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S Bakou, Lydie Guigand, Radu Apreutese, A Apreutese, Monique Wyers, et al.. Myocytes changes and satellite cells proliferation following denervation of ALD (Anterior Latissimus Dorsi) and PLD (Posterior Latissimus Dorsi) muscles in two strains of turkey (Meleagris gallopavo). Journal of Veterinary Anatomy, 2014, 7 (1), pp.89-111. hal-01190007

HAL Id: hal-01190007 https://hal.science/hal-01190007

Submitted on 1 Sep 2015

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Myocytes changes and satellite cells proliferation following denervation of ALD (*Anterior Latissimus Dorsi*) and PLD (*Posterior Latissimus Dorsi*) muscles in two strains of turkey (*Meleagris gallopavo*)

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With 3 tables & 4 figures

Received July 2013, Accepted for publication March 2014

Abstract

Morphological features and chronology of myocytes changes after denervation were studied over 35 days period in 2 heavy (HW) and light-weight (LW) strains of 5-weekmale turkeys. Denervation old caused progressive atrophy in posterior latissimus dorsi (PLD). By day 28, the weight of the PLD muscle had reached about 62% of the nondenervated contralateral muscle weight in both strains. On the contrary, ALD muscle mass increase progressively after denervation. Thus the maximum hypertrophy of the ALD, expressed as a percentage of contralateral muscle, was respectively about 67% and 37%, day 21 in the HW strain and day 28 in LW strain after denervation. ALD hypertrophy ceased apparently after day 21 (HW strain) and day 28 (LW strain). Morphometric analysis revealed that fast twitch (type II) fibers were atrophied after denervation, whereas slow tonic (type III) fibers were hypertrophied from day 7. Coagulative necrosis with fragmentation and lysis associated with moderate infiltration of inflammatory cells, were similar in both strains. Irregularities in mitochondrial distribution occurred mainly in type III fibers of ALD muscle at day 7. Seven and 14 days after denervation, immunolabelling of proliferating cell nuclear antigen (PCNA) revealed satellite cell activation in denervated muscles. The number of activated satellite cells was greater in the LW than HW mainly in ALD muscles.

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Keywords: Skeletal muscle / fiber size / denervation / satellite cells / turkey

Introduction

Avian skeletal muscle fibers can be classified in fast-twitch (type II), slow-twitch (type I) and slow-tonic (type III) on the basis of several physiological and histochemical criteria, including metabolic enzyme and myofibrillar myosin ATPase activities (Barnard et al., 1982). The metabolic and contractile properties of skeletal muscle are determined by their motor innervation (Hess, 1961 ; Gordon and Vrbova, 1975 ; O'brien and Vrbova. 1978 Dubowitz, 1985). Fast-twitch fibers are focally innervated and adapted for rapid, intermittent contractions, slow twitch fibers are multiinnervated and adapted for slow, intermittent contractions and slowtonic fibers are multi-innervated and adapted for slow, continuous contractions.

The anterior latissimus dorsi (ALD) and posterior latissimus dorsi (PLD) are two muscles of the wing of birds : ALD is solely composed of slowtonic fibers and used for postural tasks whereas the PLD, a synergistic muscle, consists exclusively of fast-twitch fibers. Classically, denervation is the most dramactic way to demonstrate the trophic role of the motor nerve on muscle. Twitch skeletal muscle fibers are known to react to denervation by atrophy. The extent of denervation atrophy varies in these fibers.

Although all authors agree that type II (fast-twitch) fiber atrophy occurs after denervation, findings on the behavior of type I (slow-twitch) fibers differ widely. Some investigators claim that type I (slow-twitch) fibers undergo less marked atrophy than type II (fast-twitch) fibers (Bajuzs, 1964; Melichna and Gutmann, 1974; Niederle and Mayr, 1978; Dubowitz, 1985). Others suggest that all fibers (type I and II) atrophy to the same extent (Romanul and Hogan, 1965; Mayer et al., 1984).

