovascular disease. Although estrogen may have cardioprotective effects,[150] it is important to determine inflammatory and atherothrombotic indices (e.g., homocysteine and C-reactive protein) that determine the health impact of prohormones on athletes. A future area of study is applying nutrient interventions to treating side effects of anabolic steroid misuse, some which may be lifelong.

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