THE INFLUENCE OF STRESS ON SUBSTRATE UTILIZATION IN SKELETAL MUSCLE FIBRES OF REINDEER (*RANGIFER TARANDUS* L)

Av stress påverkat substratutnyttjande i skeiettmuskelfibrer hos ren

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Abstract: Moderate stress in connection with handling, sampling and herding of reindeer caused a very pronounced depletion of glycogen in mainly type IIA and IIB fibres. Also intramuscular triglyceride levels decreased but mainly in type I fibres. Muscle lactate levels increased in all animals but not to the levels found in pigs exposed to stress or exertion. Reindeer muscles appeared to have a great capacity to oxidize both carbohydrates and lipids. All animals showed increased cortisol, urea and ASAT values.

A marked depletion of glycogen and lipids in many of the fibres may be a factor involved in the development of skeletal muscle degeneration in connection with mental stress and exertion as there seems to be a correlation between high ASAT values and substrate depleted musclefibres. A connection may therefore exist between high instramuscular substrate stores and the ability of a muscle to tolerate stress.

Key words: Reindeer, stress, skeletal muscle, glycogen, triglycerides.

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ESSÉN-GUSTAVSSON, B. & REHBINDER, C. Av stress påverkat substratutnyttjande i skeiettmuskelfibrer hos ren

Sammanfattning: Mättlig stress betingad av hantering, provtagning och drivning av ren orsakade en mycket kraftig minskning av muskelglykogen i främst typ IIA och typ IIB fibrer. Även triglycerider minskade framför allt i typ I fibrer. Muskellaktatniväerna ökade i samtliga undersökta djur, men inte till nivåer som ses hos gris utsatta för stress eller fysisk ansträngning.Renens muskler uppvisade en mycket hög kapacitet att oxidera, förbränna, både kolhydrat och fett.

Alla djur uppvisade förhöjda cortisol, urea och ASAT värden.

Den mycket kraftiga tömningen av kolhydrat och fett i mänga muskelfibrer kan vara en faktor medverkande till muskeldegeneration i samband med mental stress och ansträngning dä höga ASAT-värden synes vara korrelerade till uttömda muskelfibrer. Ett samband mellan hög instramuskulär substratupplagring och förmäga att tåla stress kan säledes föreligga.

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ESSÉN-GUSTAVSSON, B. & REHBINDER, C. 1984. Stressin vaikuttaneen poron substraattihyväksikäyttö luurangon lihaksiston kuiduissa.

Yhteenveto: Kohtuullinen stressi edellyttäen käsittelyä, kokeenottoa ja poronajoa aiheutti hyvin voimakkaan lihasglykogeenin vähentymisen etenkin II A ja II B tyyppisissä fiibereissä. Myöskin triglyseriidit vähentyivät kaikissa tutkituissa eläimissä, muttei kuitenkaan niihin tasoihin asti kuin sijoissa, jotka ovat joutuneet alttiiksi stressiin tai fyysilliseen rasitukseen. porojen lihakset osoittivat hyvin korkeaa kapasiteettia sekä hiilihydraatin että rasvan palamiseen.

Kaikki eläimet osoittivat kohonnutta cortisoolin, urean ja ASATin arvoa.

Hyvin voimakas hiilihydraatin ja rasvan tyhjentyminen monissa lihaskuiduissa voi olla vaikutin lihasrappeutumiseen henkisen stressin ja rasituksen yhteydessä, jolloin korkea ASAT-arvo näyttää olevan vastaavuussuhteessa tyhjentyneisiin lihaskuituihin. Lihaksiston korkean substraattivarastoimisin ja stressin sietokyvyn suhde voi siis olla olemassa.

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INTRODUCTION

It is well-known that stress situations can markedly effect skeletal muscle of different animals. Poor meat-quality and malignant hyperthermia in pigs are both related to the excessive glycogenolysis and lactic acid production which occur in skeletal muscle in connection with different stress situations (for ref. see Frøystein et al. 1981). A marked glycogenolysis has also been observed during a period of behavioral stress in cattle (McVeigh et al. 1982).Furthermore, various conditions of stress have been shown to induce degenerative changes in muscle and myocardia in domestic and wild animals (Johansson & Jönsson 1977, Bartsch et al. 1977, Rehbinder et al. 1982.)

The management methods used for semi-domestic reindeer today include several mentally and physiologically stressful periods such as herding into corrals, capture restraint, and manual handling for individual identification, and slaughter (Rehbinder et al. 1982). The purpose of this study was to investigate how the skeletal muscle of reindeer was affected by exposure to stress.

