FIBRE COMPOSITION AND ENZYME ACTIVITIES IN SIX MUSCLES OF THE SWEDISH REINDEER *(RANGIFER TARANDUS TARANDUS)*

Fibersammansättning och enzymaktiviteter i sex muskler från svensk tamren *(Rangifer tarandus tarandus)*.

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Abstract: Six skeletal muscles have been studied as regards fibre properties and enzyme activities. The muscles are cranial part of *M. gluteobiceps, M. semitendinosus, M. semimembranosus, M. longissimus dorsi, M. brachiocephalicus* and *M. sternocephalicus.*

Two histochemical methods were used for fibre identification, one based on myosin ATPase activities after preincubation at pH 4.3 and 4.6 and the other on oxidative capacity measured as NADH dehydrogenase activity. The two methods gave slightly differing results but allowed the general conclusion that of the three fibre types (I, II A and II B) the type II B fibres, which are fast-twitch, glycolytic, make up some 40 - 60 % (mean 50 %) of the muscles. Type I fibres, which are slow-twitch, oxidative, account for 30% of the total muscle volume in the two neck muscles but for only 20% or less in the rest. The third type, II A, which is fast-twitch, oxidative, glycolytic, accounts for only 20% of the volume in the neck muscles but as much as 40% in *M. longissimus dorsi.*

Oxidative capacity is high throughout. This is valid also to the capacity to oxidize fatty acids, though reaching only half the activity previously found in the Svalbard reindeer (Kiessling and Kiessling, 1983). Lactate dehydrogenase activity is comparatively low in all muscles.

The high respiratory chain activity and fatty acid oxidation and the low lactate dehydrogenase activities do not fit at all well with the high content of type II B fibres in the muscles. This high II B content is also unexpected when considering the activity pattern of the reindeer. An altogether different role for the type II B fibres, besides the traditional one, is therefore discussed.

Key words: *reindeer, muscle fibres, muscle metabolism.*

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Sammandrag: Sex skelettmuskler har undersökts med avseende på fiberegenskaper och enzymaktiviteter. De sex musklerna är kranial del av *M. gluteobiceps. M. semitendinosus, M. semimembranosus, M. longissimus dorsi, M. brachiocephalicus och M. sternocephalicus.*

Två histokemiska metoder har använts för att identifiera fibrerna, den ena baserad på myosin ATPas aktivitet efter förinkubering vid pH 4,3 och 4,6, den andre på oxidativ kapacitet mätt som NADH dehydrogenas aktivitet. De två metoderna gav något olika resultat men tillåter den generella slutsatsen att av de tre fibertyperna (I, II A och II B) typ II B fibrerna, som är snabbt kontraherande, glykolytiska, utgör 40 - 60 % (medelvärde 50 %) av muskelvolymen. Typ I fibrerna, som år långsamt kontraherande, oxidativa, svarar för 30% av totala muskelvolymen i de två halsmusklerna men bara för 20% eller mindre i övriga muskler. Den tredje typen, II A, som är snabbt kontraherande, oxidativ och glykolytisk, svarar bara för 20 % av volymen i halsmusklerna men enda upp till 40% i *M. longissimus dorsi.*

Den oxidativa kapaciteten år genomgående hög. Detta gäller även för kapaciteten att oxidera fettsyror, även om denna bara uppgår till halva den aktivitet som tidigare påvisats i Svalbardrenens muskler (Kiessling & Kiessling 1983). Laktatdehydrogenas aktiviteten är jämförelsevis låg i alla muskler.

Den höga aktiviteten hos andingskedjan och fettsyraoxidationen och den låga laktatdehydrogenas aktiviteten stämmer dåligt med den höga halten av typ II B fibrer i musklerna. Denna höga II B halt år också oväntad med tanke på renens aktivitetsmönster. Därför diskuteras en alternativ roll för typ II B fibrerna förutom den traditionella att fungera som kontraktil vävnad.