According to Jaweed et al. (1975), the response to denervation of fibers might be different in slow and fast muscles. In mammals, a transient hypertrophy of diaphragm muscles fibers occurs after unilateral phrenic denervation (Feng and Lu, 1965; Stewart, 1968; Stewart et al., 1972; Hopkins et al., 1983; Zhan and Sieck, 1992; Gosselin et al., 1994; Zhan et al., 1995). According to Zhan et al. (1995), all fiber types appear initially to hypertrophy after denervation. By 2 weeks after denervation, differences in morphological adaptations appear among fiber types, with type I fibers remaining hypertrophied whereas type II fibers

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atrophy (Zhan and Sieck, 1992; Zhan et al., 1995). Extraocular muscles, unlike other mammalian skeletal muscles, contain a population of slow-tonic, multiinnervated fibers. Asmussen and Kiessling (1975) detected hypertrophy of these slowtonic, multi-innervated fibers in denervated extraocular muscles of the rabbit at day 34. Porter et al. (1989) noted an atrophy of all fiber types in extraocular muscles in the monkey at 28, 56 and 112 days postdenervation. Most fiber types hypertrophied after spontaneous reinnervation occurred at day 167. Christianssen et al. (1992) reported hypertrophy of multi-innervated extraocular muscle fibers in the dog between 4 and 12 weeks postdenervation. Others authors established that the slow-tonic (type III) fibers of anterior latissimus dorsi muscle show long lasting hypertrophy after denervation (Feng and Lu, 1965; Jirmanova and Zelena, 1970 ; Sola et al., 1973 ; Cullen et al., 1975).

In a previous work on denervated tibialis cranialis, gastrocnemius lateralis and plantaris muscles in two strains of Turkey, we showed significant atrophy of type II fibers in these muscles. Type I fibers in tibialis cranialis and gastrocnemius lateralis were hypertrophied and type III fibers in plantaris displayed a transient atrophy then hypertrophied. If type II fiber atrophy is similar in both Turkey strains, postdenervation hypertrophy of type I and III fibers occured much more quickly in the light strain than in the heavy one (Bakou et al., 1996).

The causes of these differences are still unknown, but it would appear that muscle stretching plays a significant role as a stimulus for denervation hypertrophy (Feng et al., 1963; Stewart, 1968; Jirmanova and Zelena, 1970; Goldspink, 1976; Gosselin et al., 1994; Sakakima and Yoshida, 2003).

The effects of denervation on satellite cells have been studied by many authors, and some have claimed that it stimulates their proliferation (Ontell, 1974; 1975; McGeachie and Allbrook, 1978; Murray and Robbins, 1982; Snow, 1983; McGeachie, 1985; 1989; Gosselin et al., 1994; Rodrigues and Schmalbruch, 1995; Bakou et al., 1996; Lu et al., 1997; Viguie et al., 1997; Schmalbruch and Lewis, 2000; Borisov et al., 2005). For instance, in Turkey Heavy Weight Strain (HW), activation of satellite cells began on day 10 postdenervation and progressively increased until day 21. Thus, on day 21 postdenervation, the value reached 80 activated satellite cells per 100 fibers in tibialis cranialis and plantaris muscle and 60 in

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gastrocnemius muscle. In the Light Weight Strain (LW), number of activated satellite cells was still lower than 10% for the tibialis cranialis muscle and stay similar to the control. It was constant at about 30% for the plantaris muscle and reached the same percentage for gastrocnemius after 10 days (Bakou et al., 1996). The purpose of the present study was to compare the morphological features and chronological occurrence of postdenervation ALD and PLD muscle modifications in two different strains of Turkey, a heavy- (HW) and a light-weight (LW) ones.

Materials and Methods

Birds

Fifty five-week-old male turkeys (Meleagris gallopavo) belonging to two strains were used in this study. The HW strain was a commercial BUT-T9 strain (British United Turkeys Limited, Warren Hall, Broughton, Chester, CH4OEW, UK). These heavy-medium-line turkevs are raised in light-controlled units for 14 weeks before slaughtering and then used as butchered turkey. They were obtained from breeders who are selected at 15 weeks of age on criteria of weight, muscle mass and absence of leg weakness. The LW strain was a commercial Betina strain (Grimaud Frères, La Corbière,

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49450 Roussay, France) exclusively used as whole roasted Christmas turkeys. No weight selection criterion was applied to these birds. Growth patterns differ in the two strains. In slow muscles like ALD, postnatal hyperplasia contributes to growth in the HW weight strain, whereas only hypertrophy is observed in the LW strain (Cherel et al., 1994).

The birds were purchased from commercial hatcheries and reared on litter in pens. Food and water were provided *ad libitum*. They were fed on a starter diet with crude protein (28%) and metabolizable energy (2,800 kcal/kg). French National Institute of Agronomy Research's guide for the care and use of laboratory animals was followed.

