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CHARACTERIZATION OF HYPERCONTRACTED FIBERS IN SKELETAL MUSCLE OF DOMESTIC TURKEY (MELEAGRIS GALLOPAVO)

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Abstract

Several different muscles from a population of thirty Large White male turkeys were studied by histological and histochemical methods, and a high incidence of hypercontracted fibers was noted. The fibers were characterized in cross-section by an apparent swelling, a rounded rather than polygonal shape and a homogenous appearance. They were eosinophilic, positive to Gomori-trichrome and appeared to have an elevated lipid content. An unusual histochemical profile was noted. It was postulated that the hypercontracted fibers were real, not artifactual, and resulted from some change occurring in the muscle.

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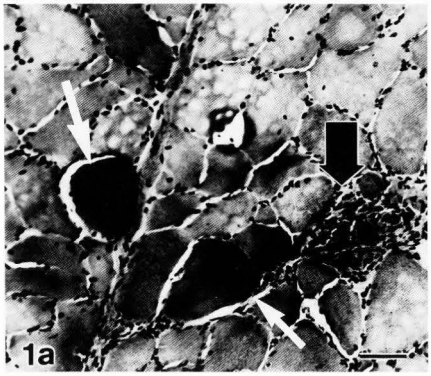
Introduction

Hypercontraction is an excessive contraction of myofibrils and is reflected by abnormally short sarcomeres (Carpenter and Karpati, 1984). Hypercontracted fibers in cryostat cross sections appear enlarged and rounded ("giant" fibers). Because the normal myofibrillar and intermyofibrillar staining pattern is lost, the terms "hyaline", "waxy" or "opaque" have also been used to describe these fibers (Wohlfart, 1937; Schmalbruch, 1973; 1975; Cullen and Fulthorpe, 1975; Carpenter and Karpati, 1984; Uchino and Araki, 1986). Although the occurrence of hypercontracted fibers in muscle tissue in human and domestic mammals (pig, cattle, sheep) has been noted previously by many authors, the underlying causes of the phenomenon are controversial.

Carpenter and Karpati (1984) pointed out that hypercontraction of fibers seen in biopsies from Duchenne muscular dystrophy patients takes place during biopsy. On the other hand, Cullen and Fulthorpe (1975) and Schmalbruch (1973, 1975) have proposed that hypercontraction in human diseased muscle is a pathological reaction of muscle cells. In animals, "giant" fibers have been found in normal muscle of wild and domestic mammals in both pre-rigor and post-rigor conditions (Weatherspoon, 1969; Linke, 1972; Dutson et al., 1978; Schmidt and Dumont, 1981; Handel and Stickland, 1986; Sink et al., 1986; Salomon and Eastridge, 1987). Scheper (1979) and Schmidt and Dumont (1977) observed more of this type of fiber in muscle of cattle or sheep which exhibited gross enlargement or hypertrophy. Likewise, "giant" fibers were often found in pigs having the conditions of Porcine Stress Syndrome (PSS), Stress-myopathy or Pale, Soft and Exudative (PSE) meat (Cassens et al., 1969; Dutson et al., 1978; Sosnicki and Domanski, 1983; Sosnicki, 1987).

Description of the histochemical profile of hypercontracted fibers has also varied significantly. They were observed as myosin Ca^{++}-ATPase positive (Cassens et al., 1969) or negative (Fenichel, 1963); succinic dehydrogenase (SDH) positive (De Bruin, 1971; Handel and Stickland, 1986) or variable (Dutson et al., 1978, Sink et al., 1986, Sosnicki, 1987); lactic acid dehydrogenase (LDH) variable (Sosnicki, 1987) or positive (Hraste et al., 1980).

Klosowska et al. (1980) have noted the occurrence of "giant" fibers in muscle of chicken. Grey et al., (1986) and Seemann et al., (1986) observed what they termed "large rounded fibers" in a study of turkey breast muscle and thought they were



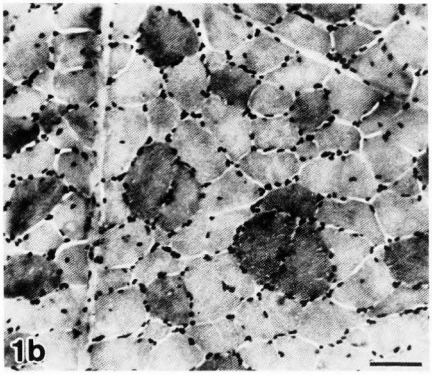


Figure 1 (a). Cross section showing muscle fiber undergoing necrosis (large arrow) and several hypercontracted fibers (small arrows). (b). Cross section showing normal appearing fibers. M. biceps femoris (Group A). H+E. Bar = 0.05 mm.

associated with toughness of the meat.

