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In Vivo MRI Quantification of Individual Muscle and Organ Volumes for Assessment of Anabolic Steroid Growth Effects

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Abstract

This study aimed to develop a quantitative and in vivo magnetic resonance imaging (MRI) approach to investigate the muscle growth effects of anabolic steroids. A protocol of MRI acquisition on a standard clinical 1.5 Tesla scanner and quantitative image analysis was established and employed to measure the individual muscle and organ volumes in the intact and castrated guinea pigs undergoing a 16-week treatment protocol by two well-documented anabolic steroids, testosterone and nandrolone, via implanted silastic capsules. High correlations between the in vivo MRI and postmortem dissection measurements were observed for shoulder muscle complex ($R = 0.86$), masseter ($R=0.79$), temporalis ($R=0.95$), neck muscle complex ($R=0.58$), prostate gland and seminal vesicles ($R=0.98$), and testis ($R=0.96$). Furthermore, the longitudinal MRI measurements yielded adequate sensitivity to detect the restoration of growth to or towards normal in castrated guinea pigs by replacing circulating steroid levels to physiological or slightly higher levels, as expected. These results demonstrated that quantitative MRI using a standard clinical scanner provides accurate and sensitive measurement of individual muscles and organs, and this in vivo MRI protocol in conjunction with the castrated guinea pig model constitutes an effective platform to investigate the longitudinal and cross-sectional growth effects of other potential anabolic steroids. The quantitative MRI protocol developed can also be readily adapted for human studies on most clinical MRI scanner to investigate the anabolic steroid growth effects, or monitor the changes in individual muscle and organ volume and geometry following injury, strength training, neuromuscular disorders, and pharmacological or surgical interventions.

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Keywords

Body composition; MRI; anabolic steroids; growth effects; testosterone; nandrolone

INTRODUCTION

In vivo quantification of individual muscle and organ volumes is important in study of various diseases and many pharmacological interventions that involve changes in body compartments [1]. A number of non-destructive techniques exist for body composition measurement, including hydrodensitometry, dual-energy X-ray absorptionmetry (DXA), and computed tomography (CT) [2, 3]. However, these techniques are either semi-quantitative, lacking individual muscle and organ measurement, or involving ionizing radiations. Magnetic resonance imaging (MRI) yields truly three-dimensional anatomy with high spatial resolution and unparalleled soft tissue contrast, thus offering the potential to produce the most accurate body composition analysis. Earlier MRI studies have demonstrated the high accuracy in measuring body adipose tissue in animal models when compared with chemical analysis [4, 5], leading to MRI quantification of adipose tissue in humans [6, 7]. High-resolution MRI was also shown to be capable of accurately tracking the individual organs (such as brain, liver, kidney and spleens), total skeletal muscle and adipose tissue mass longitudinally during aging and obesity development [8]. High-resolution MRI was also evaluated recently for its ability to measure individual muscles in human cadaver forearms [9]. Development of noninvasive MRI approach for more comprehensive body composition measurement is particularly valuable in assessing the long-term biological effect of various drug interventions or abuses in a highly quantitative manner.

One of the widely abused drugs is anabolic steroid [10, 11]. Anabolic-androgenic steroids, or anabolic steroids, are widely used by professional and recreational athletes, weight lifters and bodybuilders, and non-athletes wishing to enhance their appearance and physical performance despite their known adverse effects, unknown long-term risks, and abuse potential [10–14]. To accurately document the potency of these compounds, therefore, it is imperative to develop a quantitative procedure as a platform technology to assess the long-term growth effects and body composition changes.

The effects of classical anabolic steroids on muscle growth and body composition have been well studied in guinea pigs [15–19]. These studies examined the effect of testosterone and castration on the growth rate of different muscles and found that muscles of the guinea pig respond in a highly region-specific manner. These findings indicate that the guinea pig corresponds rather closely to humans with respect to the role of anabolic steroids in muscular development following androgen administration, suggesting that the castrated guinea pig is an appropriate animal model for the study of anabolic steroid effects on muscle growth in humans. In these previous animal studies, muscle growth was typically assessed by dissection techniques. In human studies of anabolic steroid effects, DXA and measurement of skinfolds and circumferences have been often used [20–22] while in one study MRI was used to assess the cross-sectional areas of triceps and quadriceps muscles [23].

