

Sustanon induces dose-independent hypertrophy and satellite cell proliferation in slow oxidative fibers of avian skeletal muscle

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Summary. Sustanon is a well-known anabolic drug that is used to treat hypogonadism and restore muscle mass and bone density. As research to date has been limited to its effects in glycolytic fibers, this study aimed to investigate the dose-related effects of Sustanon on the oxidative fibers of avian skeletal muscle. Adult female chickens were randomly divided into 4 groups: control (C), received a dose of 100 μ l normal saline per injection; and Sustanon-1, -2, and -3 (S1, S2, and S3), that received a dose of 12.5, 25, or 50 mg/kg Sustanon per injection, respectively. Each bird received 4 injections at weekly intervals (1 injection/week). Robust histochemical and immunofluorescent techniques along with morphometric analyses were applied to determine the oxidative activity and morphological variations of the oxidative muscle fibers in all groups. Sustanon-treated groups exhibited significant increases in fiber size and numbers of satellite cells and myonuclei compared to the control group. However, no significant variations were found between Sustanon-treated groups in the aforementioned indices. In conclusion, Sustanon induced oxidative fiber hypertrophy that was associated with satellite cell proliferation and myonuclear accretion in avian skeletal muscle. Furthermore, the effects of Sustanon appeared to be dose-independent.

Key words: Anabolic steroids, Hyperplasia, Hypertrophy, Myonuclei, Pax7

Introduction

Skeletal muscle fibers are multinucleated cells with a well-established regenerative capacity that is dependent on the presence of a specific population of adult myogenic stem cells known as satellite cells (SCs) (Yablonka-Reuveni, 2011). These cells are easily distinguished by their characteristic location between sarcolemma and basal lamina of the muscle fiber and by their specific expression of paired box transcription factor 7 (Pax7) (Halevy et al., 2004; Allouh et al., 2008). Upon the need for muscle growth or repair, SCs become active and start to proliferate, where they finally differentiate by fusing to preexisting muscle fibers or together to form new fibers. At the end of this process, the SC nuclei become new myonuclei (Zammit et al., 2006; Allouh and Rosser, 2010).

Skeletal muscle fibers are classified based on their metabolic activity into glycolytic, oxidative, or both oxidative-glycolytic (Singh et al., 2002). Glycolytic fibers are only used to produce high force for a short period of time because they fatigue rapidly. Oxidative-glycolytic fibers are characterized by an intermediate level of fatigue resistance. However, oxidative fibers show high resistance to fatigue and thus are the main fibers used in routine activities that require constant contraction for long periods of time, such as posture maintenance and walking (Torrella et al., 1998).

Anabolic androgenic steroids (AASs) comprise synthetic drugs that resemble the male sex hormone, testosterone. Among these, Sustanon is a well-known AAS that is produced for the treatment of several clinical conditions including hypogonadism, reduced

male libido, and osteoporosis (Allouh and Aldirawi, 2012a). However, this drug is also widely abused by athletes participating in power sports to increase their skeletal muscle mass and strength (Bin Bisher, 2009).

Several previous studies have reported the effects of different AASs, including Sustanon, on the glycolytic skeletal muscle fibers (Allouh and Rosser, 2010; Allouh and Aldirawi, 2012a,b). However, no studies have been published regarding the effects of these drugs on oxidative fibers or whether such effects might be dose related. A better understanding of the role of AASs in oxidative muscle fibers is of clinical importance in cases of sarcopenia (age-related muscle atrophy) and cachexia (disease-associated muscle wasting). Accordingly, this study aimed to investigate the effects of the AAS Sustanon on the oxidative capacity and structural morphology of oxidative fibers in adult avian skeletal muscle, a commonly utilized model in AAS studies (Lone et al., 1995; Lin and Hsu, 2002; Allouh and Rosser, 2010; Allouh and Aldirawi, 2012a,b).

Materials and methods

Experimental model

Adult female white leghorn egg-laying chickens (*Gallus gallus*), weighing between 1.2–1.3 kg, were raised under standard conditions in the animal house unit at Jordan University of Science and Technology (JUST). All chicken care, treatments and sacrifice procedures were conducted with the approval of the Animal Care and Use Committee at JUST and in accordance with the guidelines of the U.S. National Institutes of Health on the use and care of laboratory animals (ethical approval # 16/3/3/579). The birds were randomly divided into 4 groups: control (C), which received a dose of 100 µl/injection of normal saline; Sustanon-1 (S1), which received a dose of 12.5 mg/kg/injection of Sustanon (Organon Int., Oss, The Netherlands); Sustanon-2 (S2), which received a Sustanon dose of 25 mg/kg/injection; and Sustanon-3 (S3), which received a Sustanon dose of 50 mg/kg/injection. Each group contained 4 birds and each bird received 4 injections at weekly intervals (1 injection/week).

