

Prolonged food restriction and mild exercise in Shetland ponies: effects on weight gain, thyroid hormone concentrations and muscle Na^+, K^+ -ATPase

P Suwannachot¹, C B Verkleij^{1,3}, S Kocsis¹, E Enzerink^{2,4}
and M E Everts¹

¹Department of Anatomy and Physiology, Faculty of Veterinary Medicine, Utrecht University, PO Box 80-157, 3508 TD Utrecht, The Netherlands

²Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, PO Box 80-157, 3508 TD Utrecht, The Netherlands

³Academical Medical Center, Department of Endoscopy, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

⁴Veterinary Centre Someren, Slieven str. 16, 5711 PK Someren, The Netherlands

(Requests for offprints should be addressed to M E Everts, Department of Anatomy and Physiology, Faculty of Veterinary Medicine, Utrecht University, PO Box 80-157, 3508 TD Utrecht, The Netherlands; Email: M.Everts@vet.uu.nl)

Abstract

We determined the effects of food supply and low-intensity training on growth, serum thyroid hormone levels and the Na^+, K^+ -pump concentration in equine skeletal muscle. Twenty-two Shetland ponies were subjected to two different feeding regimes for 2½ years (11 ponies per group): food restriction (body condition score kept at 2) or *ad libitum* fed (body condition score kept at 8). Five ponies in each group underwent low-intensity training. Gluteus medius muscle and serum samples were obtained in April 1998. Subsequently, all ponies were fed *ad libitum* and the training programme was stopped. Muscle biopsies and serum samples were collected again in November 1998.

Food restriction was associated with a 30–50% reduction of body weight gain. While the total thyroxine (T_4) level was increased, the free T_4 remained at the control level. The serum total tri-iodothyronine (T_3) and free T_3 were

reduced by 30% and 49% respectively. After 6 months of refeeding there were no differences in any of the hormone levels between the *ad libitum* fed and the food-restricted groups. Food restriction produced a minor, but not significant, decrease in the Na^+, K^+ -pump concentration in the gluteus medius muscle of the Shetland ponies. Low-intensity training reduced weight gain of the *ad libitum* fed group by 25%, but had no detectable effect on the concentration of the Na^+, K^+ -pumps.

We conclude that prolonged food restriction in Shetland ponies results in a weight gain reduction of 30–50%, and is associated with similar decreases in serum total and free T_3 . The reduction in serum T_3 only slightly influenced the Na^+, K^+ -ATPase concentration in skeletal muscle, indicating that muscle tissue of different species may respond differently to changes in circulating thyroid hormones.

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Introduction

Excitability of skeletal muscle is critically dependent on the concentration gradients of Na^+ and K^+ across the sarcolemma, which are regulated by the membrane-bound Na^+, K^+ -pump (i.e. Na^+, K^+ -ATPase) (for review see Nielsen & Overgaard 1996). Regulation of the Na^+, K^+ -pump in skeletal muscle occurs by changes in either its activity (short-term) or its concentration (long-term). Long-term regulation of the concentration of Na^+, K^+ -ATPase is primarily exerted by thyroid hormones, which are the most potent stimulus for the synthesis of Na^+, K^+ -pumps (Clausen & Everts 1989, Clausen 1998).

The effects of thyroid hormones on the concentration of Na^+, K^+ -pumps in skeletal muscle have been reported in several studies both in rats and in humans (Kjeldsen *et al.*

1984, Brodie & Sampson 1988, Everts *et al.* 1990). In addition, it has been shown that either partial or complete food restriction decreased the concentration of the Na^+, K^+ -pumps in skeletal muscle due to the reduction of plasma thyroid hormone (tri-iodothyronine (T_3) and thyroxine (T_4)) levels. Conversely, refeeding or T_3 administration normalised the concentration of Na^+, K^+ -pumps (Swann 1984, Kjeldsen *et al.* 1986a, Matsumura *et al.* 1992). In horses, food deprivation for 2 days has been shown to induce a significant reduction in serum thyroid hormone levels (Messer *et al.* 1995). On the other hand, Sticker *et al.* (1995) reported that dietary energy and/or protein restriction for 33 days did not alter circulating thyroid hormone levels in horses.