Surgical procedures

Twelve turkeys of each strain were anesthetized by a mixture of Ketahydrochloride mine (Imalgene 500ND) and Xylazine hydrochloride (RompunND), using doses of 10 mg/kg and 1 mg/kg IM respectively. The common branch of the right nerve (What is the name of this right nerve?) supplying the anterior and posterior latissimus dorsi muscles was isolated, then recut over a length of about 1 cm to avoid any risk of reinnervation. For each strain, five animals birds were used as controls (sham-operated).

Four animals birds of each strain were weighed and sacrified by intravenous injection of pentobarbital (DoléthaIND) at 7, 14, 21, 28 and 35 days post-denervation. Both denervated and non denervated ALD and PLD muscles were thoroughly removed andweighed.

Histology and histochemistry

Transverse sections (about 0.5 cm thick) were performed at the muscle midbelly and frozen for 30 seconds in isopentane previously cooled with liquid nitrogen. Serial sections of 12 μ m were cut using a cryostat and then stained with hematoxylin-eosin (HE) and Gomori Trichrome (GT) to evaluate possible changes in muscle morphology.

Sections were treated with histoenzymological techniques. Acid phosphatase (AP) was used to identify macrophages in necrotic fibers, Nicotinamide adenine dinucleotide tetrazolium-reductase (NADH-TR) reaction to characterize fiber metabolism and myofibrillar adenosine triphosphatase (ATPase) reaction after acid preincubations (pH = 4.35and 4.6) and basic preincubation (pH = 10.4) to detect ATPase activities within fibers.

Immunocytochemistry

Muscle samples (about 1 cm thick) were fixed in 10% formalin and embedded in paraffin. Transversal secBakou et al.,

tions (4 µm) were cut in these blocks, using a microtome and then treated by immunocytochemistry in order to reveal proliferating cell nuclear antigen (PCNA / Cyclin) which is expressed during the G1 and Sphase of the cell cycle. The primary antibody was a PCNA monoclonal antibody (DAKO, PC10). Sections were treated with a 1:50 dilution of primary antibody for 1 hour at 37°C in a humid box. The routine streptavidin-biotin, alkaline phosphatase method (K682 DAKO, LSAB (R) kit) was used for immunohistochemical staining. Cryostat-cut sections (10 µm thick) were used for immunocytochemistry in order to revealed the ventricular myosin heavy chain (MHC) isoform. Sections were treated with a 1:2500 dilution of primary antibodies in phosphatebuffered saline (PBS) for 1 hour at 37°C in a humid box. The primary antibodies were monoclonal antibodies (MAb) directed against myosin heavy chain (MHC) isoforms of chicken muscle. The specificity of HV11 MAb for ventricular MHC have previously been documented (Bandman 1985, bandman et al, 1988, 1989).

These chicken specific MAb react with turkey myosin isoforms (Maruyama et al, 1990). Sections were washed in PBS, and the secondary antibody (fluoresceinconjugated goat anti-mouse IgG1 (EU-

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ROMEDEX, AP 106F), diluted 1:100 in PBS) was laid on sections for 1 h at room temperature.

Histomorphometrical analysis

Different morphometric parameters were assessed using a VIDS IV semiautomatic image analyzer Measuring Systems, (Analytical London Road, Pampisford, Cambridge CB2 4EF, UK.). The mean (± SD) diameter of type II and III fibers was determined from an average of 100 fibers for each type in denervated muscle as well as in contralateral (non-denervated) muscle used as a control. The relative proportion of the different fiber types (II and III) in denervated and non-denervated muscles was determined from an count of 500 fibers. Diameter measurements and determination of the proportion of different muscle fiber types were done on sections of frozen muscles treated with acid ATPase (pH = 4.6).

On transverse sections of frozen muscles treated with acid phosphatase reaction (AP), necrotic fiber quantification was done in 20 microscopic fields, each representing around 150 fibers. On transverse sections of muscles treated immunocytochemically with PCNA antibody, the quantifications of activated satellite cells and of the total number of fibers were performed in 15 microscopic fields (150 fibers each). Results are expressed as the number of cells labeled with PCNA antibody per 100 muscle fibers. Analysis of variance (ANOVA) and Fischer PLSD tests were used to compare body and muscle weights according to the ratio of muscle to body weight and the diameter and proportion of different muscle fiber types between and within the 6 groups (sham-operated turkeys and those denervated at day 7, 14, 21, 28 and 35).