The overall goal of this study was to develop an in vivo and non-destructive MRI quantification approach to accurately and longitudinally characterize the individual muscle and organ volumes, evaluate and validate the protocol using the guinea pig model treated with well-documented anabolic steroids. Development of such quantitative MRI analysis will enable accurate assessment of the growth effects of various anabolic steroids, and generally benefit the study of muscle and organs volume changes related to other pharmacological interventions or diseases.

MATERIALS AND METHODS

Experiment Design

The aim of this experimental study was to establish and validate a comprehensive MRI approach using an experimental model of castrated pigs treated with known anabolic steroids. A whole-body high-resolution MRI acquisition and image analysis protocol was first developed. The effects of silastic implant administration of two potent and widely used anabolic steroids (testosterone and nandrolone) on specific muscle and organ growth, and on SM and fat mass content in the whole body of intact and castrated guinea pigs, were determined using the MRI protocol and confirmed by dissection measurements at the study endpoint. All following animal procedures were approved by the institutional ethics committee of Columbia University.

12-week old male Hartley guinea pigs (Covance Research Products, New York) had implantation of the subcutaneous silastic capsules for steroid administration or as sham control. Six groups of animals were employed as follows: (1) intact animal with empty capsule, N=16; (2) castrated with empty capsule, N=17; (3) intact with nandrolone capsule, N=18; (4) castrated with nandrolone capsule, N=15; (5) intact with testosterone, N=19; and (6) castrated with testosterone, N=14. Appropriate dosages were pre-calibrated and used to produce normal or slightly higher than normal circulating levels of testosterone and nandrolone.

All animals were imaged at three time points, baseline, week-8 and week-16 post implantation. The animals were first imaged and had their blood drawn at 12 weeks of age (baseline measurements). Castration or sham surgery and silastic capsule implantation was carried out within 3 to 5 days following imaging. Silastic capsules containing testosterone or nandrolone in crystalline form were implanted subcutaneously in the dorsal intrascapular area. The groups were treated for 16 weeks, up to 28 weeks of age, so that maximal effects of the steroids on muscle growth could be observed. At 20 weeks of age (8 weeks of treatment) a second MRI image and blood sample was obtained (midpoint measurements), and the already implanted silastic capsules were removed and replaced by an equal number of fresh capsules. Final (endpoint) images were obtained after 16 weeks of treatment (28 weeks of age), following which a final blood sample was obtained, and the animals were euthanized with sodium pentobarbitol, perfused with saline, and dissected.

Specific muscles and organs were weighted, and their volumes were measured by fluid displacement in a calibrated cylinder of 0.1 cc accuracy. These include the masseter and temporalis muscles of the head, the rhomboideus and splenius muscles of the neck (termed

neck muscle complex), the shoulder muscle complex, the prostate gland and seminal vesicles, and the testes (in intact animals). The volumes of the same muscles and organs obtained from MRI analysis were compared with dissection measurements for validation.

MRI Acquisition and Quantitative Image Analysis Procedures

MRI Acquisition—A 1.5 Tesla whole-body clinical MRI scanner with maximum gradient of 3 G/cm (Intera, Philips Medical Systems, Netherlands) was used to acquire the high-resolution 3D image sets of guinea pigs. Guinea pigs were anesthetized by intravenous (i.p.) injection of sodium pentobarbital (28 mg/kg) prior to imaging. To facilitate the image analysis and reduce potential segmentation error in the longitudinal study, a cylindrical stereotaxic positioning device was used to fix the guinea pigs in the identical position so all MRI image shared similar anatomical orientation. The standard quadrature RF coil (10 cm inner diameter) for human knee imaging was employed in the imaging experiment to achieve the maximum possible image signal to noise ratio (SNR). A T1 weighted spin echo sequence was chosen to acquire the image data as it offers the best and most robust contrast to delineate muscle and fat tissue, as well as simplicity [24]. No respiratory and cardiac gating was used. As the transverse relaxation time (T1) of fat tissue is relatively shorter, the fat tissue can be clearly distinguished from other tissues such as muscle by high image intensity in T1 weighted image analyses [24]. The resulting uniform intensity of fat and muscle tissue, as well as the clear image intensity difference between fat and muscle areas, greatly facilitates their delineation and segmentation. Organ tissues such as testis and prostate have a similar intensity range as muscle, but would be clearly differentiated from the surrounding adipose tissue. Note that although gradient echo sequence may yield more rapid 3D data acquisition, it was not chosen for the current study because the magnetic susceptibility artifacts would likely occur in the abdominal regions and prevent reliable tissue segmentation and quantitation.