Apart from thyroid hormones, training was found to increase the skeletal muscle Na^+, K^+ -pump concentration

Table 1 Body condition score of individual animals in the four groups of Shetland ponies (April 1998)

	Treatment group			
	F+/T-	F+/T+	F-/T-	F-/T+
	8	8	2	2
	7	7	2	2
	7	7	2	2
	8	8	2	2
	7	8	2	2
	8	—	2	—
Mean ± s.e.	7.5 ± 0.2	7.6 ± 0.2	2.0 ± 0	2.0 ± 0

For explanation of the symbols, see legend to Fig. 1.

both in man and in animals (Knochel *et al.* 1985, Kjeldsen *et al.* 1986b, Green *et al.* 1993, Madsen *et al.* 1994, McCutcheon *et al.* 1999, Suwannachot *et al.* 1999). In addition, hyperkalaemia during exercise was blunted in trained subjects (Knochel *et al.* 1985, Green *et al.* 1993, McKenna *et al.* 1993, McCutcheon *et al.* 1999), suggesting improved K^+ clearance during exercise.

The Shetland pony is a breed that is well-adapted for survival under the hard environmental conditions present on the Shetland Islands, i.e. low temperature, little and poor quality of food. However, nowadays, Shetland ponies are, in the vast majority of cases, raised as companion animals and under much more favourable climatic conditions; they are increasingly used in equine sport competitions. One of the nutritional problems of the Shetland pony is that under these circumstances they easily grow too fat, especially when they are fed a high-energy diet during growth. The question arises whether a low- or high- level of energy intake during growth influences the thyroid hormone levels and the Na^+, K^+ -pump concentration, and thereby the contractile capacity of its muscles.

The objective of the present study was to explore how the factors referred to above (i.e. food supply combined with a low-intensity training programme) affect the growth, the thyroid hormone levels, and the concentration of Na^+, K^+ -pumps in skeletal muscle of Shetland ponies.

Materials and Methods

Animals

Twenty-two Shetland ponies (six months old), 53 to 106 kg initial body weight (mean 81.4 ± 0.2 kg), were allotted (11 ponies per treatment) to one of two dietary treatments. Dietary treatments were formulated with the aim of total digestible energy meeting either 1000 VEP (restricted) or 2500 VEP (*ad libitum* fed) (VEP=Voeder Eenheid Paard=Feed Unit Horse), resulting in a low (2) and high (8) body condition score for restricted and *ad libitum* fed animals respectively (Table 1). Body condition

score was estimated using the semi-quantitative method described by Henneke *et al.* (1983). In brief, the amount of stored fat in the body was estimated from the areas of the body where fat cover was visible and could be palpated. The areas selected as being indicative of changes in stored body fat were the lumbar spinous processes, ribs, tailhead, area behind the shoulder, neck and withers. Condition was evaluated on a scale of 1 to 9, with 1 being extremely emaciated and 9 being extremely fat. Within each dietary treatment group, five ponies were subjected to a training regimen, which consisted of 40 s walk (1.4 m/s), 10 s trot (3.3 m/s), and 15 s gallop (6.1 m/s). The ponies were trained five days a week, and the exercising bout was alternated between 6 and 16 bouts a day every week. The ponies were kept separately in two groups of 11 animals (according to the feeding regimen) in large loose stalls (13 × 13 m); therefore, they could move freely. From six months until one year of age, the ponies were fed three meals per day (1 × hay and 2 × concentrates). After that they were fed twice daily (1 × hay and 1 × concentrates). Fresh water was available *ad libitum*. The experiment was started in August 1995. The first-time samples (muscle biopsy and serum) were obtained in April 1998, then the dietary treatment and exercise regimen were stopped. All ponies were fed *ad libitum* until November 1998, when the second-time samples were collected. The ponies were under intensive veterinary control. They received worm prophylaxis (dewormed) on a regular basis, and were checked by a veterinarian at least every seven weeks for general welfare and judgement of their exterior and the testicles. Between those periods the ponies were under the daily observation of experienced animal keepers. The experimental protocol has been approved by the Utrecht University Ethical Committee.

Sampling procedure

Blood samples were collected from the left jugular vein. Serum clot tubes were centrifuged; serum was harvested, frozen and stored at -80°C until analysed. The muscle samples were all taken from the left gluteus medius muscle percutaneously using the needle biopsy technique under local anaesthesia, according to the method described by Snow & Guy (1976). To minimise variation in the Na^+, K^+ -ATPase concentration due to the nonhomogeneous distribution of fibre types in skeletal muscle, all biopsies were taken by the same investigator, and the second biopsy was taken on the same side (left) within 2 cm of the first sample. The biopsy locations were identified using consistent anatomical landmarks and were at two-thirds of the distance along an imaginary line running from the tuber coxae to the tuber sacrale. Samples were taken in a direction perpendicular to the skin. The sampling depth varied from 4 to 6 cm (according to the muscle size and the body condition score). Samples were thus taken from the deep part of the dorsal compartment of

the gluteus medius muscle (Valette *et al.* 1999). Biopsies (weighing around 70–100 mg) were immediately frozen in liquid nitrogen and stored at -80°C until analysed.