Results

Body and muscle weights

Table I indicates body and muscle weights and non-denervated muscle to body weight ratios for Anterior latissimus dorsi (ALD) and Posterior dorsi (PLD). latissimus Body weights increased with age during the experimental period. At the opposite, the nondenervated (contralateral) muscle-to-body-weight ratio remain constant during the experimental period, warranting the use of the contralateral muscle as an internal control in our study. Figure 1 shows plots for variations in the weight of denervated muscles compared with the contralateral muscles. Sectioning the common nerve branch supplying the ALD and PLD muscles caused progressive atrophy in the PLD muscle. Thus 28 days after denervation, the weight of PLD was about half of the con-

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trolateral nondenervated muscle in both strains. On the contrary, ALD muscle mass increase progressively after denervation. Thus the maximum hypertrophy of the ALD, expressed as a percentage of contralateral muscle (nondenervated), was respectively about 167% (day 21 in the HW strain) and 137% (day 28 in LW strain). ALD hypertrophy ceased apparently after day 21 and day 28 respectively in HW and LW strains. However ALD denervated muscles remain heavier than controlaterals muscles at day 35 (147% and 125%, respectively for HW and LW strain).

Fiber size and number

Table II shows the mean diameter values of muscle fiber types for denervated and contralateral muscles. The figure 2 indicates the relative decrease (PLD) and increase (ALD) of mean fiber diameter. Type II fibers diameter decreased from day 7 to day 35 after denervation. Decreases in type II fibers diameter ranged from 40 to 45% and type II fiber atrophy was earlier in HW strain compare to LW strain At day 21, no significant differences were noted between HW and LW strains in the degree of type II atrophy (Fig. 2A). Type III fibers diameter of the ALD increased from day 7 to day 35 after denervation. Increases in type III fiber diameter ranged from 12%

to 17%. No significant differences were noted between HW and LW strains in the severity of type III fiber hypertrophy or in the interval before its occurence (Fig. 2B).

The total number of type II fibers (PLD) and type III fibers (ALD) is shown in Table III. No significant differences between denervated and contralateral (nondenervated) muscles were noted in the total number fiber of ALD and PLD in the both strains during the experimental period.

Muscle fiber pathology

Histopathological changes were identical in both strains but were observed at day 7 and day 14 respectively in ALD and PLD muscles (Fig. 3). Thus, at day 7, nuclear changes such as hypertrophy centrally nuclei were noted in ALD muscle At day 7 ALD type III fiber hypertrophy became perceptible (Fig. 3A - B) and the number of fibers presenting central nuclei increased. In denervated muscles, necrotic fibers were observed at the same period. The number of necrotic fibers remainded low, ranging from 0.1% to 1.5% of total fibers number. The necrotic fibers showed coagulative cytoplasm and moderate infiltration by macrophages (Fig. 3F) on and after day 14 postdenervation. Non-denervated contralateral muscles never exhibited ne-

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crotic fibers. On day 14, a slight proliferation of perimysial and endomysial connective tissue was observed. The NADH-TR reaction intensity revealed many abnormalities in some fibers. Some fibers of normal size showed diffuse increased reactivity to NADH-TR, whereas others exhibited focal zones of intense reactivity (Fig. 3E) mixed with zones of weak reactivity. The abnormalities to NADH-TR reactivity revealed peripheral or central mitochondria aggregations. Few fibers appeared as 'motheaten' fibers. Abnormal reactivity to NADH-TR occured mainly in type III fibers of ALD muscle at day 7.

Satellite cells Proliferation

After immunolabelling for PCNA / cyclin, nuclei in G1 and S-phase of cell cycle (activated satellite cells) were labeled. Proliferation of satellite cells was about three times greater in ALD than in PLD muscle. In the LW strain, activation of satellite cells was earlier and greater than in HW strain. In PLD, satellite cells activation began on day 7 in LW strain with 18 activated cells per 100 fibers, then declines to 5% (similar to control) at day 28. In HW strain the number of activated satellite cells was weak (Fig. 4A). In ALD muscle, satellite cells activation began on day 7 and progressively increased until day 14. On day 14

post denervation, the value reached 77 activated satellite cells per 100 fibers in LW strain. After day 14, the number of activated satellite cells decline progressively, reaching 35% at day 35. Satellite cells activation only began on day 14 in HW strain and remained constant at about 14% during all the experimental period (Fig. 4B).

Myotubes generated by denervation

The reaction to the basic myofibrillar adenosine triphosphatase (ATPase) after basic preincubation (pH 10.4) reveals the presence of neoformed myotubes (neomyotubes) in the denervated muscles from the day 7 in the ALD and from the day 14 in the PLD (Fig. 3G). These neomyotubes are disseminated on the section of the muscular tissue and react strongly with the ventricular isoform (Mab HV11), an early marker of muscle regeneration (Fig. 3H). The number of neomyotubes are greater in the ALD than the PLD.