MATERIAL AND METHODS

Five female reindeer, two 4-years old (52, 994), two 1 year old (758, 759) and one calf (1 month) corralled at the National Veterinary Institute, Stockholm were used in this study. The three youngest animals were born in the corral (758, 759, calf) and the other two had been there for the last three years. The animals were fed a commercial reindeer fodder (Renfor, SLR) and water ad lib.

On the day of the experiment, 4 hours before the start of the sampling, the reindeer were enclosed in a central pen and fed. No. 994, however, refused to enter the pen and was captured with a lasso after an approximately 20 min. chase in the corral.

At the start of the experiment the animals were manually restrained in the pen and blood samples and muscle biopsies from the semitendinosus muscle were obtained from each reindeer. They were then set free in the corral and kept moving by a person walkie behind them for a 2 hour period. The animals were recaptured with a lasso and blood and muscle sampling were repeated. Thus the stress factors applied were, manual handling and restraint during sampling (2 x 10 min. per animal), the sampling itself, the chase and physical exercise for 2 hours and the recapture with lasso. The stress applied was mild to moderate compared with conventional reindeer herding methods. (Rehbinder & Edqvist 1981, Rehbinder et al 1982).

Blood samples

Two blood samples from each stressed reindeer were collected from the jugular vein, before and after the 2 hour chase, using 10 ml vacutainer tubes (Becton-Dickinson) containing either heparin or no anticoagulant. Cortisol concentrations in plasma were determined by a competitive protein binding technique, utilizing horse plasma as described by Lundström et al. (1975). Urea values were determined by means of a glucose/urea/creatinine analyser (IL 919; Instrumentation Laboratories), using reagents and procedures recommended by the manufacturer. Aspartate aminotransferase (ASAT) was determined by a kinetic technique on a LKB Reaction Rate Analyser according to the recommendations of the Scandinavian Committee on Enzymes (1974).

Muscle samples

Muscle biopsies were obtained from the semitendinosus muscle by means of a Bergström-needle (Bergström 1962) before and after the 2 hour chase. Two pieces were obtained from each reindeer after local anaesthesia of the skin (Carbocain 2%) and cutting through the fascia with a scalpel blade. One piece was immediately frozen in liquid nitrogen and stored at -80°C until biochemical analyses were performed. The second piece was mounted on a chuck with tissuetek and frozen in isopenthane pre-cooled in liquid nitrogen and then stored at -80°C until histochemical analyses were performed.

Biochemical analyses

The muscle tissue was freeze-dried for 24 hours and then put under a dissection microscope so that connective tissue, fat and blood could be removed. Glycogen and lactate analyses were performed on 1-2 mg of muscle tissue (Lowry and Passoneau 1970), while 5-10 mg tissue was used for analysing the enzyme activities (citrate-synthase (CS), 3-OH-acyl coH dehydrogenase (HAD), triose-phosphate dehydrogenase (TPDH) and lactate dehydrogenase (LDH)) according to Essén et al. (1980). For triglyceride analyses (Essén 1978) two or three samples weighing 0.5 - 1.5 mg of each muscle biopsy were used.

Histochemical analyses

Serial sections (10 µ) were cut in a cryostat and stained for myofibrillar ATPase after both acid and alkaline preincubation (Brooke and Kaiser 1969). Thicker sections (20 µ) were taken for the PAS (glycogen) and OIL RED (lipid) stains (Pearse 1961). The stained ATPase sections were then photographed and fibre types were identified as type I, IIA, and IIB fibres (Brooke and Kaiser 1970). Between 400 - 800 fibres were identified from each sample. After the fibres were identified on the photos the PAS and OIL RED stains were put under a «visopan» microscope and the staining of each fibre was thus rated as either dark, medium or light.

RESULTS

Fibre type composition (Table 1)

The mean percentage of type I fibres in semitendinosus was low (11%) while the mean percentage of type IIB fibres (63%) was more than twice as high as that of type IIA fibres (26%).

Enzyme activities (Table 1)

The mean activity for CS, analysed as a marker for citric acid cycle activity, was 88 µmol/g/min. For HAD, analysed as a marker for lipidoxidation, the mean activity was 56 µmol/g/min. TPDH, analysed as a marker for glycolytic capacity had an activity of 1680 µmole/g/min and LDH showed 1172 µmole/g/min when analysed as a marker for lactate production.

