Histol Histopathol (2010) 25: 133-140

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Nandrolone decanoate increases satellite cell numbers in the chicken pectoralis muscle

Mohammed Z. Allouh[1,2] and Benjamin W.C. Rosser[1]

[1]University of Saskatchewan, College of Medicine, Department of Anatomy and Cell Biology, Saskatoon, Canada.

[2]Present address: Jordan University of Science and Technology, Faculty of Medicine, Department of Anatomy, Irbid, Jordan

Summary. The anabolic androgenic steroid nandrolone decanoate has minimal androgenic effects and, thus, is widely used to induce muscle hypertrophy in both patients and athletes. Although increases in satellite cell numbers and satellite cells giving rise to new myonuclei are associated with hypertrophy in many experimental models, the relationship between nandrolone and satellite cells is poorly understood. Here we test the hypothesis that nandrolone administration is associated with an increase in satellite cell numbers in muscle. Nandrolone was injected at weekly intervals for four weeks into the right pectoralis muscle of female white leghorn chickens aged 63 days post hatch. Age/size/sex matched control birds received saline injections. The contralateral pectoralis was excised for study from each control and nandrolone treated bird. An antibody against Pax7 and immunocytochemical techniques were used to identify satellite cells. Nandrolone significantly increased mean pectoralis mass by approximately 22%, and mean fiber diameter by about 24%. All satellite cell indices that were quantified increased significantly in chicken pectoralis with administration of nandrolone. Nandrolone injected birds had on average higher satellite cell frequencies (#SC nuclei/all nuclei within basal lamina), number of satellite cells per millimeter of fiber, and satellite cell concentrations (closer together). Myonuclei were further apart (less concentrated) in nandrolone injected muscle. However, an overall increase in myonuclear numbers was revealed by a significantly greater mean number of myonuclei per millimeter of fiber in nandrolone injected muscle. Our results suggest that satellite cells may be key cellular vectors for nandrolone induced muscle fiber hypertrophy.

Key words: Satellite cell, Myonuclei, Nandrolone, Hypertrophy, Muscle

Offprint requests to: Dr. B.W.C. Rosser, University of Saskatchewan, College of Medicine, Department of Anatomy and Cell Biology, 107 Wiggins Road, Saskatoon, Saskatchewan, S7N 5E5 Canada. e-mail: ben.rosser@usask.ca

Introduction

Nandrolone decanoate (also called Deca-Durabolin or 19-nortestosterone) is a commonly used anabolic androgenic steroid (Kuhn, 2002; Kicman, 2008). Anabolic steroids exert their actions on muscle in a complex fashion, via several diverse mechanisms. These mechanisms include directly binding to androgen receptors, which then undergo conformational changes and translocation into the nucleus to bind to DNA and/or interact with other proteins to regulate transcription (Wiegel and Moore, 2007). Other mechanisms include an anti-catabolic effect due to interference with glucocorticoid receptor expression, and by activating pathways in the CNS which result in behavioural changes (Kicman, 2008). Nandrolone has a higher affinity for androgen receptors than does testosterone (Saartok et al., 1984; Bergink et al., 1985). In androgenic tissue, testosterone is converted to a more potent metabolite than is nandrolone (Toth and Zakar, 1982; Sundaram et al., 1995). Consequently, nandrolone has higher anabolic and lower androgenic effects than testosterone (Shahidi, 2001). Nandrolone has been used to restore muscle mass in patients afflicted with a wide variety of clinical conditions, such as chronic obstructive pulmonary disease (Schols et al., 1995; Creutzberg et al., 2003), osteoporosis (Flicker et al., 1997; Frisoli et al., 2005), chronic renal failure (Johansen et al., 2006) and acquired immune deficiency syndrome (Dudgeon et al., 2006; Gold et al., 2006). Nandrolone is also the most

widespread anabolic steroid used to increase muscle mass by athletes and, especially, bodybuilders (Lenehan, 2003; Van Marken Lichtenbelt et al., 2004). Despite its widespread use, however, the effects and mechanisms of action of nandrolone at the cellular level are poorly understood.

Satellite cells are mononuclear stem cells located between plasmalemma and basal lamina of the skeletal muscle fiber (Zammit et al., 2006; Figeac et al., 2007). Satellite cells have the ability to become active, proliferate and differentiate into new myonuclei during the postnatal maintenance, growth, regeneration or repair of muscle fibers (Kuang and Rudnicki, 2008). Satellite cell entry into the cell cycle can be stimulated by a variety of influences including mechanical stressors, such as exercise or stretch, growth factors and hormones (Anderson, 2006; Brack and Rando, 2007). It is thought that satellite cells differentiating into myonuclei will maintain the ratio between cytoplasmic volume and number of myonuclei (Alway and Sui, 2008). The administration of testosterone, the most widely studied anabolic androgenic steroid, has been shown to increase satellite cell numbers in muscle. Joubert et al. (1994) applied ultrastructural techniques to study rat levator ani muscle, and showed a positive correlation between testosterone levels and an increase in the number of satellite cells (total number of satellite cell nuclei/total number of fiber cross-sectional profiles) of males at puberty and females treated with testosterone. Subsequently, by incorporating autoradiographic techniques, Joubert and Tobin (1995) deduced that testosterone induced satellite cell proliferation in female rat levator ani muscle. Sinha-Hikim et al. (2003) employed ultrastructural techniques to study human male vastus lateralis muscle, and demonstrated that testosterone supplementation produced an increase in satellite cell numbers (mean number of satellite cells per unit length of muscle fiber). Using the same experimental model with immunocytochemical and immunoelectron microscopy methods, Sinha-Hikim (2004) showed that satellite cells were the predominant site of androgen receptor expression. By comparison, however, the relationship between nandrolone administration and satellite cell numbers is poorly understood.

