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THE NECESSITY OF SATELLITE CELL PROLIFERATION FOR TURKEY SKELETAL MUSCLE GROWTH

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Background

Skeletal muscle mainly consists of long multinucleate cells (myofibers) that are incapable of further mitotic division (Stockdale and Holtzer, 1961). Post-hatch skeletal muscle growth occurs through a cellular hypertrophy accompanied by the gain of additional DNA units (myonuclei) through satellite cell fusions (Moss and Leblond, 1971), without significant increases in myofiber number (Smith, 1963).

The DNA unit size (cytoplasmic volume to nucleus ratio (CNR)) is the amount of cytoplasm theoretically controlled by each myonucleus (Cheek, 1985). Some authors have suggested that new myonuclei are added at a rate sufficient to maintain a constant CNR during post-hatch myofiber growth (Moss, 1968; Winchester and Goynea, 1992). A ramification of a constant CNR is that the rate of myofiber growth would be principally dependent upon the rate of myonuclear accretion. However, the results of other authors have suggested that increases in CNR also play a role in myofiber growth (Landing *et al.*, 1974).

Irradiation may be a useful tool to study the ability of myofibers to grow through increases in CNR because it is generally accepted that irradiation kills mitotically active cells, such as satellite cells, while leaving postmitotic cells, such as myofibers, unaltered (Coggle, 1983). It has been suggested that an irradiation dose of 25 Gy will sterilize satellite cells from mammalian muscle (Rosenblatt and Parry, 1993). The elimination of satellite cells, while leaving normal myofibers, in growing muscle provides an excellent model to study myofiber growth and adaptation without the addition of satellite cell derived myonuclei.

Objectives

The first objective of these studies was to examine the relative contributions of myonuclear increases through satellite cell fusions and increases in CNR to myofiber growth in rapidly growing tom turkeys (Mozdziak *et al.*, 1994a). The second objective was to determine the effect of an irradiation dose of 25 Gy on turkey skeletal muscle growth, satellite cell mitotic activity, and CNR. The rationale of the second study was to reduce the satellite cell population during the early post-hatch skeletal muscle growth period to examine myofiber compensation for the resultant deficit in myonuclear accretion by increasing CNR.

Methods

Characterization of Turkey Myofiber Growth

Tom turkeys (Nicholas strain) at 3 (n = 5), 6 (n = 5), 9 (n = 3), 18 (n = 5), and 26 (n = 3) weeks of age were injected with 5-bromo-2'-deoxyuridine (BrdU) at a dose of 100 μg/g body weight. BrdU is only incorporated into nuclei that are synthesizing DNA. Toms were killed 1 hour after BrdU injection and thin tissue strips (0.5 cm^2 X 4 cm) were harvested from the right *Pectoralis thoracicus* and *Biceps femoris*, immersed in Carnoy's fixative (overnight), and prepared for BrdU immunohistochemistry as described below.

Irradiation

The left *Pectoralis thoracicus* of 2-week-old tom turkeys (Nicholas strain) were exposed to a 6 MeV electron beam to receive a dose of 25 Gy. The right *Pectoralis thoracicus* served as a non-irradiated control. Toms were implanted (subcutaneously) with mini-osmotic pumps (2ML1, Alzet®, Palo Alto, CA) 1 week prior to euthanasia (0.25 mg BrdU/hour per kg body weight). Toms were killed 1, 4, 7, and 15 weeks following irradiation. Immediately following euthanasia, the left (irradiated) and right (non-irradiated) *Pectoralis thoracicus* were removed from the birds and weighed. Thin tissue strips (0.5 cm^2 X 4 cm) were taken from the irradiated and non-irradiated muscles, immersed in Carnoy's fixative (overnight), and prepared for BrdU immunohistochemistry as described below.

Immunohistochemistry and Image Analysis

Immunohistochemical and image analysis procedures were carried out following the procedures of Mozdziak *et al.* (1994b). Briefly, labeled satellite cells were identified on enzymatically isolated myofiber segments using a mouse primary monoclonal antibody (anti-BrdU) followed by fluorescein-5-isothiocyanate (FITC) conjugated goat anti-mouse IgG secondary antibody. Myofiber nuclei (satellite cell nuclei + myonuclei) were counterstained with propidium iodide (25-50 μg/mL PBS).