We have often observed hypercontracted fibers during the course of our histochemical studies of muscle of rapidly growing turkeys. The purpose of this work is to present frequency data about the hypercontracted fibers together with some possible explanation for their occurrence, and a description of histological and histochemical features is also provided.

Materials and Methods

Histological and histochemical studies of "giant" fibers were carried out on 30 Large White turkey males that were 14 (group A), 16 (group B) and 18 (group C) weeks of age (10 in each group). The birds were selected at random from commercial flocks which had been raised in light controlled houses and fed a standard corn/soybean meal poultry diet.

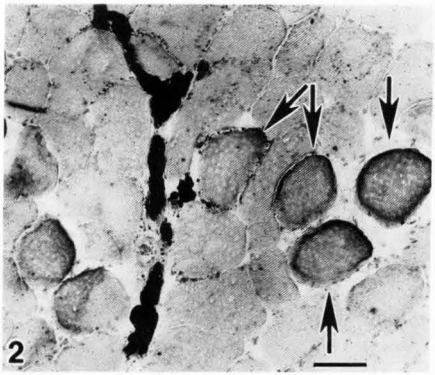
Samples for analysis were collected immediately post slaughter from 5 skeletal muscles: m. pectoralis thoracicus (superficialis), m. supracoracoideus (pectoralis deep), m. biceps femoris, m. semitendinosus and m. femorotibialis medius. To prevent trauma, muscle samples were held at rest length by forceps and then rapidly frozen in isopentane cooled with liquid nitrogen.

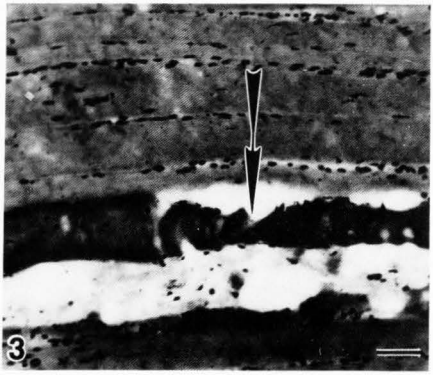
Serial cross and longitudinal sections 8 micrometers thick were obtained with a cryostat. In the analysis of histological traits, hematoxylin and eosin (H&E), modified Gomori-trichrome and Oil red O staining techniques were used. Histochemical reactions were conducted in order to show activity of myosin Ca^{++}-ATPase (Guth and Samaha, 1970), succinic dehydrogenase (SDH) (Barka and Anderson, 1963), alkaline and acid phosphatases and phosphorylases a and b (Chayen et al., 1973).

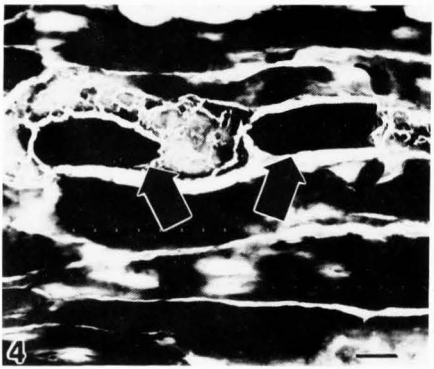
Results

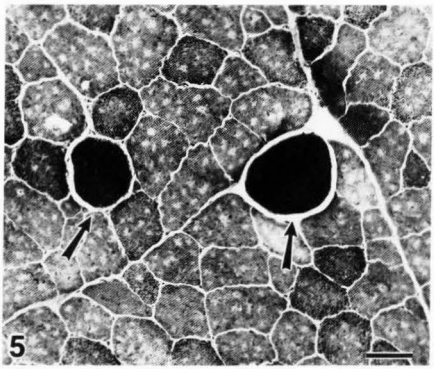
While an exact quantification was not made, the muscle incidence was considered positive if hypercontracted fibers were present in a low power (25x) of cross-sectioned muscle (1.68 mm^2 area). A uniform pattern of incidence of the hypercontracted fibers in the particular birds was not seen nor were certain muscles more prone to fiber hypercontraction than others. They appeared to be distributed randomly throughout any given muscle bundle and were not located preferentially in either peripheral or central areas of the bundle. In some instances, only a few single, scattered hypercontracted fibers were seen, but, in other cases, they appeared in close proximity (being almost grouped) in numbers of three or more. In rare instances, up to twenty-five percent of the fibers in a small area appeared affected. In view of this distribution, we merely counted a bird positive if one or more hypercontracted fibers were present (see above for area viewed). From Table 1, it appears that the older birds had a higher incidence.

