

Modulation of Muscle Fiber Type

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Introduction

Feral fallow deer have existed in New Zealand for over 100 yr, but only in the last 10 yr has this species been farmed. Deer farmers have obtained animals from feral populations and have not as yet selected for live-animal or carcass traits. Nevertheless, fallow deer carcasses (Gregson and Purchas, 1983; Asher, 1983), like those from red deer (Drew and Greer, 1977; Tan and Fennessey, 1981) have a fat content only one-third that of cattle and sheep.

As the mating season for deer approaches, neck muscles of male deer enlarge. In male sheep, cattle and red deer, the splenius neck muscle has one of the highest sex-related growth ratios (Jury et al., 1977; Tan 1981; Tan and Fennessey, 1983; Butterfield et al., 1983). A major difference between male deer and cattle or sheep is that necks of deer enlarge prior to the rut and then regress to their prerut size, while necks of bulls and rams remain enlarged. Seasonal neck development in deer and the appearance of other secondary sex characteristics are accompanied by a dramatic 1000-fold increase in plasma testosterone levels (Lincoln, 1971; Lincoln and Kay, 1979; Muir et al., 1982). Testosterone causes this neck enlargement in deer by stimulating muscle fiber growth, and might also influence the distribution of muscle fiber types (Young and Bass, 1984). In addition, there may be a change in fiber type with growth which is independent of testosterone (Ashmore et al., 1972; 1973; Moody et al., 1980). Because considerable debate has focused on fiber type change, it was felt that data obtained on a muscle with rapid seasonal growth that is accompanied by a rapid rise in testosterone could be useful. Testosterone and/or rapid muscle growth could affect muscle quality as well as muscle mass because muscle fiber type determines the biochemistry of muscle and therefore dictates the ultimate properties of the meat (Cassens, 1977).

The objectives of this study were to determine the influence of season and/or testosterone levels on muscle growth, fiber occurrence and fiber area in the splenius neck muscle of male fallow deer.

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Experimental

Ten male fallow deer about 25 mo old, all from the same farm, were used. Five were slaughtered in the New Zealand summer on January 18 and five were slaughtered the following autumn on April 6. The autumn slaughter date was 1 wk before mating began in a comparable breeding herd of fallow deer and it was at a time when neck muscle enlargement and plasma testosterone concentrations of male fallow deer peak (G.W. Asher, personal communication). The summer slaughter date was shortly after plasma testosterone levels and neck circumference began to increase (Field et al., 1985). Therefore, the summer and autumn slaughter dates represent prerut and start of the rut conditions, respectively.

Immediately after slaughter, samples of splenius muscle were removed from the same anatomical location for each deer and frozen in iso-pentane cooled to partial freezing in liquid nitrogen. Two frozen transverse serial sections (10 μ m thick) were cut from each of the samples in a cryostat set at -18° C. Myofibrillar ATPase activity at pH 9.4 was examined in one serial section after preincubation in formaldehyde fixative (Guth and Samaha, 1969), and NADH-tetrazolium oxidoreductase activity (Dubowitz and Brooke, 1973) was examined in the other. Representative areas of each section were photographed through a Leitz Orthoplan microscope in normal transmission mode, and fiber areas were measured on enlarged photomicrographs with a digitizer (model EDT11, Houston Instruments, Austin, Texas), linked to a computer.

Classification of fibers in sections was determined by ATPase activity. Nearly all fibers stained unequivocally dark or light, and no further classes were resolved at pH values above or below 9.4. In the sections stained for NADH-tetrazolium oxidoreductase activity, all fibers were either high or medium in staining intensity, but no fibers low in staining intensity were present. Therefore, type I (slow contracting, oxidative) and type IIA (fast contracting, oxidative-glycolytic) fibers dominated the splenius and type IIB (fast contracting, glycolytic) fibers were absent (Young, 1984).