$[^3\text{H}]$ Ouabain binding

The Na^+, K^+ -ATPase concentration was quantified by measuring $[^3\text{H}]$ ouabain binding capacity in the presence of vanadate (VO_4) as described by Nørgaard *et al.* (1983). This method allows the quantification of the total concentration of Na^+, K^+ -ATPase in small samples of muscle. Furthermore, studies in rat and human skeletal muscle have shown that the values obtained correspond to the total population of functional Na^+, K^+ -pumps (Clausen 1998). Since there was not enough tissue to make a complete standard curve with ouabain concentrations in the range 10^{-8} M to 10^{-6} M, an ouabain concentration of 10^{-6} M was used, since this has been shown to be above that required for saturation in rat (Nørgaard *et al.* 1983) and in foal (Suwannachot *et al.* 1999) muscle tissue. In brief, frozen biopsies were gently thawed and cut into small pieces weighing 5–10 mg, and were incubated in baskets with the bottom attached to a gas inlet allowing continuous gassing with air to ensure agitation. The specimens were pre-washed twice for 10 min at 37°C in unlabelled buffer solution to remove any Na^+ and K^+ present, to avoid interference with the binding of ouabain and/or vanadate. The unlabelled buffer solution contained Tris (24 mM), MgSO_4 (3 mM), vanadate (1 mM) and sucrose (250 mM). The final pH was adjusted to 7.3 with HCl. Incubation took place at 37°C in buffer containing $0.6 \mu\text{Ci/ml}$ $[^3\text{H}]$ ouabain and unlabelled ouabain added to a final concentration of 10^{-6} M for 120 min under continuous gassing with air. One set of specimens was incubated at an ouabain concentration of 10^{-3} M to allow correction for the unspecific uptake of $[^3\text{H}]$ ouabain. Incubation was followed by the washout of unbound $[^3\text{H}]$ ouabain, which took place in unlabelled buffer solution for 4×30 min on ice. After the last washout period, the specimens were gently pressed between two pieces of filter paper. Each specimen was put into a counting vial and weighed. Then 0.5 ml 5% trichloroacetic acid (TCA), containing 0.1 mM ouabain, was added to each specimen. Finally, after 16 h extraction in the refrigerator, 3 ml scintillation cocktail (Optifluor) were added and the ^3H -activity was measured by liquid scintillation counting (Minaxi Tri-carb, 4000 series; Packard Bioscience B.V., Groningen, The Netherlands). On the basis of the specific activity of $[^3\text{H}]$ ouabain in the incubation medium, the amount of $[^3\text{H}]$ ouabain taken up and retained in the muscle samples was calculated and after correction (for unspecific uptake and isotopic purity) was expressed as pmol/g wet weight.

Serum thyroid hormones

Serum 3,5,3'-tri-iodothyronine (T_3) and thyroxine (T_4) were measured using standard RIAs in unextracted serum.

Equilibrium dialysis was performed to estimate the serum T_3 and T_4 dialysable fractions. Free T_3 (FT_3) and free T_4 (FT_4) were calculated as the product of either total T_3 (TT_3) or total T_4 (TT_4) and their dialysable fractions (Sterling & Brenner 1966).

Serum K^+

Serum K^+ was determined using air-acetylene flame atomic absorption spectrometry (Varian SpectraAA-250 plus; Varian Australia Pty. Ltd, Victoria, Australia). Serum samples were diluted to 1:275 in 5.5 ml deionised water containing 0.1% Cs to suppress partial ionisation in the air-acetylene flame. Calibration was performed with 6 standard solutions (containing the same amount of Cs as in the diluted samples) ranging from 0 to 0.038 mmol/l potassium.

Chemicals

All chemicals were of analytical grade. TCA and K^+ standard (titrisol) were from Merck KGaA, Darmstadt, Germany. CsCl, sucrose and HCl were from Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA. Tris, MgSO_4 , ouabain, and vanadate were from Sigma Chemical Co., St Louis, MO, USA. Scintillation cocktail (Optifluor) was from Packard Bioscience B.V. $[^3\text{H}]$ ouabain (35.0 Ci/mmol) was from Amersham International, Amersham, Bucks, UK. According to the radiochemical batch analysis of Amersham, the 95% purity of $[^3\text{H}]$ ouabain gave a correction factor of 1.05 for isotope impurity.