The treatments started at the age of 26 weeks post-hatching by intramuscular injection into the right pectoralis muscle of the birds. At the age of 30 weeks post-hatching, the birds were sacrificed by cervical dislocation. Total body weight was directly measured and the left anterior latissimus dorsi (ALD) muscle was dissected out, trimmed free of fat, and weighed. Chicken ALD is a small muscle that helps in keeping the wing folded against the body and consists almost entirely of slow oxidative fibers (Kirkpatrick et al., 2008). In addition, the posterior latissimus dorsi (PLD) muscle was dissected from the control birds to serve as a control in the histochemical analysis for oxidative capacity as it is composed mostly of fast glycolytic fibers (Kirkpatrick et al., 2008).

Tissue preparation and sectioning

Each muscle was transversely excised through the middle into two halves. Muscle samples were coated with optimal cutting temperature compound (OCT; Bio-Optica, Milano, Italy) and frozen in 2-methylbutane cooled via liquid nitrogen. All samples were then stored at -80°C.

Serial cross sections of 10 µm thickness were cut from OCT coated samples at -20°C using a cryostat (SLEE-MNT, SLEE Medical GmbH, Mainz, Germany). Each pair of serial sections was picked up on positively charged microscopic slides (AM-1800, AmLabs West LLC, Boulder, CO, USA). Two sections were collected on each slide to increase the possibility of choosing better fields for imaging. Serial slides bearing sections were numbered and stored at -40°C. Along with these cross sections, some longitudinal sections were also obtained in order to measure the lengths of SCN and myonuclei.

Histochemistry

The oxidative capacity of the muscle fibers was determined by measuring succinate dehydrogenase (SDH) activity. Five slides each from PLD-C, ALD-C, ALD-S1, ALD-S2, and ALD-S3 muscle groups were removed from -40°C storage and air dried for 10 min. Slides were then placed in a staining jar containing the incubation medium for 30 min. Incubation medium consisted of 15 ml 1× phosphate-buffered saline (PBS), 15 ml sodium succinate solution prepared in a concentration of 54 mg sodium succinate dibasic (S5047, Sigma-Aldrich GmbH, Steinheim, Germany) per 1 ml distilled water, and 15 ml staining solution prepared in a concentration of 2 mg nitroterazolium blue chloride (N6876, Sigma-Aldrich GmbH) per 1 ml distilled water. At the end of the incubation period, the reaction was stopped by washing the slides twice in 1× PBS solution for 5 min each. Slides were then mounted with cover slips using an aqueous mounting medium (SC-24941, Santa Cruz Biotechnology Inc., Dallas, TX, USA) and sealed.

Immunofluorescence

Slides were removed from -40°C storage, air-dried for 10 min, fixed with 4% formaldehyde in 1× PBS for 4 min, and washed twice in fresh PBS for 5 min each. After that, slides were incubated in ice-cold methanol for 15 min for permeabilization followed by two 5 min washes with PBS. Blocking solution consisting of 5 mM ethylenediaminetetraacetic acid, 5% goat serum, and 10% bovine serum albumin in PBS was applied over the sections for 20 min. The blocking solution was then drained from each slide and combined anti-Pax7 and anti-laminin primary antibodies diluted in blocking solution were applied at a volume of 150 µl per slide. Anti-Pax7 (Developmental Studies Hybridoma Bank,

Iowa City, IA, USA) is a mouse monoclonal antibody developed against chicken Pax7 and was used at a dilution of 1:100 to detect SC nuclei (Allouh et al., 2008). Anti-laminin (L9393, Sigma-Aldrich GmbH) is a rabbit polyclonal antibody developed against the glycoprotein laminin of mouse origin and was used at a dilution of 1:200 to label the basal laminae of skeletal muscle fibers (Allouh, 2011). After applying the primary antibodies, slides were incubated overnight at 4°C.