Table 1. Fibre type composition and enzyme activities (CS = citrate synthase, HAD = 3-OH-acyl-coA dehydrogenase, TPDH = triose-phosphate dehydrogenase and LDH = lactate dyhydrogenase) in semitendinosus muscle of reindeer exposed to stress.

Fibertypsammansättning och enzymaktivitet (CS = citrat synthas, HAD = 3-OH-acyl-coA dehydrogenas, TPDH = trios-fosfat dehydrogenas och LDH = laktat dehydrogenas) i Musculus semitendinosus hos renar utsatta för stress.

	Fibre type composition (%) *Fibertyp sammansättning (%)*			Enzyme activities (µmol/g/min) *Enzymaktivitet (µmol/g/min)*			
	I	IIA	IIB	CS	HAD	TPDH	LDH
Calf	14	26	60	112	56	1416	1300
759	8	21	71	80	60	1628	1136
758	12	30	58	76	52	1460	1064
52	12	28	60	84	60	2380	1452
994	8	27	65	80	52	1464	900
Mean value	11	26	63	88	56	1680	1172
± S.D.	3	3	5	16	4	396	212

Glycogen (Table 2, Table 3) (Plate 1)

After the 2 hour chase the amount of glycogen had decreased in all reindeer with a mean of 46µmole/g. The PAS stain showed that the glycogen in the muscle was stored in type IIA and IIB fibres as these were all dark or medium stained while some type I fibres were lightly stained. It was type IIA and IIB fibres that showed a larger percentage of lightly stained fibres after the 2 hour chase.

Triglyceride (Table 2)

Intramuscular triglyceride levels decreased in all animals after the 2 hour chase with a mean of 9.1µmole/g. Initially triglycerides were found largely in type I and IIA fibres as most of these were dark or medium stained by OIL RED whereas half of the type IIB fibres were lightly stained. After the chase more type I fibres were lightly stained.

Lactate (Table 2)

Muscle lactate levels had increased in all animals after the 2 hour chase with a mean of 16 µmole/g.

Cortisol, urea, and ASAT (Table 2)

All reindeer had, after the 2 hour chase, markedly higher values for cortisol, urea and ASAT compared with the initial samples.

Table 2. Glycogen, triglyceride, lactate (µmol/g) in semitendinosus muscle of stressed reindeer and cortisol (nmol/l), ASAT (µkat/l) and urea (mmol) levels in blood. I = Initial, F = Final sampling.

Glykogen, triglycerid, laktat (µmol/g) i musculus semitendinosus bos stressade renar samt cortisol (nmol/l), ASAT (µkat/l) och urea (mmol) i blod. I = Initialprovtagning, F = Slutlig provtagning.

	Glycogen		Triglyceride		Lactate		Cortisol		ASAT		Urea	
	I	F	I	F	I	F	I	F	I	F	I	F
Calf	174	164	15.9	5.6	26	47	90	106	2.3	5.7	13.4	16.8
759	217	155	26.3	13.6	40	58	30	97	1.9	4.5	9.3	10.7
758	256	187	17.1	10.4	34	65	48	89	2.1	4.2	11.7	13.9
52	120	32	30.0	29.7	23	36	99	100	3.4	6.2	13.9	16.3
994	17	15	36.3	20.9	15	16	97	112	5.6	13.8	14.8	17.9
Mean value	157	111	25.1	16.0	28	44	73	101	3.1	6.9	12.6	15.1
± S.D.	93	81	8.7	9.4	10	19	32	9	1.5	4.0	2.2	2.9

Table 3. In each fibre type (I, IIA, IIB) the staining intensity of OIL RED and PAS was rated as dark, medium and light. The values are given as per cent of dark, medium and light stained fibres within type I, IIA, and IIB fibre type population. I = Initial, F = Final sampling.

Hos varje fibertyp (I, IIA, IIB) har färgningsintensiteten graderats som mörk, medium, ljus. Värdena är angivna som procent mörka, medium och ljust färgade fibrer av det totala antalet undersökta fibrer av typ I, IIA och IIB.

