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MRI and Image Quantitation for Drug Assessment - Growth Effects of Anabolic Steroids and Precursors

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Abstract- **MRI and image quantitation play an expanding role in modern drug research, because MRI offers high resolution and non-invasive ability, and provides excellent soft tissue contrast. Moreover, with development of effective image segmentation and analysis methods, in-vivo and serial tissue growth measurements could be assessed. In the study, MR image acquisition and analysis protocol were established and validated for investigating the effects of anabolic steroids and precursors on muscle growth and body composition in a guinea pig model. Semi-automatic and interactive segmentation methods were developed to accurately label the tissue of interest for tissue volume estimation. In addition, a longitudinal tissue area outlining procedure was proposed for study of tissue geometric features in relation to tissue growth. Finally, a fully automatic data retrieval and analysis scheme was implemented to facilitate the overall huge amount of image quantitation, statistical analysis, as well as study group comparisons. As a result, highly significant differences in muscle and organ growth were detected between intact and castrated guinea pigs using the selected anabolic steroids, indicating the viability of employing such protocol to assess other anabolic steroids. Furthermore, the anabolic potential of selected steroid precursors and their effects on muscle growth, in comparison with that in respective positive control groups of castrated guinea pigs, were evaluated with the proposed protocol.**

I. INTRODUCTION

Androgens, or anabolic steroids were observed increase muscle mass and were 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. Concurrent with the use of classical anabolic steroids, a new phenomenon is the parallel use of 'dietary supplements', some of which are actually potent steroid precursors such as androstenedione and bolandiol, or active steroid analogs such as nandrolone.

Experimentally, the effects of anabolic steroids on muscle growth and body composition were previously investigated in intact and castrated the guinea pigs using dissection method, revealing an increase of muscle mass [1,2]. Recently, MRI method became a desirable body composition measurement means, because it has the in-vivo and non-invasive advantages, and offers the ability to assess growth longitudinally. The viability of MRI measurement has been validated in a rat model developed for normal growth, aging, and obesity in our previous study [3].

The overall goal of this study is to establish and employ an MRI protocol to evaluate and characterize the growth effects of various anabolic steroids, and evaluate anabolic potential of selected precursors in a guinea pig model. First, MRI protocol was validated in intact and castrated guinea pigs in a 16-week treatment protocol using nandrolone and testosterone [4]. Highly significant differences in muscle and organ growth were detected between intact and castrated guinea pig groups, indicating the viability of employing such protocol to assess other anabolic steroids. Then, the MRI protocol was further applied to evaluate the anabolic potential of the selected steroid precursors, namely 4-Androstene-3, 17-dione (androstenedione) and 19-Nor-4-Androstene-3β-17β-diol (bolandiol), and the effects on muscle growth in groups of castrated male guinea pigs over a 10-week period, in comparison with growth in respective positive control testosterone and nandrolone groups were determined and confirmed with image analysis. MRI measurement appears more sensitive to potential differences in muscle growth than the dissection method.

II. METHODS

A. Animals for validation study

12-wk old male Hartley guinea pigs (550-600g) were employed. All were implanted with subcutaneous silastic capsules for steroid administration or as sham control. Animals were grouped (n=6) and imaged at three time points, i.e., baseline, wk-8, and wk-16 post the implantation. They were (1) Intact animal with empty tube, N=16; (2) Castrated with empty capsule, N=17; (3) Intact with nandrolane capsule, N=18; (4) Castrated with nandrolane capsule, N=18; (5) Intact with testosterone, N=19; and (6) Castrated with testosterone, N=14. The dosage schedule of nandrolane and testosterone was first determined in a pilot study using blood sampling and analysis over 80-day period.

B. Animals for accessing growth effects of steroid precursors

Animals were grouped (n=8) for each of the two steroid precursors (androstenedione and bolandiol) and the respective positive control (testosterone and nandrolone) steroids. They were (1) Intact androstenedione, n = 14; (2) Castrated androstenedione, n = 15; (3) Intact bolandiol, n = 16; (4) Castrated bolandiol, n = 16; (5) Intact testosterone, n = 9; (6) Castrated testosterone, n = 10; (7) Intact nandrolone, n = 10; and (8) Castrated nandrolone, n = 8. Images were taken at baseline and 10-wk treatment period.

