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In vivo MRI evaluation of anabolic steroid precursor growth effects in a guinea pig model

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Abstract

Anabolic steroids are widely used to increase skeletal muscle (SM) mass and improve physical performance. Some dietary supplements also include potent steroid precursors or active steroid analogs such as nandrolone. Our previous study reported the anabolic steroid effects on SM in a castrated guinea pig model with SM measured using a highly quantitative magnetic resonance imaging (MRI) protocol. The aim of the current study was to apply this animal model and in vivo MRI protocol to evaluate the growth effects of four widely used over-the-counter testosterone and nandrolone precursors: 4-androstene-3 17-dione (androstenedione), 4-androstene-3 β 17 β -diol (4-androsdiol), 19-nor-4-androstene-3 β -17 β -diol (bolandioli) and 19-nor-4-androstene-3 17-dione (19-norandrostenedione). The results showed that providing precursor to castrated male guinea pigs led to plasma steroid levels sufficient to maintain normal SM growth. The anabolic growth effects of these specific precursors on individual and total muscle volumes, sexual organs, and total adipose tissue over a 10-week treatment period, in comparison with those in the respective positive control testosterone and nandrolone groups, were documented quantitatively by MRI.

Keywords

MRI; Growth effects; Androstenedione; 4-Androsdiol; Bolandioli; 19-Norandrostenedione

1. Introduction

One of the widely abused drugs in the modern world is anabolic steroid [1–10]. For decades, anabolic-androgenic steroids, or anabolic steroids, have been known to promote skeletal

muscle (SM) growth. They are widely used by athletes and non-athletes wishing to enhance their appearance and physical performance. More importantly, a new phenomenon is the parallel use of 'dietary supplements' for building SM mass. Although widely available over the counter, some of these supplements include potent steroid precursors such as androstenedione and bolandiol, or active steroid analogs such as nandrolone. However, their growth effects, long-term risks and abuse potential are not clearly documented.

High-resolution magnetic resonance imaging (MRI) provides an accurate and *in vivo* platform to measure body composition, including quantification of skeletal muscles [11–17]. In our previous study, we developed a quantitative MRI approach to investigate the muscle growth effects of anabolic steroids *in vivo* [18]. A protocol of MRI acquisition using a standard clinical 1.5 T scanner and a quantitative three-dimensional (3D) image analysis procedure was established and validated. It was then employed to measure the individual muscle and organ volumes in an experimental model of intact and castrated male guinea pigs [19–23] that underwent a 16-week treatment protocol by two well-documented anabolic steroids, testosterone and nandrolone, via implanted silastic capsules. The results demonstrated that quantitative MRI provides accurate and sensitive measurement of individual muscles and organs, constituting an effective experimental paradigm to investigate the longitudinal and cross-sectional growth effects of various anabolic steroids and their precursors.

The aim of the present study was to employ this *in vivo* MRI approach to evaluate the anabolic potential of several common steroid precursors using the experimental guinea pig model. Four steroid precursors that are widely found in dietary supplements were investigated, including two testosterone precursors (4-androstene-3 17-dione (androstenedione) and 4-androstene-3 β 17 β -diol (4-androstenediol)) and two nandrolone precursors (19-nor-4-androstene-3 β -17 β -diol (bolandiol) and 19-nor-4-androstene-3 17-dione (19-norandrostenedione)). Specifically, the growth effects of silastic implant administration of the four steroid precursors on specific muscle compartments, sexual organs, total skeletal muscle, and total adipose tissue over a 10-week period, in the whole-body of the intact and castrated guinea pigs, in comparison with those in the respective positive control testosterone and nandrolone groups, were documented quantitatively.

2. Materials and methods

2.1. Experiment design

Certified virus free male Hartley guinea pigs weighing 450–500 g at 8–9 weeks (Covance Research Products, New York) were acquired for this study. The appropriate silastic capsule doses of the steroid precursors required to maintain target levels of testosterone and nandrolone, for study of growth effects in castrated and intact guinea pigs, were determined in a pilot study prior to imaging. The guinea pigs were grouped for each of the four steroid precursors (intact androstenedione, $n=14$; castrated androstenedione, $n=15$; intact 4-androstenediol, $n=14$; castrated 4-androstenediol, $n=15$; intact bolandiol, $n=16$; castrated bolandiol, $n=16$; intact 19-norandrostenedione, $n=14$; and castrated 19-norandrostenedione, $n=15$), their positive control steroids (intact testosterone, $n=9$; castrated testosterone, $n=10$; intact nandrolone, $n=10$; and castrated nandrolone, $n=8$), and

the empty capsule (intact empty, $n = 9$; castrated empty, $n = 10$) groups. Images obtained prior to treatment (baseline), and at the conclusion of treatment (endpoint, 10 weeks), were analyzed to assess the growth increments of masseter muscle, temporalis muscle, neck muscle complex (rhomboideus and splenius), shoulder muscle complex, testes (intact groups), prostate and seminal vesicles, total skeletal muscle, and total adipose tissue.