			TYPE I			TYPE IIA			TYPE IIB		
OIL RED			Dark *Mörk*	Medium *Medium*	Light *Ljus*	Dark *Mörk*	Medium *Medium*	Light *Ljus*	Dark *Mörk*	Medium *Medium*	Light *Ljus*
	758	I	44	54	2	38	62	0	5	54	41
		F	16	60	24	16	83	1	2	61	37
	759	I	21	72	7	15	85	0	1	58	41
		F	17	35	48	25	74	1	5	82	13
	994	I	39	46	15	0	100	0	0	57	43
		F	7	80	13	3	97	0	5	68	27
	$\bar{x}$	I	35	57	8	18	82	0	2	56	42
		F	14	58	28	15	85	1	4	70	26
PAS											
	758	I	0	59	41	0	100	0	10	90	0
		F	0	41	59	4	92	4	17	79	4
	759	I	0	74	26	0	97	3	10	90	0
		F	0	72	28	0	96	4	6	89	5
	994	I	0	8	92	2	25	72	3	24	73
		F	0	13	87	0	0	100	2	18	80
	$\bar{x}$	I	0	47	53	1	74	25	8	68	24
		F	0	42	58	1	63	36	8	62	30

DISCUSSION

The larger percentage of type II fibres, particularly type IIB and the high activities of enzyme markers for oxidative capacity (CS and HAD)seen in this study of reindeer semitendinosus muscle agree with two other reports on reindeer muscle (Kiessling and Rydberg 1983, Essén-Gustavsson and Rehbinder submitted). However, in this study the glycogen levels observed (256 µmol/g) at the initial sampling were remarkably much lower than those found in the semitendinosus of two unstressed reindeer, 523-464 µmole/g (Essén-Gustavsson and Rehbinder submitted) and also those found in other animals such as the pig (Essén et al. 1980), horse (Lindholm et al. 1974) and bull (McVeigh et al. 1982). The reason for this pronounced difference in glycogen levels is most likely that the samples with the higher glycogen levels were obtained from reindeer shot in the free state when resting or ruminating while the reindeer in this study were exposed before biopsying to mental and physical stress. Further explanations for low glycogen levels are differences in diet and increased physical activity before sampling. The reindeer in this study ate similar fodder as the unstressed reindeer and all animals were sampled during the same season, thus different diets were not a factor here. Furthermore, the reindeer in this study were kept in a corral and had not been exposed to a great amount of physical activity before sampling. One exception was no. 994 who refused to enter the pen and had to be captured with a lasso after a 20 min. chase. Notably, this reindeer had very low glycogen levels in all fibre types, likely due to the excessive stress and physical activity before capture. The elevated urea and ASAT values are indicative of myodegeneration and comparable to those found in reindeer with confirmed myodegeneration, (Rehbinder et al. 1982). The initial low glycogen content in the muscles of all reindeer in this study therefore must indicate that a marked glycogenolysis occured in many of the fibres, because of both mental and physical stress, during the handling and sampling. The blood values for cortisol, urea and ASAT obtained at the first sampling event (before the chase) support this conclusion. The PAS-stains revealed that many type I fibres were completely depleted and that most of the type II fibres were moderately depleted (Plate 1).

Not only did glycogen levels fall after the 2 hour chase and lasso capture but triglycerides also noticably decreased. Evaluation of the degree of fibre depletion from the PAS- and OIL RED-stains, obtained from 3 reindeer, was not always easy to determine. If there was only a partial depletion of glycogen or lipids from medium stained fibres, the intensity of staining changes was difficult to evaluate.

A higher percentage of type IIA and IIB fibres were depleted, compared to type I, in the post chase PAS-stains, indicating that these fibre types were recruited and have utilized glycogen (Plate 1). As the reindeer muscle contain more type II fibres (89 %) than type I fibres (11 %), small changes in glycogen levels in type II fibres greatly effect the level of depletion of the muscle as a whole. In contrast when type I fibres also appeared to have been recruited they utilized lipids to a greater extent as many type I fibres stained lightly by OIL RED after the chase. The histochemical investigations thus indicate that all fibre types have been recruited but that type I and IIA fibres seem to have utilized both lipids and glycogen whereas type IIB fibres mainly utilized glycogen. After the chase the lactate levels in muscle had increased but not to the level seen in pigs (for ref. see Frøystein et al. 1981) where 2-3 times higher lactate levels are often recorded after stress and/or intense physical activity. The very high oxidative capacity of all fibre types in reindeer muscle probably explains the low lactate levels and further more the lactate dehydrogenase activity is also 2-3 times lower than that found in pigs and horses (Essén et al. 1980, Essén et al. 1980). It thus seems that reindeer have a great capacity to oxidize both carbohydrates and lipids.