C. MR image acquisition

3D High-resolution images were acquired to cover the entire body of a guinea pig with a coronal view using T1W fast spin echo sequence, with TR/TE=500ms/ 17ms, voxel size 0.5×0.5×1.5mm^3, and a slice gap 0.3mm, on a whole-body 1.5T Philips Intera scanner, equipped with a 10cm I.D. quadrature coil. Such images provide optimal anatomical definition and adequate soft tissue contrast for delineation of muscle, fat, and organs. To facilitate the image analysis and reduce the potential segmentation error in the longitudinal study, a cylindrical stereotaxic positioning device was customized to fix the guinea pigs in the identical position, so that all MRI image sets would share similar anatomical orientation. All animals were anesthetized with IP injection of 30 mg/Kg sodium pentobarbital during imaging.

D. MR image segmentation

Images were analyzed using an IDL based customized software package [5] developed mainly for segmentation and quantitation of MR body composition images. It features semi-automatic and interactive segmentation using a combination of histogram based thresholding of ROI, 2D&3D region growing, and active contouring algorithms for effective expert-guided tissue separation and labeling. Nine tissue compartments were labeled and quantified based on boundaries detectable in MR images, which included the mass of five skeletal muscles in specific segments (temporalis, masseter, neck complex, shoulder complex, and the remaining muscle), three sexual organs (prostate, seminal vesicles, and 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 [6]. Figure 1 shows a slice of a typical coronal (longitudinal) view of a T1W MRI image (a), image segmentation (b), and a 3D visualization of the segmented masseter, neck, and should muscles, as well as prostate & seminal vesicles and testis in an intact guinea pig (c). Tissue volumes were then estimated from voxel number and voxel size, and the growth effects were statistically analyzed and compared between and within groups. Figure 2 illustrates a flowchart of the image processing scheme.

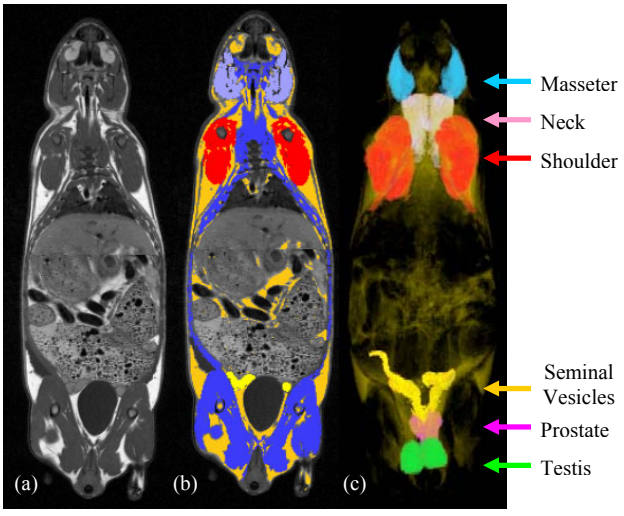


Fig. 2. Typical coronal view of a slice of whole body guinea pig image a. original T1W MR image; b. image segmentation; c. 3D visualization

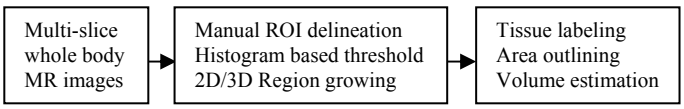


Fig. 1. Flow chart of image processing scheme

E. MRI data analysis

Since image slices were segmented into respective tissue type/organ components, the areas obtained from every slice were integrated to yield total tissue/organ volumes. Mean and standard deviation of tissue volumes for different experimental groups (intact, castrated, intact & treatment, and castrated & treatment), as well as percent change of tissue/organ volume during growth were calculated and used for multiple group comparisons and tissue growth measurements.

The volume of specific tissue compartments could also be obtained based on area outlining, where the areas from slices were plotted against axial distance to produce a longitudinal "area profile" reflecting tissue distribution from rostral to caudal. A profile alignment was then employed to register tissue area profiles of an individual subject when calculating the average "area profile" of a specific experimental group. The profile of shoulder muscle was used as reference in the alignment because its shape and position were stable and consistent across the guinea pigs. The geometric parameters such as tissue volume, tissue length, and tissue center could be derived from the area profile, and thus be compared across groups to reflect the total volume and shape changes as a function of treatment time. Figure 3a shows an example of specific muscle areas distribution in the Intact Empty capsule group. Total muscle mass distribution after treatment in Castrated Empty capsule group is shown in Figure 3b.

