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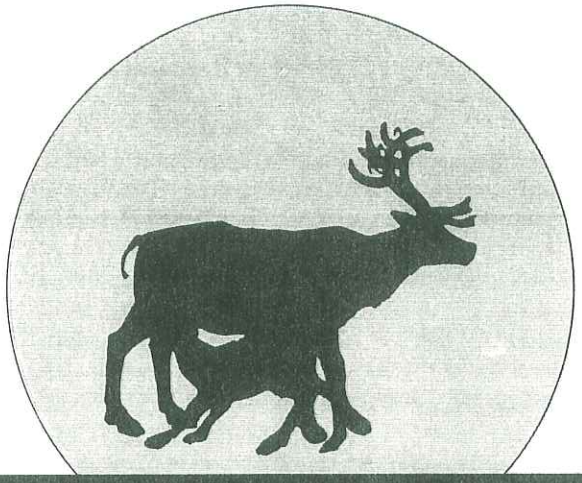

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Histochemical and enzymatic differences in skeletal muscle from Svalbard reindeer during the summer and winter

Histokemiska och enzymatiska skillnader i skelettmuskulatur från Svalbardren mellan sommar och vinter.

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Abstract: Enzyme activities and fibre properties in four muscles from Svalbard reindeer, collected during the summer, have been compared with corresponding muscles during the winter. In two muscles, gluteobiceps and semimembranosus, oxidative capacity is higher in winter than in summer; in the other two muscles, semitendinosus and longissimus dorsi, there is no difference with time of the year. The capacity to oxidize fatty acids is low in winter compared with summer, especially in semitendinosus and longissimus. These changes are similar in both sexes.

Histochemical studies of the three main fibre types, I (ßR), IIA (∝R) and IIB (∝W), from the four muscles show that in male reindeers the muscle fibres are narrower at the end of the winter season than during the summer. The decrease of muscle tissue amounts to about one third of the total volume (33%), of which I accounts for 5%, IIA for 2% and IIB for 26%.

The results indicate that the Svalbard reindeer use lean tissue in general, and IIB fibres in particular, in order to survive the hostile arctic winter period at Svalbard.

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Sammandrag: Enzymaktiviteter och fiberegenskaper i fyra av Svalbardrenens muskler, insamlade under sommaren, har jämförts med motsvarande muskler insamlade under vintern. I två muskler, gluteobiceps och semimembranosus, är oxidativa kapaciteten högre under vintern än under sommaren; i de andra två musklerna, semitendinosus och longissimus dorsi, föreligger ingen skillnad i detta avseende. Kapaciteten att oxidera fettsyror är låg under vintern jämfört med sommaren, speciellt i semitendinosus och longissimus. Inga könsskillnader föreligger i dessa avseenden.

Histokemiska studier av de tre huvudtyperna av muskelfibrer, ßR (I), ∝R (IIA) och ∝W (IIB), från de fyra musklerna visar att hos handjuren är fibrerna tunnare vid slutet av vintersäsongen jämfört med sommaren. Denna minskning i muskelvävnad uppgår till en tredjedel av totala volymen (33%). Härav svarar ßR för 5%, ∝R för 2% och ∝W för 26%.

Resultaten antyder att Svalbardrenen använder muskelvävnad, speciellt ∝W fibrer, för att överleva under den hårda arktiska vinterperioden på Svalbard.

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INTRODUCTION

In a previous study fibre composition and enzyme activities were investigated in skeletal muscle from Svalbard reindeer during the summer season (Kiessling and Kiessling 1984). The most striking characteristics observed were the high oxidative capacity and the high capacity to metabolize fatty acids despite the comparatively large content of type IIB fibres. Furthermore, histochemical staining revealed only small differences in oxidative capacity between the three fibre types I, IIA and IIB, indicating a tendency to bring the three fibre types closer together as regerds metabolic activities. This could indicate additional rôles for the IIB muscle fibres in the Svalbard reindeer besides their classical function as an anaerobically active muscle. One such rôle may be to serve as an extra energy supply during starvation. Furthermore, the IIB fibres may be able to contribute to physical activity by means of their comparatively high oxidative capacity in spite of the tranquil life of the Svalbard reindeer.