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Fiiberien kokoonpano ja entsyymien aktiivisuudet kuudessa lihaksessa ruotsalaisessa porossa. *(Rangifer tarandus tarandus)*

Yhteenveto: On tutkittu kuutta luurangon lihasta mitä fiiberien ominaisuuksiin ja entsyymien aktiivisuuksiin tulee. Lihakset ovat craniaalinen osa seuraavista: *M. gluteobiceps, M. semitendinosus, M. semimembranosus, M. longissimus dorsi, M. brachiocephalicus* ja *M. sternocephalicus.*

Fiiberien identifioitiin käytettiin kahta histokemiallista menetelmää, ensimmäinen perustui myosin ATPase aktiivisuuksiin preinkubaation jälkeen pH 4,3:ssa ja 4,6:ssa ja toinen oksidatiiviseen kapasiteettiin mitattuna NADH dehydrogenase aktiivisuutena. Nämä kaksi menetelmää antoivat hienoisen eron, mutta antaa aihetta yleiseen johtopäätökseen, että niistä kolmesta fiiberityypistä (I, II A ja II B) tyyppi II B fiiberit, jotka ovat nopeasti supistavia, glycolyyttisiä, koostuvat suunnilleen 40 - 60 % (keskimäärin 50 %) lihaksista. Tyyppi I fiiberit, jotka ovat hitaasti supistavia, oksidatiivisiä, tekee 30 % lihaksen kokonaismäärästä niissä kahdessa niskalihaksessa, mutta vain 20 % tai vähemmän muissa. Kolmas tyyppi, II A, joka on nopeasti supistava, oxidatiivinen, glycolyyttinen, tekee vain 20 % niskalihasten määrästä, mutta niin paljon kuin 40 % *M. longissimus dorsi:sta.*

Oksidatiivinen kapasiteetti on läpeensä korkea. Tämä pätee myöskin kapasiteettiin oksidoida rasvahappoa, vaikkakin saavuttaa vain puolet aktiivisuudesta aikaisemmista löydöistä Huippuvuorten poroissa (Kiessling ja Kiessling, 1983). Lactate dehydrogenase aktiivisuus on verrattain alhainen kaikissa lihaksissa.

Korkea respiraatioketju aktiiviteetti ja rasvahappo oksidaatio sekä alhainen lactate dehydrogenase aktiivisuudet eivät sovi laisinkaan hyvin yhteen korkea sisältöisten tyyppi II B fiiberien kanssa lihaksissa. Tämä korkea II B sisältöinen on myöskin odottamaton huomioonottaen poron aktiivisuus käyttäytymisen. Aivan erilainen tehtävä tyyppi II B fiibereille, traditionaaliseen verrattuna, on siksi keskusteltu.

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INTRODUCTION

The fibre types in mature muscle can be described as slow twitch oxidative (Type I), fast twitch glycolytic (Type II B), and fast twitch oxidative-glycolytic (type II A) (Peter et al., 1970). Studies on the recruitment of muscle fibres during different types of activity (Gollnick et al., 1973 a, 1973 b, 174) suggest that these various types of fibres have differing physiological roles. The physiological properties of a muscle should therefore depend on the number, size, and type of its constituent fibres.

According to Thomson (1971, 1973) the adult reindeer spends about 97 % of its time grazing, lying, standing and walking, both in summer and in winter. These activities should involve the use of mainly type I fibres. Our intention has therefore been to ascertain whether this activity pattern of the adult reindeer is reflected in the histochemical and biochemical characteristics of its skeletal muscles. In order to do so we have examined fibre composition and enzyme activities in six skeletal muscles of the Swedish reindeer.

MATERIAL AND METHODS

The animals were Swedish domestic forest reindeer from Arvidsjaur in Northern Sweden. They were slaughtered in September 1981-82. Thirteen reindeer steers and two reindeer cows, age 2 to 7 years, were included.

Muscles

Muscle pieces were taken by surgical biopsy directly after slaughter. The following muscles were studied: *M. gluteobiceps, M. semitendinosus, M. semimembranosus, M. longissimus dorsi, M. brachiocephalicus* and *M. sternocephalicus.*

Histochemistry

For histochemistry, muscle specimens were taken as surgical biopsies, trimmed, oriented, mounted in Cryoform, frozen in isopentane cooled by liquid nitrogen, and stored at —80°C until analysed. Transverse sections (10 pm) were cut with a cryotome and stained for myofibrillar ATPase after preincubation at pH 4.3 - 4.6 (Padykula and Herman, 1955; Guth and Samaha, 1969; Brooke and Kaiser, 1970) and for NADH dehydrogenase (Novikoff et al., 1961). The fibres were classified as I, II A and II B.