A coronal slice view of the whole body was chosen to provide an optimal anatomical definition of specific tissues such as masseter, neck complex, and shoulder complex, and seminal vesicle structure, which greatly improved the efficiency and accuracy of the segmentation of these tissues. As the length of the guinea pig's body is longer than the RF coil, two contiguous sets of coronal image data representing the anterior and posterior part of body were acquired to cover the entire body. The two sets of coronal data were first combined to generate the whole-body guinea pig image. The sequence parameters were TR/TE = 500/17 ms, NEX = 3. The image matrix for data acquisition was 360×360 within a 180 mm × 180 mm field of view, and 40 coronal slices were acquired from dorsal to ventral to cover the upper/lower part of the body. The slice thickness was 1.5 mm with a 0.3 mm inter-slice gap. The total scan time was approximately 20 minutes per animal. Note that animals were wrapped in blanket to maintain body temperature during the MRI scans.

Image Segmentation Toolkit—Due to the large amount of volumetric image data to be analyzed in the current study, an image analysis software package (available at <http://www.hku.hk/bisp/intranet.htm>) was first designed and developed for generic 3D regional and global tissue quantification. It was a general-purpose software toolkit that runs on standard PCs or Unix workstations with IDL version 5.2 platform (ITT Visual Information

Solutions, CO, USA) that provides all standard graphic functions required for the analysis in the current study [8, 25]. To facilitate the rapid tissue segmentation and quantification, several functions were incorporated into a graphical user interface. They included (i) multi-slice and multi-planar visualization of MRI data in standard formats including DICOM format; (ii) coarse histogram-based semi-automatic segmentation of local structures (muscle, fat and organs) by selecting specific range of image intensities within the image intensity histogram [8]; (iii) fine segmentation by 2D and 3D region growing from the initial histogram-based segmentation results to take advantage of tissue connectivity and structural similarity within the same or adjacent image slices [26]; (iv) manual segmentation and correction of segmentation errors; (v) labeling of various segmented structures by different colors; and (vi) volume and mass estimation of segmented structures by voxel sizes, numbers and densities assumed; (vii) data output to the standardized EXCEL files for statistical analysis. The integration of these seven functions into a single graphical interface led to minimal operator interaction and much improved segmentation efficiency, with flexibility to accommodate certain level of image quality variations that were slight but expected in high throughput MRI experiments.

MRI Tissue and Organ Quantitative Analysis—Nine tissue compartments and organs were segmented, labeled and quantified based on boundaries detectable in the whole-body MR images, which included the mass of five SMs in specific segments (temporalis, masseter, neck complex, shoulder complex, and the remaining SM), three sexual organs (prostate and seminal vesicles, testis), and whole-body adipose tissue. The boundaries of these regions were defined stereotaxically based on anatomical landmarks identified using a standard guinea pig anatomy atlas [27]. The volumes of muscle, organ, and adipose tissue were calculated from voxel number and voxel size. Tissue weight/mass and volume were then computed using the respective densities of each tissue/organs [28]. Mean and standard deviation of tissue volumes for the six different experimental groups as well as percent change of tissue/organ volume during growth were calculated and used for multiple group comparisons and tissue growth measurements. The general image analysis flow is shown in Figure 1. The entire analysis took approximately 2 hours for each image data set using the IDL image segmentation toolkit described above. Note that all segmentation work in the current study was performed by an individual reader who received a brief training in software usage and guinea pig anatomy. Intra-reader reliability was assessed by three repeated segmentations of six animals from the intact empty group. Single-score, one-way intraclass correlation coefficients for agreement were calculated (with 95% confidence intervals) for the shoulder, testis, masseter, prostate, and temporalis volumes [29].

Data Analysis and Power Calculations—The accuracy of MRI volume measurements using the acquisition and analysis described above were assessed by comparing their absolute values of each muscle and organ with the respective volumes obtained by postmortem dissection using linear regression analysis. To investigate the level of agreement of the MRI volumes with those found by dissection, Bland-Altman analyses were carried out [30] that plotted the difference (MRI volume - dissection volume) against the average value of the two measurements for each subject. The 95% confidence limits of difference were calculated.