Classification of fibers was determined by two different methods. First, 85 adjacent fibers in the center of each of two photomicrographs were classed as type I or type IIA. In the second method, percent occurrences of fiber types were measured with transect lines struck at random across the photomicrograph and all fibers that touched the lines were classified. This latter method was questioned by those who reasoned that larger fibers would touch the transect line more often than smaller fibers. Therefore, the percentage of small-

er type I fibers would be underestimated and the larger type IIA fibers overestimated. Data obtained in this study are based upon 85 adjacent fibers in each of two photomicrographs for each animal. Nevertheless, data from the transect line method are also mentioned for comparative purposes.

From the right side of each carcass, six muscles were dissected and weighed so that growth of individual muscles could be compared. Splenius, semispinalis capitis and obliquus capitis caudalis were dissected from the neck, longissimus from the back, and semitendinosus and semimembranosus were dissected from the hind leg.

Muscle Growth

The effect of season on muscle weights is shown in Table 1. Whether expressed as weight or weight with the effect of carcass weight removed, two of the three neck muscles were significantly ($P < 0.01$) larger in autumn. The weight of the splenius and obliquus capitis caudalis muscles increased from summer to autumn but increased weight of the semispinalis capitis in the autumn was not significantly different when the effect of carcass weight was removed. The longissimus, semitendinosus and semimembranosus muscles tended to be heavier in autumn, but when the effect of carcass weight was removed, weights tended to decrease from summer to autumn, indicating that during this time these muscles grew more slowly than other carcass components, particularly the neck muscles. The proportion of subcutaneous fat and intermuscular fat in the neck region and in other parts of the carcass did not change with season (data not shown) so the increase in neck circumference of male deer as the rut approaches is undoubtedly a result of muscle growth. The neck muscle that enlarges the most is the splenius.

The disproportional growth of the splenius compared to other muscles is due to testosterone (Lincoln, 1971; Fletcher, 1978; Muir et al., 1982). This neck muscle can be considered a target organ for testosterone, similar to the temporalis muscle of guinea pigs, which also enlarges in response to testosterone (Kochakian et al., 1966, Bass et al., 1971).

Fiber Type Occurrence

Fiber type occurrence in splenius muscle by season is shown in Table 2. In spite of the rapid growth of the splenius muscle and the dramatic increase in plasma testosterone levels (Lincoln, 1971; Lincoln and Kay, 1979; Field et al., 1985), splenius muscle fiber types did not change significantly ($P < .05$) over the 11 wk period from summer to autumn. The findings are in line with Field et al. (1985). They found that 34.8% and 34.3% of the fibers in splenius muscle of fallow deer in the spring and autumn respectively were type I.

Whereas testosterone did not affect fiber occurrence in deer splenius, there are reports describing effects of testosterone on fiber occurrence in other species. Young and Bass (1984) reported that castration of bulls, which reduces plasma testosterone concentration, resulted in a variable but significant change in ATPase staining. This effect was particularly obvious in the longissimus muscle. In other species, androgens also influence fiber type distribution and myosin ATPase activity. For example, in the soleus muscle of the mouse, intact males have significantly higher percentages of glycolytic fibers (high ATPase) than castrates or females (Vaughan et al., 1974). In male frogs, fibers with low ATPase activity are present during the mating season, but in females, all fibers have a relatively high ATPase activity (Melichna et al., 1972). Therefore, testosterone does not affect the distribution of fiber types in all muscles of all species the same way.

The lack of change in fiber types of splenius muscle from prerut (summer) to the start of the rut (fall) also has implications with regard to exercise. Neck muscles of the male fallow deer immediately prior to the start of the rut are used more than they are earlier in summer. Pre-mating activities in the fall include fraying branches and horning fence posts with the antlers, vocalizing and defending a "stand." The present data support the contention of little or no convertibility with exercise between types I and II fibers in humans (Gollnick, 1982). Nevertheless, a few studies have suggested that exercise results in a conversion between these two fiber types (Howald, 1982). It is well documented in human sub-