Discussion

In this study, we show that the ALD and PLD muscles deprived of their innervation motor undergo different morphological modifications. The PLD muscle atrophies progressively after the denervation whereas the ALD muscle hypertrophies. Thus, 35 days after the denervation, the

weight of the PLD had reached 40% to 64% of the controlateral muscle one (respectively in the LW and HW strains). This finding is in agreement with numerous authors in the literature, who reported that morphological modifications following the denervation is an atrophy of the muscular masses (Stewart et al., 1972; Jaweed et al., 1975; Dhoot and Perry, 1982; Buhr, 1990; Bakou et al., 1996). For instance, Buhr (1990) reported atrophy of the pectoralis muscle [fast twitch (type II) fibers] of the chicken, which reached 48% to 56%, 4 weeks after the denervation. In a previous study realized in turkey, we showed that the section of the sciatic nerve causes an atrophy of the tibilais cranialis, the gastrocnemius lateralis and plantaris muscles from 45 to 60% of the weight of the controlateral muscle 21 days after

denervation (Bakou et al., 1996). Nevertheless, it would appear that all muscles do not have the same behavior after denervation. Some muscles respond to the denervation by a transient hypertrophy. This occurs with the hemidiaphragm of the hamster, the bat (Stewart et al., 1972) and the rat (Turner and Manchester, 1973; Gundersen and Bruusgaard, 2008).

Unlike the postdenervation hypertrophy of the hemidiaphragm which is transient, the postdenervation hypertrophy of the ALD (Feng et al., 1963; Jirmanova et Zelena, 1970 ; Stewart et al., 1972 ; Sola et al., 1973) is permanent even if the weight of the denervated muscle declines slowly during the second month after denervation in chicken (Jirmanova et Zelena, 1970) or from the 21st postdenervation day in turkey in our study. However, the denervated ALD muscles remain 47% (HW strain) and 25% (LW strain) heavier than the controlateral mus-

cles 35 days after denervation.

The denervation has a selective action on the morphological nature of the muscle fibers according to their type. Generally, in mammals (Bajuzs, 1964; Melichna and Gutmann, 1974; Asmussen and Kiessling, 1975; Jaweed et al., 1975; Gutmann, 1976; Niederle and Mayr, 1978; Rubinstein and Kelly, 1978; Dhoot and Perry, 1982; Mayer et al., 1984; Dubowitz, 1985; Christiansen et al., 1992) just like with birds (Feng et al, 1963; Jirmanova et Zelena, 1970; Cullen et al., 1975 ; Bakou et al., 1996) denervation causes an atrophy of type II (fast twitch, phasic contraction, focal innervation). In our study, type II fibers of the PLD muscle undergo a progressive atrophy that will begin at the 7th post-denervation day and reach 40% to 45%, 35 days after denervation. Our results are in

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agreement with those authors. Moreover, the post denervation atrophy of type II fibers in turkey presents similar features in homogeneous muscles (ex PLD) and heterogeneous ones (ex the tibialis cranialis, mix of type I and II fibers or plantaris, mix of type III and II) (Bandman, 1985).

In our study, the slow fibers with tonic contraction and multiple innervation (type III) of the ALD hypertrophy after the denervation. Our observations are similar to previous studies that reported that type III fibers hypertrophy after denervation (Feng et al., 1963; Jirmanova et Zelena, 1970; Sola et al., 1970 1973; Cullen et al., 1975). In the chicken, for example, Cullen et al. (1975) mentioned that 7 days after the denervation, there was an increase of the diameter of the ALD fibers of 15% in comparison with the diameter of the muscle fibers of the control muscle. Differences of behaviour between the fibers types remain unexplained. The mechanism of the post-denervation hypertrophy are yet to be elucidated, but it probably involves several factors such as muscle inactivity (Reid et al., 1989; Connold et al., 1993; Zhan et Sieck, 1992; Zhan et al., 1995), the discharge of growth factors (Allen et al., 1995) and the mode of innervation of the differents types of fibers which could act as intrinsic factors (Bakou et al., 1996). Sakakima and Yoshida (2003) show that static stretching could prevent atrophy of type I denervated fibers. Moreover it seems that only the stretched muscles are able to hypertrophy after denervation (Feng et al., 1963; Feng and Lu, 1965; Stewart ; 1968 ; Jirmanova et Zelena, 1970 ; Goldspink, 1976). In our study, the animal's wing weight carries out a spontaneous stretching of the muscles.