Conventional statistical methods were used to compare the mean values of the experimental groups, and to analyze relationships between variables. Specifically, a 3-factor ANOVA model with repeated measures was fitted for each tissue or organ. The fixed effects were time point (with levels 8 weeks and 16 weeks), surgery (with levels castrated and intact), and steroid treatment (with levels empty, nandrolone, and testosterone), which were treated as factor variables. Mixed effects models with animal identity as the random effect, and compound symmetry used for the covariance structure, were employed for the analysis, and the Tukey multiple comparison correction was applied on the set of contrasts that were tested. For each fixed time point, pairs of surgery/treatment cohorts were compared; for instance, at 8 weeks, the intact (no surgery) empty (no hormone) group was compared to the castrated nandrolone group.

To observe the effects of two standard anabolic steroids on muscle and organ growth, the selection of experimental group size was based on the following power calculations [31]. Power calculations were performed using mean and standard deviation values for weights of various head, neck and shoulder muscles of groups of castrated vs. intact male guinea pigs 12 weeks following castration [17]. At 80% power, with $\alpha = 0.05$ and $n = 15$, we would be able to detect muscle weight differences, on average, 38% of the observed difference between the muscle weights of castrated vs. intact male guinea pigs [17]. Moreover, in the case of the prostate gland and seminal vesicles, a group size of $n = 15$ at the same statistical criteria would allow us to detect a difference of only 16% of the difference observed between prostate weights of castrated vs. intact male guinea pigs at 12 weeks post-castration [17].

Animal Procedures

Castration Surgery—Guinea pigs were castrated at approximately 12 weeks of age. Animals were anesthetized with ketamine-xylazine (30 mg/kg i.p., 5.0 mg/kg i.m., respectively), supplemented with isoflurane anesthesia (2–5%). A 1.5-cm horizontal scalpel incision was made across the midline of the lower abdomen, and the testicles were gently retrieved through the midline incision using forceps. The spermatic cord and blood vessels were ligated according to published procedures, and the testicles severed distal to the ligature [32, 33].

Silastic Tube Implantation and Steroid Dosages—Subcutaneous silastic implants have been used for the administration of steroids to experimental animals for many years [34–36]. The use of pure compounds packed in silastic tubing was tested and validated by AM Matsumoto (Univ. of Washington, private communication, 2001) as a reliable means of elevating circulating steroid levels in rodents. Silastic tubing (Dow Corning, Medical Grade 602-285, 0.62 in. OD and 0.125 in. ID) was cut to the desired length plus 10 mm. One end of the tube was plugged with 5 mm of silastic medical adhesive (Dow Corning #891). The tubes were loaded using a long Pasteur pipette and a latex bulb. Steroids and steroid precursors were obtained from Steraloids, Inc. (Newport, RI, USA).

Silastic capsules were first primed by soaking in sterile saline at 4° C for 24 hr prior to insertion. A small (~1.0 cm) transverse incision was made in the mid-scapula scapula. The

capsules were then inserted, the opening closed with wound clips. Four 4.0-cm silastic capsules containing testosterone, or three 4.0-cm capsules containing nandrolone in crystalline form, were implanted. To avoid the impairment of steroid release caused by the tissue overgrowth, the already implanted silastic capsules were removed and replaced by an equal number of fresh capsules at the midpoint during treatment. Note that such dosing scheduling was chosen to maintain the levels of testosterone and nandrolone at or slightly above physiological levels in castrated guinea pigs over extended periods of time, as determined from a pilot study using blood sampling and assay analysis over an 80-day period.

Blood Sampling and Steroid Level Analyses—Blood was sampled percutaneously from the animals at baseline, midpoint and endpoint during the experiment. Approximately 1–2 ml of blood was drawn from the inferior vena cava in the region of the anterior rib cage while being anesthetized with isoflurane (2–5%). Assays of plasma testosterone, nandrolone and 4-Androstene-3, 17-dione were carried out using specific ELISA or RIA kits (DSL Laboratories, Inc., TX, or Neogen Corp., KY, USA).

