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Pectoralis and semimembranosus muscle fiber alterations in migrating bird (*Branta leucopsis*) during embryonic, neonatal and adult stages

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ABSTRACT: Some migrating birds, although young, develop musculature capable of sustaining prolonged flights. Such physical capacitation involves muscle fibers alterations which occur in a short space of time between hatching and migration. Considering that myosin is the major contractile protein of the muscle fiber, the myosin heavy chain (MHC) behaviour in pectoralis and semimembranosus muscles of a migratory bird (barnacle goose - *Branta leucopsis* Bechstein) is evaluated in this paper, using different monoclonal antibodies. The EB165 and B103 monoclonal antibodies were used for the immunocytochemical recognition of MHC isoforms of fast-twitch fibers of two populations (captive and wild) of the barnacle goose during different stages of development. Distinct fiber types of the pectoralis and semimembranosus muscles were recognized through variations in the reactions of the antibodies with the

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MHC during the embryonic, neonatal and adult stages of the migrating and captive barnacle geese. Differences regarding the antibodies reactivity between the two populations were not observed.

Key words: *Branta leucopsis*, myosin heavy chain, pectoralis and semimembranosus, monoclonal antibodies

INTRODUCTION

Some species of migrating birds, although very young, develop musculature capable of sustaining prolonged flights. For instance, the barnacle goose (*Branta leucopsis* Bechstein), with approximately 12 weeks old, fly about 2.500 km from the european Artic (its breeding grounds) to Scotland, where it winters. Such capacitation involves muscle fiber alterations in a short space of time, comprising adaptations of the components involved in the process of muscle contraction and its regulation.

Myosin is the major contractile protein of the skeletal muscle, consisting of a large molecule composed of two heavy chains (200 000 Da) and four light chains (18 000 - 26 000 Da) (ALBERTS et al., 1989). In vertebrates, myosins are part of a complex multigene family (BUCKINGHAM, 1985; ROBBINS *et al*., 1986) that by expressing different genes during muscle development promotes the sequential appearance of different isoforms (GAUTHIER *et al.*, 1982; WADE & KEDES, 1989; MOORE *et al*., 1992). The transitional expression of distinct isoforms occurs during the embryonic, neonatal and adult stages and reflects the gradual achievement of full mobility and load-bearing capabilities in the musculoskeletal system (WHALEN *et al*., 1981).

In this paper, the myosin heavy chain (MHC) expression in the pectoralis and semimembranosus muscles of two populations of barnacle goose is evaluated during different stages of development.

MATERIALS AND METHODS

Fragments of the pectoralis and semimembranosus muscles of two populations of barnacle goose were used: a captive/non-migrating population kept at the University of

Birmingham (Birmingham - England) and a wild/migrating one captured on the western coast of the island of Spitsbergen, Svalbard, artic Norway. The geese were killed by injection of sodium pentobarbitol or inhalation of halothane.

The muscle fragments were obtained at selected stages of development, using at least three samples of each stage: prehatch, comprising "in ovo" samples, approximately four days before hatching; hatch, samples of nestlings obtained few hours after birth; goslings of 1, 3, 5, 7, 11 weeks and adults.

The immunocytochemical study of the MHC isoforms involved two primary monoclonal antibodies EB165 and B103 (kindly provided by BANDMAN, E. and DAVIS, C., University of California, Davis, USA). These monoclonal antibodies recognize MHC isoforms of fast-twich muscle fibers of birds such as chicken (CERNY & BANDMAN, 1987; BANDMAN & BENNETT, 1988; TAYLOR & BANDMAN, 1989; MOORE *et al*., 1992) and turkey (MARUYAMA & KANEMAKI, 1991; MARUYAMA *et al*., 1993). Specifically, the EB165 antibody is reactive to the embryonic and adult isoforms and the B103 antibody is reactive to the embryonic and neonatal isoforms in the chicken pectoralis muscle.

The secondary antibody anti-mouse IgG FITC was used to identify the primary antibodies.

Control samples were treated with PBS/0.1% BSA in place of primary antibodies and were then incubated with the secondary antibody. The unspecific fluorescence detected in the control groups was evaluated by its low intensity reaction and served as base for the characterization of specific fluorescence of the reactive fibers, done by direct examination under the fluorescent microscope and by analysis of photomicrographies.

RESULTS

The immunocytochemical reactions observed in the pectoralis and semimembranosus muscles of captive and wild populations of the barnacle goose indicate that myosin heavy chain (MHC) of fast-twitch fibers has epitopes which are recognized by the EB165 and B103 antibodies in all stages of development analysed.