In the present work muscle samples from Svalbard reindeers were collected in April when the animals were extremely starved due to a long period with over-icing-conditions. Fibre composition, fibre size and enzyme activities have been investigated. The results are discussed in relation to corresponding values for the reindeer during the summertime.

MATERIAL AND METHODS

Animals

Six female and seven male reindeers were killed by shooting during late April on Kap Linné, Nordenskiøld land. The summer animals, eight females and four males, were killed during early July in the same area.

Muscles

Samples were taken from the following four muscles: M. gluteobiceps, M. semitendinosus, M. semimembranosus and M. longissimus dorsi.

Histochemistry

The muscle specimens for histochemistry were taken as surgical biopsies and treated as described earlier (Kiessling and Kiessling 1984). Transverse sections were stained for myofibrillar ATP-ase and for NADH-dehydrogenase and the fibres were classified as I, IIA and IIB (ßR, ∝R and ∝W).

Enzyme activities

Three enzymes were chosen to represent the important pathways in energy metabolism: the respiratory chain by cytochrome oxidase (cytox; EC 1.9.3.1.), fatty acid ß-oxidation by 3-hydroxyacyl-CoA dehydrogenase (HAD; EC 1.1.1.35.) and lactate fermentation by lactate dehydrogenase (LDH; EC 1.1.1.27.). The analyses have been described earlier (Kiessling and Kiessling 1984).

RESULTS

Body and carcass weight

Table 1 shows that both live weight and carcass weight differ between the summer and winter season in male animals while this is only found for live weight as regards females. The small difference in female carcass weight between summer and winter animals is explained by the fact that they were killed early in the food-rich season. Full muscle mass is obtained during August — September.

Enzyme activities

Figure 1 shows the activities of cytox, HAD and LDH in four muscles from Svalbard reindeer, collected from 15th to 30th of April

Table 1. Live and carcass weights of Svalbard reindeers in summer and winter.

Tabell 1. Levande- och slaktvikt för Svalbardren på sommaren och vintern.

	Live weight, kg		Carcass weight, kg	
	♂	♀	♂	♀
Summer	95.7±3.5 n=4	43.1±2.5 n=8	43.4±2.5 n=4	19.5±1.5 n=8
Winter	44.9±3.6 n=7	35.0±0.8 n=6	20.6±1.9 n=7	17.5±0.3 n=6
P	<0.001	0.02-0.01	<0.001	n.s.

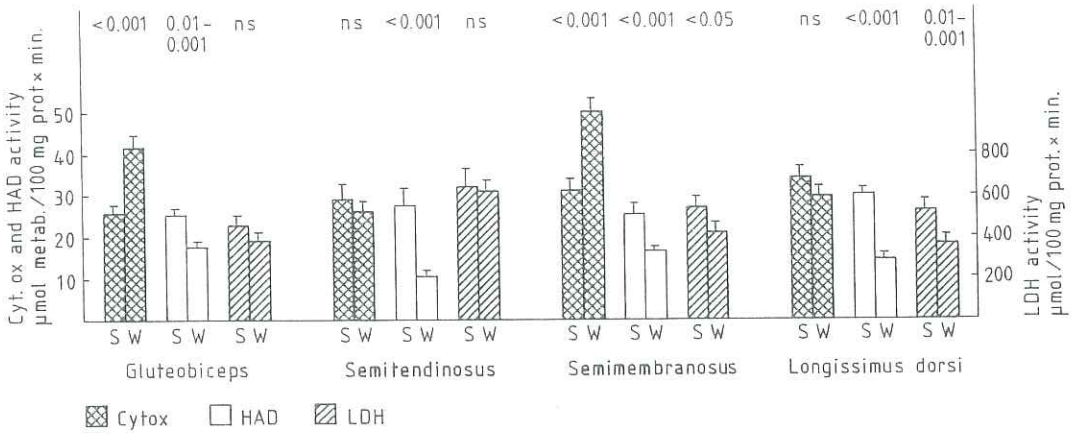

Figure 1. Enzyme activities in four skeletal muscles from male and female reindeers during summer (s) and winter (w) season.
The columns are mean values of four to seven summer and seven winter animals. Above each couple of columns are given statistical significance of the differences between summer and winter animals as regards enzyme activity