This current study tests the hypothesis that nandrolone administration is associated with an increase in satellite cell numbers in muscle. Robust morphometric methods applied in our previous works (Allouh et al., 2008; Kirkpatrick et al., 2008) were used to quantify parameters associated with satellite cell and myonuclear numbers. The experimental model used is the superficial region of the pectoralis (breast) muscle of the chicken, as it consists exclusively of one fiber type (fast-twitch glygolytic; Rosser et al., 1996; Bandman and Rosser, 2000) and has been shown to respond to anabolic androgenic steroids (Lone et al., 1995; Lin and Hsu, 2002). Some of the preliminary findings of this study have been presented as a conference abstract (Allouh and Rosser, 2007).

Materials and methods

Experimental model

Female White Leghorn chickens (*Gallus gallus*; Hy-Line W-36, Clark Hy-Line, Brandon, Canada) were hatched at the same time and raised under identical conditions at the University of Saskatchewan, College of Veterinary Medicine. Hens were utilized to avoid the influences of the comparatively higher levels of endogenous testosterone found in roosters. The birds were fed *ad libitum* and raised in a large room with aspen shaving bedding on the floor. They were exposed to 24 hours light for the first three days and then kept at 12 hours light and 12 hours dark cycle. Initial room temperature was 35°C. Subsequently, it was decreased 2.5°C at weekly intervals until 20°C was reached when the birds were 42 days old. At age 63 days, injections into the right pectoralis (M. pectoralis pars sternobrachialis and pars thoracobrachialis; Vanden Berge and Zweers, 1993) muscle of each bird were begun. There were four birds in the control group, and four birds in the nandrolone group. Each bird received one injection per week, at weekly intervals, for four weeks. Each of the four animals in the control group received 300 ml normal saline per injection, while each of the four in the nandrolone group received 300 ml (30 mg) nandrolone decanoate (Organon Int., Oss, Holland) per injection. As the four birds in the nandrolone group weighed on average 839g (±26, SE) at 63 days, a dosage of 30 mg per week was somewhat comparable with levels administered intramuscularly to rats by other researchers (Carson et al, 2002; Cunha et al. 2005). Also, pilot studies (unpublished) in which we injected 5, 10, 20 or 30 mg of nandrolone/week for four weeks showed that the highest dosage yielded the greatest pectoralis weights. Four control birds were size matched to the nandolone group, weighing on average 847 g (±18, SE) at 63 days. The control group accounted for continued growth of the birds that would normally occur during the ensuing four weeks of injection. Following the Canadian Council on Animal Care Guidelines, and with the approval of the University of Saskatchewan Committee on Animal Care and Supply, the four birds from each group were killed by cervical dislocation at the age of 91 days post-hatch.

Tissue preparation and sectioning

The left pectoralis muscle of each bird was dissected free and weighed. Several samples, each approximately 0.5x0.5x2-4 cm, were excised from the cranial half of the superficial region of the main part (pars sternobrachialis) of the muscle of each bird. This region of the muscle consists exclusively of fast-twitch glycolytic fibers (Rosser et al., 1996; Bandman and Rosser, 2000). Our pilot study (unpublished) confirmed that this was the case for the nandrolone treated birds. Each sample was coated with Tissue-Tek OCT Compound (Sakura Finetek USA Inc., Torrance,

California) and immediately frozen in isopentane cooled via liquid nitrogen (Dubowitz and Sewry, 2007). Samples were stored at -80°C. Serial cross sections of samples from each chicken were cut at 10 microns thickness using a cryostat at 20°C. Tissue sections were placed on ProbeOn Plus charged microscope slides (Fisher Scientific Ltd., Nepean, Canada) and stored at -20°C.

Immunohistochemistry

Immunohistochemical labeling was performed using slides from each muscle, according to our previously published methodologies (Allouh et al., 2008; Kirkpatrick et al., 2008). Briefly, the blocking solution consisted of 5% goat serum, 1% bovine serum albumin and 5mM ethylenediaminetetraacetic acid in phosphate-buffered saline (PBS; 0.02 M sodium phosphate buffer, 0.15 M sodium chloride, pH 7.2). Satellite cells are routinely identified by immunolabelling methods that localize the expression of Pax7 (Zammit et al., 2006; Day et al., 2007). The primary antibody anti-Pax7 (Developmental Studies Hybridoma Bank, Iowa City, Iowa), a mouse monoclonal, was used to label satellite cells. Anti-laminin (L9393, Sigma Chemical Co., St. Louis, Missouri), a rabbit polyclonal, labeled basal laminae. The primary labeling cocktail, consisting of 1:200 anti-laminin and 1:100 anti-Pax7 in block solution, was applied overnight in the dark at 4°C.

Alexa Fluor 488 goat anti-mouse IgG (A-11001, Invitrogen Corporation, Carlsbad, California) was used to label anti-Pax7 green, and Alexa Fluor 546 goat anti-rabbit IgG (A-11010, Invitrogen Corp.) anti-laminin red when viewed with epifluorescent microscopy. The secondary labeling cocktail, containing both secondary antibodies each diluted 1:200 in PBS at pH 7.2, was applied to each slide for 30 minutes at room temperature in the dark. Hoechst 33258 (Bisbenzimide; Sigma Chemical Co., St. Louis, Missouri) was applied to each slide for 5 minutes at a dilution of 1:1,500,000 in PBS to label the DNA in nuclei blue under epifluorescent microscopy. Slides were fixed in 4% formaldehyde, and then mounted in Geltol (Thermo Scientific, Pittsburgh, Pennsylvania) and stored at 4°C in the dark.