Table 1. Weights of Six Muscles from Male Fallow Deer in Summer and Autumn (n=5 for each season)

| Item | Weight | | | Weight with the effect of carcass weight removed | | |
|----------------------|--------|--------|-------|--|--------|-------|
| | Summer | Autumn | SE | Summer | Autumn | SE |
| Carcass kg | 26.2 | 31.8 | 1.6** | | | |
| Muscles, g | | | | | | |
| Splenius | 28 | 73 | 6.2** | 37 | 64 | 7.3** |
| Semispinalis capitis | 79 | 105 | 6.8** | 88 | 96 | 8.2 |
| O. capitis caudalis | 46 | 76 | 3.2** | 50 | 72 | 4.3** |
| Longissimus | 893 | 934 | 53.5 | 978 | 850 | 45.5* |
| Semitendinosus | 192 | 202 | 8.3 | 204 | 191 | 9.6 |
| Semimembranosus | 631 | 696 | 33.7 | 680 | 648 | 35.4 |

*Means are significantly different $P < .05$.

**Means are significantly different $P < .01$.

Table 2. Means for Occurrence and Area of Muscle Fibers in the Splenius of Male Fallow Deer in Summer and Autumn (n = 5 for each season)

| <i>Item</i> | <i>Summer^a</i> | <i>Autumn^a</i> | <i>SE</i> |
|-----------------------------|---------------------------|---------------------------|-----------|
| Fiber occurrence, % | | | |
| Type I | 37.2 | 34.2 | 1.8 |
| Type IIA | 62.8 | 65.8 | 1.8 |
| Fiber area, μm^2 | | | |
| Type I | 649 | 1228 | 70.6** |
| Type IIA | 776 | 1980 | 185.1** |

^a Tissues for fiber typing and fiber area were removed from the same anatomical location of the splenius muscle at time of slaughter. Dates were January 18 (summer) and April 6 (autumn). In NZ fallow deer begin matings in early April.

**Means are significantly different $P < 0.01$.

jects that long-distance runners have a larger percentage of type I fibers than controls or weight lifters (Staron et al., 1984) but whether or not the predominance of the type I fibers in these endurance athletes is induced through training or is genetically predetermined remains to be resolved.

The same general pattern of fiber types in the splenius of fallow deer in this study and in cattle (Young and Bass, 1984) exists. That fiber type distribution is similar for a domesticated species (cattle) and a wild species (fallow deer) is unexpected. Rahelic and Puac (1981) reported that two out of three wild pigs had 100% oxidative fibers in the longissimus muscle. Glycolytic fibers in the longissimus of the third wild pig were attributed to cross breeding with domestic pigs. The similar distribution of about 65% type IIA and the remainder type I fibers in fallow deer and cattle may result from the splenius neck muscles of these two species having comparable work loads and movements. In contrast, the work load and movement of the longissimus muscles in domesticated pigs and wild pigs would be very different because of differences in shape of the pigs' backs and because of differences in the amount of exercise.

A number of factors cause variability of results with regard to changes in fiber types. For example, muscle training by endurance swimming in young but not older rats may cause an increase in the percentage of strong ATPase fibers in slow-contracting muscles but not in fast-contracting muscles (Syrový et al., 1972). Anabolic derivatives of testosterone enhance the muscle response in female rats to isometric exercise training but not in male rats (Exner et al., 1973a, 1973b). In rats, treadmill running causes a decrease in biochemically determined ATPase activity in fast-contracting aerobic muscle, but not in fast-contracting anaerobic muscle (Baldwin et al., 1975). In addition, muscle fiber types vary among muscles and within cross-sectional areas of muscle (Hunt and Hedrick, 1977) and failure to recognize this could result in variability of results. After a convincing review of the literature showing that in some instances fiber types do change, Swatland (1984) asks whether muscle fibers really change from one histochemical fiber type to another, or do the percentages of fiber types change because of fiber losses or rearrangement?