Enzyme acitivities

Three enzymes were chosen to represent the important pathways in energy metabolism: the respiratory chain by cytochrome oxidase (cytox;

E.C. 1.9.3.1.), fatty acid ß-oxidation by 3hydroxyacyl-CoA dehydrogenase (HAD; E.C. 1.1.1.35.) and lactate fermentation by lactate dehydrogenase (LDH; E.C. 1.1.1.27.). Cytox activity was estimated according to Whereat et al. (1969), HAD and LDH by the methods of Bass et al. (1969). For enzyme activity determinations, biopsy samples (25-50 mg) were homogenized with 19-fold amounts (w/v) of ice-cold potassium bicarbonate, 62 mM, pH 7.4, containing 0.15 M KCl and 6 mM EDTA in a small, ice-cooled, all-glass Potter-Elvehjem homogenizer. The resulting crude homogenate was kept ice-cold and was diluted appropriately prior to activity determinations. The biopsies had been kept frozen in liquid nitrogen during transport to the laboratory in Uppsala.

RESULTS

Fibre composition

Fibre composition expressed as relative area of each fibre type, e.g. the area as percentage, that they occupy in transverse section, is shown in Fig. 1. The identification of the fibres is based either on their NADH dehydrogenase activity or ATPase activity after preincubation at pH 4.3 and 4.6. The two methods give only slightly differing results, except for longissimus, where the type II A fibres constitute 50% of the total fibre area with the ATPase staining and only 30% with the NADH dehydrogenase staining. In the three leg muscles the type I fibres make up 20% or less of the total fibre area and in the two neck muscles as much as 30%. In the latter muscles a corresponding decrease in the type II A fibre area occurs.

Enzyme activities

Fig. 2 shows the activity of three enzymes in the six muscles. Oxidative capacity, measured as cytox activity, is very similar in all muscles-except the two neck muscles, where it is only two-thirds of that in the other muscles. The capacity to metabolize fatty acids varies from 13 µmol (per minute and 100 mg protein) in the small neck muscle to 18 µmol in longissimus. Also LDH activity is lowest in the small neck muscle.

DISCUSSION

Muscle fibre classification

Muscle fibres are commonly classified, histochemically, into three types by their staining intensity for myosin ATPase combined with staining for metabolic enzymes. Preincubation at pH 4.6 shows as a rule three staining intensities of myosin ATPase which can be used for fibre typing. In the present study the NADH dehydrogenase activity has been used as a measure of metabolic activity, in this case oxidative capacity.

The two staining methods are based on completely different properties of the muscle, the pH sensitivity of the contractile proteins on the one hand and the oxidative capacity coupled to energy production on the other. Theoretically it would seem that the two properties should be correlated. In practice, however, it cannot be taken for granted that the results will be mutually consistent. Analysis of nine muscles from pig revealed very close agreement between the two methods (Kiessling and Hansson, 1983). In muscles from the Svalbard reindeer, NADH dehydrogenase staining showed more type II B fibres than did ATPase staining (Kiessling and Kiessling, 1983), whereas with rat muscle the two techiques gave the converse result, that is, more fibres were identified as II B when staining for ATPase than for NADH dehydrogenase activity (unpublished results). This problem has recently been subjected to a renewed discussion (Nemeth et al., 1979; Nemeth and Pette, 1980; 1981 a, 1981 b, Green et al., 1982).

Fibre recruitment

Histochemical studies have shown a primary involvement of type I fibres during low-intensity exercise (Gollnick et al., 1973 a, b, 1974). When such activity rises above a certain level, there is a gradual involvement of type II fibres, with II A being involved first, until, if the exercise extends to exhaustion, all fibres are involved (Gollnick et al., 1973 a, b, 1974). In rodents it has been shown that type II A fibres are recruited when the animals perform all types of phasic activities, such as walking, running, sprinting (Baldwin et al., 1975, 1977).

Grazing in summertime involves standing or slow walking, in wintertime digging for feed hidden under the snow. The annual migrations from one biotope to another involve walking and trotting. During the mating season there is a period of increased physical activity, especially among the males. All these various activities no doubt involve muscle fibre types which are adapted to maintaining posture (isometric tension) and for carrying out slow repetitive movements (mainly type I fibres and to some extent type II A fibres). The

brief activity burst, which may be an initial phase when escaping predators, can be accomplished by type II B fibres, which are adapted for high power output, and are recruited only when very rapid movement is required. As they suffer fatigue very rapidly, further flight has to be accomplished by fibres adapted for reasonably fast movements of a repetitive nature (II A fibres).