Imaging and Analyses

Images of cross-sections were captured using a Sony digital still camera (Sony Corporation, Tokyo, Japan) attached to a Zeiss microscope (Carl Zeiss AG, Oberkochen, Germany) equipped for epifluorescence. The different coloured fluorescent images from each field of view were superimposed and their contrast improved using Adobe Photoshop (Adobe System Inc., San Jose, California).

These images were used to identify and count the number of satellite cells and myonuclei per muscle fiber. Two hundred adjacent extrafusal fibers from each sample were analyzed in this way. The ellipse minor axes of these fibers were determined using Scion Image 1.63 (developed by US National Institute of Health and available on the internet by anonymous FTP from Zippy.nimh.nih.gov, Frederick, Maryland). Ellipse minor axis is identical to lesser fiber diameter (Rosser et al., 2000). Lesser fiber diameter is defined as the maximum aspect across the lesser aspect of a fiber, and is routinely used to overcome distortion that may result when a muscle fiber is cut obliquely rather than transversely (Dubowitz and Sewry, 2007).

Calculations and statistics

Values for each of the following parameters, were obtained from the fibers of the left pectoralis each of the eight birds (four control and four nandrolone) studied. The mean frequency of satellite cells was calculated using the average number of satellite cell nuclei (SCN) and myonuclei (MN) per fiber, and the formula SCN frequency = (SCN/([SCN + MN]) x 100% (Schmalbruch and Hellhammer, 1977). The mean number of SCN or MN per unit length (Z) was determined using the formula $Z = NxL/(d + l)$ where N = number of nuclear profiles (SCN or MN) per fiber profile, L = unit length of fiber (1mm), d = section thickness (10 microns), and l = average SCN or MN length (Schmalbruch and Hellhammer, 1977). The average lengths of satellite cell nuclei and myonuclei used for these calculations were 10.28 and 10.58 microns, respectively, based upon our previous studies. We had shown a uniform length of 10.28 microns for satellite cell nuclei (Allouh et al., 2008) and 10.58 microns for myonuclei (Rosser et al., 2002) throughout the posthatch development of the pectoralis of the same strain of white leghorn chickens used in the current study. The mean concentration or surface area of sarcolemma per satellite cell (S) was calculated using the formula $S = \pi EL / Z_{SC}$, where E = mean ellipse minor axis, L = unit length of fiber (1mm), and Z_{SC} = number of satellite cell per unit length of fiber. The mean cross sectional area (C) was quantified according to the formula $C = \pi(E/2)^2$ where E = mean ellipse minor axis of fiber. The mean volume of sarcoplasm per myonucleus (myonuclear domain; V) was calculated using the formula $V = CxL / Z_{MN}$ where C = mean cross sectional area of fiber, L = unit length of fiber (1 mm), and Z_{MN} = number of MN per unit length of fiber (Rosser et al., 2002).

Data for each parameter measured were blocked into two groups; control vs. nandrolone. Levene's test for equality of variances was first applied to determine the homogeneity of variance. Data were then evaluated by independent sample t-tests at the 5% level of significance. Statistical analyses were performed using SPSS program (standard version 12.0.0, SPSS Inc., Chicago, Illinois).

Results

The immunohistochemical labeling obtained is entirely consistent with our previous work on the same muscle and strain of chicken (Halevy et al., 2004;

Allouh et al., 2008). Muscle fiber basal laminae are outlined by an antibody against laminin, which is labelled red by a fluorescent secondary antibody (Fig. 1A-D). Fibers are the large, red, round to oval-shaped outlines (Halevy et al., 2004; Allouh et al., 2008). Nuclei within these outlines are either myonuclei or satellite cell nuclei. While both myonuclei and satellite cell nuclei are labelled blue by Hoechst 33258 (Fig. 1A,C), only satellite cell nuclei are also labelled green by a fluorescent secondary antibody against the Pax7 antibody (Fig. 1B,D). Fibers in the nandrolone injected pectoralis (Fig. 1C,D) appear larger than those in control pectoralis (Fig. 1A,B). All numeric results are summarized in Table 1.

Body weight at the end of the experiment was not significantly (p=0.9775) different between nandrolone treated and control groups; ranging from 1110 to 1300 grams in nandrolone birds and 1088 to 1302 g in controls. The pectoralis muscle was significantly (p=0.0002) heavier by about 22% in the nandrolone treated than control birds. Wet muscle weights ranged from 63.74 to 67.76 g in the nandrolone group, and from 52.14 to 57.06 g in controls. Similarly, muscle fibers were also significantly (p=0.0047) larger in the nandrolone treated than control birds. Mean ellipse minor axis of the fibers ranged from 36.39 to 42.32 microns and was approximately 24% larger in the nandrolone group than the controls, which ranged from

Table 1. Nandrolone versus control group.