The remarkable depletion of glycogen and lipids in many of the fibres may be a factor involved in the development of reindeer skeletal muscledegeneration in connection with exertion and mental stress (Rehbinder et al. 1982).

Furthermore, it appears that a relation between substrate depleted fibres and high serum ASAT values exists, indicating that degenerative changes do occur in these fibres. A connection may therefore exist between high intramuscular substrate stores and the ability of a muscle to tolerate stress.

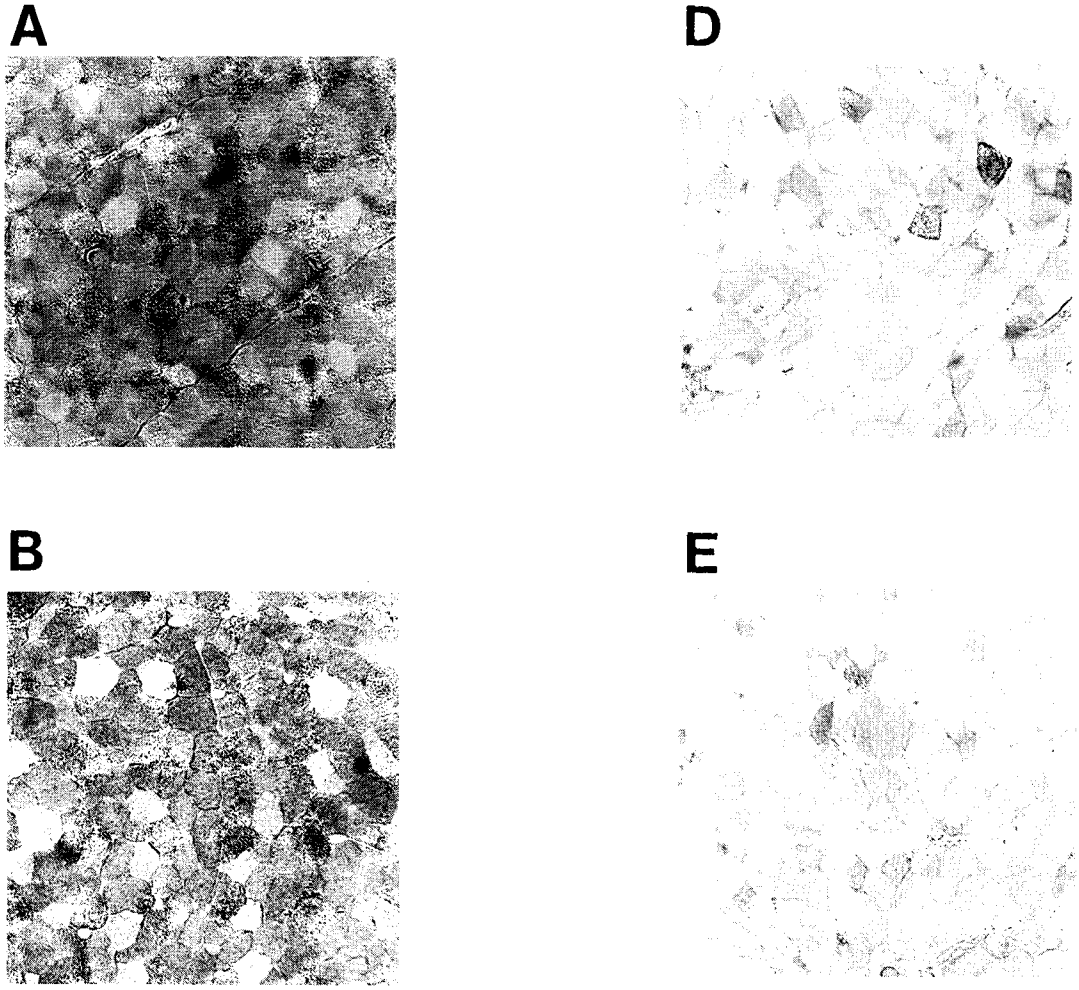

Plate 1. Sections of semitendinosus muscle of reindeer stained by PAS.

Unstressed reindeer (A) (Musclesample from study by Essén and Rehbinder, submitted)

Initial (B) and final (C) samlple of reindeer 758 and initial (D) and final (E) sample of reindeer 994.

Snitt av semitendinosus muskel hos ren färgad med PAS.

Ostressad ren (A) (Muskelprov från studie av Essén & Rehbinder, för publicering).

Initialt (B) och slutligt prov (C) från ren 758 och initialt (D) och slutligt (E) prov från ren 994.

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