At 12 weeks of age, baseline MRI images were obtained. Basal blood sampling and castration (or sham surgery) were performed. The silastic capsules containing the four steroid precursors, in amounts sufficient to achieve target levels of the steroid products, were implanted in the intact and castrated experimental groups. The positive control steroid capsules containing testosterone or nandrolone sufficient to maintain normal growth over the 10-week treatment period were implanted in the intact and castrated control groups. Blood was also sampled from the groups at midpoint (week 5), followed by a silastic capsule replacement after approximately 5 weeks to prevent the impairment of drug release due to tissue overgrowth around the capsule. At the endpoint, the guinea pigs were imaged a second time, and then euthanized following a final blood draw.

2.2. Animal procedures

2.2.1. Animal handling—All animals were housed singly or doubly in hanging cages. They were maintained on a timer-controlled 12:12 light/dark cycle in an air and temperature controlled (23–24 °C) room. The animals were fed with Purified Guinea Pig Diet #50001 (Purina, Inc.) and water ad libitum. All following animal procedures were approved by the Institutional Ethics Committee of Columbia University.

2.2.2. General surgical schedule—Two days following baseline imaging, guinea pigs were anesthetized with ketamine-xylazine (30 mg/kg i.p., 5.0 mg/kg i.m., respectively), supplemented with isoflurane inhalation (2–5%), and underwent both castration and silastic capsule implantation procedures in the same surgical session. Intact animals received sham surgery only, plus silastic capsule implants. Two doses of antibiotic (Sulfatrim, 0.3 ml s.c.) were administered, one prior to, and one following surgery. In addition, two doses of buprenorphine (0.05 mg/kg s.c.) were administered post surgery, to minimize potential pain and distress.

2.2.3. Silastic capsules and implantation for steroid administration—The results from our validation study [18] have confirmed that the use of crystalline (powdered) steroid compounds packed in 4-cm silastic capsules provides a reliable means of elevating circulating steroid levels in guinea pigs [24]. Silastic is semi-permeable, and interstitial fluid slowly and continuously dissolves and releases the steroid at a steady rate. However, we have found that tissue overgrowth can begin to impair steroid release starting at approximately 4–5 weeks post implantation. Therefore, fresh silastic capsules were implanted at the midpoint (5 weeks) of the experimental period. Steroid precursors to be tested were obtained from Steraloids, Inc. (Newport, RI). Details of silastic capsules, compound loading and implantation procedures were described in our previous study [18].

To enable testing of steroid precursor effects over a wide range of potential capsule doses, the ability to implant more than four intrascapular silastic capsules was needed. In the

current study, a bilateral dorsal subcutaneous “pocket” technique was developed, with which four capsules on each side of the animal, for a total of eight capsules, could be safely implanted with minimum adverse effects. Specifically, the silastic capsules were implanted in the dorsal mid-trunk area of the back in two parasagittal subcutaneous pockets located 1.5 cm from the midline.

2.2.4. Blood sampling and steroid level analyses—Blood was sampled at multiple time points during the experiments. Approximately 2.0 ml of blood was drawn from the inferior vena cava in the region of the midline anterior rib cage, and was immediately placed in 3.0 ml EDTA tubes and spun at 4 °C for collection of plasma which was stored at –20 °C. Assay of plasma testosterone and androstenedione (RIA), and nandrolone (ELISA) were carried out with kits purchased from DSL Laboratories (Webster, TX), and Neogen Corp. (Lexington, KY), respectively. Required sample size for these assays was 100 µl. Levels of bolandiol and nandrolone were measured by chromatographic/MS analysis in the Department of Toxicology at the University of Utah.

2.3. Silastic capsule dose determinations

2.3.1. Target doses—The appropriate doses of the selected steroid precursors, specifically the number of 4-cm silastic capsules capable of delivering levels of the steroid agents that would result in circulating steroid levels within the target range in castrated guinea pigs over a 10-week period was determined prior to the imaging study. The androstenedione and 4-androstenediol are testosterone precursors. The target level is a dose which could result in circulating testosterone levels approximately 1.5–2 times of normal in the castrated animals. This level was selected as target based upon the results obtained in the validation study [18], in which the replacement of testosterone to normal levels in castrated guinea pigs did not completely normalize the bodyweight gain and muscle growth. As normal adult levels of testosterone in guinea pigs vary within a range of 1.5–3.0 ng/ml [25], our target level was 3.0–4.0 mg/dl with response to the administered precursors. The bolandiol and 19-norandrostenedione are not endogenous steroids, but are nandrolone precursors [26]. The dose of bolandiol to be administered was determined by the target level of nandrolone achieved in the validation study (approximately 3.5 ng/ml [18]). This level of nandrolone normalized the muscle growth in castrated guinea pigs.

2.3.2. Capsule doses design—12-week-old male guinea pigs in intact and castrated treatment groups received different doses of steroid precursors. The implantation of 2, 4, 6, and 8 silastic capsules were selected to examine the effects of the androstenedione and bolandiol doses; and the implantation of 3, 6, and 9 silastic capsules were selected for 4-androstenediol and 19-norandrostenedione doses. Following the baseline blood sampling and silastic implantation at 12 weeks of age, blood samples were taken from the androstenedione, 4-androstenediol, bolandiol, and 19-norandrostenedione groups at weeks 1, 3, and 7 post implantation. The samples were analyzed for testosterone and nandrolone concentrations to determine the capsule doses.