Figur 1. Enzymaktiviteter i fyra skelettmuskler från han- och honrenar under sommar- (s) och vinter- (w) säsongen.
Staplarna är medelvärden av fyra till sju sommar- och sju vinterdjur. Ovanför varje stapelpar anges statistisk signifikans för skillnaderna mellan sommar- och vinterdjur vad beträffar enzymaktivitet.

1985. In the same table figures are given from corresponding muscles collected during the summer (1st to 15th of July, 1982). There are no obvious differences in enzyme activities between male and female animals and therefore they are treated as one group.

The low HAD activity during the winter compared with the summer is most striking. Thus HAD activity is only 40% in semitendinosus and about 50% in longissimus in winter. Less pronounced differences are seen in the remaining two muscles. The more than 50% increase in cytox activity in gluteobiceps and semimembranosus simultaneous with unchanged activity in semitendinosus and longissimus is also striking. All male muscles except semitendinosus show a decrease in LDH activity during the winter.

Fibre properties

Table 2 shows number (%) and diameter of the three fibre types in male summer and winter reindeers. As to diameter there is a general difference between male summer and winter animals in so far as that the fibres are narrower during the winter. This difference is significant in IIA and IIB fibres from biceps and longissimus but not in the other two muscles, probably because these animals were shot too early in the summer. All four muscles are made into one group (Table 3). A significant decrease in muscle volume, on average 32.6% (mean value of at least ten variables), is obtained for male winter animals compared with male summer animals. I is responsible for 5% of this decrease in volume, IIA for 2% and IIB for 26%. No corresponding differences in muscle size could be observed in female reindeer (Table 3), again probably mainly due to the early summer «harvesting» time.

Figure 2 shows that in semitendinosus and semimembranosus in males, and in biceps and semimembranosus in females, the relative area occupied by IIA and IIB fibres is different during the winter and the summer. Thus the relative area of IIB fibres in a transverse section decreases and that of IIA fibres increases during the winter. The reason for this is partly the already mentioned decrease in fibre diameters which is more pronounced in IIB than in IIA fibres, but also a transformation of IIB into IIA fibres, an observation based on histochemical staining for NADH dehydrogenase activity.

DISCUSSION

In a previous paper (Kiessling and Kiessling 1984) we have tried to explain the Svalbard reindeer's muscle functions in the light of the

Table 2. Fibre diameters and relative fibre numbers in four muscles from male Svalbard reindeers during summer (s) and winter (w).

Tabell 2. Fiberdiametrar och relativa fiberantal i fyra muskler från hanliga Svalbardrenar under sommaren (s) och vintern (w).

		Diameter, μm			Number, %		
		I	IIA	IIB	I	IIA	IIB
Gluteo-biceps	S	37.1±2.6	40.6±3.5	44.7±3.5	20±2	35±4	45±3
	W	33.2±1.0	33.7±1.3	34.3±1.7	19±2	38±2	43±2
	p	ns	<0.05	<0.02	ns	ns	ns
Semiten-dinosus	S	34.7±4.8	40.1±0.3	44.0±4.2	15±5	23±3	63±5
	W	30.4±1.6	36.2±2.5	39.5±3.0	7±1	45±2	48±2
	p	ns	ns	ns	ns	<0.001	0.01
Semimem-brano-sus	S	36.0±1.2	35.9±2.2	37.7±3.5	5±1	30±4	65±3
	W	33.2±2.1	31.7±2.3	31.5±2.8	8±1	49±2	43±2
	p	ns	ns	ns	ns	<0.001	<0.001
Long-issimus dorsi	S	41.4±2.8	42.3±2.5	42.5±5.1	15±3	36±3	56±2
	W	31.2±3.1	32.1±2.2	31.4±2.1	11±1	41±1	48±1
	p	ns	<0.05	<0.05	ns	ns	0.01-0.001

extreme conditions to which these animals must adapt in order to survive.