Methods for Determining Fiber Occurrence

Apparent changes in fiber types may also occur because of methods used. For example, when transect lines were struck across photomicrographs in the present study, occurrence of type I fibers was two percentage points lower and that for type IIA fibers was two percentage points higher than the values listed in Table 2. Therefore, when transect lines are used, those fiber types which enlarge the most during growth would appear to increase in number. Counting total number of fibers within specific fiber bundles could also be misleading because the smaller type I fibers tend to be found in the center of fiber bundles and smaller fiber bundles could vary in percentage of type I fibers when compared to larger bundles. Nevertheless, Moody et al. (1980) concluded that muscle bundle size in the longissimus muscle of lambs had no significant relationship to percentage fiber types. The conclusion may not be true for all muscles of all species. Hedrick et al. (1971) and Miller et al. (1975) found that fiber occurrence did change with bundle size when Sudan Black B was used. Miller et al. (1975) found 13.7% dark fibers in small bundles compared to 12.6% in larger bundles. We believe that counting a specific number of adjacent muscle fibers in a circular area of a specific anatomical region in the muscle is the best way to determine fiber type occurrence. Begin by picking a fiber at random, then move one fiber to the right because a bigger fiber is more likely to be picked than a smaller one. Begin classification in a spiral manner until the n^{th} cell is struck. Edge effects, which could select large fibers, are nonexistent with this technique.

Fiber Growth

Whereas fiber occurrence did not change with season, fiber area changed markedly. Between summer and autumn, type I fibers virtually doubled in area and type IIA fibers increased by a factor of about 2.6. The season by fiber type interaction for fiber area was highly significant ($P < .01$), indicating that during splenius enlargement, type IIA fibers grew faster than type I fibers.

In autumn, the splenius enlarged because fiber area increased. Type IIA fibers played a greater part in this

enlargement than type I fibers, both because IIA fibers enlarged to a greater extent and because they were more prevalent (Table 2). Fibers in the splenius and in other muscles that enlarge before the rut may contain testosterone-binding proteins (Jung and Baulieu, 1972) to mediate testosterone's action. Within the splenius, IIA fibers may have more testosterone-binding proteins than type I fibers, accounting for the greater growth of IIA fibers. Alternatively, testosterone may act by stimulating the release of other hormones that directly cause neck muscle growth. Neural factors may also be involved, because thyroid hormone can only affect a skeletal muscle if its nerve supply is intact (Johnson et al, 1980).

More rapid growth in type IIA fibers than in type I fibers with muscle enlargement has not consistently been reported. Moody et al. (1980) and Solomon et al. (1981) studied the longissimus muscle of lambs. Type I fibers increased in diameter faster than other fiber types as weight increased. In contrast, Swatland and Cassens (1972) concluded that the greater part of the extra muscle mass of rats originated from the enlargement of fibers which are specialized for anaerobic metabolism rather than for aerobic metabolism. It appears that the contribution of fiber types to muscle growth varies with muscle and species of animal.

A final discussion topic about muscle fiber growth relates to growth of large and small fibers within a fiber type. Field et al. (1985) have shown that within fiber types, fibers are normally distributed in the splenius muscle of fallow deer both before and at the start of the rut and that coefficients of variation of both times are similar. These results strongly

suggest that all fibers within a type are equally liable to grow. For example, small type IIA fibers are not likely to grow more than larger type IIA fibers as the muscle enlarges from summer to autumn.

Summary

Carcass and splenius muscle fiber characteristics of farmed male fallow deer were studied. Five deer approximately 25 mo old were slaughtered in summer and five were slaughtered 11 wk later just before the mating season (rut) began. From summer to autumn, total muscle weight increased in concert with carcass weight. However, the increases in individual muscle weight were not the same: two of the three neck muscles studied grew more rapidly than the average, whereas the loin and two hind leg muscles grew more slowly. Cryosections of splenius, the neck muscle which grew the most, were stained for myofibrillar ATPase and for NADH-tetrazolium oxidoreductase activity. Slow oxidative fibers (type I) were less prevalent than fast oxidative-glycolytic fibers (type IIA) and fast glycolytic fibers (type IIB) were absent. From summer to autumn, fiber types did not change in occurrence, but fiber area increased markedly, with type I fibers almost doubling in area and type IIA fibers increasing by a factor of about 2.6. Contrasting results on changes, or lack of changes, in fiber types, and on growth or lack of growth of specific fiber types reported in the literature emphasize the importance of choice of species, sex and age of animal, type of exercise employed, and type of muscle for interpretation and comparison of results. Method of determining fiber type occurrence is also important.