According to Gosselin et al. (1994), the transient hypertrophy observed 3 days after denervation of the hemidiaphragm of the rat would corresponds to an important protein synthesis initiated by the incorporation of the nucleus of satellite cells activated after denervation. Seven days (LW strain) and fourteen days (HW strain) after the denervation, we noted satellite cells activation in denervated muscles, revealed by PCNA/cyclin. Other authors mentioned similar observations (Ontell, 1974: Snow. 1983: McGeachie. 1985; 1989; Gosselin et al., 1994; Bakou et al., 1996; Schmalbruch and Lewis, 2000; Borisov et al., 2005).

The origin of the stimulus and the mechanism by which the satellite cells reaches the state of mitosis is not entirely known, but the dener-

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vation per se could play a major role in this mechanism (Christiansen et al., 1992). The nervous but butt in degeneration seems to discharge mitogenic substances during the first 36 hours (Murray and Robbins, 1982). Thus, satellite cells proliferation would be due to a complex mechanism in which the modification of the electric activity at the surface of the combined denervation muscle fibers and / or the nervous but should play an important role. In fact Bischoff (1990), by in vitro study, suggested that the hypothesis according to which the discharge of mitogenic substances

combined to the removal of the inhibition of contact due to the rupture of the plasmalemma of necrotic fibers could be a determinant factor for the state of mitosis reached by the satellite cells. This hypothesis is backed up by the fact that the satellite cells associated to with viable muscle fibers remain quiescient. In rats, Jimena et al. (1993) reported that the injection of denervated muscle extract in the healthy muscle causes a muscle hyperplasia and hypertrophy due to the proliferation and the fusion of satellite cells.

The results of our study shows that activated satellite cells after denervation differentiate in myotubes 7 days after denervation, mainly in the ALD muscles. Snow (1983) shows that the multinucleated myotubes are present at 30 days in the extensor digitorum longus and soleus muscle in rat. Schultz (1978), observesd a hyperplasia 3 weeks after denervation, which he attributesd to the proliferation of satellite cells. Nevertheless, authors such as Murray and Robbins (1982) reported

that the proliferation of cells in the denervated muscles do did not lead to muscle degeneration.

In our study, myotubes generated by denervation in the ALD muscle are in greater number and have a bigger size in HW strain than the LW strain. This observation suggests that the HW strain is able to produce muscular hyperplasia after denervation as during the growth (Cherel et al., 1994). However we were not able to objectivize this hypothetical hyperplasia, since the total number of muscle fibers in the denervated muscles is not statistically different to the total number of fibers in controlateral muscles.

This difference of behaviour observed in the differentiation of myotubes suggests that satellite cells in both turkey strains have different merging capacities. The substantial and earlier proliferation of satellite cells in the LW strain and their weak differentiation in myotubes versus the most important differentiation of activated satellite cells in the HW strain, despite a weaker and late proliferation, brings us to set down

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the possibility of the existence of different populations of satellite cells, an early one, and another one which appears later or that the different conditions of the microenvironement of satellite cells could modulate their destination.

Other differences between the two strains are to be noted in our study. The atrophy of type II fibers of the PLD muscle takes place earlier in the HW strain than in the LW one. The LW strain seems therefore to present a lesser sensibility to postdenervation atrophy of type II fibers. Gundersen and Bruusgaard (2008) show that atrophy is not accompanied by any loss of nuclei. However, atrophy of the fibers of the PLD in the LW strain, may be delayed by the incorporation of nuclei by fibers, as the proliferation of satellite cells after denervation takes place earlier and is more significant in the LW strain. These differnces between two strains of the same species show that selection of the animals based on muscle mass

have induced modifications on the type of muscle satellite cells and their behavior.

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Table I: Body (g) and muscle weights (g) (means \pm S.D.) of anterior and posterior latissimus dorsi, and muscle to body weight ratios for heavy-weight (HW) and light-weight (LW) strains 7, 14, 21, 28 and 35 days after denervation (4 animals for each time-point)