The following day, slides were washed twice in fresh 1× PBS for 5 min each and secondary antibodies diluted at 1:200 in PBS were applied over the sections on each slide for 45 min at room temperature. Alexa Fluor 488-conjugated goat anti-mouse (A-11001, Invitrogen Corp., Carlsbad, CA, USA) and tetramethylrhodamine isothiocyanate-conjugated goat anti-rabbit (T6778, Sigma-Aldrich GmbH) secondary antibodies were used to label Pax7 green and laminin orange, respectively, when viewed with epifluorescent microscopy. Slides were then washed twice in PBS for 5 min each. UltraCruz Mounting Medium containing DAPI (SC-24941, Santa Cruz Biotechnology Inc.) was then applied and cover slips were placed over the sections and sealed.

Image analyses and data collection

Images of SDH staining were captured using a brightfield microscope (AxioScope.A1, Carl-Zeiss, Jena, Germany) and a digital camera (AxioCam ICc5, Carl-Zeiss). The differences in the staining intensity resulting from SDH activity were determined from the captured images using Image J software (<https://imagej.nih.gov/ij/>). The mean gray value (MGV) measurements were used to indicate the intensity of staining. Images were first converted to gray scale and MGV was then measured for 100 contiguous fibers from each bird. Black and white were automatically assigned MGVs of 0 and 255, respectively, at the ends of an arithmetic linear regression ($r^2=1.00$). Images from the PLD muscles of control birds were used to determine the MGV for glycolytic fibers.

Images from the immunofluorescence slides were captured using an AxioScope.A1 microscope equipped with epifluorescence and an AxioCam ICc5 digital still camera. Four different fields of view were obtained from each slide. From each field, three epifluorescent images, each viewed through a different wavelength filter, were acquired. The resultant images were all nuclei labeled in blue, SC nuclei in green, and basal laminae in orange. The images were subsequently transferred to a PC computer (ProDesk 600 G1, Hewlett-Packard, Palo Alto, CA, USA) and the three images of each field were superimposed using Adobe Photoshop software (Adobe System Inc.; San Jose, CA, USA).

The ellipse minor axis (lesser fiber diameter) was used to assess fiber size (Allouh et al., 2008; Allouh and Rosser, 2010). The minor axis was chosen to surmount the distortion in the muscle fiber cross section when it was cut at an angle other than transversely. Minor axes

of 400 contiguous fiber cross-sections were measured from each bird. The basal laminae images were used to measure the ellipse minor axis of individual fibers with the Image J program.

The numbers of SC nuclei and myonuclei per each fiber cross-sectional profile were counted for 400 contiguous fibers from each bird. The mean SC nuclei (MSCN) and mean number of myonuclei (MMN) per fiber profile were then calculated. The frequency of SCs was calculated according to the equation: Frequency = $[\text{MSCN}/(\text{MSCN} + \text{MMN})] \times 100\%$. In addition, the numbers of SCs and myonuclei per unit length of fiber (1 mm) were calculated using the equation: Number = $A/(\text{Ln} + M)$, where A is the mean number of nuclei per fiber cross-sectional profile (MSCN or MMN); Ln is the mean nuclear length; and M is the thickness of the tissue section (Allouh and Rosser, 2010; Allouh and Aldirawi, 2012a; 2012b). Longitudinal sections were used to measure the lengths of both SC nuclei and myonuclei. The same immunofluorescence technique as described above was applied to study these longitudinal sections. The lengths of 100 SC nuclei and 100 myonuclei were measured from each bird using Image J.

The surface area of the sarcolemma per unit length of fiber was calculated for each bird using the equation: Area = πEU , where E is the ellipse minor axis and U is the unit length of fiber (1 mm). The area of sarcolemma per SC was then determined by dividing the surface area measurement by the number of SCs per mm in each bird. In addition, the size of the myonuclear domain (MD) i.e., the volume of sarcoplasm per myonucleus, was determined by dividing the volume of sarcoplasm per unit length of fiber by the number of myonuclei in that unit length (1 mm). The volume of sarcoplasm per unit length was computed according to the equation: Volume = $\pi(E/2)^2 \times U$.

Statistics

Samples were divided into five groups for histochemical analysis (PLD control and three Sustanon-treated ALD groups plus the ALD control) and four groups (ALD treated and control) for immunofluorescence analysis. Levene's test was first applied to determine the homogeneity of variance among the groups. Data were then evaluated by one-way analysis of variance (ANOVA) at 1% and 5% levels of significance. If a significant difference was found, Fisher's least significant difference (LSD) *post hoc* test was performed to examine the statistical differences between groups.