Statistical analysis

All data are given as mean values \pm s.e. The differences between the paired data were tested using the paired sample *t*-test. The differences among group means for the effects of food restriction, refeeding and training on the serum thyroid hormones, the $[^3\text{H}]$ ouabain binding sites, weight gain and serum K^+ in either April or November were subjected to ANOVA, and Levene's test was used to verify the homogeneity of variances. Multiple comparison (post-hoc test) was performed using the Tukey's honestly significant difference test or, when data had unequal variances, using the Games-Howell pairwise comparison test. The differences were considered to be significant if $P < 0.05$.

Results

Weight gain

At the start of the investigation (6 months of age), mean body weights of the four groups were not significantly

Table 2 Body weights and weight gain of Shetland ponies. Values are means \pm s.e. The values in parentheses indicate the number of animals in each group

Group	Body weight (kg)			Weight gain (kg/day)	
	Aug. 1995	Apr. 1998	Nov. 1998	Aug. '95–Apr. '98	Apr. '98–Nov. '98
F+/T- (6)	84.0 \pm 2.6	209.3 \pm 5.8*	212.8 \pm 7.0	0.127 \pm 0.003†	0.016 \pm 0.010‡
F+/T+ (5)	76.2 \pm 5.9	169.9 \pm 10.2	179.6 \pm 12.4	0.095 \pm 0.006§	0.046 \pm 0.014
F-/T- (6)	81.3 \pm 4.4	135.3 \pm 13.6	172.5 \pm 16.7	0.055 \pm 0.010	0.172 \pm 0.016
F-/T+ (5)	83.6 \pm 7.3	148.2 \pm 9.7	188.4 \pm 12.5	0.066 \pm 0.004	0.186 \pm 0.014

* $P < 0.005$ F+/T- vs F-/T- and F-/T+; † $P < 0.02$ F+/T- vs F+/T+, F-/T- and F-/T+; ‡ $P < 0.001$ F+/T- vs F-/T- and F-/T+; § $P < 0.05$ F+/T+ vs F-/T- and F-/T+; || $P < 0.001$ F+/T+ vs F-/T- and F-/T+.

different, but a large variation among the individual animals existed. The mean body weights in April 1998 showed an even larger variation. As shown in Table 2, weight gain from August 1995 until April 1998 of the two *ad libitum* fed groups was significantly (30–50%) higher than that of the two restricted diet groups ($P < 0.05$ or better). This reflected the lower energy supply available for growth in the restricted groups. On the other hand, the growth rate from April until November 1998, which is the re-feeding and detraining period, of the restricted groups was significantly (3- to 10-fold) higher than that of the *ad libitum* fed groups ($P < 0.001$ for food restricted/untrained (F-/T-) and food restricted/trained (F-/T+) vs *ad libitum* fed/untrained (F+/T-) and *ad libitum* fed/trained (F+/T+)

groups). Low-intensity training reduced the weight gain from August 1995 until April 1998 by 25% ($P < 0.02$) in the *ad libitum* fed group.

Thyroid hormone status

Results for serum concentrations of total T_4 (TT₄) and free T_4 (FT₄), and total T_3 (TT₃) and free T_3 (FT₃) are presented in Figs 1 and 2 respectively. It can be seen that food restriction increased serum TT₄ by 40% compared with the *ad libitum* fed groups ($P < 0.01$), while serum FT₄ was the same in all groups (Fig. 1A and 1B, solid bars). This was associated with a 30% and 49% reduction of serum TT₃ and FT₃; $P < 0.05$ and $P < 0.01$ respectively