The animals prepare themselves for the long winter period (with only very limited access to feed) among other things by building up large energy depots (Reimers and Ringberg 1983) of highly saturated fatty acids (Ringberg et al. 1979) and by increasing their insulation substantially (Nilssen et al. 1984). The Svalbard reindeer furthermore minimizes the energy expenditure by a very tranquil life (Nilssen 1984). Despite these precautionary measures not only their fat stores but also considerable amounts of lean tissue are lost (Reimers and Ringberg 1983). The male animals studied in April had a mean weight of less than 50% of that of the summer animals (Table 1). According to Reimers and Ringberg female reindeer may lose up to 30% of their autumn lean body weight and males around 42% during the winter. Food restriction to Svalbard reindeer was shown by Larsen et al. (1985) to cause an immediate rise in plasma levels of free

Table 3. Fibre-size and -composition in summer and winter reindeers expressed as values of four muscles.

Tabell 3. Fiberstorlek och fibersammansättning hos sommar- och vinterren uttryckt som medelvärden för fyra muskler.

	Diameter, μm			Number, %			Relative area		
	I	IIA	IIB	I	IIA	IIB	I	IIA	IIB
♂									
Summer	37.7±1.5	40.1±1.8	42.5±2.1	14±3	32±2	54±3	29	33	38
Winter	32.9±1.0	33.5±1.1	34.3±1.3	11±1	43±1	46±1	31	34	35
p	<0.01	<0.01	0.01	ns	<0.001	<0.01			
♀									
Summer	30.0±0.8	32.8±0.7	33.9±0.9	12±1	37±2	51±2	29	34	37
Winter	31±9	33.8±1.2	34.5±1.5	11±1	42±1	47±1	30	34	36
p	ns	ns	ns	ns	<0.05	ns			