Relationship between fibre type composition and normal functional usage

Whether the relative volume of the three fibre types was distinguished by means of myosin ATPase activity or by means of oxidative capacity, some 40 - 60% of the muscle volume in the six muscles is identified as type II B fibres. As these fibres produce energy by means of anaerobic glycolysis they are usually recruited only when very rapid movements are required. It is therefore difficult to understand why these fibres predominate in all reindeer muscles investigated.

The same situation was found in the Svalbard reindeer (Kiessling and Kiessling, 1983). Two circumstances could, at least partly, justify this fibre distribution in the Svalbard animal. One is that their type II B fibres show an unusually high oxidative capacity and are, in this respect, sometimes difficult to distinguish from type II A and even from type I fibres by means of the histochemical staining technique for NADH dehydrogenase activity. The other is that during wintertime the Svalbard reindeer metabolizes 30% or even more of its lean tissue (Reimers and Ringberg, in press) which thus contributes considerably to its energy supply. If mainly type II B fibres are used up, as is indicated by experiments on rodent muscle (Goldspink and Ward, 1979), this may ascribe a quite different role to the type II B fibre besides the traditional one, namely to function as an enormous energy reserve supply. This hypothesis has yet to be established.

Great similarities occur between the Svalbard and the Swedish reindeer in these respects. Oxidative capacity, measured as mean value for all muscles, is the same in the two species and so too is the amount of type II B fibres. Preliminary results indicate that also in the Swedish reindeer there is a decrease in muscle tissue during the winter season, amounting to 10% from mid-September to early January (Rydberg, 1982).

The two neck muscles show a slightly different pattern compared with the other muscles studied.

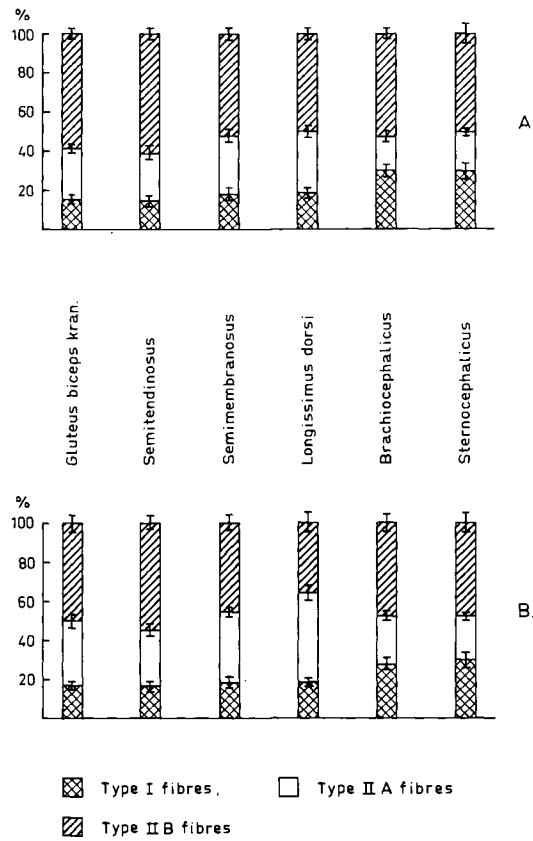

Fig. 1. Fibre composition of six skeletal muscles from Swedish reindeer. The proportion of each fibre type is expressed as a relative area, i.e. the area as a percentage, that they occupy in transverse sections. The columns are mean values from 12 - 15 animals ± standard error (except for *M. sternochephalicus* where only five animals were used).
(A) After staining for NADH dehydrogenase activity.
(B) After staining for myosin ATPase activity.

Fig. 1. Fibersammansättning i sex skelettmuskler från svensk tramren. Mängden av varje fibertyp uttrycks som relativ yta, d.v.s. den yta i procent som den upptar i ett muskeltvärsnitt. Staplarna är medelvärden av 12 - 15 djur ± standard error (med undantag för *M. sternocephalicus* där endast 5 djur använts).
(A) Efterfärgning med avseende på NADH dehydrogenas aktivitet.
(B) Efterfärgning med avseende på myosin ATPas aktivitet.