	Mean Body Weight (grams)	Mean Weight Pectoralis (grams)	Mean Fiber Diameter (microns)	Mean Frequency Satellite Cells (percent)	Mean Number Satellite Cells per millimeter of fiber	Mean Area Sarcolemma per Satellite Cell (microns^2)	Mean Number Myonuclei per millimeter of fiber	MeanVolume Sarcoplasm per Myonucleus (microns^3)
Control (n=4)	1209±78	53.80±1.13	31.59±0.82	6.36±0.29	5.61±0.31	17,778±622	81.51±2.30	9623±355
Nandrolone (n=4)	1207±90	65.50±0.93*	39.28±1.56*	8.15±0.49*	8.32±0.50*	14,931±764*	95.24±3.40*	12743±696*

*: significant (p<0.05; see text for more detailed p-values) difference between Nandrolone and Control Groups. Values are expressed as mean ± SE (standard error)

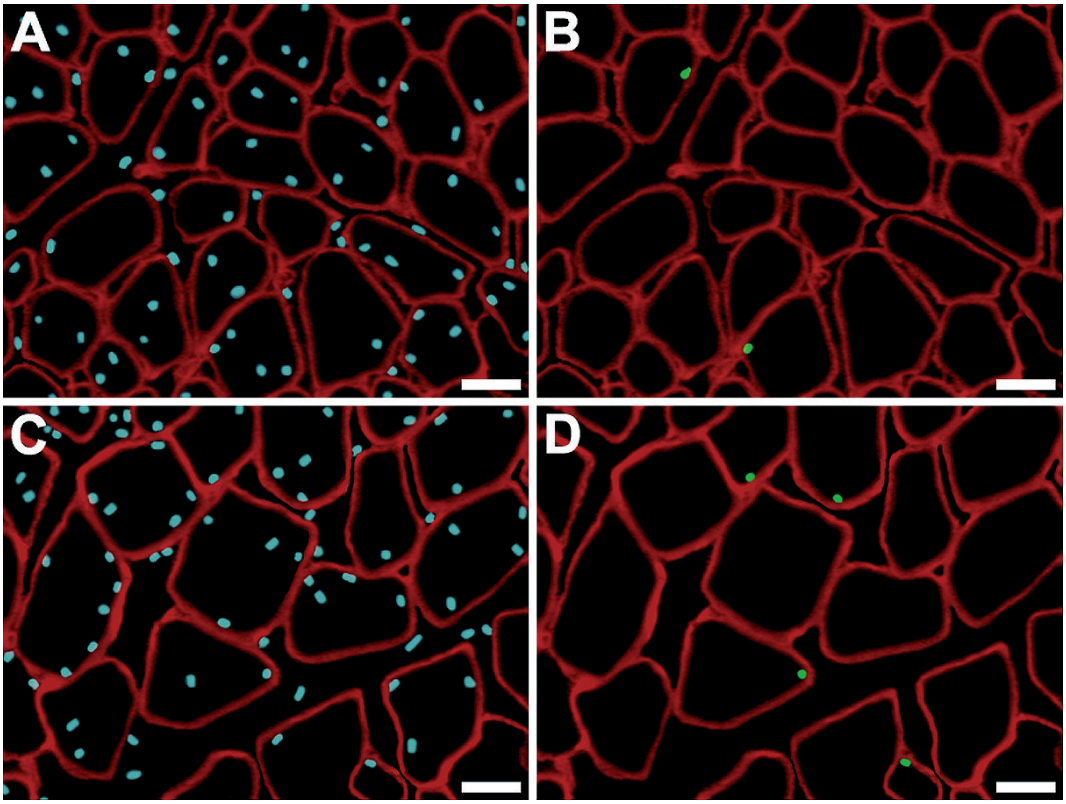

Fig. 1. Immunohistochemical labeling of cross-sections of pectoralis. Control **(A, B)** and nandrolone injected **(C, D)**. **A and B** are one cross-section, as are **C and D**. In all images, laminin is coloured red. In **A and C**, all nuclei are coloured blue. In **B and D**, nuclei expressing Pax7 are green. Within basal laminae of muscle fibers, nuclei that are blue (in **A or C**) but not green (in **B or D**) are myonuclei. Nuclei within basal laminae that are both blue (in **A or C**) and green (in **B or D**) are satellite cells. Scale bars: 30 µm.

30.70 to 34.04 microns.

Satellite cell frequency in the pectoralis was on average about 28% and significantly (p=0.0199) greater in the nandrolone treated than control birds. Mean satellite cell frequency in the nandrolone group ranged from 7.16 to 9.01%, and from 5.50 to 6.82% in the controls. The mean number of satellite cells per millimeter of fiber was on average nearly 50% and significantly (p=0.0038) higher in the nandrolone treated than control birds. Values in nandrolone birds ranged from 7.15 to 9.62 satellite cells per millimeter of fiber, and controls from 4.93 to 6.41. The area of sarcolemma per satellite cell was approximately 16% and significantly (p=0.0276) less in the nandrolone birds. Mean area of sarcolemma per satellite cell ranged from 13,588 to 16,333 square microns in the nandrolone treated animals, and from 16,674 to 19,518 square microns in controls.

Myonuclei per mm of fiber length was significantly (p=0.0156) different between nandrolone and control birds. Values averaged from 87.46 to 103.98 myonuclei per mm of fiber length in nandrolone injected animals, and from 75.80 to 86.49 in controls. Myonuclear domain size, determined by calculating the average amount of sarcoplasm per myonucleus, was about 33% and significantly (p=0.0071) larger in the nandrolone group. Mean volume of sarcoplasm per myonucleus ranged from 11,085 to 14,199 cubic microns in the nandrolone birds, but from 8,824 to 10,517 in the controls.