					Time after operation (days)			
			S.O.	7	14	21	28	35
ALD	нw	Den	15450±1322b	14623±2303 ^a	15812±2265ª	16732±1461bc	17685±2776bc	18175±3011¢
Total number		Con	15512±1495 ^{ab}	13583±1948 ^a	15751±1588 ^{ab}	16760±1251 ^{ab}	17827±2334 ^b	17566±2774 ^b
of slow-tonic fibres	LW	Den	13295±1920 ^a	16235±1371bc	15397±1824 ^{ab}	13682±1114bc	15027±1126¢	13290±2372abo
		Con	13846±2745ª	17183±1229 ^a	15460±1867 ^a	14491±1831ª	15284±1698ª	12802±1807 ^a
PLD	HW	Den	29060±3725 ^a	28555±4521ª	26115±3934 ^a	27735±3033 ^a	26639±3158 ^a	28721±1847 ^a
Total number		Con	28586±5023ª	27373±5127ª	26358±2473ª	27390±3558 ^a	26059±3753 ^a	27681±3753ª
of fast-twitch fibres	LW	Den	28982±3179 ^a	26085±4005 ^{ab}	26982±3429 ^b	26569±1816 ^a	26170±2804 ^a	28161±1800
		Con	28591±3546 ^a	29023±4385 ^a	26123±3958 ^a	28921±2058 ^a	25737±4887 ^a	28019±2408 ^a

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Table II: Diameter (μ m) (means ± S.D.) of fast twitch (type II) fibers of posterior latissimus dorsi (PLD) and slow tonic (type III) fibers of anterior latissimus dorsi (ALD) in heavyweight (HW) and light-weight (LW) strains 7, 14, 21, 28 and 35 d after denervation

		and the second	and the second second		Time after operation (days)			
			S.0.	7	14	21	28	35
ALD	нw	Den	39.6±5.0ab	38.3±1.1ª	45.5±1.3 ^b	45.5±6.1 ^b	54.0±1.4 ^c	54.3±7.8°
Diameter (µm)		Con	37.4±7.5ª	37.5±2.1ª	41.5±2.9ab	39.8±3.0 ^a	47.2±3.0bc	49.9±4.6°
of slow-tonic fibres	LW	Den	36.5±8.1 ^b	28.5±1.1 ^a	39.1±3.4bc	38.0±1.1b	40.4±2.8bc	45.5±5.7°
		Con	33.2±5.5b	28.4±1.3 ^a	35.6±1.9bc	33.3±0.5 ^b	36.4±3.2 ^{bc}	38.5±2.5¢
PLD	нw	Den	36.6±5.4 ^b	26.0±1.5ª	22.3±1.7 ^a	22.5±2.8 ^a	26.3±9.9ª	23.1±2.9ª
Diameter (µm)		Con	36.5±6.8abc	31.2±3.0 ^a	34.6±4.0ab	35.9±3.4abc	40.1±3.4bc	41.8±0.8¢
of fast-twitch fibres	LW	Den	26.7±4.4 ^c	22.1±3.1 ^b	22.6±2.3bc	19.0±2.2 ^{ab}	15.6±1.7 ^a	19.1±3.2ª
		Con	25.1±3.7ªb	22.9±1.7ª	28.0±0.9bc	29.2±2.8°	28.6±0.4 ^c	32.9±2.6d

Table III. Total number (means \pm S.D.) of fast twitch (type II) fibers of posterior latissimus dorsi (PLD) and slow tonic (type III) fibers of anterior latissimus dorsi (ALD) in heavyweight (HW) and light-weight (LW) strains 7, 14, 21, 28 and 35 d after denervation

	Time after operation (days)								
			I ime after operation (days)						
	3		S.O.	7	14	21	28	35	
Body weight (g)	нw		1837±561b	1284±102 ^a	1682±291ab	1938±249bc	2393±179¢	2954±345d	
	LW		1494±389b	992±24 ^a	1342±115 ^b	1530±58bc	1660±220°	2127±127d	
ALD weight (g)	нw	Den	01.3±0.3ª	01.2±0.1ª	01.8±0.3ab	02.3±0.6bc	02.8±0.5¢	02.9±0.8°	
		Con	01.3±0.4 ^b	00.9±0.1ª	01.2±0.2ab	01.4±0.3 ^b	01.8±0.2 ^c	02.0±0.2 ^c	
	LW	Den	01.1±0.4ab	00.8±0.1ª	01.1±0.2ab	01.4±0.2bc	01.6±0.2 ^c	01.8±0.3¢	
		Con	01.0±0.2 ^a	01.2±0.6 ^{ab}	00.9±0.7 ^a	01.0±0.1ab	01.1±0.1 ^{ab}	01.4±0.1 ^b	
PLD weight (g)	нw	Den	01.4±0.3 ^b	00.6±0.1ª	00.5±0.2ª	00.7±0.2ª	00.8±0.4ª	00.6±0.2 ^a	
		Con	01.2±0.4abc	00.8±0.2 ^a	01.0±0.2 ^{ab}	01.3±0.3bcd	01.5±0.2 ^{cd}	01.7±0.5 ^d	
	LW	Den	00.6±0.2b	00.4±0.1ª	00.5±0.2ab	00.4±0.0 ^a	00.3±0.1ª	00.5±0.2 ^{ab}	
		Con	00.7±0.2 ^b	00.5±0.4 ^a	00.8±0.1bc	00.9±0.0 ^{bc}	00.9±0.2 ^{bc}	01.0±0.2 ^c	
Non denervated ALD	НW		.07±.00 ^a	.07±.00 ^a	.07±.00 ^a	.07±.00 ^a	.08±.01ª	.07±.00 ^a	
weight / body weight (%)	LW		.07±.00 ^a	.12±.07 ^b	.07±.00 ^a	.07±.00ª	.07±0.00 ^a	.07±.00ª	
Non denervated PLD	нw		.07±.00 ^a	.06±.01ª	.06±.01 ^a	.07±.00 ^a	.06±.01 ^a	.06±.01ª	
weight / body weight (%)	LW		.05±.01ab	.05±.00 ^b	.06±.00bc	.05±.00 ^b	.05±.00ab	.05±.01ª	