2.4. MRI data acquisition and quantitative analysis

2.4.1. MRI image acquisition and analysis—As described in our validation study [18], a 3D high-resolution image data set covering the entire body of guinea pig (from rostral to caudal) was acquired using a multi-slice spin-echo T1-weighted sequence (0.5 mm × 0.5 mm × 1.5 mm voxel size, 0.3 mm slice gap) on a whole-body 1.5 T clinical scanner (Intera, Philips Medical Systems, Netherlands). A 10-cm quadrature RF coil for clinical human knee imaging was employed. Such an MRI protocol provides adequate soft tissue contrast for delineation of the tissues of interest (see Fig. 1). Prior to imaging, the animals were anesthetized with sodium pentobarbital (28 mg/kg i.p.). Total imaging time for each animal was approximately 20 min.

Image segmentation and quantitation were conducted using a customized software package in a semi-automatic manner. The procedures were similar to those used in our previous study [18]. In brief, each 3D MRI image data set was first segmented for nine tissue compartments, including five skeletal muscles (temporalis, masseter, neck complex, shoulder complex, and the remaining skeletal muscle), three sexual organs (prostate, seminal vesicles, and testes), and whole-body adipose tissue. The mean and standard deviation of these tissue volumes for various groups (intact, castrated, intact treatment, and castrated treatment) were then calculated.

To assess the spatial distribution of the growth effects, longitudinal tissue “area profile,” expressed as the cross-sectional muscle area distribution along the rostral to caudal body axis, was measured for each animal to reflect the tissue distribution from rostral to caudal. The geometric parameters such as tissue length and tissue center were derived to monitor the tissue distribution changes over time. Note that a stereotaxic registration of the area profiles was performed to align the individual area profiles from different animals by aligning the center of the shoulder tissue in each animal.

2.4.2. Statistical data analysis—To evaluate the growth effects of the selected steroid precursors, complete statistical analyses were applied to volume measurements of the selected muscle and organs, 4-muscle index representative of upper body muscle (defined as the sum of temporalis, masseter, neck complex, and shoulder complex), total skeletal muscle, and total adipose tissue. Conventional statistical methods were used to compare the mean values of the experimental groups, and to analyze relationships between variables. Specifically, a mixed-effects ANOVA model for repeated measures was fitted for each tissue compartment. The fixed effects were time point (with levels baseline and 10 weeks), surgery (castrated and intact), and steroid treatment (empty, and various steroid precursors), all of which were treated as factor variables. All their interactions were included, and a common baseline mean for all groups was assumed due to randomization [27]. At the 10-week endpoint, pairs of surgery/treatment cohorts were compared. Also, in each fixed surgery/treatment cohort, the change from baseline was assessed.

3. Results

3.1. Steroid and precursor silastic capsule doses determination

3.1.1. Testosterone levels in androstenedione and 4-androstenediol groups—

The testosterone level of the castrated androstenedione groups over the 7-week post-implantation period is shown in Fig. 2(a). Well pronounced dose–response effects of androstenedione capsules on circulating plasma testosterone levels in castrated guinea pigs were observed at weeks 1, 3 and 7 post implantation, with the exception of the 8-capsule group, in which testosterone appeared to level off, rather than increase, at weeks 3 and 7, suggesting a biphasic dose–response at these time points. Therefore, the decision was made to implant the androstenedione groups with 6 capsules, which are better tolerated than 8 capsules as a function of time. Moreover, testosterone levels of the 6-capsule group over the 7-week period achieved the target level of 3.0–4.0 mg/dl or greater.

The testosterone levels of the 4-androstenediol groups are shown in Fig. 2(b). A dose–response of increasing capsule number on testosterone level in the castrated groups at week 1 was clearly demonstrated. Following week 3, the testosterone levels of the 9-capsule group fell to 2.0 ng/ml or less. Testosterone levels of the 6-capsule group, in contrast, ranged between 2.5 and 3.0 ng/ml over the 7-week period. As a result, a dose of 6 capsules was selected for the castrated 4-androstenediol group for the target steroid level. A dose higher than this may risk a reduction of testosterone levels as the experiment progresses beyond 3–5 weeks.