Results

Body and muscle weights

Both total body and ALD muscle weights increased significantly ($P<0.05$) following Sustanon administration (Table 1). However, no significant differences ($P>0.05$) were identified in either weight measure among the three

Table 1. Anterior latissimus dorsi weight and morphology in control and Sustanon-treated chickens.

Group	Body weight (kg)	Muscle weight (g)	Muscle area (mm ²)	Fiber area (μm ²)	Estimated # of fibers
C	1.34±0.04	0.32±0.02	6.48±0.29	959.81±118.33	6959±594
S1	1.66±0.06**	0.46±0.04*	13.09±1.42**	1720.30±89.76**	7545±419
S2	1.63±0.03**	0.43±0.04*	11.94±0.59**	1612.70±121.68**	7460±335
S3	1.66±0.01**	0.50±0.03**	12.61±0.81**	1775.42±144.61**	7146±259

C: control group; S1, S2, and S3: groups treated with 12.5, 25, and 50 mg Sustanon injection/week for 4 weeks, respectively. Values represent the means ± standard error of the mean (SE). *P<0.05, ** P<0.01 significantly different from the control group (ANOVA, LSD post hoc).

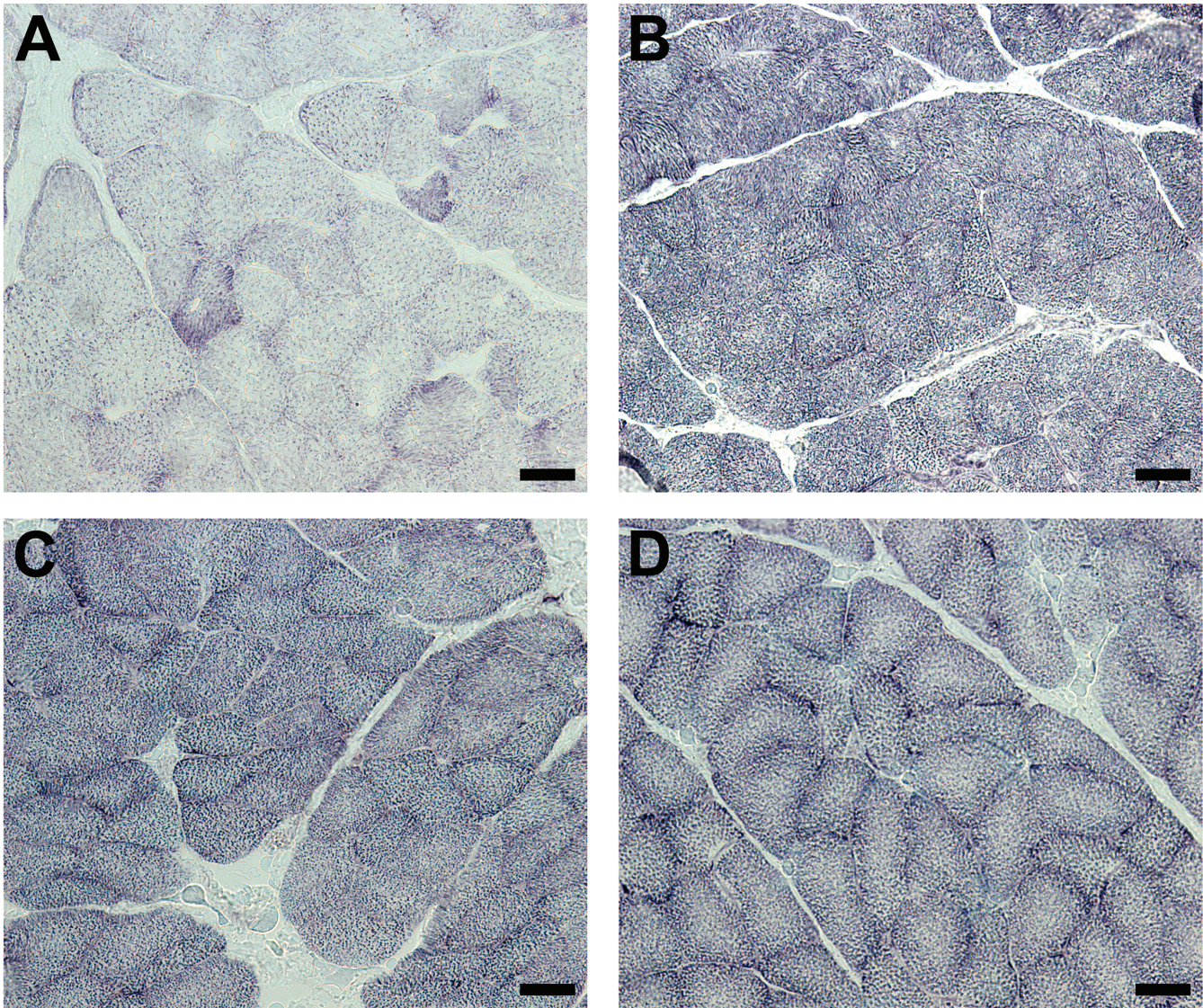


Fig. 1. Histochemical labeling for succinate dehydrogenase activity in skeletal muscles from control and Sustanon-treated chickens. Representative cross-sections from the posterior latissimus dorsi muscle (**A**) and anterior latissimus dorsi (**B**) of a control bird, and from the anterior latissimus dorsi of a bird treated with 12.5 mg (**C**) and 50 mg (**D**) Sustanon injection/w for 4 wks. Scale bars: 40 μm.

Sustanon effects on oxidative muscle fibers

Sustanon-treated groups. The percent increase in ALD wet weight in the Sustanon-treated groups ranged between 34-56% when compared to the wet weight of control ALD muscle.

SDH activity

Histological sections from ALD muscle of the different bird groups stained for SDH activity are shown in Fig. 1. The MGV measurements for SDH staining intensity revealed a significant difference ($P<0.01$) between the PLD group and the ALD muscle groups. However, no significant difference ($P>0.05$) was found between the control and Sustanon-treated ALD muscle groups (Table 2). This indicates that Sustanon treatment has no effect on the SDH oxidative activity of the chicken ALD muscle fibers.

Size and number of fibers