Typical hypercontracted and normal appearing muscle fibers are illustrated in Figures 1a and 1b respectively. In comparison to normal muscle cells, an apparent swelling of hypercontracted fibers was observed, and in cross-section they had a rounded rather than polygonal shape. They had a homogeneous appearance (described typically as "waxy" or "hyaline"), were eosinophilic and stained a uniform dark-red with the Gomori-trichrome procedure. The content of intracellular fat was also higher in comparison to that present in normal muscle cells (Figure 2). However, interpretation of this may be confused by a change of the histological staining appearance due to the contraction (an apparent stronger staining intensity may result from the denseness due to contraction). The hypercontracted fibers also had a characteristic appearance in longitudinal section (Figure 3). In some areas a strong contraction was obvious with associated tearing and separation of the contents of the fibers. Open areas were observed in many cases surrounding the hypercontracted fibers both in longitudinal (Figure 3) and cross









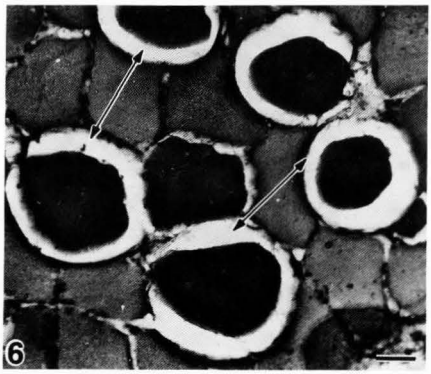


Figure 2. A higher content of intracellular fat in hypercontracted fibers (arrows) as shown with oil Red O staining. M. pectoralis thoracicus (Group A).

Figure 3. Longitudinal section showing a "compresse" appearance of hypercontracted fibers (arrow). M. pectoralis thoracicus (Group C). H+E.

Figure 4. Multifocal hypercontraction (arrows) within a fiber undergoing necrosis. M. supracoracoideus (Group C). Gomori-trichrome.

Figure 5. Hypercontracted fibers showing high SDH reaction (arrows); other muscle fibers show a weak activity. M. femorotibialis medius (Group B).

Figure 6. Ca^{++}-ATPase reaction (pH 4.60) showing a high activity in hypercontracted fibers (arrows). M. semitendinosus (Group B).

Bar (for all Figures) = 0.05 mm.

Table 1. Number of birds in which the hypercontraction of muscle fibers was recorded

Muscle	Groups[1]/Age in weeks A / 14	B / 16	C / 18
Pectoralis thoracicus	7	7	9
Supracoracoideus	8	7	10
Biceps femoris	7	7	9
Semitendinosus	7	7	9
Femorotibialis medius	7	6	9

[1] All groups contained 10 birds

sections (see also Figure 6). In other instances an apparent degeneration was observed in association with the hypercontracted fibers (Figure 4).

Because the reaction pattern of the hypercontracted fibers was not typical, the histochemical profile was somewhat confusing (Table 2). A strong positive SDH reaction was exhibited (Figure 5) and a positive reaction was found for myosin Ca^{++}-ATPase after either alkaline (pH 10.4) or acid preincubation (pH 4.6) (see Figure 6). For phosphorylase a and b, some cases of weak reaction were found but the majority of hypercontracted fibers possessed high activity. Therefore, a simple classification of the hypercontracted fibers as slow-oxidative (Type I), fast-glycolytic (Type IIb) or fast oxidative-glycolytic (Type IIa) was not possible. Positive, but diffuse, reactions for acid and alkaline phosphatase were observed.

Discussion

The question of artifact always deserves consideration, and hypercontracted fibers are a case in point - are they artifactual or reflective of an in vivo pathological reaction?

Carpenter and Karpati (1984) reported that hypercontracted fibers, seen in almost all biopsies from Duchenne dystrophic patients, did not show features of necrosis, rarely occurred in groups, and transitional stages between hypercontraction and true necrosis were not observed. Conversely, Cullen and Fulthorpe (1975) described the process of hypercontraction as resulting in the contractile filaments forming a homogenous mass (hyaline degeneration). The next step of degeneration was described as true necrosis of hypercontracted fibers (Cullen and Fulthorpe, 1975). Likewise, Schmalbruch (1975) reported some of the hypercontracted fibers contained phagocytes and macrophages, and they showed closely attached regenerating fibers with normal sarcomere spacing.

There is the question if the hypercontracted fibers we observed are an intermediate stage between normal fibers and those undergoing a necrotic change. We did not observe obvious regenerative changes. However, parts of a hypercontracted fiber in close proximity to a necrotic fiber and an area of mononuclear cell invasion were often seen. In addition, we noted a positive acid phosphatase activity in hypercontracted fibers which can be taken as evidence of necrosis. In the final step of breakdown of hypercontracted fibers fatty tissue replacement usually occurred (Cullen and Fulthorpe, 1975). Similar symptoms were observed in our study.