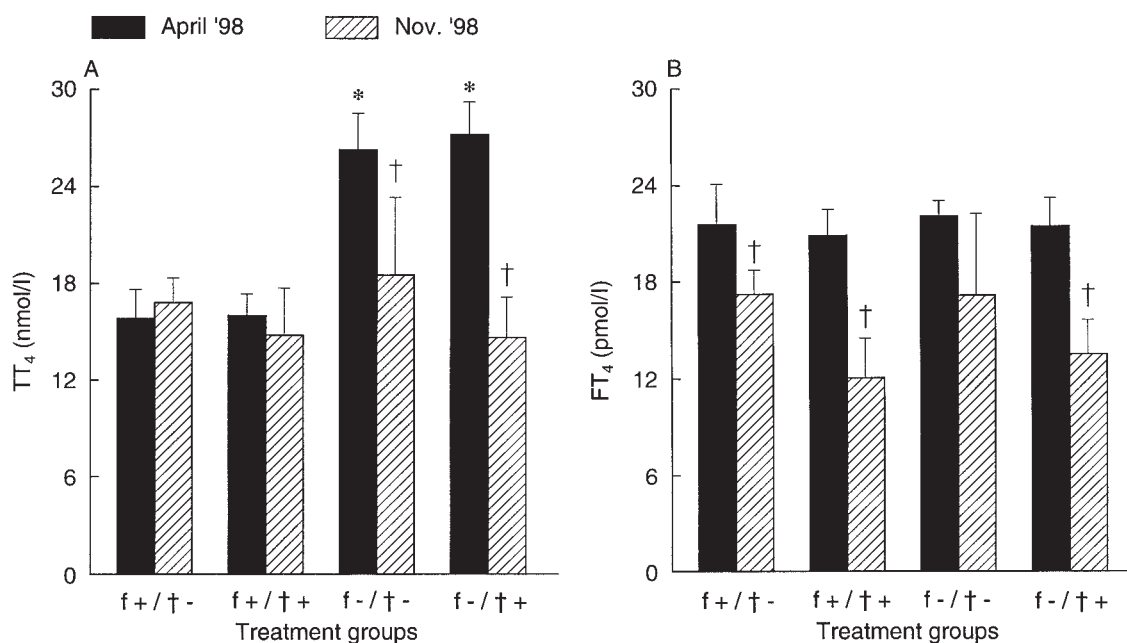


Figure 1 Serum thyroxine levels (TT₄: 1A, FT₄: 1B) in the four groups of Shetland ponies (f+/t-: *ad libitum* fed/untrained, f+/t+: *ad libitum* fed/trained, f-/t-: restricted/untrained, f-/t+: restricted/trained) in April (solid bars) and in November (hatched bars). Each column shows the mean \pm s.e. of 5–6 ponies. * Indicates a significant ($P < 0.01$) difference between food restriction and *ad libitum* fed. † Indicates a significant ($P < 0.05$) difference between April and November 1998.

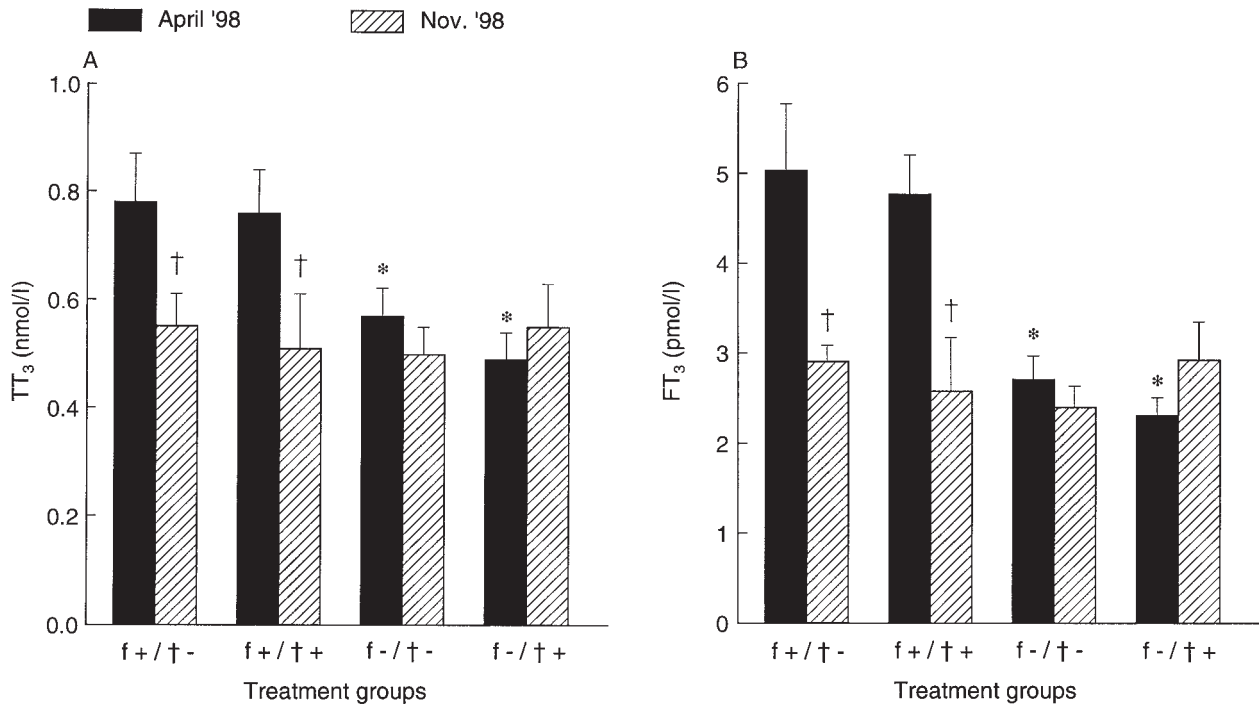


Figure 2 Serum 3,5,3'-tri-iodothyronine levels (TT₃: 2A, FT₃: 2B) in the four groups of Shetland ponies. For explanation of the symbols, see legend to Fig. 1. Each column shows the mean \pm S.E. of 5–6 ponies. * Indicates a significant ($P < 0.05$) difference between food restriction and *ad libitum* fed. † Indicates a significant ($P < 0.05$) difference between April and November 1998.