In the prehatch and hatch stages, the pectoralis and semimembranosus muscle fibers reacted strongly with the B103 (Figure 1A) and EB165 (Figure 2A) antibodies, however, it was

also evident the presence of negative fibers, that is, fibers which were not recognized by the B103 and EB165 antibodies, distributed throughout the samples.

In subsequent stages of development, from the neonatal (first week of postnatal life) up to the adult stage, it has been observed that the pectoralis and semimembranosus muscle fast-twich fibers of the barnacle goose reacted differently with the B103 and EB165 antibodies.

The majority of fibers of both muscles reacted strongly with the B103 antibody from the first week up to the fifth week (Figure 1B) when the semimembranosus and later (around the seventh week) the pectoralis started to show less strongly reactive fibers. It was also observed a clear reduction in the number of non-reactive fibers. In the 11-week and adult samples, different fiber types showing strong, moderate and weak reactivity were observed. Specifically, the 11-week (Figure 1C) and adult (Figure 1D) semimembranosus muscles presented fibers with strong, moderate and weak reaction, while the 11-week pectoralis muscle (Figure 1E) showed strongly and moderately reactive fibers, and the adult (Figure 1F) showed at least four fiber types: fibers with strong, moderate and weak reaction and non-reactive fibers, corresponding possibly to the amount of epitopes present in each fiber. These remarks suggest that the transition of the adult isoform began around the fifth week posthatch on the semimembranosus muscle and around the seventh week on the pectoralis muscle.

Similar to what was observed with the B103 antibody at the beginning of postnatal development, the majority of the pectoralis and semimembranosus fibers also reacted with the EB165 antibody. However, this specific and uniform recognition of fibers was equally observed in more advanced stages of the development (Figure 2B). All fibers of the 11-week and adult pectoralis muscle showed strong reaction with the EB165. Distinctly, on the 11-week semimembranosus muscle (Figure 2C) few fibers reacted strongly with the EB165 and the presence of a large number of non-reactive fibers was detected. On the adult semimembranosus muscle, all fibers presented again a strong and uniform reaction with this antibody (Figure 2D).

During the evolutionary process, the pectoralis and semimembranosus fibers change their pattern of reaction with the B103 and EB165 antibodies. However, when we analyse,

comparatively, the behavior of these fibers in the migrating and non-migrating populations of the barnacle goose, distinct patterns of reaction between these populations could not be identified.