S.O., sham operated; Den, denervated; Con, controlateral. Means with common letters are not significantly different (P<0.05)

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Fig (1): Muscle weights following denervation of anterior latissimus dorsi and posterior latissimus dorsi expressed as a percentage of control muscle weights.

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Fig (2): Diameter changes in type III fibers [anterior latissimus dorsi (ALD)] or type II fibers [posterior latissimus Dorsi (PLD)]. Data are expressed as a percentage of controls. S.O., sham operated.

(*) indicated a significant difference between both strains.

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Fig (3): bar: 30 micrometers

A: Histological appearance of the ALD contralateral muscle. Frozen section, Hematoxillin eosin, x400.

B: Histological appearance of the ALD denervated muscle of the same animal. Day 28. Frozen section, Hematoxillin eosin, x400. A mild hypertrophy of the myofibers could be observed.

C: Histological appearance of the PLD contralateral muscle. Frozen section, Hematoxillin eosin, x400.

D: Histological appearance of the PLD denervated muscle of the same animal. Day 28. Frozen section, Hematoxillin eosin, x400. A severe atrophy of the myofibers is observed.

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Fig (3): bar: 30 micrometers

E: NADH-TR reaction on a denervated ALD, Day 28 x400: The distribution of the mitochondria is modified with less enzyme activity in the center of the fibers.

F: Acid Phosphatase reaction on a denervated ALD; x400. A necrotic fiber is phagocyted by macrophagic cells (colored in red by their Acid phosphatase lysosomial content)

G: Basic ATPase reaction on a denervated ALD, Day 28. Small dark stained fibers are myotubes resulting of the proliferation and fusion of satellite cells.

H: Immunohistofluorescence against Ventricular isomyosin: ALD Day 35 x500. Only small myotubes exhibit this developmental isomyosin.

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Figure 4. Percentage (means \pm S.D.) of activated satellite cells in denervated muscles. S.O., sham operated. (*) indicate a significant difference between both strains

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Animals of this issue

Domestic Fowl (Gallus gallus domesticus)



Kingdom: Animalia & Phylum: Chordata & Class: Aves & Order: Galliformes & Family: Phasianidae & Subfamily: Phasianinae & Genus: *Gallus &* Species: *G. gallus &* Subspecies: *G.g.domesticus*

The **chicken** (*Gallus gallus domesticus*) is a domesticated fowl, a subspecies of the Red Junglefowl. As one of the most common and widespread domestic animals, with a population of more than 24 billion in 2003, there are more chickens in the world than any other species of bird. Humans keep chickens primarily as a source of food, consuming both their meat and their eggs.

Chickens are omnivores. In the wild, they often scratch at the soil to search for seeds, insects and even larger animals such as lizards small snakes or young mice.

Roosters can usually be differentiated from hens by their striking plumage of long flowing tails and shiny, pointed feathers on their necks (*hackles*) and backs (*saddle*), which are typically of brighter, bolder colours than those of females of the same breed. The identification can be made by looking at the comb, or eventually from the development of spurs on the male's legs. Adult chickens have a fleshy crest on their heads called a comb, or cockscomb, and hanging flaps of skin either side under their beaks called wattles. Both the adult male and female have wattles and combs, but in most breeds these are more prominent in males.

Source: Wikipedia, the free encyclopaedia