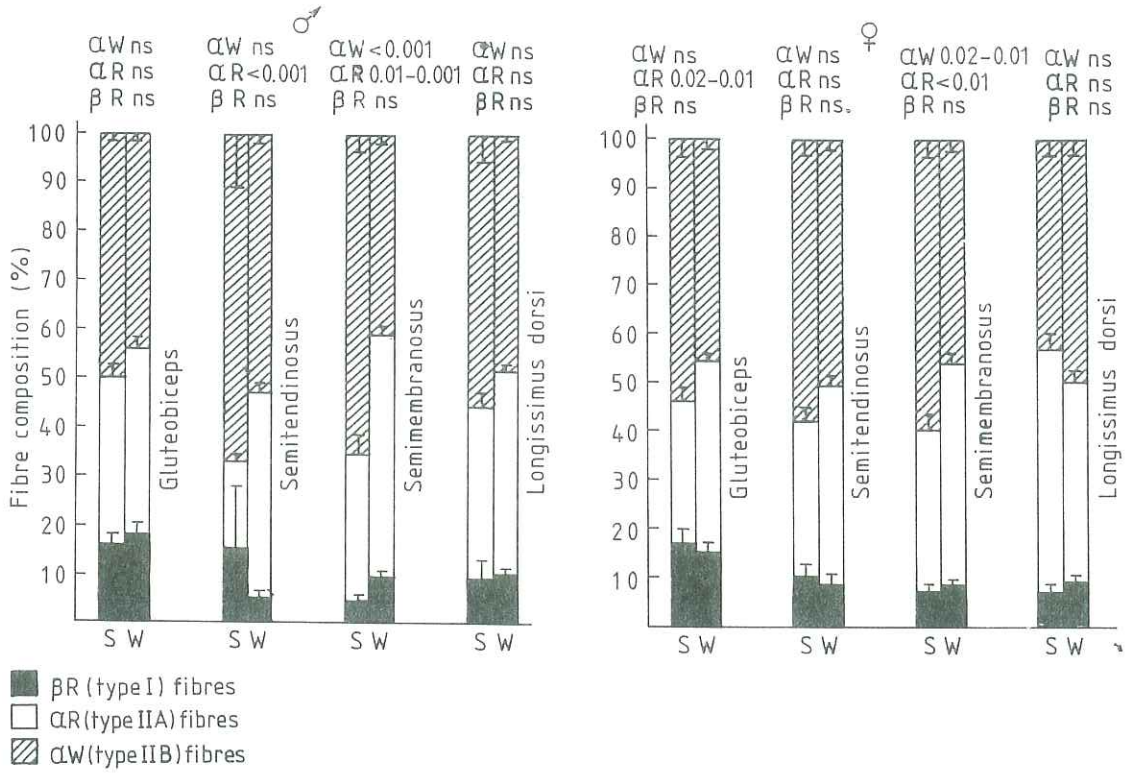

Figure 2. Fibre composition of four skeletal muscles from male and female reindeers during summer (s) and winter (w) season.
The proportion of each fibre type is expressed as a relative area, e.g. the area as a percentage that they occupy in transverse sections. The columns are mean values of four to seven summer and seven winter animals. Above each couple of columns are given statistical significance of the differences between summer and winter animals as regards fibre composition.

Figur 2. Fibersammansättning i fyra skelettmuskler från han- och honrenar under sommar- (s) och vinter- (w) säsongen.
Andelen av varje fibertyp är uttryckt som relativ yta, d.v.s. den yta i procent som den upptar i ett tvärsnitt. Staplarna är medelvärden av fyra till sju sommar- og sju vinterdjur.
Över varje stapelpar anges statistisk signifikans för skillnaderna mellan sommar- och vinterdjuren vad beträffar fibersammansättning.

fatty acids and glycerol whereas protein degradation, measured as plasma urea, increased steadily only from day eleven. Our hypotesis, based on the unexpectedly high content of IIB fibres in the skeletal muscles of Svalbard reindeers, was that particularly these fibres may constitute an energy reserve to be utilized when fat depots are consumed (Kiessling and Kiessling 1984).

The results in Figure 1 and Table 2 reveal obvious differences between muscle from summer and winter reindeers, metabolically as well as regarding fibre composition. Thus oxidative capacity (cytox) has increased about 50% in two muscles, biceps and semitendinosus, but not at all in the other two. Beta-oxidation of fatty acids (HAD), on the other hand, has decreased considerably in all four muscles, but most dramatically in longissimus and semitendinosus, that is, the two muscles with no decrease in oxidative capacity. These changes are valid for male as well as for female reindeer and may reflect adaptations to a nutritional situation with more or less exhausted fat stores and an increasing demand for protein as an energy store.

In all four male animal muscles studied there is a tendency towards a reduced fibre size during the winter compared with the summer (Tables

2 and 3) which, on average, amounts to one third (32.6%) of the muscle size. This shows that muscle protein has been used up during the winter and that all three fibre types, but IIB in particular, have contributed in males. These results are on the whole in agreement with our suggestion that especially the IIB fibre can serve as an energy source.

Staining for NADH-dehydrogenase, a histochemical measure of the oxidative activity, reveals only minor differences between the three fibre types. In some muscles IIB fibres are even darker than IIA fibres, which are mostly looked upon as highly oxidative compared with IIB. This means that from a metabolic point of view the three fibre types in certain muscles are rather similar as regards oxidative capacity. Besides being a potential energy reserve IIB fibres may thus also perform work by means of aerobic metabolism.

In contrast to male reindeers the results show no decrease in female muscle fibre size (Table 3). The explanation is most likely that the female animals were shot before any significant muscle mass regeneration could have taken place. Thus, the difference between summer and winter muscle fibre size would propably also have been greater in the males if the summer animals were shot later in the summer. The Svalbard reindeer may therefore survive the long food-depleted winter season partly by consuming muscle tissue.

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