(Fig. 2A and 2B, solid bars). Refeeding for 6 months normalised serum TT₄ levels of the restricted groups to the baseline value (Fig. 1A, hatched bars). Surprisingly, serum TT₃ and FT₃ of the restricted groups did not increase after the period of refeeding, but serum TT₃ and FT₃ levels in the *ad libitum* fed groups were decreased to the same level as in the restricted groups (Fig. 2A and 2B, hatched bars). There was no difference in serum TT₃ and FT₃ levels between the period of food restriction and refeeding in the restricted groups (Fig. 2A and 2B, hatched bars). Serum FT₄ in all four groups was decreased in November compared with the values in April (Fig. 1B, hatched bars). No effects of training or detraining were found on the thyroid hormone levels in the restricted and the *ad libitum* fed groups.

[³H]Ouabain binding site concentration

The concentration of Na⁺,K⁺-ATPase expressed per gram wet weight in gluteus medius muscle is presented in Fig. 3. Neither food intake nor training induced any significant change in the Na⁺,K⁺-ATPase concentration between groups during the food restriction or the refeeding period. However, during the food restriction period, the mean values of the Na⁺,K⁺-ATPase concentration in the *ad libitum* fed groups were 12–15% higher than those of the restricted groups. After 6 months of refeeding, the

mean values of the Na⁺,K⁺-ATPase concentration of the restricted groups showed a slight increase (6–16%) compared with the food restriction period. Conversely, the mean values of the Na⁺,K⁺-ATPase concentration of the *ad libitum* fed groups were slightly decreased by 12–15%.

Serum K⁺

Results of the serum K⁺ concentration are presented in Fig. 4. Neither food restriction nor training resulted in any significant change in serum K⁺ among the groups in April. The serum K⁺ in the restricted/untrained group was significantly increased after the 6-month refeeding period ($P < 0.05$).

Discussion

Undernutrition and/or starvation including caloric restriction are accompanied by a reduction in thyroid hormone levels, which subsequently may lead to a reduction in the Na⁺,K⁺-ATPase concentration in skeletal muscle as shown for rats and pigs (Swann 1984, Kjeldsen *et al.* 1986a, Dauncey & Burton 1989, Matsumura *et al.* 1992, Harrison *et al.* 1996). Skeletal muscle contains the largest pool of Na⁺,K⁺-ATPase in the body, representing a considerable capacity for the clearance of K⁺ from the

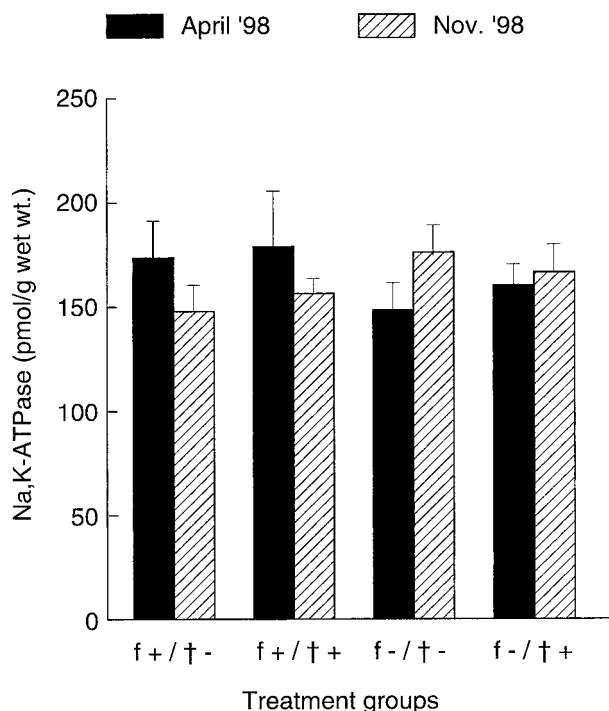


Figure 3 Effects of food restriction, low-intensity training and refeeding on the concentration of Na^+, K^+ -ATPase in gluteus medius muscle of Shetland ponies. For explanation of the symbols, see legend to Fig. 1. Each column shows the mean \pm S.E. of 5–6 ponies in each group. There were no significant differences between groups or months.