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References

- Asher, G.W. 1983. Carcass yield and fatness of mixed age, farmed fallow bucks slaughtered in summer. In: K.R. Drew, P.F. Fennessy (Ed.) International Symposium on the Biology of Deer, Dunedin. Invermay Agric. Res. Centre (Mosgiel, N.Z.).
- Ashmore, C.R.; Tompkins, G.; Doerr, L. 1972. Postnatal development of muscle fiber types in domestic animals. *J. Anim. Sci.* 34:37.
- Ashmore, C.R.; Addis, P.B.; Doerr, L. 1973. Development of muscle fibers in the fetal pig. *J. Anim. Sci.* 36:1088.
- Baldwin, K.M.; Winder, W.W.; Holloszy, J.O. 1975. Adaptation of actomyosin ATPase in different types of muscle to endurance exercise. *Am. J. Physiol.* 229:422.
- Bass, A.; Gutmann, E.; Hanzlikova, V.; Syrový, I. 1971. Sexual differentiation of enzyme pattern and its conversion by testosterone in the temporal muscle of the guinea pig. *Physiol. Bohemoslov.* 20:423.
- Butterfield, R.M.; Zamora, J.; James, A.M.; Thompson, J.M.; Williams, J. 1983. Changes in body composition relative to weight and maturity in large and small strains of Australian Merino rams. 2. Individual muscles and muscle groups. *Anim. Prod.* 36:165.
- Cassens, R.G. 1977. Muscle biochemistry: the importance of myofiber type. *Food Technol.* 31:76.
- Drew, K.R.; Greer, G.J. 1977. Venison production and carcass composition of red deer. *N.Z. Agric. Sci.* 11:187.
- Dubowitz, V.; Brooke, M.H. 1973. *Muscle Biopsy: A Modern Approach.* W.B. Saunders Co., London.
- Exner, G.U.; Staudte, H.W.; Pette, D. 1973a. Isometric training of rats – Effects upon fast and slow muscle and modification by an anabolic hormone (nandrolone decanoate). I. Female rats. *Pflügers Arch.* 345:1.
- Exner, G.U.; Staudte, H.W.; Pette, D. 1973b. Isometric training of rats – Effects upon fast and slow muscle and modification by an anabolic hormone (nandrolone decanoate). II. Male rats. *Pflügers Arch.* 345:15.
- Field, R.A.; Young, O.A.; Asher, G.W.; Foote, D.M. 1985. Characteristics of male fallow deer muscle at a time of sex-related muscle growth. *Growth* (In press).
- Fletcher, T.J. 1978. The induction of male sexual behavior in red deer (*cervus alaphus*) by the administration of testosterone to hinds and estradiol-17 to stags. *Hormones and Behavior.* 11:74.