RESULTS

Validation of MRI Measurement of Individual Muscle and Organ Volumes

A typical coronal view of a T1 weighted MRI image, the 2D segmentation, and 3D visualization is shown in Figure 2, illustrating the ability of high-resolution MRI and semi-automatic segmentation to visualize the volume and geometry of individual tissues and organs. Scatter plots of individual postmortem dissection vs. in vivo MRI measurements for each muscle and organ from all of the groups are shown in Figure 3. Table 1 summarizes the comparisons between dissection and MRI measurements. In general, excellent agreement between the volumes measured by the two techniques was observed for all muscles and organs, with the exception of the neck muscle complex. The MRI neck muscle volumes were systematically lower than those from dissection, and the degree of disagreement decreased with volume. For the five tissues examined (testis, shoulder, prostate and seminal vesicles, masseter, and temporalis), the bias was less than 10% and the half-width of the 95% confidence band ranged from 10% to 20%. Difficulties in separating (dissection) and segmenting (MRI) the muscles selected for dissection (rhomboideus and splenius) from other over- and underlying muscles in the neck complex contributed to lack of a greater agreement between measurements obtained by the two procedures. As for the intra-reader reproducibility assessed by three repeated segmentation of six animals for five tissues/organs, intraclass correlation coefficients were found to be 0.90, 0.95, 0.91, 0.97, 0.98 for shoulder, testis, masseter, prostate and temporalis, respectively, indicating the excellent segmentation reproducibility.

Longitudinal MRI Characterization of Testosterone and Nandrolone Induced Growth Effects

Individual Muscle and Organ Volume Changes—The castration and steroid replacement effects were successfully detected. The mean values of tissue and organ volumes measured by MRI for all six experiment groups are shown in Figures 4 and 5. The

mean values of various tissue and organ volume differences measured between various surgery/treatment groups, their standard errors, and the tests of significance using a 3-factor repeated-measure ANOVA, are summarized in Table 2. As shown in Table 2, the in vivo MRI measurements in conjunction with the appropriate statistical tests are powerful enough to detect the relevant differences that we expect. Because of the large number and complexity of the pair wise tests, the results of these tests were not represented graphically.

Significant differences were observed for all of the comparisons made between the intact and castrated empty capsule groups ($p<0.01$), at 8 and 16 weeks. Similarly, steroid replacement in the castrated groups resulted in significant growth to or towards normal for all muscles and organs ($p<0.01$) in comparison with the castrated empty capsule group, with the exception of the shoulder muscle complex at both 8 and 16 weeks. The prostate and seminal vesicles, shoulder muscle complex, neck muscle complex, masseter muscle, and temporalis muscle, were all significantly reduced in the castrated empty group, in comparison with the intact empty capsule group. Thus, castrated male guinea pigs not given steroid replacement had significantly inhibited growth of prostate and seminal vesicles, as well as several SMs in the anterior part of the body.

In contrast to the castrated empty capsule group, the castrated testosterone and nandrolone capsule groups, which received replacement steroids at physiological (testosterone) or above physiological (nandrolone) levels, showed normal growth of most muscles, in comparison with the intact empty capsule group ($p>0.05$). Mild but significant reductions of prostate and seminal vesicles, and temporalis muscle volumes were observed in the castrated testosterone and nandrolone groups, in comparison with the intact empty capsule group, at 8 and 16 weeks. However, the prostate and seminal vesicles, and temporalis muscle in these groups grew more significantly than in the castrated empty group. Thus, steroid replacement in castrated growing male guinea pigs restored the growth to, or towards normal in the case of all muscles and organs measured in this study.

Additionally, a comparison of the same measurements, for the three intact groups showed significant reductions in testis volume for the intact testosterone and nandrolone groups at both 8 and 16 weeks, in comparison with the intact empty capsule group. This result is consistent with the known effects of exogenous steroid administration on testes growth that long-term anabolic steroid exposure results in the atrophy of the testes. All other muscles, as well as the prostate and seminal vesicles, grew normally in these groups.

For each surgery/treatment cohort, we found significant ($p<0.01$) increases from baseline to 8 weeks and from baseline to 16 weeks, except in the temporalis in the castrated empty group for both time points. For the prostate and seminal vesicles, the castrated empty group had a significant decrease, rather than increase, relative to baseline, at both time points. We also tested whether the changes from baseline at 8 and at 16 weeks were different from each other. In most cases the differences were significant ($p<0.01$). The exceptions were the percent increases of prostate and seminal vesicle, masseter, and temporalis volumes of the castrated empty group; the shoulder muscle volumes of the intact nandrolone, castrated nandrolone, and castrated testosterone groups; and the testis volumes of the intact nandrolone and intact testosterone groups.