3.1.2. Nandrolone levels in bolandiol and 19-norandrostenedione groups—Fig.

2(c) shows the nandrolone levels of the bolandiol groups. A dose–response of increasing capsule number on nandrolone level is seen at week 1, and only up to the level of the 6 capsules. The lower nandrolone level obtained in the 8-capsule group suggests a biphasic dose–response at week 1. By week 3, no clear dose-dependent effect can be seen, and nandrolone levels are in most cases lower. However, by week 7, a linear dose–response appears as a function of bolandiol capsule number, with nandrolone level rising to an average of 0.81 ± 0.28 ng/ml in the 8-capsule group. Thus, a slower, but steadily increasing rate of bioconversion from bolandiol to nandrolone was achieved as exposure time and capsule number increases. As a result, decision was made to implant 8 bolandiol capsules in the groups. Nonetheless, the overall nandrolone levels in these groups were lower than the target level of 3.0–3.5 ng/ml. The highest nandrolone level achieved was that of the castrated 6-capsule group at week 1, with a mean level of 1.35 ± 0.52 ng/ml, followed by the intact 6-capsule group at the same time point. Several factors may contribute to the low bolandiol-induced nandrolone levels. They include the decreased rate of diffusion of bolandiol from the capsule; the interference in the Neogen nandrolone assay from bolandiol contained in the samples; and the biological factors such as reduced rate of bolandiol conversion to nandrolone. Thus, the refinements of the “pocket” capsule implantation technique were made during the study, which improved the wound healing and capsule retention. Although nandrolone levels did not achieve the target range, the 8 capsules offered the greatest potential for approaching this range over the 10-week treatment period.

Fig. 2(d) shows the nandrolone levels of the 19-norandrostenedione groups. The 3-capsule group maintained a stable circulating nandrolone that is at least two times the normal level over the 7-week period, meeting the target value of 3.5 ng/ml in castrated guinea pigs. Thus, 3 capsules of 19-norandrostenedione were selected for implantation.

3.1.3. Steroid levels in testosterone and nandrolone control groups—Castrated and intact testosterone and nandrolone control groups were studied to confirm the predicted effects of the selected number of capsules. As shown in Fig. 2(e), 6 testosterone capsules elevated the circulating testosterone in both groups to 1.5–2 times the normal level at week 1, which is largely consistent with that observed in our validation study [18], and levels remained slightly above normal at week 3. By week 7, testosterone levels of both groups returned to normal. Such levels would insure normal growth in these groups.

The nandrolone levels of the castrated and intact nandrolone groups with 3 capsules implanted bilaterally are demonstrated in Fig. 2(f), which also closely replicated the results of the nandrolone implantation in the validation study [18]. The nandrolone levels at weeks 1, 3 and 7, for both groups, with the exception of the intact group at week 7, remained well within the range of 2.5–5.0 ng/ml.

3.2. Steroid levels induced by steroid precursors in the experimental groups

3.2.1. Androstenedione and 4-androstenediol—Circulating testosterone levels at baseline, week 5, and week 10 for the intact and castrated androstenedione and 4-androstenediol groups were compared with that of the empty capsule and testosterone groups as shown in Fig. 3(a). As expected, castration immediately following baseline led to highly significant decreases of circulating testosterone at weeks 5 and 10 ($p < 0.01$). Barely measurable levels of testosterone following castration have been observed previously [28], and are attributed to conversion of natural steroid precursors in the body to testosterone by other tissues, such as the adrenal glands [29]. In contrast, testosterone levels of the intact empty group remained well within the normal range across the entire treatment period.

Implantation of 6 testosterone capsules increased circulating testosterone levels in the intact and castrated testosterone groups to above normal by week 5 (4.23 and 3.96 ng/ml, respectively). With capsule replacement at week 5, testosterone levels remained elevated in the castrated testosterone group at week 10. In the intact testosterone group, one may speculate that down regulation of testosterone production in response to exogenous testosterone administration resulted in restoration of normal testosterone levels in these animals.

The androstenedione and 4-androstenediol groups had baseline testosterone levels within the normal range for growing male guinea pigs (1.5–2.5 ng/ml). Implantation of 6 androstenedione capsules resulted in highly significantly elevated testosterone levels in both intact and castrated groups at weeks 5 and 10 ($p < 0.01$). Testosterone levels of these groups ranged from 4.52 to 5.75 ng/ml over the 10-week experimental period. Thus, androstenedione administration at a dose of 6 capsules was able to elevate the testosterone levels to the target range for both castrated and intact guinea pigs, and promote normal levels of growth in castrated guinea pigs.

Implantation of 6 4-androstenediol capsules in the castrated group succeeded in maintaining testosterone at the level of intact group up to week 5. This effect weakened by week 10 when the testosterone level had fallen to 1.61 ng/ml and was significantly decreased compared to that of the intact 4-androstenediol group. However, neither group had testosterone levels significantly above baseline at week 10. The reduction of testosterone levels during the second half of the treatment period had no effect on body weight gain in the castrated 4-androstenediol group, and as shall be seen later had virtually no effect on rates of muscle growth.

3.2.2. Bolandiol and 19-norandrostenedione—Nandrolone levels of the intact and castrated bolandiol and 19-norandrostenedione groups are shown in Fig. 3(b). Implantation of 3 nandrolone capsules led to highly significant elevations of circulating nandrolone at week 5 in both intact and castrated groups ($p < 0.01$), with a significant elevation maintained in the castrated group at week 10 ($p < 0.01$). Observed elevations of nandrolone in the castrated nandrolone group spanned a range of 2.3–3.4 ng/ml, which were comparable to that of the validation study [18], and proved sufficient to promote normal muscle growth in castrated guinea pigs.