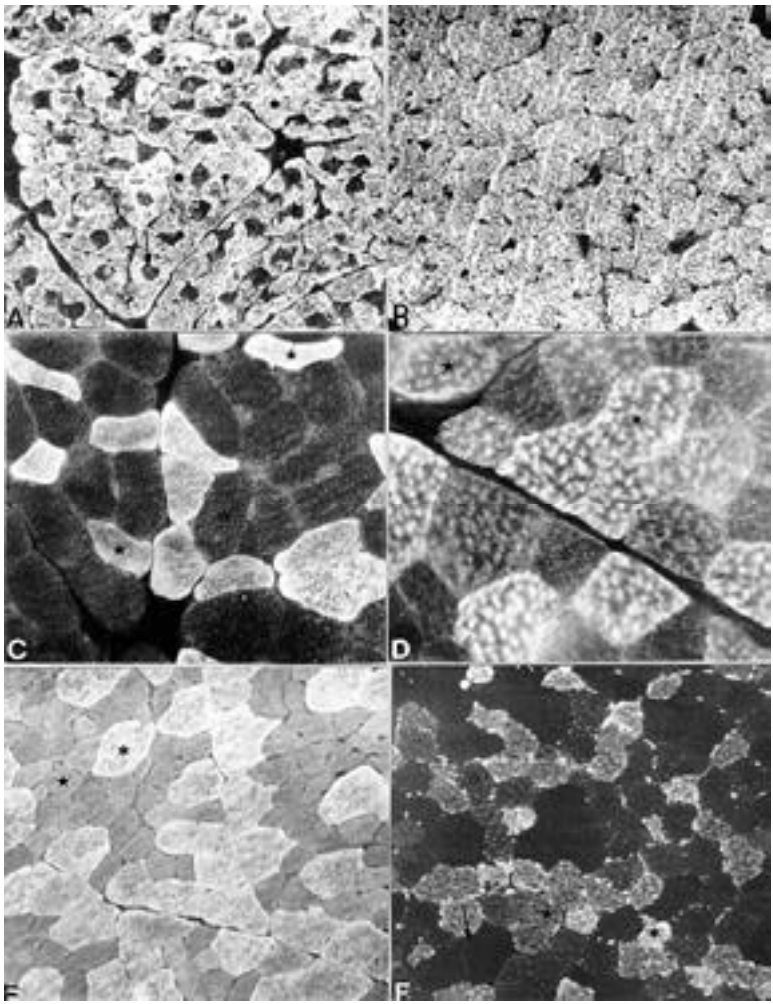

Figure 1 – Immunocytochemical staining of captive Barnacle goose muscles reacted with B103 antibody. **(A)** Prehatch semimembranosus muscle showing reactive (❋) and negative fibers (arrow) (836,5X). **(B)** 3-week posthatch semimembranosus muscle with strong and uniform reaction in almost all fibers (821,4X). **(C)** 11-week posthatch semimembranosus muscle with strong (✱), moderate (★) and weak (☆) reacted fibers (800X). **(D)** Adult semimembranosus muscle with strong, moderate and weak reacted fibers (819X). **(E)** 11-week posthatch pectoralis muscle with strong and moderate reacted fibers (857,1X). **(F)** Adult pectoralis muscle with strong, moderate, weak and negative fibers (854,2X).

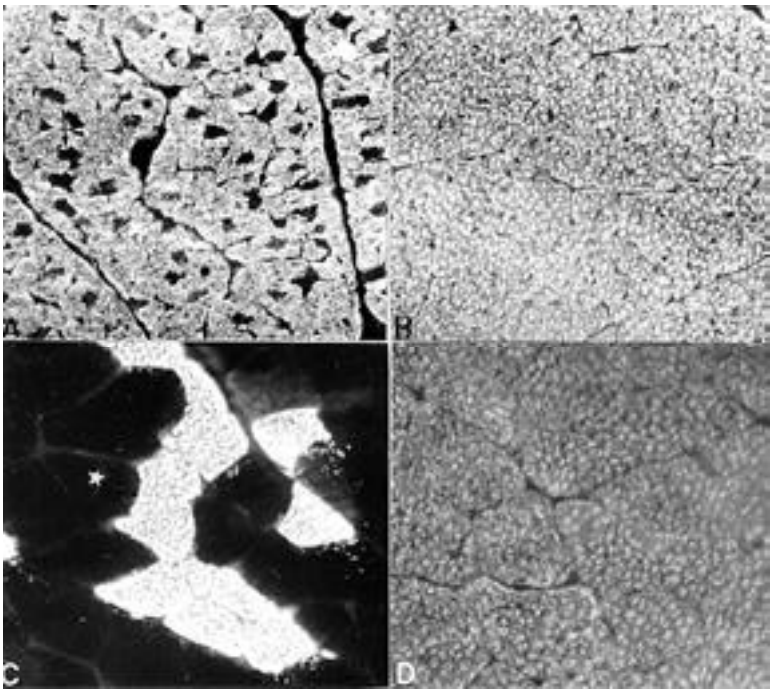

Figure 2: Immunocytochemical staining of captive Barnacle goose muscles reacted with EB165 antibody. **(A)** Prehatch semimembranosus muscle showing reactive (✳) and negative fibers (arrow) (850X). **(B)** 5-week posthatch pectoralis muscle with strong and uniform reaction of fibers (825,8X). **(C)** 11-week posthatch semimembranosus muscle showing a large number of negative fibers (★) (839,3X). **(D)** Adult semimembranosus muscle with strong and uniform reaction of fibers (821,4X).

DISCUSSION

During muscle development, it can be observed, among other miofibrillar constituents, the sequential appearance of different MHC isoforms, characterized as embryonic, neonatal and adult, depending, respectively, on the stage of development which they appear (CERNY & BANDMAN, 1987). Many aspects involved in the isoforms transition have not been cleared yet but the complexity of MHC isoforms expression is supported

by their multigenic origin (ROBBINS *et al.*, 1982, 1986) and by the influence of hormonal and neural factors (CERNY & BANDMAN, 1987; GARDAHAUT *et al.*, 1992).

In this paper, with the use of two specific chicken MHC antibodies, B103 and EB165, it was attempted to characterize the MHC isoforms of the pectoralis and semimembranosus muscle fast-twitch fibers of two populations of the barnacle goose in various stages of its development.