There may be a local inability of the sarcomeres to relax, which implies a defect of mitochondria or sarcoplasmic reticulum (Cullen and Fulthorpe, 1975). In other words, hypercontraction may be caused by an influx of extracellular calcium into the muscle fibers (Carpenter and Karpati, 1984). Indeed, the high enzyme reaction of hypercontracted fibers seen in the previous, and present study (see Table 2), may result from fragment breaks in the surface membrane in the vicinity of the observed hypercontraction (Carpenter and Karpati, 1984; Handel and Stickland, 1986, Levin et al., 1981, Sink et al., 1986). Furthermore, functional stressing of muscle fibers (i.e. a strong exercise) has been shown to affect calcium ion uptake and muscle relaxation, and consequently the fibers are thought to be more prone to post-mortem lysis (Salomons and Henriksson, 1981). On the other hand, evidence is also available to support the concept that the subcellular components of abnormal fibers (porcine PSE muscle) are more susceptible to the effects of freeze-thaw contraction (Cloke et al., 1981).

Finally, regarding an explanation for the basis of occurrence of hypercontracted fibers, we cannot make a definite conclusion. We favor the explanation that they are real and may be associated with actual changes occurring in the muscle. We conclude this because we did observe some areas of apparent degeneration and fatty tissue replacement in longitudinal sections of hypercontracted fibers. Moreover, the positive reaction for acid phosphatase in the hypercontracted fibers and an unusual histochemical profile support the idea that they are real and are associated with a developing or present muscle pathology.

Acknowledgements

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Table 2. The characteristic staining and reaction pattern of normal and hypercontracted muscle fibers

Type of staining or reaction	Type of muscle fibers		
	I slow-oxidative	II fast-glycolytic	hypercontracted
H & E	positive (pink)	positive (pink)	eosinophilic (red)
Gomori-trichrome	positive (green)	positive (green)	positive (dark red)
Oil Red O	high	low	high
ATPase, pH 9.4	low	high	high
ATPase, pH 4.6	high	low	high
SDH	high	low	diffuse or high
Alkaline phosphatase	no activity	no activity	diffuse
Acid phosphatase	no activity	no activity	diffuse
Phosphorylases a, b	low	high	high

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Discussion with Reviewers

Reviewer I: Was all the biopsied material frozen in isopentane cooled with liquid nitrogen? Although frozen tissue is necessary for histochemical studies and an H&E stain done on this material provides a "control" for histochemical studies, the basic morphology of the muscle seen by H&E stain might better be done on formalin fixed tissue.
Authors: All the muscle samples studied in this experiment were frozen in isopentane cooled with liquid nitrogen. We agree that a better morphology would undoubtedly have been seen if H&E staining had been done on formalin fixed tissue rather than on unfixed frozen tissue. However, formalin fixed muscle fibers usually have a more rounded shape in contrast to the more angular or polygonal shape of muscle fibers in frozen section. The rounded shape might have caused some confusion in interpretation of the hypercontracted fibers.

Reviewer II: Some experiments to be considered would be the effect of trauma at the time of slaughter where one might compare a lethal dose of Nembutol to exsanguination. Also, to answer the question of growth hypertrophy to the appearance of hypercontracted fibers, one might supplement the turkey's diet with steroids.
Authors: We did not study sacrifice by Nembutol injection or the effect of steroids in this experiment. Trauma during transport and slaughter is always a concern. The reasons for believing that the hypercontracted fibers are not artifactual are explained in the text.

Reviewer III: In regard to quantitation of hypercontracted fibers, how many fields per muscle per bird were counted and what was meant by "a slightly higher incidence of these fibers"?

Reviewer IV: How many hypercontracted fibers are found per bird and does the frequency increase with time?
Authors: We have not done a statistical quantification but merely reported that the hypercontracted fibers appeared quite frequently in the birds examined. Our work was directed more at an examination of the properties of the fibers in an attempt to determine if they were real or artifactual. One field under low power magnification (25x) is equal to about 1.65 mm^2, and the approximate area of our cryostat sections was 1 cm^2. Therefore, we usually evaluated about 60 fields for a given muscle and because the samples were collected from 5 different muscles, about 300 fields were evaluated per bird.

Reviewer III: If necrosis has occurred, would one expect to find regenerative changes?
Authors: Yes, the degeneration and regeneration processes usually occur together and we would expect to observe evidence of regeneration--but such was not the case. Necrosis is not always accompanied by regeneration, and, for example, is unlikely under ischemic conditions.

Reviewer III: What about the possibility of lysosomal proteases degrading the sarcolemma?
Authors: This is not an easy question because there is no direct evidence on the matter. One explanation for hypercontraction is an increase of available calcium in the fiber which could result from damage to the fiber during the preslaughter time or during collection and freezing of the sample. Our observations on acid phosphatase showed a positive but diffuse activity. We believe lysosomal proteases do not directly cause hypercontraction of fibers but may act in a secondary way.

Reviewer IV: Were the muscles restrained before removal from the carcass?
Authors: No. They were removed and then an attempt was made to adjust the sample to approximate rest length and restrain it with forceps during freezing.