Nandrolone levels of the bolandiol groups were significantly elevated, with the exception of the castrated bolandiol group at week 10. However, nandrolone elevations were milder in the bolandiol groups than those in the nandrolone groups, spanning a much narrower range of 1.20–1.86 ng/ml. Thus, although significant circulating nandrolone levels could be induced in intact and castrated guinea pigs with bolandiol capsule implantation, these levels did not reach the target range (3.0–3.5 ng/ml) identified as capable of promoting normal growth. Nevertheless, nandrolone levels in the castrated bolandiol group appeared sufficient to maintain an almost normal growth, due perhaps to the high potency of nandrolone as a testosterone agonist *in vivo*.

As shown in Fig. 3(b), treatment of both intact and castrated guinea pigs with 3 capsules of 19-norandrostenedione resulted in extremely high levels and significant increases of the steroid product nandrolone at weeks 5 and 10 ($p < 0.01$). Nandrolone levels reached as high as 6.8–9.0 ng/ml, and were well above the nandrolone target levels for this precursor.

3.3. Growth effects of steroid precursors on muscle and organ by MRI

To study the four selected steroid precursors, 430 guinea pig MRI datasets underwent the 3D image segmentation and data analysis. Fig. 4 summarizes the growth effects of the castration, control steroid, and steroid precursor replacements on the specific muscle compartments, as well as the prostate and seminal vesicle tissues. The tissue volumes of the androstenedione and 4-androstenediol groups were compared with that of the control testosterone group, and the tissue volumes of the bolandiol and 19-norandrostenedione groups were compared with that of the nandrolone group. In addition to the analysis of individual muscles and organs, a combined muscle score 4-muscle index was also tested. Fig. 5 demonstrates the growth effects of the castration, control steroids, and steroid precursors on the total skeletal muscle, total body adipose tissue, and total body weight. The

growth effects on testes tissue in intact groups are shown in Fig. 6. Tables 1 and 2 summarize the percent tissue growth in all groups over the 10-week period.

3.3.1. Growth effects of steroid precursors in castrated groups—The growth rates of different muscles and organs may be affected by castration differently. All groups experienced muscle growth from baseline to week 10, except that in the castrated empty group, the temporalis shrank and the neck muscle had no significant increase. The prostate and seminal vesicles also shrank in the castrated empty group. Significantly lower ($p < 0.01$) tissue volumes were detected in the castrated empty group compared to that of the intact empty group at week 10, except in total adipose tissue where it was significantly higher ($p = 0.049$). The steroid and precursor replacements in the castrated guinea pigs resulted in significant tissue growth to or towards normal for all muscles at week 10 (Table 2). Such treatment restored the tissue growth to the level of the intact empty group, and in some cases stimulated even faster growth (androstenedione, nandrolone, 19-norandrostenedione, and testosterone) than that of the intact empty group. Significant differences were observed for almost all of the comparisons made between the castrated steroid or steroid precursor group and the castrated empty group ($p < 0.01$). The growth effects on muscle compartments suggested that the selected steroid precursors stimulated normal or towards normal muscle growth in castrated guinea pigs over a 10-week period.

The growth of the prostate and seminal vesicles in the castrated bolandiol and nandrolone groups showed little or no treatment effect over the 10-week period (Fig. 4). This is a known result of nandrolone administration, and a result of the fact that 19-norandrogens, when 5 α -reduced in the sex organs, including the prostate gland, have significantly reduced androgenic potency due to decreased binding to androgen receptors in these tissues [30]. Although castrated empty group showed significant total skeletal muscle growth between baseline and week 10 ($p < 0.01$), the percent total skeletal muscle growth of this group was significantly less than that of the intact (Table 1) and castrated (Table 2) steroid or steroid precursor treatment groups.

3.3.2. Growth effects of steroid precursors on muscle compartments—Similar growth effects were observed on muscle compartments among the androstenedione, 4-androstenediol, and control testosterone groups, as well as among the bolandiol, 19-norandrostenedione, and nandrolone groups, with almost no significant differences in percent tissue growth between the intact and castrated groups. Androstenedione and 4-androstenediol significantly elevated circulating testosterone levels in both intact and castrated guinea pigs. As a result, significantly greater percent growth was detected in these groups compared to that in the intact and castrated testosterone groups. Thus, maintenance of testosterone at these levels augmented the growth of specific muscles to above normal levels in intact animals over the 10-week period.

Despite a milder but significantly greater percent growth of shoulder muscle detected in the castrated 19-norandrostenedione group, the growth of other muscle compartments (masseter, 4-muscle index) in either the 19-norandrostenedione groups was greater than normal compared to that in the intact empty group, on both absolute and percent basis. When percent growth was examined, differences of the growth effects between nandrolone and

bolandiol were observed. Castrated bolandiol group showed less percent growth of neck, masseter, and temporalis muscles compared to that in the castrated nandrolone or intact bolandiol groups. The small but significant reductions in muscle growth over the 10-week period in castrated bolandiol group suggested that bolandiol administration was not as effective in promoting normal growth of muscles in castrated guinea pigs as nandrolone administration, probably due to the lower nandrolone levels achieved with bolandiol administration. In summary, the steroid precursors androstenedione, 4-androstenediol, and 19-norandrostenedione were capable of increasing overall and/or individual muscle growth to above normal levels in both intact and castrated guinea pigs.