Distinct fiber types of the pectoralis and semimembranosus muscles were recognized through variations in the reactions of the antibodies with the MHC during the embryonic, neonatal and adult stages of the migrating and captive barnacle geese.

The presence of fibers that showed strong reaction with the B103 and EB165 was detected in all stages of development. Some fibers, however, presented variation in the intensity of reaction or even complete lack of reaction with these antibodies.

In the prehatch and hatch samples of the pectoralis and semimembranosus muscles of the wild and captive populations, it was observed that some fibers did not react with the B103 and EB165 antibodies. The analysis of the samples suggests a larger proportion of negative fibers on the semimembranosus muscle. However, such observation becomes improbable if we consider the size of the semimembranosus muscle fibers which, although they have not been measured, is clearly larger than the pectoralis muscle fibers. It is more likely that the proportion of negative fibers be approximately the same between the two muscles and that the larger size of the semimembranosus muscle fibers possibly indicates a precocious differentiation of this muscle in comparison with the pectoralis.

Another aspect to be questioned is if these non-reactive fibers could be indicating the non recognition of the embryonic isoform of the barnacle goose by the chicken B103 and EB165 antibodies, while the isoform recognized would be the neonatal one which was already being detected at this stage of development. That is, considering that the barnacle goose is a precocial and nidifugous species, it is possible that the embryonic MHC isoform in the fast-twitch fiber was expressed at an earlier stage of development and from the "in ovo" stage on (around four days before hatching) used in this study its

presence would seem to be much reduced. In this case, the fast-twitch fibers of the prehatch and hatch stages of the barnacle goose would be expressing the neonatal isoform, recognized by the B103 and EB165 antibodies, which in the chicken specifically react with the embryonic isoform (CERNY & BANDMAN, 1987; BANDMAN & BENNETT, 1988). Taking into account this considerations, it was not possible to reach a conclusive opinion regarding the transitional appearance of the embryonic and neonatal isoforms of fast-twitch fibers in the *Branta leucopsis* species, although it was possible to observe the clear reduction of the number of negative fibers from the first week posthatch, which, in a certain way, strengthens the hypothesis that these negative fibers have the embryonic isoform.

Reactive fibers showing different intensities of reaction in the semimembranosus (from the fifth week) and the pectoralis (from the seventh week) muscles demonstrated the recognition of distinct fiber types by the B103 antibody in both populations of the barnacle goose. However, the fast-twitch fibers of these muscles showed a strong and uniform reaction with the EB165 antibody from the first week posthatch up to the adult stage. Only on the 11-week semimembranosus muscle was detected a large number of negative fibers contrasting with the presence of strongly reactive fibers.

There is no definite explanation which justifies the presence of a large number of negative fibers in the 11-week samples, but it is suggested that the neonatal MHC isoform, maybe still present on this stage of development, was not being recognized by the EB165 antibody anymore. Another hypothesis would be that the EB165 antibody would not be recognizing some other transitional type of the adult isoform that apparently disappeared when the goose reached the adult stage.

These results differ partially from the studies performed in the chicken pectoralis muscle, incubated with the B103 and EB165 antibodies. It was observed that in this animal the fast-twitch fibers of the adult pectoralis did not react with the B103 (BANDMAN & BENNETT, 1988) and fibers of the neonatal pectoralis did not react with the EB165 (CERNY & BANDMAN, 1987). Contrary to what was expected, that is, the lack of reaction of the adult MHC with the B103 and the neonatal MHC

with the EB165, in the barnacle goose the fast-twitch fibers of the neonatal pectoralis and adult semimembranosus muscles reacted with these antibodies. Regarding these facts, it is questioned whether the expression of extra isoforms could not be occuring in the MHC of the neonatal and adult pectoralis and semimembranosus muscles of the barnacle goose which would not be present in the chicken neonatal and adult pectoralis.

In this paper, by use of immunocytochemestry, it was not possible to identify characteristcs related to the B103 and EB165 antibodies reactivity that distinguished the migrating and non-migrating populations of the barnacle goose. However, it was possible to show that different fiber types were detected by use of specific antibodies on the pectoralis and semimembranosus muscles of both populations. It was also shown the presence of distinct patterns of the immunoreactivity distribution among the embryonic, neonatal and adult stages and between the pectoralis and semimembranosus muscles, which in a certain way, proves the expression of different MHC isoforms on the fast-twitch fibers during the development of the species *Branta leucopsis*.

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