Discussion

Increased muscle mass via nandrolone administration has been a goal of both physicians and athletes. In concurrence with studies of mammalian muscle, we demonstrate that nandrolone administration increases the mass of an avian muscle. The chicken pectoralis consists almost entirely of fast-twitch fibers (Rosser et al., 1996; Bandman and Rosser, 2000). We show that nandrolone injection increases the mean diameter of these fast-twitch fibers. Testosterone injections increased the size of both slow (type 1) and fast (type 2) contracting fibers in the vastus lateralis muscle of healthy young men (Sinha-Hikim et al., 2002) and the trapezius muscle of trained power lifters (Kadi et al., 1999). Nandrolone injection, however, did not affect lesser fiber diameter in either slow or fast contracting fibers of the deltoid muscle of male strength-trained atheletes (Hartgens et al., 2002). Alternatively nandrolone administration did increase the cross-sectional areas of the fast, but not slow, contracting fibers of the diaphragm muscle of female rats (Lewis et al., 2002). McClung et al. (2005) and Thompson et al. (2006) found that nandrolone administration increased the frequency of larger (>5000 or >4800 square microns), but not smaller (<500 square microns), fibers in the soleus (slow contracting) muscles of, respectively, young and older rats. However, neither of the preceding two works studying the effects of nandrolone on rat soleus showed a change in mean fiber diameter. The literature indicates that nandrolone administration may differentially affect fast and slow contracting fibers (Joumma and Leoty, 2001; Bouhlel et al., 2003). Furthermore, like testosterone (Bhasin et al., 2001; Sinha-Hikim et al., 2002; Chen et al., 2005), the effects of nandrolone on skeletal muscle may be dose dependent. Earlier studies of the effects of nandrolone on fiber diameter may have produced contradictory or mixed findings due to the different dosages administered.

Ours' is the first work to show that nandrolone administration is associated with an increase in satellite cell numbers. All satellite cell indices (frequency, number per millimeter of fiber, and concentration) quantified were higher in the chicken pectoralis with the administration of nandrolone. Whereas substantive evidence demonstrates that testosterone administration increases satellite cell numbers (Joubert et al., 1994; Joubert and Tobin, 1995; Sinha Hikim et al., 2003, 2004; Chen et al., 2005), to date comparable results have not been obtained from studies of nandrolone. An earlier study of rat soleus found that while nandrolone treatment of functionally overloaded muscle appeared to increase satellite cell activity, there was no increase in satellite cell activity between control and non-overloaded nandrolone treated animals (McClung et al., 2005). Bovine muscle cell cultures responded to nandrolone treatment by increasing the expression of androgen receptors (Lee et al. 2007), and satellite cells are the predominant site of androgen receptor expression (Sinha-Hikim et al. 2004).

It is held that satellite cells in mature muscle are converted to myonuclei to maintain the ratio between cytoplasmic volume and number of myonuclei (Allen et al., 1999; Alway and Sui, 2008). This concept has been challenged by others who indicate that constant myonuclear domains are displayed only under certain circumstances, and that the more typical condition is a loose association between cytoplasmic volume and myonuclear numbers (Gundersen and Bruusgaard, 2008). Both views may be accurate, as whether the mean size of the myonuclear domain is maintained appears to depend upon the experimental model employed (Hikida, 2007). Testosterone administration to humans produced muscle fiber hypertrophy and an increase in the size of myonuclear domains (Eriksson et al., 2005). Our results show that nandrolone injection also yields fiber hypertrophy and an increase in myonuclear domain size. An increase in myonuclear domain size suggests that no new myonuclei are added to the muscle during hypertrophy. However, we also demonstrated that nandrolone treated muscle showed a significant increase in the mean number of myonuclei per millimeter of fiber. Thus, new myonuclei must have been added to the fibers of nandrolone treated muscles. Presumably the source for these new myonuclei was the increased numbers of satellite cells that we also quantified.

Whether satellite cells are required for hypertrophy

of mature muscle is a point of contention in the literature (Rehfeldt et al., 2007). Whereas some researchers have determined that the generation of new myonuclei by satellite cells is necessary for this hypertrophy (Rosenblatt and Parry, 1992; Kadi and Thornhill, 2000; O'Connor and Pavlath, 2007; Hyatt et al., 2008), others have contended that satellite cell activity is not always a prerequisite (Nash et al., 1994; Lowe and Alway, 1999; McCarthy and Esser, 2007). The role that satellite cells play in the hypertrophy of post-natal muscle may depend upon the type of stimulus used to induce the hypertrophy, the magnitude of the growth response, the muscle studied, the age of the animal, the species studied, and when the muscle is sampled after the applied stimulus (O'Connor et al., 2007). It is, nonetheless, widely held that testosterone enhances satellite cell proliferation as a prelude to an increase in myonuclear numbers and muscle hypertrophy (Joubert and Tobin, 1995; Kadi, 2000; Chen et al., 2005). We demonstrate that nandrolone induced hypertrophy is associated with increases in satellite cell and myonuclear numbers.

In conclusion, our results suggest that satellite cells may be key cellular vectors for nandrolone induced muscle fiber hypertrophy. Administration of nandrolone can lead to increased muscle mass and muscle fiber hypertrophy. These increases appear to be dependent upon the activation of satellite cells, some of which differentiate into new myonuclei. The new myonuclei are most probably required for increased protein synthesis associated with muscle hypertrophy (Allen et al., 1999). The mechanisms and effects of nandrolone injection into skeletal muscle appear similar to those of testosterone treatment. Androgen receptors located primarily on satellite cells seem to be the means by which both testosterone (Sinha-Hikim et al., 2004; Chen et al., 2005) and nandrolone (Lee et al., 2007) effect changes in muscle. An alternate explanation for our results, however, is that nandrolone indirectly affects satellite cells by initiating protein synthesis and fiber hypertrophy which then signal satellite cell activation. Additional research is needed to further discern whether nandrolone acts directly or indirectly on satellite cells, the dose dependent relationships, differences in fiber type responses and the mechanism(s) of satellite cell activation in nandrolone induced hypertrophy.