3.3.3. Growth effects of steroid precursors on sexual organs—The selected steroids and their precursors produced significant increments of growth of prostate and seminal vesicles in intact and castrated guinea pigs, with the exception of the bolandiol and nandrolone capsule replacements, with which a significant reduction of volume or growth of the prostate and seminal vesicle was observed over the 10-week period. Bolandiol in particular was found to suppress prostate and seminal vesicles growth in both intact and castrated animals. No differences in percent growth were found between the intact empty group, and the intact/castrated testosterone group or one of the other steroid precursors groups (androstenedione, 4-androstenediol, and 19-norandrostenedione). This indicates that the growth effects of these steroid precursors on prostate and seminal vesicles were potent even in castrated guinea pigs.

More dramatic reductions of normal growth were seen in the testes (Fig. 6). Except for the intact 4-androstenediol, bolandiol, and 19-norandrostenedione groups, testes of the other intact steroid and steroid precursor groups failed to grow normally on an absolute or percent basis (greater than 50% reduction), compared to that of the intact empty group. The percent growth of testis in bolandiol and 19-norandrostenedione groups was much less than that of the intact empty and 4-androstenediol groups. This is consistent with the study evaluating the efficacy of the anabolic-androgenic steroids over prolonged periods for male contraception [31], in which the normal men were found become azoospermic or severely oligozoospermic on high dosages of testosterone enanthate.

3.3.4. Growth effects of steroid precursors on total muscle—Significant increments in total skeletal muscle over a 10-week period, expressed in both volume (Fig. 5) and percent increases (Table 2), were observed for all castrated steroid and steroid precursor replacements groups, compared to that of the castrated empty group. Although the castrated empty group showed significant total skeletal muscle growth between baseline and week 10 ($p < 0.01$), the percent total skeletal muscle growth of this group was less than that of the intact (Table 1) and castrated (Table 2) steroid or steroid precursor treatment groups.

Longitudinal skeletal muscle profiles are shown in Fig. 7 to demonstrate the effects of steroid precursors on the skeletal muscle distribution along the body length. Pronounced increments in skeletal muscle distribution were observed in castrated steroid and steroid precursor replacements groups, compared to that in the castrated empty group which had significantly decreased overall muscle growth. The testosterone and nandrolone replacements in the castrated guinea pigs apparently maintained a normal muscle growth

over the 10-week period. Very similar skeletal muscle distribution was found between the castrated groups with steroid precursor and control steroid replacements. The muscle growth and distribution in all castrated steroid precursor groups were close to or in some cases above normal, compared to the castrated control steroid and intact empty groups.

3.3.5. Growth effects of steroid precursors on whole-body adipose tissue and body weight—The growth effects of the steroids on the whole-body adipose tissue and body weight are demonstrated in Fig. 5. In contrast to the reduction in total skeletal muscle growth, the total adipose tissue growth in the castrated empty capsule group was significantly higher than that in the intact empty capsule group ($p < 0.05$). Although reductions of the percent growth in total adipose tissue were found in the intact and castrated testosterone, nandrolone, androstenedione, bolandiol, and 19-norandrostenedione capsule groups, the growth of total skeletal muscle in these groups were near or greater than that in the intact empty capsule group. Finally, although steroid precursor may result in changes in body composition, no significant differences were found in body weight gain between the intact and castrated empty capsule groups at week 10, as well as among the intact and castrated steroid and steroid precursor replacement groups.

4. Conclusion and discussion

In this study, a castrated male guinea pig experimental model was developed, and *in vivo* MRI was utilized, to quantitatively document the anabolic potential and growth effects of four widely used over-the-counter testosterone and nandrolone steroid precursors. The testosterone levels in castrated androstenedione and 4-androstenediol groups, and the nandrolone levels in castrated 19-norandrostenedione group were sufficient to maintain a normal skeletal muscle growth over a 10-week treatment period, compared to that of the castrated control testosterone and nandrolone groups, respectively. Significant muscle growth equal to or in some cases greater than normal, in response to the administrations of these steroid precursors, over a 10-week period in intact and castrated guinea pigs, were observed by MRI analysis. The growth of the selected muscles and organs, especially the temporalis muscle compartment, the prostate, and seminal vesicles was sensitive to castration, steroid, and steroid precursor replacement.