The cross-sectional area of the ALD fibers was significantly ($P<0.01$) greater in all Sustanon-treated groups when compared to the control group, increasing

by 68-85% (Table 1). However, no significant ($P>0.05$) difference in the cross-sectional area was detected between the three Sustanon-treated groups. This indicates that Sustanon administration induced ALD muscle hypertrophy in a dose-independent manner.

In addition, no significant ($P>0.05$) difference was found in the estimated number of ALD fibers among all groups (Table 1). This indicates that Sustanon administration did not induce hyperplasia in the avian ALD muscle.

Distribution of satellite cells

Immunofluorescence sections labeled for SC nuclei from the ALD muscles of control and Sustanon-treated groups are shown in Fig. 2. The mean length of SC nuclei in the ALD muscle groups ranged between 9.73 ± 0.47 and 10.92 ± 0.64 μm . No significant ($P>0.05$) difference in this length was observed among all experimental groups. The frequency of SCs was significantly ($P<0.05$) greater in all Sustanon-treated groups compared to the control group as was the mean number of SCs per unit length of fiber (1 mm) ($P<0.01$). The surface area of sarcolemma per SC was significantly ($P<0.05$) smaller in Sustanon-treated groups compared to the control group, which indicated that SCs were more concentrated (closer to each other) in Sustanon-treated versus control groups. However, there were no significant ($P>0.05$) differences in all SC indices among the Sustanon-treated groups (Table 3).

Distribution of myonuclei

Immunofluorescence sections labeled for myonuclei within the fiber basal laminae of ALD muscles from control and Sustanon-treated groups are shown in Fig. 2. The mean length of myonuclei in the ALD muscle groups ranged between 10.06 ± 0.33 and 10.97 ± 0.41 μm . No significant ($P>0.05$) difference in this length was observed among all experimental groups. The mean numbers of myonuclei per fiber cross-sectional profile and per unit length of fiber were significantly ($P<0.05$) higher in all Sustanon-treated groups compared to the

Table 2. Mean gray value (MGV) measurements for succinate dehydrogenase activity in skeletal muscle fibers obtained from control and Sustanon-treated chickens.