Total Body Skeletal Muscle and Adipose Tissue Volume Changes—As shown in Figure 5 and Table 2, castration led to significant reductions of total SM, but not total adipose tissue, at midpoint and endpoint in the castrated empty capsule group, in comparison with the intact empty capsule group. In contrast, hormone replacement in the castrated testosterone capsule group completely normalized the growth of total SM at these points. These whole-body SM measurements provide a global assessment of the effects of testosterone removal and steroid replacement on muscle growth in castrated guinea pigs.

Steroid Level Measurements—Plasma levels of testosterone and nandrolone are shown in Table 3. A comparison of testosterone levels of the intact and castrated empty capsule groups shows normal levels of the hormone for both groups at baseline [37], with no significant change of testosterone levels across the 16-week treatment period for the intact empty capsule group. In contrast, a dramatic reduction to a mean level of 0.11 ng/ml at 8 weeks was seen in the castrated empty capsule group, which increased to 0.36 ng/ml at 16 weeks. Extremely low but measurable levels of testosterone were previously observed in castrated male guinea pigs within 2 weeks following castration [34], attributable to low-level steroid production by the adrenal glands [38]. The administration of testosterone in silastic capsules to the intact testosterone group resulted in significant elevations of circulating testosterone at 8 and 16 weeks of treatment, in comparison with the group's own baseline. This was not the case for the castrated testosterone capsule group, whose testosterone levels were not different from baseline at the two later time points. It should be noted that the testosterone level of the intact testosterone group at midpoint was significantly higher than the respective testosterone level at the same time point in the castrated testosterone group. Nandrolone levels shown at baseline for these groups represent crossreactivity of the assay antibody with testosterone, which occurred at a level of 13.2% in the current study. True nandrolone levels, shown at midpoint and endpoint for both intact and castrated groups, were highly elevated relative to baseline, with no significant differences of nandrolone levels between the groups. These steroid level measurements demonstrated that subcutaneous silastic capsules employed in the current study were capable of maintaining levels of testosterone and nandrolone at or slightly above physiological levels in castrated guinea pigs over extended periods of time in this study.

CONCLUSION AND DISCUSSION

Our results demonstrated the ability of high-resolution MRI to quantify specific muscle and organ volumes in intact and castrated guinea pigs *in vivo*. In this study, overall high correlations and good agreement were observed between muscle and organ volumes as determined by postmortem dissection and *in vivo* MRI analysis using a standard clinical 1.5 Tesla scanner and semi-automatic analysis toolkit. Three-dimensional and high-resolution MRI approach developed is highly quantitative. Such MRI quantification of body composition is valuable because it allows for observing changes in body composition and tissue volumes over time in the same subject without having to performing with a high number of subjects and animal-to-animal variability.

A number of other MRI approaches are also available to measure the fat and muscle. Dixon method can provide direct and separate high-resolution water and fat images, allowing more

accurate measurement of local fat and water content, but at cost of longer MRI scan time (twice or more) [39]. Chemical shift imaging (CSI) measures various proton compounds including intra- and extramyocellular lipids [40], producing more biochemical information. However, high-resolution 2D and 3D CSI is prohibitively time-consuming and not suitable for body composition measurement at large region or whole body level. Because CSI is based on the magnetic resonance frequency difference between water and various lipids, it requires relatively high magnetic field (above 1 Tesla) and good magnetic shimming. Both Dixon and CSI approaches are also vulnerable to local magnetic inhomogeneities that are often seen in the chest and abdominal areas due to the tissue-air interfaces. In contrast, the high-resolution spin echo T1 weighted imaging approach utilized in the current study is simple and easy to implement on virtually all standard clinical MRI scanners. The image data acquisition is fast. With semi-automatic analysis toolkit and procedure developed and demonstrated in the current study, it can provide efficient and robust measurement of individual muscle and organ volumes and geometries to monitor the body composition changes at both whole body and individual muscle/organ level. Note that error in tissue and organ volume quantification can occur depending on the structural contrast and image resolution (because of the partial volume effect). For specific application, however, it is possible to reduce such error by (i) optimizing MRI tissue contrast, spatial resolution and image orientation; (ii) incorporating additional MRI data for better visualization and segmentation of the tissue/organ structures and pathologies of interest.