Acknowledgements. A Discovery Grant awarded to BWCR from the Natural Sciences and Engineering Research Council (NSERC) of Canada provided funds for this study. The Jordan University of Science and Technology and the College of Medicine, University of Saskatchewan, provided funds for MZA. We thank Lisa J. Kirkpatrick and Tonya McGowan of our Department for assistance in preparing the final images for publication, and LJK for comments on the manuscript. The antibody against Pax7 developed by A. Kawakami was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA 52242.

References

Allen D.L., Roy R.R. and Edgerton V.R. (1999). Myonuclear domains in muscle adaptation and disease. Muscle Nerve 22, 1350-1360.

Allouh M.Z. and Rosser B.W.C. (2007). The effect of Nandrolone decanoate on the frequency and concentration of satellite cells in skeletal muscle. FASEB J. 21, A203.

Allouh M.Z., Yablonka-Reuveni Z. and Rosser B.W.C. (2008). Pax7 reveals a greater frequency and concentration of satellite cells at the ends of growing skeletal muscle fibers. J. Histochem. Cytochem. 56, 77-87.

Alway S.E. and Siu P. M. (2008). Nuclear apoptosis contributes to sarcopenia. Exerc. Sport Sci. Rev. 36, 51-57.

Anderson J.E. (2006). The satellite cell as a companion in skeletal muscle plasticity: currency, conveyance, clue, connector and colander. J. Exp. Biol. 209, 2276-2292.

Bandman E. and Rosser B.W.C. (2000). Evolutionary significance of myosin heavy chain heterogeneity in birds. Microsc. Res. Tech. 50, 473-491.

Bergink E.W., Janssen P.S.L., Turpijn E.W. and Vander Vies J. (1985). Comparison of the receptor-binding properties of nandrolone and testosterone under in vitro and in vivo conditions. J. Steroid Biochem. Mol. Biol. 22, 831-836.

Bhasin S., Woodhouse L., Casaburi R., Singh A.B., Bhasin D., Berman N., Chen X., Yarasheski K.E., Magliano L., Dzekov C., Dzekov J., Bross R., Phillips J., Sinha-Hikim I., Shen R., and Storer T.W.T (2001). Testosterone dose-response relationships in healthy young men. Am. J. Physiol. Endocrinol. Metab. 281, E1172-E1181.

Bouhlel A., Joumaa W.H. and Claude L. (2003). Nandrolone decanoate treatment affects sarcoplasmic reticulum Ca^{2+} ATPase function in skinned rat slow- and fast-twitch fibres. Eur. J. Physiol. 446, 728-734.

Brack A.S. and Rando T.A. (2007). Intrinsic changes and extrinsic influences of myogenic stem cell function during aging. Stem Cell Rev. 3, 226-237.

Carson J.A, Lee W.J., McClung J. and Hand G.A. (2002). Steroid receptor concentration in aged rat hindlimb muscle: effect of anabolic steroid administration. J. Appl. Physiol. 93, 242-250.

Chen Y., Zajac J.D. and MacLean H.E. (2005). Androgen regulation of satellite cell function. J. Endocrinol. 186, 21-31.

Creutzberg E.C., Wouters E.F.M., Mostert R., Pluymers R.J. and Schols A.M.W.J. (2003). A role for anabolic steroids in the rehabilitation of patients with COPD? Chest 124, 1733-1742.

Cunha T.S, Moura M.J.C.S., Bernardes C.F., Tanno A.P. and Marcondes F.K. (2005). Vascular sensitivity to phenylephrine in rats submitted to anaerobic training and nandrolone treatment. Hypertension 46, 1010-1015.

Day K., Shefer G., Richardson J.B., Enikolopov G. and Yablonka-Reuveni Z. (2007). Nestin-GFP reporter expression defines the quiescent state of skeletal muscle satellite cells. Dev. Biol. 304, 246-259.

Dubowitz V. and Sewry C.A. (2007). Muscle biopsy: A practical approach. Elsevier Ltd. Philadelphia.

Dudgeon W.D., Phillips K.D., Carson J.A., Brewer R.B., Durstine J.L. and Hand G.A. (2006). Counteracting muscle wasting in HIV-infected individuals. HIV Med. 7, 299-310.

Eriksson A., Kadi F., Malm C. and Thornell L.E. (2005). Skeletal muscle morphology in power-lifters with and without anabolic steroids. Histochem. Cell Biol. 124, 167-175.

Figeac N., Daczewska M., Marcelle C. and Jagla K. (2007). Muscle stem cells and model systems for their investigation. Dev. Dyn. 236, 3332-3342.

Flicker L., Hopper J.L., Larkins R.G., Lichtenstein M., Buirski M. and Wark J.D. (1997). Nandrolone decanoate and intranasal calcitonin as therapy in established osteoporosis. Osteoporosis Int. 7, 29-35.

Frisoli A., Chaves P.H.M., Pinheiro M.M. and Szejnfeld V.L. (2005). The effect of nandrolone decanoate on bone mineral density, muscle mass, and hemoglobin levels in elderly women with osteoporosis: a double-blind, randomized, placebo-controlled clinical trial. J. Gerontol., Series A, Biological Sciences and Medical Sciences 60, 648-653.