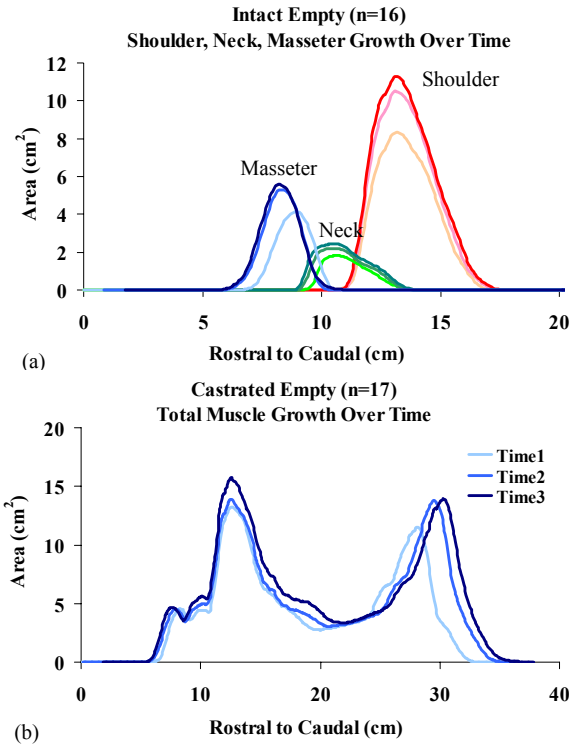


Fig. 3. Muscle area profile from rostral to caudal

F. Validation of MRI Protocol

All animals for validation study were sacrificed at the end point (wk-16) and dissection analyses were performed. Accuracy of MRI acquisition and analysis protocol was validated by comparing MRI and actual dissection volume.

G. Statistical analysis

Conventional statistical analysis including t-tests and ANOVA were used to make comparisons of tissue volumes between MRI and dissection measurements, across groups, and detect tissue volume changes before and after treatment. The completed statistical analysis will allow us to validate MRI and data analysis, establish androgen effects on muscle/ organ/adipose tissue mass, and their structure/shape.

H. Automatic data retrieval and analysis scheme

Since hundreds of guinea pigs were scanned at multiple time points in this 2-year study, it is crucial to develop an automatic data retrieval and analysis scheme to facilitate the huge amount of data analysis for sufficient group comparisons and understanding of the androgen effects on muscle/organ growth. A Microsoft Excel-based data retrieval, organization, processing, statistical analysis, graphical representation, and group comparison scheme was implemented using Excel's Macro development tool for the study. Given the names and locations of the segmentation output files organized by study groups and treatment time points, the multiple-time area outlining profile information of selected tissue compartments of individual guinea pigs in a group could be retrieved and imported into the Excel data analysis tool, which was then followed by an automatic procedure of area profile alignment, group mean volume/profile calculation, geometric parameters estimation, and graph generation. The group mean values were alternatively linked to an automatic analysis module designed for various treatment group comparisons, statistical analysis, and tracking tissue growth over time. Figure 4 illustrates the automatic data analysis scheme.

III. RESULTS

A. Validation study

MRI protocol employed in this study permits the delineation and quantitation of target muscles and organs. Excellent correlations were found between in vivo MRI measurement and postmortem dissection analyzes at the end point (figure 5), in temporalis muscle, masseter mascle, and shoulder muscle complex (fig. 5a), as well as in prostate & seminal vesicle tissue (fig. 5b), and testis (fig. 5c).

Significant differences were observed for all of the comparisons made between the Intact and Castrated Empty capsule groups ($p<0.01$). Steroid replacement in the castrated groups resulted in significant muscle growth to or towards normal for all muscles and organs with the exception of the shoulder complex at both wk-8 and wk-16 ($p<0.01$).

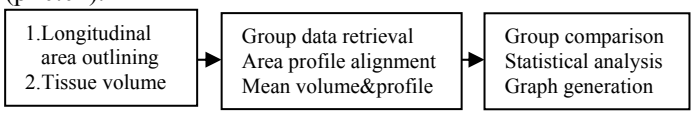


Fig. 4. Flow chart of automatic data analysis scheme

B. Total Body Skeletal Muscle and Fat Growth Effects

Castration led to significant reductions of total skeletal muscle, 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 and Nandrolone capsule groups completely normalized the growth of skeletal muscle at these points. Figure 6 shows total muscle mass distribution in Intact Empty, Castrated Empty, Castrated Nandrolone, and Castrated Testosterone capsule groups at 16-week of treatment.