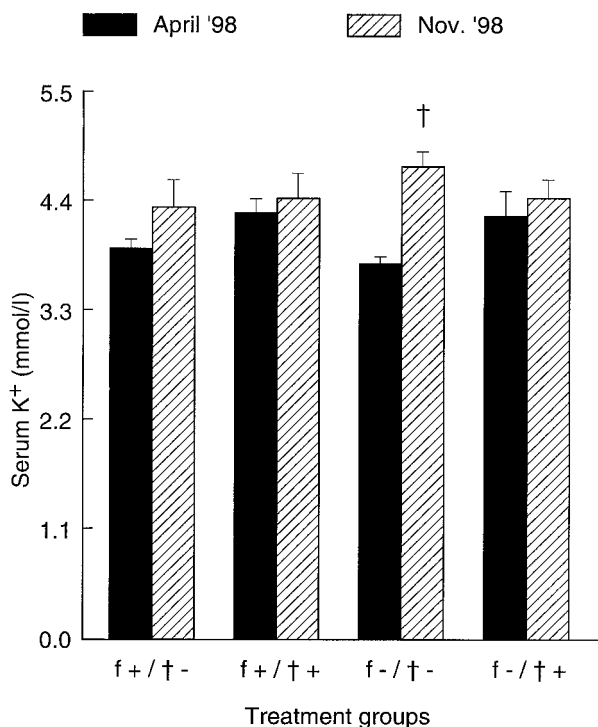


Figure 4 Histogram showing the concentration of serum K^+ for the four groups of Shetland ponies as described in the legend to Fig. 1. † Indicates a significant ($P < 0.05$) difference between April and November.

plasma, for restoration of the membrane potential and maintenance of contractile performance (Clausen & Everts 1989, Nielsen & Overgaard 1996). Since the equine species is considered to be a great athletic species, restricted energy intake, especially during growth, may affect the Na^+, K^+ -ATPase concentration due to changes in thyroid hormone levels as well as the development of the muscle, and thus diminish potential athletic performance.

At variance with other reports in horses showing that neither plasma T_3 nor T_4 concentrations were altered due to restriction of the food supply (Glade *et al.* 1984, Glade & Reimers 1985, Sticker *et al.* 1995), the present results show that prolonged dietary caloric restriction in Shetland ponies is not unexpectedly associated with a diminished body weight gain, but is also accompanied by changes in thyroid hormone metabolism. As a consequence of dietary energy restriction, serum TT_4 increased (40%) while serum TT_3 and FT_3 decreased (30% and 49%). This difference may be due to variation in the severity and the period of food restriction. In our study food restriction was rather severe, resulting in a body condition score of 2 on a scale ranging from 1 (extremely emaciated) to 9 (extremely fat). Yambayamba *et al.* (1996) found that the TT_3 and TT_4 concentration of food restricted heifers did not

differ from those of *ad libitum* fed heifers on day 20, but a difference existed after 48 days. Thus food restriction to 50–80% of the daily requirement (Glade *et al.* 1984, Glade & Reimers 1985, Sticker *et al.* 1995) for ≤ 1 month may not be long enough for horses to adapt their metabolic hormonal regulation. However, short-term food restriction in horses may influence cellular metabolism since caloric-restricted horses showed reduced levels of plasma glucose and insulin, and increased plasma nonesterified fatty acid concentrations (Sticker *et al.* 1996). The changes in thyroid hormone levels in the present study, with elevated TT_4 and decreased TT_3 and FT_3 are similar to those seen in caloric-restricted cats (Fettman *et al.* 1998) as well as in humans suffering from malnutrition (Wartofsky & Burman 1982). This may represent an adaptive response to minimise the basal metabolic rate during conditions of a negative caloric balance. The low T_3 may be the result of either reduced T_4 to T_3 conversion (Aláez *et al.* 1992) or reduced transport of T_4 into the liver (Hennemann *et al.* 1998). The finding of an increased serum TT_4 with normal FT_4 values in the ponies fed a restricted diet suggests that most of the rise in serum TT_4 is accounted for by elevation of the protein-bound hormone.

The changes in the thyroid hormone levels we observed following the refeeding period are not easy to explain.

Although serum TT_4 in the restricted groups was normalised, serum TT_3 and FT_3 remained the same as in the restriction period. It could be that not only food intake but also environmental factors, such as temperature and/or seasonal variation, influenced the thyroid hormone levels during this period. This is supported by the fall in serum TT_3 , and FT_3 in the *ad libitum* fed groups. In addition, the FT_4 level which did not differ between the groups during the restriction period (April), tended to decrease in November. Seasonal variation in thyroid hormone levels has been reported in several mammalian species including horses, and this variation is thought to be dependent on environmental temperature and/or photoperiod (Johnson 1986, Clariget *et al.* 1998, Rhind *et al.* 1998). However, it should be noted that a difference in species and/or geographic locations (latitudes of $40^{\circ}30'N$, Johnson 1986; $57^{\circ}N$, Rhind *et al.* 1998; and $52^{\circ}N$, the present results), causing a difference in temperature and daylight period, may influence the month of hormone peak throughout the year.