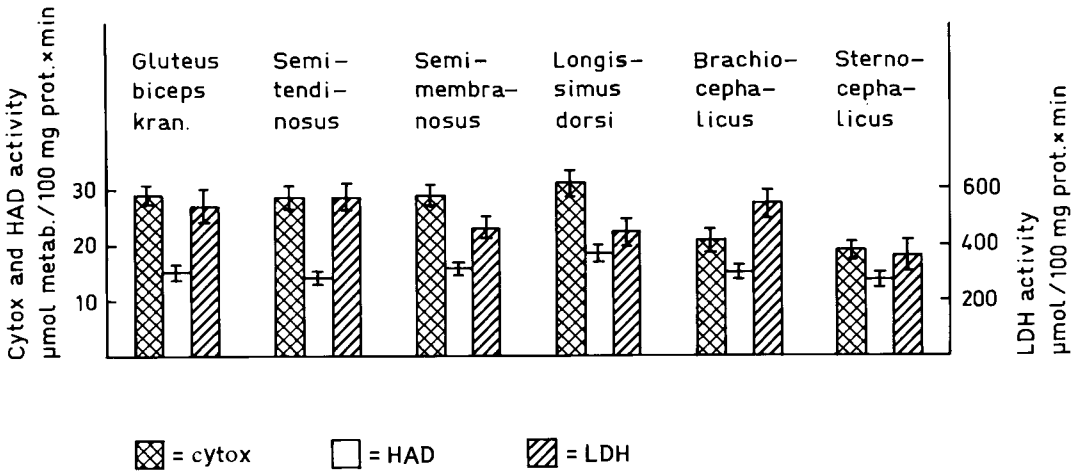

Fig. 2. Enzyme activities in six skeletal muscles from Swedish reindeer. The columns are mean values from 12 - 15 animals ± standard error (except for *M. sternocephalicus* where only five animals were used). The enzymes are cytochrome oxidase (cytox: oxidative capacity), 3hydroxyacyl-CoA dehydrogenase (HAD: fatty acid oxidation capacity) and lactate dehydrogenase (LDH: lactate fermentation).

Fig. 2. Enzymaktivitet i sex skelettmuskler från svensk tamren. Staplarna är medelvärden av 12 - 15 djur (med undantag av *M. sternocephalicus* där endast 5 djur använts). Enzymerna är cytochrome oxidas (cytox: oxidativ kapacitet), 3dehydroxyacyl-CoA dehydrogenas (HAD: kapacitet att oxidera fettsyror) och lactat dehydrogenas (LDH: lactatfermentation).

The ratio between white and red fibres, that is between II B on the one hand and I and II A on the other, is unaltered. The type I fibre part, however, nearly d ɔles at the expense of the type II A fibres. This is probably one prerequisite for the isometric contraction needed to carry heavy antlers and to fight competitors successfully.

Enzyme activities

Oxidative capacity, measured as cytochrome oxidase (cytox) activity, is comparatively high relative to lactate dehydrogense activity. This is especially pronounced in semimembranosus and longissimus. The activities are roughly the same as in the Svalbard reindeer muscle. A remarkable difference between the Swedish and the Svalbard reindeer exists, however, in fatty acid oxidation capacity, measured as the activity of 3hydroxyacyl-CoA dehydrogenase in the muscles. The Svalbard reindeer is roughly twice as active as the Swedish reindeer.

The explanation for this difference may be that the Svalbard reindeer is forced to use fat as an energy source to a much greater extent during the winter season than is the Swedish reindeer. The latter migrates to areas where circumstances for survival during winter are favourable. Thus the relative importance of winter pasture is far greater for the Swedish than for the Svalbard reindeer which has no corresponding pattern of behaviour.

In conclusion: There is no obvious and straightforward correlation, in conventional terms, between the activity pattern of the reindeer and its muscle properties.

Thus the Swedish reindeer, which spends most of its time (97%) in comparatively sedentary activities, has muscles dominated by fibre types conventionally associated with rapid movements (II B). This phenomenon is even more pronounced in the Svalbard reindeer (Kiessling and Kiessling, 1983).

A hypothesis is put forward to account for the seemingly inexplicable presence of the large amount of muscle rich in II B fibres. It is attributed to the reindeer's need of this tissue for survival, as

its energy reserve, during the forced starvation in the winter. This hypothesis is supported by the findings of the extreme reduction in lean tissue during the winter season in the Svalbard reindeer and a corresponding tendency in the Swedish reindeer.

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