The androstenedione and 4-androstenediol were particularly effective in stimulating above normal muscle growth, significantly increasing the volume of individual muscle compartments, and total skeletal muscle, in both intact and castrated guinea pigs. This was true, but to a lesser extent, for the nandrolone precursor 19-norandrostenedione. The nandrolone levels achieved with bolandiol administration in castrated guinea pigs were not sufficient to promote a normal growth during the 10-week treatment period. Significant reductions in the growth of specific muscles in the castrated bolandiol group were observed. Failure to elevate nandrolone levels sufficiently may be due to reduced efficiency of bolandiol diffusion from the silastic capsules, or slow rates of conversion of bolandiol to nandrolone *in vivo*. The ability of 4-androstenediol to enhance muscle growth above normal may arise from the activity of intermediary steroid products, since testosterone levels themselves were not elevated markedly in 4-androstenediol implanted groups.

Besides the skeletal muscle growth was the study of the effects of steroid agents on the growth of sexual organs and tissues. Administration of 4-androstenediol had no detrimental effect on growth of these tissues. However, administration of the control steroids and other precursors (testosterone, androstenedione) either significantly reduced or completely eliminated the testicular growth in intact male guinea pigs over the 10-week experimental period. Moreover, administration of nandrolone and bolandiol stunted the growth of prostate and seminal vesicle tissue. While reduced or eliminated testicular growth is a known effect of nandrolone administration, the basis for arrested testicular growth in the case of testosterone and androstenedione is not well known. It has been shown in a human study that the use of anabolic steroids decreases the production of testosterone by the testes via negative feedback to the hypothalamus [32]. Although no reported cases of anabolic steroid use resulting in irreversible sterility in men, decrease in the size and firmness of testes was observed with extended use of anabolic steroids, and sperm production also falls significantly on high dosages of anabolic-androgenic steroids [31]. The steroid and steroid precursor administrations manipulated the growth of muscle compartments, sexual organs, and total adipose tissue, but had least effect on the body weight gain during the growth period.

3D high-resolution MRI was employed in this study to quantify the specific muscle compartments and organ volumes. The results demonstrated that the MRI imaging technique is extremely sensitive in detecting differences in individual muscle compartments in the growing guinea pigs, offering the ability to assess growth at multiple time points in the same set of animals, and confirmed the growth effects of various potential steroid precursors. Thus, such in vivo protocol can accurately and sensitively evaluate the anabolic effects of various steroid agents. The established imaging and analysis protocol are translatable to future human studies.

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Fig. 1. High-resolution T1-weighted MR imaging provides sufficient soft tissue contrast for detection and quantification of various tissues and organs of interest to evaluate the growth effects of selected anabolic steroids and steroid precursors.

Fig. 2.

The circulating steroid levels (in mean $\pm$ standard deviation) observed over 7 weeks post capsule implantation in various groups for study of dosing effect (i.e., number of capsules) prior to in vivo MRI experiment. Animal sample sizes were 3–4 for each steroid precursor group and control steroid group, respectively.

Fig. 3.

The steroid levels measured at baseline, week 5, and week 10 in different experimental groups during the in vivo MRI study. Difference from baseline: * $p < 0.05$ and ** $p < 0.01$.

Abbreviations: Testo: testosterone; Andros: androstenedione; 4-Andros: 4-androstenediol; Nandro: nandrolone; 19-Nor.: 19-norandrostenedione.

Fig. 4.

The tissue and organ volumes measured at baseline and 10-week time points for the intact and castrated groups in response to the empty, control steroid, and respective steroid precursor replacement, demonstrating the growth effects of the steroid precursor replacements on the muscle compartments, and the prostate and seminal vesicle tissues. Difference from baseline: * $p < 0.05$ and ** $p < 0.01$.

Fig. 5.

The total skeletal muscle, adipose tissue, and body weight measured at baseline and 10-week time points for the intact and castrated groups in response to empty capsule, steroid, and respective steroid precursor capsule replacement. Difference from baseline: * $p < 0.05$ and ** $p < 0.01$.

Fig. 6.

The testes volumes measured at baseline and 10-week time points for the intact groups in response to empty capsule, steroid, and respective steroid precursor capsule replacement. Difference from baseline: * $p < 0.05$ and ** $p < 0.01$.

Fig. 7. MRI muscle distribution (rostral to caudal) in intact empty, castrated empty, castrated steroid, and castrated steroid precursor capsule replacement groups at the 10-week endpoint.

Table 1

Percent tissue growth in intact groups treated with steroid precursors and positive control steroids over a 10-week period.