Gold J., Batterham M.J., Rekers H., Harms M.K., Geurts T.B.P., Helmyr P.M.E., Silva de Mendonc J., Falleiros Carvalho L.H., Panos G., Pinchera A., Aiuti F., Lee C., Horban A., Gatell J., Phanuphak P., Prasithsirikul W., Gazzard B., Bloch M. and Danner S.A. (2006). Effects of nandrolone decanoate compared with placebo or testosterone on HIV-associated wasting. HIV Med. 7, 146-155.

Gundersen K. and Bruusgaard J.C. (2008). Nuclear domains during muscle atrophy: nuclei lost or paradigm lost? J. Physiol. 586, 2675-2681.

Halevy O., Piestun Y., Allouh M.Z., Rosser B.W.C., Rinkevich Y., Reshef R., Rozenboim I., Wleklinski-Lee M. and Yablonka-Reuveni Z. (2004). Pattern of Pax7 expression during myogenesis in the posthatch chicken establishes a model for satellite cell differentiation and renewal. Dev. Dyn. 231, 489-502.

Hartgens F., van Straaten H., Fideldij S., Rietjens G., Keizer H.A. and Kuipers H. (2002). Misuse of androgenic-anabolic steroids and human deltoid muscle fibers: differences between polydrug regimens and single drug administration. Eur. J. Appl. Physiol. 86, 233-239.

Hikida R.S. (2007). Comments on Point:Counterpoint "Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy." J. Appl. Physiol. 103, 1104-1105.

Hyatt J.P.K., McCall G.E., Kander E.M., Zhong H., Roy R.R. and Huey K.A. (2008). Pax3/7 expression coincides with MyoD during chronic skeletal muscle overload. Muscle Nerve 38, 861-866.

Johansen K.L., Painter P.L., Sakkas G.K., Gordon P., Doyle J. and Shubert T. (2006). Effects of resistance exercise training and nandrolone decanoate on body composition and muscle function among patients who receive hemodialysis: a randomized, controlled trial. J. Am. Soc. Nephrol. 17, 2307-2314.

Joubert Y. and Tobin C. (1995). Testosterone treatment results in quiescent satellite cells being activated and recruited into cell cycle in rat levator ani muscle. Dev. Biol. 169, 286-294.

Joubert Y., Tobin C. and Lebart M.C. (1994). Testosterone-induced masculinization of the rat levator ani muscle during puberty. Dev. Biol. 162, 104-110.

Joumaa W.H. and Leoty C. (2001). Differential effects of nandrolone decanoate in fast and slow rat skeletal muscle. Med. Sci. Sports Exerc. 33, 397-403.

Kadi F. (2000). Adaptation of human skeletal muscle to training and anabolic steroids. Acta Physiol. Scand. 168, 2-52.

Kadi F., Eriksson A., Holmner S. and Thornell L.E. (1999). Effects of anabolic steroids on the muscle cells of strength-trained athletes. Med. Sci. Sports Exer. 31, 1528-1534.

Kadi F. and Thornell L.E. (2000). Concomitant increases in myonuclear and satellite cell content in female trapezius muscle following strength training. Histochem. Cell Biol. 113, 99-103.

Kicman A.T. (2008). Pharmacology of anabolic steroids. Brit. J. Pharmacol. 154, 502-521.

Kirkpatrick L.J., Allouh M.Z., Nightingale C.N., Devon H.G., Yablonka-Reuveni Z. and Rosser B.W.C. (2008). Pax7 shows higher satellite cell frequencies and concentrations within intrafusal fibers of muscle spindles. J. Histochem. Cytochem. 56, 831-840.

Kuang S. and Rudnicki M.A. (2008). The emerging biology of satellite cells and their therapeutic potential. Trends Mol. Med. 14, 82-91.

Kuhn C.M. (2002). Anabolic steroids. Rec. Prog. Hormone Res. 57, 411-434.

Lee E.J., Choi J., Hyun J.H., Cho K.H., Hwang I., Lee H.J., Chang J. and Choi I. (2007). Steroid effects on cell proliferation, differentiation and steroid receptor gene expression in adult bovine satellite cells. Asian-Australian J. Anim. Sci. 20, 501-510.

Lenehan P. (2003). Anabolic steroids and other performance enhancing drugs. Taylor and Francis Inc. London, UK.

Lewis M.I., Horvitz G.D., Clemmons D.R. and Fournier M. (2002). Role of IGF-I and IGF-binding proteins within diaphragm muscle in modulating the effects of nandrolone. Am. J. Physiol. Endocrinol. Metab. 282, E483-E490.

Lin C.Y. and Hsu J.C. (2002). Effects of surgical caponization on growth performance, fiber diameter and some physical properties of muscles in Taiwan country chicken cockerels. Asian-Australasian J. Anim. Sci. 15, 401-405.

Lone K.P., Hussain S., Nasreen S., Khan R. and Saeed A. (1995). The effect of age and feeding an anabolic-androgenic steroid (dimethazine) on the body and organ weights of female chicks. Punjab Univ. J. Zool. 10, 89-102.

Lowe D.A. and Alway S.E. (1999). Stretch-induced myogenin, MyoD, and MRF4 expression and acute hypertrophy in quail slow-tonic muscle are not dependent upon satellite cell proliferation. Cell Tissue Res. 296, 531-539.

McCarthy J.J. and Esser K.A. (2007). Counterpoint: Satellite cell addition is not obligatory for skeletal muscle hypertrophy. J. Appl. Physiol. 103, 1100-1102.

McClung J.M., Mehl K.A., Thompson R.W., Lowe L.L. and Carson J.A. (2005). Nandrolone decanoate modulates cell cycle regulation in functionally overloaded rat soleus muscle. Am. J. Physiol. Regul. Integr. Comp. Physiol. 288, R1543-R1552.