In this study, the MRI data acquisition and analysis protocol was successfully demonstrated for in vivo and longitudinal detection and characterization of the well-documented growth effects of two common anabolic steroids, testosterone and nandrolone, confirming the appropriateness of a castrated guinea pig model for evaluation of various anabolic steroid effects in vivo. Our results demonstrated that castration significantly reduces the growth of several muscles in the anterior part of the body, plus prostate and seminal vesicles, in male guinea pigs. Reduced muscle growth can be restored to, or significantly towards, normal in castrated guinea pigs with the administration of testosterone and nandrolone at physiological or higher levels. These experimental results clearly indicated that the in vivo MRI analysis and guinea pig model employed in this study can constitute an effective and general protocol for future testing of potential anabolic steroids.

The MRI protocol developed and validated in the current study can be readily adapted for assessing anabolic steroid effects in humans. Potentially, this quantitative MRI protocol can be also employed to study individual muscle and organ volume changes in humans in vivo following injury, strength training, adaptation to space flight, cancer, sarcopenia, and other neuromuscular disorders as well as pharmacological and surgical interventions. As muscle volume and geometry are important parameters in characterizing individual muscles, it is also potentially useful for biomechanical study of musculoskeletal system. Further development of MRI body composition approach may provide more comprehensive characterization of body composition changes in vivo. For example, fat suppression can lead to more accurate muscle quantification. The recent advance in MR diffusion tensor imaging may also enable us to determine the muscle bundle orientation for improved segmentation of individual muscles, and possibly evaluate the muscle fiber quality [41, 42]. Detailed measurement of MR properties of muscles such as absolute water content, longitudinal and

transverse relaxation rates, water diffusion rate, magnetization transfer, and structural anisotropy may provide in-depth characterization of individual muscles.

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Figure 1. MR image processing scheme. The protocol includes semi-automatic and interactive segmentation for efficient expert-guided tissue separation, labeling, and quantitative analysis.

Figure 2. Illustration of a typical T1 weighted coronal slice (a), segmented 2D image with color labeling for different tissues and organs (b), and 3D visualization of the segmented muscles and organs at two different view angles (c) in an intact guinea pig.

Figure 3. Comparison of various tissue and organ volumes measured by in vivo MRI and postmortem dissection in all groups combined at the study endpoint.

Figure 4.

Growth effects of nandrolone and testosterone on various muscles in the guinea pig model are illustrated using the mean measurement values by MRI for various surgeries, steroid treatments, and time points. Abbreviations: Nandro = Nandrolone, Testo = Testosterone.

Figure 5.

Growth effects of nandrolone and testosterone on organs, total skeletal muscle (SM) and adipose tissue in the guinea pig model are illustrated using the mean measurement values by MRI for various surgeries, steroid treatments, and time points.

Table 1

Comparison between postmortem dissection and in vivo MRI volume measurements using Bland-Altman analysis

Tissue	Correlation	Bias of difference (cm ³) (% of mean value)	STDEV of difference (cm ³)	Half-width of 95% confidence limit as % of mean value
Shoulder	0.86	0.602 (1.8%)	1.83	10.7%
Masseter	0.79	-1.07 (-8.9%)	1.27	20.7%
Temporalis	0.95	-0.013 (0.7%)	0.16	16.4%
Neck	0.58	-2.35 (-30.7%)	1.34	34.3%
Prostate + SV	0.98	-0.232 (-5.1%)	0.42	18.1%
Testis	0.96	0.110 (2.3%)	0.34	13.7%

Table 2

Values of tissue or organ volume differences measured between various surgery/treatment groups at week 8 and week 16