Group	MGV
PLD Control	$150.12\pm0.39^{**}$
ALD Control	130.03 ± 0.45
ALD S1	129.24 ± 0.32
ALD S2	130.87 ± 0.60
ALD S3	129.09 ± 0.37

Black and white were automatically assigned MGVs of 0 and 255, respectively, at the ends of an arithmetic linear regression ($r^2=1.00$). PLD: posterior latissimus dorsi muscle; ALD: anterior latissimus dorsi. S1, S2, and S3: groups treated with 12.5, 25, and 50 mg Sustanon injection/week for 4 weeks, respectively. Values represent the means $\pm$ standard error of the mean (SE). $^{**}P<0.01$ significantly different from all other groups (ANOVA, LSD post hoc).

Table 3. Satellite cell (SC) distribution in the anterior latissimus dorsi from control and Sustanon-treated chickens.

Group	SC Frequency	SC per mm	Area of Sarcolemma per SC (μm^2) $\times 10^3$
C	6.79 ± 0.38	11.38 ± 0.90	9.73 ± 0.73
S1	$13.82\pm1.43^{**}$	$27.63\pm3.18^{**}$	$5.52\pm0.63^{**}$
S2	$10.86\pm1.08^*$	$21.88\pm2.38^{**}$	$6.81\pm1.02^*$
S3	$11.67\pm0.64^{**}$	$23.50\pm1.71^{**}$	$6.46\pm0.60^*$

C: control group; S1, S2, and S3: groups treated with 12.5, 25, and 50 mg Sustanon injection/week for 4 weeks, respectively. Values represent the means $\pm$ standard error of the mean (SE). $^*P<0.05$, $^{**}P<0.01$ significantly different from the control group (ANOVA, LSD post hoc).

Table 4. Distribution of myonuclei (MN) in the anterior latissimus dorsi from control and Sustanon-treated chickens.

Group	MN per fiber profile	MN per mm	Myonuclear Domain (μm^3) $\times 10^3$
C	3.12 ± 0.11	155.75 ± 5.57	6.14 ± 0.65
S1	$3.43\pm0.05^*$	$171.62\pm2.46^*$	$10.05\pm0.63^{**}$
S2	$3.59\pm0.15^{**}$	$179.25\pm7.63^{**}$	$9.11\pm0.96^*$
S3	$3.55\pm0.05^{**}$	$177.25\pm2.32^{**}$	$10.03\pm0.84^{**}$

C: control group; S1, S2, and S3: groups treated with 12.5, 25, and 50 mg Sustanon injection/week for 4 weeks, respectively. Values represent the means $\pm$ standard error of the mean (SE). $^*P<0.05$, $^{**}P<0.01$ significantly different from the control group (ANOVA, LSD post hoc).