Nash J.E., Rocha H.J., Buchan V., Calder G.A., Milne E., Quirke J.F. and Lobley G.E. (1994). The effect of acute and chronic administration of the beta- agonist, cimaterol, on protein synthesis in bovine skin and muscle. Br. J. Nutr. 71, 501-513.

O'Connor R.S. and Pavlath G.K. (2007). Point: Satellite cell addition is obligatory for skeletal muscle hypertrophy. J. Appl. Physiol. 103, 1099-1100.

O'Connor R.S., Pavlath G.K., McCarthy J.J. and Esser K.A. (2007). Last Word on Point: Counterpoint: Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy. J. Appl. Physiol. 103, 1107.

Rehfeldt C., Mantilla C.B., Sieck G.C., Hikada R.S., Booth F.W., Kadi F., Bodine S.C. and Lowe D.A. (2007). Comments on Point: Counterpoint "Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy." J. Appl. Physiol.103, 1104-1106.

Rosenblatt J.D. and Parry D.J. (1992). Gamma irradiation prevents compensatory hypertrophy of overloaded mouse extensor digitorum longus muscle. J. Appl. Physiol. 73, 2538-2543.

Rosser B.W.C., Dean M.S. and Bandman E. (2002). Myonuclear domain size varies along the lengths of maturing skeletal muscle

fibers. Int. J. Dev. Biol. 46, 747-754.

Rosser B.W.C., Farrar C.M., Crellin N.K., Andersen L.B. and Bandman E. (2000). Repression of myosin isoforms in developing and denervated skeletal muscle fibers originates near motor endplates. Dev. Dyn. 217, 50-61.

Rosser B.W.C., Wick M., Waldbillig D.M. and Bandman E. (1996). Heterogeneity of myosin heavy chain expression in fast-twitch fiber types of mature avian pectoralis muscle. Biochem. Cell Biol. 74, 715-728.

Saartok T., Dahlberg E. and Gustafsson J.A. (1984). Relative binding-affinity of anabolic-androgenic steroids-comparison of the binding to the androgen receptors in skeletal muscle and in prostate, as well as to sex hormone-binding globulin. Endocrinology 114, 2100-2106.

Schmalbruch H. and Hellhammer U. (1977). The number of nuclei in adult rat muscles with special reference to satellite cells. Anat. Rec. 189, 169-175.

Schols A.M.W.J., Soeters P.B., Mostert R., Pluymers R.J. and Wouters E.F.M. (1995). Physiological-effects of nutritional support and anabolic-steroids in patients with chronic obstructive pulmonary-disease - a placebo controlled randomized trial. Amer. J. Resp. Crit. Care Med. 152, 1268-1274.

Shahidi, N.T. (2001). A review of the chemistry, biological action, and clinical applications of anabolic-androgenic steroids. Clinical Theraputics 23, 1355-1390.

Sinha-Hikim I., Artaza J., Woodhouse L., Gonzalez-Cadavid N., Shen R. and Bhasin S. (2002). Testosterone-induced increase in muscle size in healthy young men is associated with muscle fiber hypertrophy. Am. J. Physiol. Endocrinol. Metab. 283, E154-E164.

Sinha-Hikim I., Roth S.M., Lee M.I. and Bhasin S. (2003). Testosterone-induced muscle hypertrophy is associated with an increase in satellite cell number in healthy, young men. Am. J. Physiol. Endocrinol. Metab. 285, E197-E205.

Sinha-Hikim I., Taylor W.E., Gonzalez-Cadavid N.F., Zheng W. and Bhasin S. (2004). Androgen receptor in human skeletal muscle and cultured muscle satellite cells: up-regulation by androgen treatment. J. Clin. Endocrin. Metab. 89, 5245-5255.

Sundaram K., Kumar N., Monder C. and Bardin C.W. (1995). Different patterns of metabolism determine the relative anabolic activity of 19-norandrogens. J. Steroid Biochem. Molec. Biol. 53, 253-257.

Thompson R.W., McClung J.M., Baltgalvis K.A., Davis J.M. and Carson J.A. (2006). Modulation of overload-induced inflammation by aging and anabolic steroid administration. Exper. Gerontol. 41, 1136-1146.

Toth M. and Zakar T. (1982). Relative binding affinities of testosterone, 19-nortestosterone and their 5-alpha-reduced derivatives to the androgen receptor and to other androgen binding proteins—a suggested role of 5- alpha-reductive steroid-metabolism in the dissociation of myotropic and androgenic activities of 19-nortestosterone. J. Steroid Biochem. Mol. Biol. 17, 653-660.

Vanden Berge J.C. and Zweers G.A. (1993). Myologia. In: Handbook of avian anatomy: Nomina Anatomica Avium. Baumel J.J., King A.S., Breazille J.E., Evan H.E. and Vanden Berge J.C. (eds). Publication of the Nuttall Ornithological Club, Cambridge, MA., pp. 189-247.

Van Marken Lichtenbelt W.D., Hartgens F., Vollaard N.B.J., Ebbing S. and Kuipers H. (2004). Bodybuilders' body composition: effect of nandrolone decanoate. Med. Sci. Sports Exerc. 36, 484-489.

Weigel N.L. and Moore N.L. (2007). Steroid receptor phosphorylation: a key modulator of multiple receptor functions. Mol. Endocrinol. 21, 2311-2319.

Zammit P.S., Partridge T.A. and Yablonka-Reuveni Z. (2006). The skeletal muscle satellite cell: the stem cell that came in from the cold. J. Histochem. Cytochem. 54, 1177-1191.

Accepted July 27, 2009