C. Evaluation of the two steroid precursors

Steroid and the respective precursor replacement in the castrated groups resulted in significant growth to or towards normal for all muscles and organs at week-10. Both steroid precursors stimulated normal (androstenedione) or near-normal (bolandiol) muscle growth in castrated guinea pigs over the 10-week interval. Figure 7 shows total muscle mass distribution profiles of Intact Empty, Castrated Empty, Castrated Androstenedione, and Castrated Testosterone capsule groups at 10-week of treatment. Figure 8 illustrates the total muscle (a), temporalis muscle (b), and prostate & seminal-vesicle (c) growth in castrated groups treated with androstenedione and testosterone, comparing to their growth in empty capsule groups. Very similar growth effects were observed between the androstenedione and testosterone groups, as well as bolandiol and nandrolone groups with no significant differences in percent growth between the groups in either surgical condition (intact or castrated).

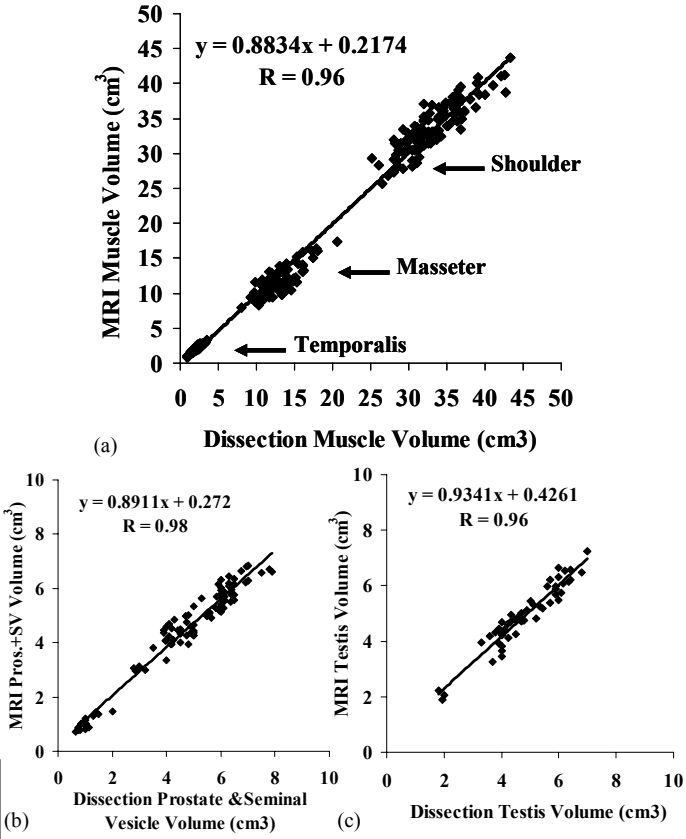


Fig. 5. Validation of MRI measurements

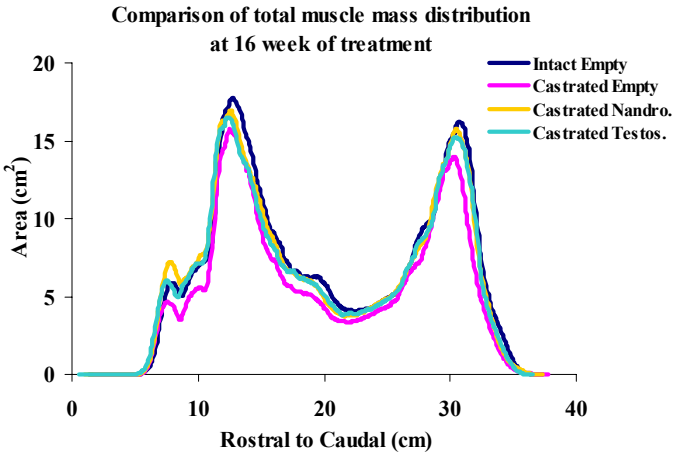


Fig. 6. Total muscle mass distribution at 16 week of treatment

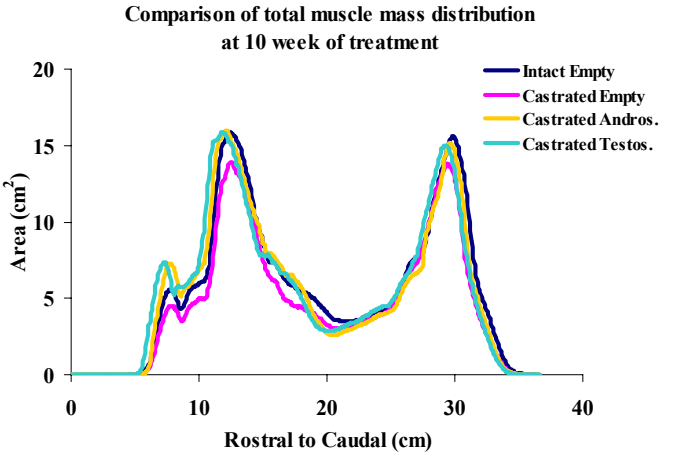
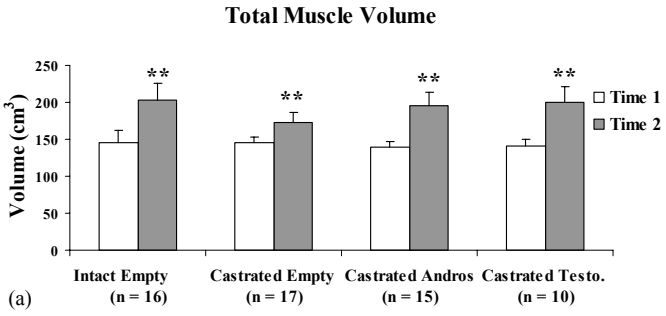
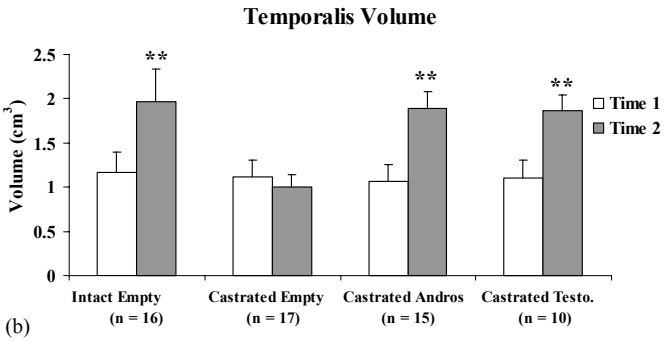


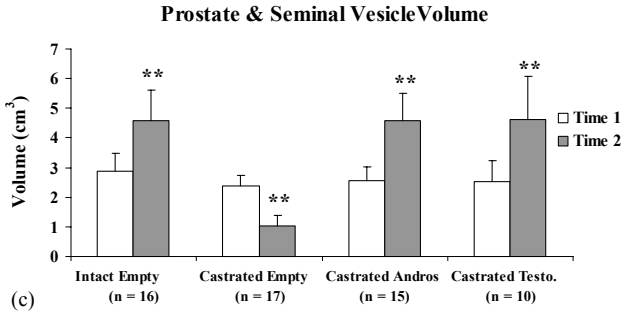
Fig. 7. Total muscle mass distribution at 10 week of precursor treatment



(a)



(b)



(c)

Fig. 8. Specific muscle and organ growth in Empty Capsule and Castrated Androstenedione-Testosterone groups over 10-week interval

An MRI acquisition and analysis protocol to quantify the body composition changes related to drug effects was established and validated. A castrated guinea pig anabolic test model was demonstrated here, showing a practical and experimental aspect of MR imaging and image analysis technology in biomedicine applications. In the study, highly significant differences of muscle and organ growth were detected between intact and castrated guinea pigs in a 16-week steroids and 10-week steroid precursor treatment protocol. MRI measurements appear more sensitive to potential differences in muscle growth than the dissection study, and offer the ability to assess growth at multiple time points in the same set of animals. MRI image analysis confirmed the growth effects of two widely used over-the-counter steroid precursors. Androstenedione administration via subcutaneous silastic capsule was found resulted in highly elevated circulating testosterone levels in both intact and castrated guinea pigs, and the maintenance of circulating testosterone at levels well above normal can augment the growth of specific muscles in intact animals. The study showed that MR imaging technique is extremely sensitive in detecting differences in individual muscle volumes in the discussed guinea pig model with growing guinea pigs. The established imaging and analysis protocol are applicable to future human studies.

ACKNOWLEDGEMENT

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