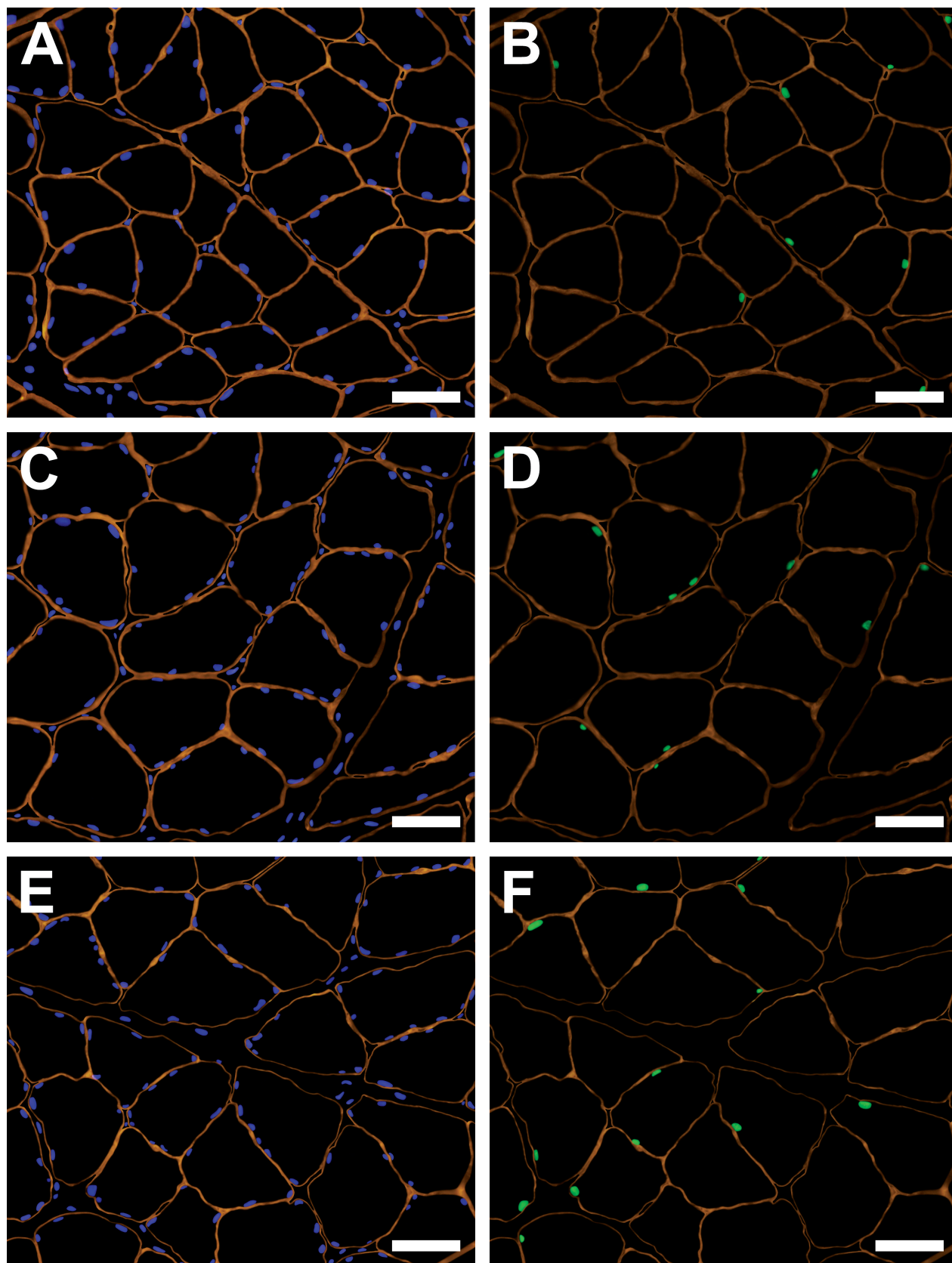


Fig. 2. Immunofluorescence labeling for basal laminae (orange), all nuclei (blue), and satellite cell nuclei (green) in anterior latissimus dorsi muscles obtained from control and Sustanon-treated chickens. Representative cross-sections from a control bird (**A and B**) and from birds treated with 12.5 mg (**C and D**) and 50 mg (**E and F**) Sustanon injection/w for 4 wks. Scale bars: 40 μ m.

control group. However, no significant ($P>0.05$) differences were found in the number of myonuclei among the Sustanon-treated groups, indicating that Sustanon administration induced myonuclear accretion in ALD muscle in a dose-independent manner. Furthermore, the size of MD was significantly ($P<0.05$) greater in all Sustanon-treated groups compared to the control group, with no significant ($P>0.05$) difference among the Sustanon-treated groups (Table 4).

Discussion

This study revealed for the first time that Sustanon administration could induce hypertrophy without hyperplasia in the oxidative fibers of adult avian skeletal muscle. In addition, it showed that Sustanon administration over a relatively short period of time could significantly induce SC proliferation in a dose-independent manner. Furthermore, the study elucidated that Sustanon administration did not appear to affect the oxidative capacity of the ALD muscle fibers as no significant changes were detected in the SDH activity of the Sustanon-treated birds.

Several previous studies have shown that AAS administration had no obvious effect on fiber type distribution and myosin heavy chain isoform content in the skeletal muscles of various species (Bisscoph et al., 1997; Hartgens et al., 2002; Isayama et al., 2006). Saborido et al. (1991) reported that treatment with AASs increased the SDH activity in the mitochondria of fast twitch muscle but that this effect was not observed in those from the slow twitch soleus muscle. However, some studies have demonstrated that AAS administration could reduce the oxidative capacity of skeletal muscles by inducing the transformation of muscle fibers from the slow oxidative to the fast glycolytic type. For example, Holmång et al. (1990) reported a decrease in the amount of type I fibers accompanied by a significant increase in type II in the soleus muscle following testosterone treatment in ovariectomized female rats. In addition, Salmons (1992) reported a decrease in the population of fast oxidative fibers with an increase in the proportion of fast glycolytic fibers in female rabbit tibialis anterior muscle treated with Nandrolone Decanoate. However, the treatment period in both studies lasted for 12 weeks, whereas treatment duration in the current Sustanon administration study extended only over four weeks. Conversely, two-related studies reported that administration of the AAS Mesterolone increased the oxidative capacity of fibers in murine skeletal muscles (Fontana et al., 2010, 2013).