Myofiber segments were observed using a Nikon inverted microscope equipped with epifluorescence illumination and a SIT video camera. Labeled satellite cells were observed using a FITC filter set, and myofiber nuclei were observed using a Texas Red filter set. Myofiber segment dimensions as well as the number of FITC and PI labeled nuclei were determined with the assistance of Image-1® (West Chester, PA) software. At least 1,000 myofiber nuclei (satellite cell nuclei + myonuclei) were counted from each muscle examined, and an index of satellite cell mitotic activity was expressed as the number of BrdU labeled satellite cell nuclei per 1,000 myofiber nuclei. The DNA unit size was calculated as the cytoplasmic volume to nucleus ratio [$CNR=\pi(\text{myofiber segment diameter})/2)^2$ (myofiber segment length)/myofiber nuclei]. Data were analyzed using the General Linear Models (GLM) procedure of SAS® (SAS Institute, 1985). Treatment means were separated on the basis of Least Significant Differences (Ott, 1988). If population variances were found to be unequal, a logarithmic transformation or a nonparametric Kruskal-

Wallis test was performed (Conover, 1980).

Results and Discussion

Characterization of Turkey Myofiber Growth

There was an age-related ($P < 0.05$) increase in body weight and myofiber diameter between each age examined indicating that myofibers were growing. Satellite cell mitotic activity was highest in 3-week-old toms (13.7 ± 1.1/1,000 *Pectoralis thoracicus*; 16.4 ± 1.7/1,000 *Biceps femoris*), and it declined as the birds aged, until it reached essentially zero in 26-week-old toms (0.3 ± 0.3/1,000 *Pectoralis thoracicus*; 0.0 ± 0.0/1,000 *Biceps femoris*). The age-related decline in satellite cell mitotic activity was similar to observations in other species (Allbrook *et al.*, 1971; Knizetova *et al.*, 1972), but satellite cells were nearly mitotically quiescent in 9-week-old turkeys that were still growing rapidly. The CNR increased ($P < 0.05$) from 3 to 6 weeks of age, but from 6 to 9 weeks of age, it did not ($P > 0.05$) change. However, between 9 and 26 weeks of age there was a steady increase ($P < 0.05$) in CNR. Myofibers in both the *Pectoralis thoracicus* and *Biceps femoris* exhibited an early growth phase dependent on satellite cell fusions and increases in CNR, and a later growth phase almost entirely dependent on increases in CNR.

Irradiation

The left (irradiated) *Pectoralis thoracicus* weighed the same ($P > 0.10$) as the right (non-irradiated) *Pectoralis thoracicus* 1 week following irradiation, but at 4, 7, and 15 weeks following irradiation, the irradiated muscles were significantly ($P < 0.05$) smaller than the non-irradiated muscles. However, there was an increase ($P < 0.05$) in irradiated muscle weight between each post-irradiation interval indicating that the irradiated muscles retained the ability to grow. Satellite cell mitotic activity was significantly ($P < 0.05$) lower in the irradiated than the non-irradiated muscles 1 and 4 weeks following irradiation because irradiation resulted in a reduced satellite cell population. Satellite cell mitotic activity was significantly ($P < 0.05$) higher in the irradiated than the non-irradiated muscles 7 weeks following irradiation suggesting a possible compensation for the previous reduction in satellite cell numbers. However, the compensatory response by the satellite cell population was abolished by 15 weeks following irradiation when satellite cell mitotic activity was the same ($P > 0.10$) in the irradiated and non-irradiated muscles. There was an age-related increase ($P < 0.05$) in CNR between all intervals examined, but there were no significant ($P > 0.10$) differences discovered between the irradiated and non-irradiated muscles at any one age indicating that irradiated myofibers did not compensate for the reduction in the satellite cell population by increasing CNR.

Conclusions

Turkey myofiber growth may be partitioned into three phases. The first phase occurred from 3 to 6 weeks of age; it was characterized by high satellite cell mitotic activity and increased CNR. The second phase occurred from 6 to 9 weeks of age and was characterized by decreased satellite cell mitotic activity and a constant CNR. Between 9 and 26 weeks of age, myofiber growth occurred through increased CNR with little contribution from new satellite cell derived myonuclei.

Irradiation at 25 Gy did not sterilize satellite cells from growing tom turkey *Pectoralis thoracicus*. More accurately, a 25 Gy dose induced a short term reduction in the satellite cell population that resulted in a long term reduction in skeletal muscle growth. The results suggest that compensatory mechanisms in the muscle modulated the mitotic behavior of the satellite cells that remained following irradiation rather than increasing CNR. The irradiated myofibers retained the capacity to increase CNR with age, but the increase did not surpass non-irradiated control levels. The results of these studies suggest that a transient reduction in myonuclear accretion during the early phase of skeletal muscle growth (before 6 weeks of age) results in a reduction in the ability of myofibers to grow, and a reduction in the yield of lean meat at market age.

Pertinent Literature

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