Volume Differences (cm ³)	Intact empty – castrated empty	Intact empty – intact nandrolone	Intact empty – castrated nandrolone	Intact nandrolone – castrated nandrolone	Intact empty – intact testosterone	Intact empty – castrated testosterone	Castrated empty – castrated testosterone	Intact testosterone – castrated testosterone
Shoulder								
Week 8	3.2±1.0**	-1.9±1.0	-0.2±1.0	-3.4±1.0**	1.7±1.0	0.4±1.0	-2.5±1.0*	0.3±1.0
Week 16	3.7±1.0**	-0.3±1.0	1.4±1.0	-2.3±1.0*	1.8±1.0	1.6±1.0	-2.0±1.0	0.06±1.0
Neck								
Week 8	1.3±0.2**	-0.2±0.2	0.3±0.2	-1±0.2**	0.5±0.2*	0.3±0.2	-0.9±0.2**	-0.05±0.2
Week 16	1.9±0.2**	-0.4±0.2	-0.02±0.2	-1.9±0.2**	0.4±0.2*	0.4±0.2	-1.5±0.2**	-0.03±0.2
Masseter								
Week 8	1.6±0.3**	-1.2±0.3**	-0.9±0.3**	-2.6±.3**	0.3±0.3	0.7±0.3	-1.2±0.4**	-0.3±0.4
Week 16	2.3±0.3**	-2.6±0.3**	-1.6±0.3**	-3.9±0.3**	1±0.3	0.8±0.3	-1.8±0.4**	-0.3±0.4
Temporalis								
Week 8	1.0±0.1**	-0.2±0.1	0.3±0.1**	-0.6±0.1**	0.2±0.1*	0.2±0.1*	-0.4±0.1**	0.4±0.1**
Week 16	1.4±0.1**	0.2±0.1**	0.5±0.1**	-0.9±0.1**	0.2±0.1**	0.2±0.1**	-0.7±0.1**	0.5±0.1**
Testis								
Week 8	N/A	1.2±0.2**	N/A	N/A	N/A	1.3±0.2**	N/A	N/A
Week 16	N/A	1.5±0.2**	N/A	N/A	N/A	1.8±0.2**	N/A	N/A
Prostate+SV								
Week 8	3.5±0.3**	0.7±0.2**	1.1±0.2**	-2.4±0.2**	0.4±0.2	0.4±0.2	-2.4±0.3**	0.7±0.3**
Week 16	4.7±0.3**	0.6±0.2*	1.3±0.2**	-3.4±0.2**	0.7±0.2**	0.5±0.2*	-3.4±0.3**	0.8±0.3**
Total SM								
Week 8	31±6.7**	-8.8±6.6	2.0±6.6	-28.5±7**	10.8±6.4	5.8±6.5	-20±6.9**	4.2±6.7
Week 16	40±6.7**	-8.6±6.6	6.8±6.6	-33.5±7**	15.4±6.4*	11.4±6.5	-30±6.9**	-0.7±6.7

Volume Differences (cm ³)	Intact empty – castrated empty	Intact empty – intact nandrolone	Intact empty – castrated nandrolone	Castrated empty – castrated nandrolone	Intact empty – intact testosterone	Intact empty – castrated testosterone	Castrated empty – castrated testosterone	Intact testosterone – castrated testosterone
TAT								
Week 8	-7.6±10	11.8±9.9	5.4±9.9	13±9.8	-2.7±9.8	20.5±10.6	28±10.4**	23±10.2*
Week 16	-3.7±10	15±9.9	22±9.9*	26±9.8**	10.7±9.8	34±10.6**	38±10.4**	23.5±10*

Values are given in means ± SEM in cm³;

ANOVA tests of significance are indicated by a single asterisk * for $p < 0.05$ and double asterisk ** for $p < 0.01$.

Table 3

Testosterone or nandrolone levels in the six groups at different time points

Group / steroid	Group size (N)	Baseline	Midpoint	Endpoint
Intact empty/testosterone	15	1.69±0.21	1.73±0.20	1.78±0.19
Castrated empty/testosterone	15	1.86±0.29	0.11±0.03**	0.36±0.05**
Intact nandrolone/nandrolone	16	0.25±0.04	3.33±0.31**	3.40±0.36**
Castrated nandrolone/nandrolone	15	0.23±0.03	3.45±0.34**	3.38±0.27**
Intact testosterone/testosterone	18	1.50±0.17	2.63±0.19**	2.20±0.20*
Castrated testosterone/testosterone	14	1.71±0.22	1.70±0.17++	1.80±0.24

Values are given in means ± SEM in ng/ml.

Difference from Baseline: * for $p < 0.01$ and ** for $p < 0.001$;

Difference from intact testosterone at the same time point ++ for $p < 0.01$.