Notably, the current study demonstrated that Sustanon administration stimulated the growth of oxidative muscle fibers mainly by hypertrophy, without exercise. Similar findings were reported with respect to the glycolytic fibers of growing avian skeletal muscles (Allouh and Aldirawi, 2012a) and an earlier study demonstrated a comparable effect of testosterone on both the oxidative and glycolytic fibers of unexercised men

(Sinha-Hikim et al., 2002). However, whether this effect is dose-dependent is still a matter of investigation. Previous studies have reported that AAS administration is associated with a dose-dependent increase in human muscle mass and strength without exercise (Bhasin et al., 2001; Saad et al., 2011). However, whether this effect is due to hypertrophy alone or both hypertrophy and hyperplasia remains unknown. In the present study, we found that Sustanon was associated with a dose-independent increase in avian ALD muscle mass that was mainly due to fiber hypertrophy. In addition, unpublished data from our laboratory showed similar results from the fast glycolytic PLD muscle.

The study showed that Sustanon administration led to significant increases in all SC indices within ALD muscle fibers in a dose-independent manner. It remains unclear, however, whether AASs increase SC numbers through enhancing their proliferation, reducing their apoptosis, or via both mechanisms. Furthermore, the exact molecular mechanisms by which AASs activate SCs also are not well understood. It is believed that the anabolic effect of AASs is mediated by their binding to the androgen receptors that are expressed by both myonuclei and SCs (Sinha-Hikim et al., 2004). Once the AASs bind to the androgen receptors in the cytoplasm, they translocate into the nucleus where they bind to their DNA response elements, which alter the transcription of several genes influencing SC activity (McKenna et al., 1999). In addition, it has been suggested that AASs also exert rapid, non-genomic effects on their target tissues (Kicman, 2008). AAS rapid action is mediated by an increase in intracellular Ca^{2+} , which leads to the activation of Ca^{2+} -dependent second messenger cascades (Estrada et al., 2003). Notably, this non-genomic action is insensitive to inhibitors of either transcription or translation (Kicman, 2008). Little is known about these non-genomic effects on SCs and muscle fibers. However, one hypothesis dictates that the activated second messenger cascades modulate the genomic expression induced by androgen receptors (Vicencio et al., 2011).

In turn, the necessity of SCs for muscle hypertrophy is also a matter of debate. In a human study, Petrella et al. (2008) showed that robust myofiber hypertrophy is driven by SC activation leading to myonuclear accretion. In addition, a recent study reported that the depletion of SCs prevents hypertrophy of the skeletal muscle fibers (Egner et al., 2016). However, other studies demonstrated that effective hypertrophy can occur without SC involvement (Raffaello et al., 2010; McCarthy et al., 2011). We speculate that skeletal muscle hypertrophy occurs concurrently by both SC differentiation and through enhanced transcriptional and translational activities within muscle fibers. This dual response was evident in the present study by the increase in numbers of SCs and myonuclei, which indicates the involvement of SCs in the hypertrophy process, and by the increase in MD size, reflecting the enhanced process of protein synthesis within the myofibers. However, one of these

two mechanisms may predominate depending on several factors including age, species, and the type and amplitude of the growth stimulus.

Previous studies suggested that myonuclear accretion presumably occurs to maintain a constant ratio of cytoplasmic volume per each myonucleus, thus preserving a relatively fixed MD size (Allen et al., 1999; Alway and Siu, 2008). However, our study showed that the increased myonuclear number was associated with a simultaneous increase in the size of the MD. This is comparable with previous studies that used AASs to induce muscle fiber hypertrophy (Allouh and Rosser, 2010; Allouh and Aldirawi, 2012a). Together, these findings suggest the presence of a loose correlation between MD size and myonuclear number, which is also supported by previous studies on muscular atrophy (Gundersen and Bruusgaard, 2008; Bruusgaard et al., 2012).

In conclusion, this study showed that the administration of Sustanon could lead to hypertrophy in avian slow oxidative fibers without affecting their oxidative capacity. This hypertrophy was associated with SC proliferation and myonuclear accretion in the oxidative fibers. These effects of Sustanon appeared to be dose-independent. Future comparative studies to evaluate the anabolic effects of different doses of Sustanon coupled with exercise adaptation will also be informative for the management of muscular atrophy disorders and for ameliorating the effects of AAS abuse.

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