Interestingly, food restriction was associated with a reduced body weight gain and reduced total and free T_3 levels, while refeeding was accompanied by a 3- to 10-fold higher growth rate without a rise in total and free T_3 . Apart from the seasonal variation in thyroid hormone levels as mentioned above, this could also suggest that growth hormone and/or insulin-like growth factor-I (IGF-I) are more important for the catch-up growth in the refeeding period (Everts *et al.* 1990). Glucocorticoids may also play a role in body weight gain during the food restriction period as well as during the refeeding period (Sticker *et al.* 1995) since they are involved in gluconeogenesis, lypolysis and protein catabolism (Irvine 1983). Unfortunately, we had insufficient serum to determine growth hormone, IGF-I and glucocorticoids.

Unlike studies in rats (Swann 1984, Kjeldsen *et al.* 1986a), the Na^+,K^+ -ATPase concentration in gluteus medius of the ponies did not show any significant change due to the low serum T_3 mediated by food restriction. However, it should be noted that the fall in serum T_3 during the restriction period did not reach a level lower than that found in *ad libitum* fed animals in November, which is presumed to be due to seasonal variation. Furthermore, the reduced body weight gain associated with food restriction will also reflect changes in the muscle, such as a relatively smaller fibre size or cross sectional area (Harrison *et al.* 1994), and thereby a greater plasma membrane surface area per unit wet weight. Since the Na^+,K^+ -ATPase concentration in the present study is expressed per unit wet weight, the reduction in Na^+,K^+ -ATPase concentration would have been greater if the change in plasma membrane area could be taken into account. As can be seen from the results, food restriction induced a decrease in TT_3 and FT_3 , and subsequently decreased the Na^+,K^+ -ATPase concentration by around 12–15%. Refeeding did not alter serum TT_3 and FT_3

levels in the restricted diet ponies, which is probably due to seasonal variation as mentioned before, but it did result in an increase of between 6 and 16% in Na^+,K^+ -ATPase concentration. In addition, the fall in TT_3 and FT_3 in the *ad libitum* fed groups in November was accompanied by a reduction of 12–15% in Na^+,K^+ -ATPase concentration. This suggests that thyroid status may have some influence on the Na^+,K^+ -ATPase concentration in Shetland ponies, but the effect of mild hypothyroidism (low T_3) is smaller than that seen in rats. This may be due to species-dependent differences in the response of skeletal muscle tissue to thyroid hormones such as Na^+,K^+ -ATPase isoform expression (Sweadner 1989, Azuma *et al.* 1993) and/or the number of T_3 receptors (Morovat & Dauncey 1995, Harrison *et al.* 1996).

Since the training intensity in the present study was rather low, we could not observe a significant rise in Na^+,K^+ -ATPase concentration. This suggests that there is a certain threshold for up-regulation of the Na^+,K^+ -ATPase concentration, and also supports a conclusion from our previous study that the increase in Na^+,K^+ -ATPase concentration following training seems to be related to the amount and intensity of exercise (Suwannachot *et al.* 1999). K^+ deficiency has been shown to down-regulate Na^+,K^+ -ATPase concentration in rat skeletal muscle (Kjeldsen *et al.* 1986a, Clausen & Everts 1989). The present results show that food restriction did not influence the serum K^+ concentration of the ponies. Although the serum K^+ in the restricted/untrained group was significantly increased after the 6-month refeeding period, it is unlikely that food restriction leads to K^+ deficiency since serum K^+ of the restricted/trained group showed no significant difference between the restricted and the refeeding period.

In conclusion the present results suggest that food restriction in Shetland ponies induces a large decrease in body weight gain, and is associated with a decrease in serum total and free T_3 . The reduction in circulating T_3 may interfere with growth, development and metabolism of muscle cells. The changes in serum T_3 concentration due to food restriction only slightly influenced the skeletal muscle Na^+,K^+ -ATPase concentration. This indicates that skeletal muscle of different species may respond differently to changes in circulating thyroid hormones. For further understanding of the influence of thyroid hormones on the Na^+,K^+ -ATPase concentration in horses, the Na^+,K^+ -ATPase isoform expression and the number of T_3 receptors in skeletal muscle should be the focus of future investigations.

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