- Gollnick, P.D. 1982. Relationship of strength and endurance with muscle structure and metabolic potential. *Int. J. Sports Med.* 3:26.
- Gregson, J.E.; Purchas, R.W. 1983. The carcass composition of male fallow deer. In: K.R. Drew, P.F. Fennessy (Ed.) *International Symposium on the Biology of Deer*, Dunedin. Invermay Agric. Res. Centre, Mosgiel, N.Z.
- Guth, L.; Samaha, F.J. 1969. Qualitative differences between actomyosin ATPase of slow and fast mammalian muscle. *Exp. Neurol.* 25:138.
- Hedrick, H.B.; Laffety, D.T.; Aberle, E.D.; Judge, M.D. 1971. Relation of porcine muscle fiber type and size to postmortem shortening. *J. Anim. Sci.* 32:57.
- Howald, H. 1982. Training-induced morphological and functional changes in skeletal muscle. *Int. J. Sports Med.* 3:1.
- Hunt, M.C.; Hedrick, H.B. 1977. Profile of fiber types and related properties of five bovine muscles. *J. Food Sci.* 42:513.
- Johnson, M.A.; Mastaglia, F.L.; Montgomery, A.; Pope, B.; Weeds, A.G. 1980. Neurally mediated effect of thyroid hormone deficiency on slow-twitch skeletal muscle. In: D. Pette (ed.) *Plasticity of Muscle*. pp. 607-615. Walter de Gruyter, Berlin.
- Jung, I.; Baulieu, E. 1972. Testosterone cytosol receptor in the rat levator ani muscle. *Nature*, 237:24.
- Jury, K.E.; Fourie, P.D.; Kirton, A.H. 1977. Growth and development of sheep IV. Growth of musculature. *N.Z.J. Agric. Res.* 20:115.
- Kochakian, C.D. 1966. Regulation of muscle growth by androgens. In: E.J. Briskey, R.G. Cassens, J.C. Trautman (Ed.) *The Physiology and Biochemistry of Muscle as a Food*. pp. 81-112. The University of Wisconsin Press, Madison.
- Lincoln, G.A. 1971. The seasonal reproductive changes in the red deer stag (*cervus elaphus*). *J. Zool. (London)* 163:105.
- Lincoln, G.A.; Kay, R.N.B. 1979. Effects of season on the secretion of LH and testosterone in intact and castrated red deer stags (*Cervus elaphus*). *J. Reprod. Fert.* 55:75.
- Melichna, J.; Gutman, E.; Herbrychova, A.; Stichova, J. 1972. Sexual dimorphism in contraction properties and fiber pattern of the flexor carpe radialis muscle of the frog (*Rana temporaria*). *Experientia*, 28:89.
- Miller, L.R.; Garwood, V.A.; Judge, M.D. 1975. Factors affecting porcine muscle fiber type, diameter and number. *J. Anim. Sci.* 41:66.
- Moody, W.G.; Kemp, J.D.; Mahyuddin, M.; Johnston, D.M.; Ely, D.G. 1980. Effects of feeding systems, slaughter weight and sex on histological properties of lamb carcasses. *J. Anim. Sci.* 50:249.
- Muir, P.D.; Barrell, G.K.; Sykes, A.R. 1982. Modification of antler growth in red deer stags by use of a synthetic progestagen. *Proc. N.Z. Soc. Anim. Prod.* 42:145.
- Rahelic, S.; Puac, S. 1981. Fiber types in longissimus dorsi from wild and highly selected pig breeds. *Meat Sci.* 5:439.
- Solomon, M.B.; Moody, W.G.; Kemp, J.D.; Ely, D.G. 1981. Effect of breed, slaughter, weight and sex on histological properties of ovine muscle. *J. Anim. Sci.* 52:1019.
- Staron, R.S.; Hikida, R.S.; Hagerman, F.C.; Dudley, G.A.; Murray, T.F. 1984. Human skeletal muscle fiber type adaptability to various workloads. *J. Histochem. Cytochem.* 32:146.
- Swatland, H.J. 1984. *Structure and Development of Meat Animals*. pp. 236-239. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.
- Swatland, H.J.; Cassens, R.G. 1972. A brief study of muscle enlargement in the rat. *J. Anim. Sci.* 34:21.
- Syrový, I.; Gutmann, E.; Melichna, J. 1972. Effect of exercise on skeletal muscle myosin ATPase activity. *Physiol. Bohemoslov.* 21:633.
- Tan, G.Y. 1980. *Carcass development and cellular growth of muscle and fat in male and female cattle*. PhD thesis. Massey University, Palmerston North, N.Z.
- Tan, G.Y.; Fennessy, P.F. 1981. The effect of castration on some muscles of red deer (*Cervus elaphus* L.). *N.Z.J. Agric. Res.* 24:1.
- Vaughan, H.S.; Aziz-Ullah; Goldspink, G.; Nowell, N.W. 1974. Sex and stock differences in the histochemical myofibrillar adenosine triphosphatase reaction in the solus muscle of the mouse. *J. Histochem. and Cytochem.* 22:155.
- Young, O.A. 1984. The biochemical basis of fiber types in bovine muscle. *Meat Sci.* 11:123.
- Young, O.A.; Bass, J.J. 1984. Effect of castration on bovine muscle composition. *Meat Sci.* 11:139.