Growth (%)	Total AT	Total muscle	Prostate + SV	4-muscle index	Neck	Masseter	Temporalis	Shoulder	Testis
Intact empty	222.2 ± 46.9	41.6 ± 12.9	105.4 ± 42.6	33.9 ± 9.0	31.5 ± 7.1	37.6 ± 13.2	81.6 ± 23.5	30.8 ± 9.2	100.3 ± 63.8
Intact Testo	139.9 ± 53.2	42.8 ± 13.3 ^{**}	82.9 ± 48.1	39.1 ± 9.8 ^{**}	50.7 ± 23.8 ^{**}	42.2 ± 14.5 ^{**}	105.8 ± 33.2	33.3 ± 10.3 [*]	23.3 ± 51.7 ^(**)
Intact Nandro	180.4 ± 77.9 ^{**}	38.1 ± 10.8 [*]	-4.7 ± 31.1 ^(**)	30.8 ± 10.4	39.8 ± 10.8 ^{**}	42.1 ± 15.9 ^{**}	49.9 ± 35.5	24.4 ± 9.8	-2.0 ± 15.2 ^(**)
Intact Andros	125.8 ± 42.0	46.9 ± 15.9 ^{**}	112.1 ± 57.7 [*]	47.0 ± 13.21 ^{**}	47.5 ± 28.0 ^{**}	63.6 ± 17.6 ^{**}	101.7 ± 40.8	39.8 ± 10.6 ^{**}	34.6 ± 41.5 ^(**)
Intact 4-Andros	222.3 ± 31.4	58.5 ± 14.4	120.4 ± 60.6	65.0 ± 12.9 ^{**}	65.3 ± 15.6	71.9 ± 17.6 [*]	114.8 ± 26.0	60.5 ± 12.6 ^{**}	139.5 ± 44.1
Intact Bolandiol	161.1 ± 52.0 ^{**}	38.6 ± 14.3 [*]	4.0 ± 36.4 ^(**)	31.4 ± 13.3	42.6 ± 16.2 ^{**}	34.2 ± 13.7	59.3 ± 39.7	27.1 ± 13.7	24.7 ± 38.8
Intact 19-Nor.	120.5 ± 45.3 ^(**)	51.1 ± 12.8 ^{**}	79.4 ± 34.4	50.8 ± 15.4 ^{**}	47.2 ± 16.2	68.8 ± 22.8 ^{**}	80.8 ± 24.6	44.2 ± 14.4 ^{**}	38.1 ± 14.2

Abbreviations: Testo: testosterone; Nandro: nandrolone; Andros: androstenedione; 4-Andros: 4-androstenediol; 19-Nor.: 19-norandrostenedione; AT: adipose tissue; SV: seminal vesicle.

Statistically significances are marked as: ^{*} $p < 0.05$ and ^{**} $p < 0.01$ for significantly higher than intact empty;

(^{*}) $p < 0.05$ and (^{**}) $p < 0.01$ for significantly lower than intact empty.

Note that the comparisons were made between the pairs of treatment and empty capsule cohorts at the 10-week endpoint, and the statistical analysis took into account the baseline mean for all groups.

Percent tissue growth in castrated groups treated with steroid precursors and positive control steroids over a 10-week period.

Table 2

Growth (%)	Total AT	Total muscle	Prostate + SV	4-muscle index	Neck	Masseter	Temporalis	Shoulder
Castrated empty	244.9 ± 35.7*	28.3 ± 9.5(**)	-18.9 ± 14.2(**)	17.3 ± 9.5(**)	7.0 ± 12.4(**)	22.1 ± 10.6(**)	-12.8 ± 9.2(**)	19.9 ± 11.4(**)
Castrated Testo	130.9 ± 52.2(&)	43.8 ± 20.5***, &&	90.4 ± 63.9&&	38.5 ± 15.1***, &&	43.2 ± 24.4***, &&	47.9 ± 8.8***, &&	75.5 ± 36.1(*), &&	33.5 ± 16.2*, &&
Castrated Nandro	152.6 ± 56.4**	43.5 ± 7.0*, &&	34.8 ± 28.0(*), &&	37.4 ± 6.9*, &&	48.0 ± 16.2*, &&	48.1 ± 11.4***, &&	44.5 ± 11.4(*), &&	31.7 ± 6.5&&
Castrated Andros	121.1 ± 32.5(&)	40.1 ± 12.5***, &&	84.8 ± 45.9&&	41.9 ± 8.3***, &&	42.2 ± 14.5***, &&	51.9 ± 10.4***, &&	81.5 ± 35.0&&	37.5 ± 9.7***, &&
Castrated 4-Andros	243.9 ± 62.1(&&)	50.4 ± 7.1&&	102.0 ± 49.9&&	53.4 ± 8.5&&	49.4 ± 13.2&&	55.2 ± 9.3&&	61.1 ± 19.0(**), &&	53.4 ± 9.2&&
Castrated Bolandiol	152.4 ± 47.3**	33.2 ± 12.4&&	-10.6 ± 21.2(**)	24.6 ± 10.3&&	30.6 ± 14.4&&	26.8 ± 9.7&&	27.5 ± 19.8(**), &&	22.9 ± 12.1&&
Castrated 19-Nor.	124.6 ± 49.8(**),(&&)	53.7 ± 13.7*, &&	88.9 ± 25.1&&	53.1 ± 15.4***, &&	49.5 ± 18.9&&	78.8 ± 19.2***, &&	77.7 ± 28.6&&	44.6 ± 14.9*, &&

Statistically significances are marked as: * $p < 0.05$ and ** $p < 0.01$ for significantly higher than intact empty;

(*) $p < 0.05$ and (**) $p < 0.01$ for significantly lower than intact empty;

&& $p < 0.05$ and && $p < 0.01$ for significantly higher than castrated empty (assessed for castrated groups only);

(&&) $p < 0.05$ and (&&) $p < 0.01$ for significantly lower than castrated empty (assessed for castrated groups only).