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Effects of semi-starvation and potassium deficiency on the concentration of [^{3}H]ouabain-binding sites and sodium and potassium contents in rat skeletal muscle

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1. Using vanadate-facilitated [^{3}H]ouabain binding, the effect of semi-starvation on the total concentration of [^{3}H]ouabain-binding sites was determined in samples of rat skeletal muscle. When 12-week-old rats were semi-starved for 1, 2 or 3 weeks on one-third to half the normal daily energy intake, the [^{3}H]ouabain-binding site concentration in soleus muscle was reduced by 19, 24 and 25% respectively. In extensor digitorum longus, diaphragm and gastrocnemius muscles the decrease after 2 weeks of semi-starvation was 15, 18 and 17% respectively. The decrease was fully reversible within 3 d of free access to the diet. Complete deprivation of food for 5 d caused a reduction of 25% in soleus muscle [^{3}H]ouabain-binding-site concentration. It was excluded that the reduction in [^{3}H]ouabain binding was due to a reduced affinity of the binding site for [^{3}H]ouabain.

2. Semi-starvation of 12-week-old rats for 3 weeks caused a reduction of 45 and 53% in 3,5,3′-triiodothyronine (T_3) and thyroxine (T_4) levels respectively. As reduced thyroid hormone levels have previously been found to decrease [^{3}H]ouabain-binding-site concentration in skeletal muscle, this points to the importance of T_3 and T_4 in the down-regulation of the [^{3}H]ouabain-binding-site concentration in skeletal muscle with semi-starvation. Whereas potassium depletion caused a decrease in K content as well as in [^{3}H]ouabain-binding-site concentration in skeletal muscles, semi-starvation caused only a tendency to a decrease in K content. Thus, K depletion is not a major cause of the reduction in [^{3}H]ouabain-binding-site concentration with semi-starvation.

3. Due to its high concentration of Na,K pumps, skeletal muscle has a considerable capacity for clearing K from the plasma as well as for the binding of digitalis glycosides. Semi-starvation causes a severe reduction in the total skeletal muscle pool of Na,K pumps and may therefore be associated with impairment of K tolerance and increased digitalis toxicity.

It has been demonstrated that potassium deficiency leads to a selective loss of K and Na,K-ATPase from muscle cells (Nørgaard *et al.* 1981, 1985; Clausen *et al.* 1982; Kjeldsen *et al.* 1984; Brown *et al.* 1986). These changes are associated with a considerable gain in muscle sodium content and may become quite severe within 1 or 2 weeks, particularly in young animals. K deficiency leads to fatigue, wasting and cellular necrosis in muscle as well as impaired protein synthesis (Alexis *et al.* 1971; Knochel, 1982). Furthermore, severe K deficiency may cause gastrointestinal paralysis with ileus and reduced absorption of nutrients (Lindeman & Pederson, 1983). In human subjects, K deficiency is often associated with reduced energy intake and inadequate protein supply. It is therefore of interest to compare the effects of K deficiency and semi-starvation.

Starvation is associated with a decrease in the plasma levels of thyroid hormones (Vagenakis *et al.* 1975; Burman *et al.* 1977; Schussler & Orlando, 1978; Germain & Galton, 1985) and hypothyroidism is associated with a decrease in skeletal muscle Na,K-ATPase concentration (Asano *et al.* 1976; Lin & Akera, 1978; Biron *et al.* 1979; Kjeldsen *et al.* 1984, 1985, 1986*a*). Thus, it was of interest to determine thyroid hormone levels and Na,K-ATPase concentration in skeletal muscles during semi-starvation.

Based on measurements made on membrane fractions of rat skeletal muscle, the Na,K-ATPase activity after 5 d of total fasting has been reported to decrease by 50% (Swann, 1984). We have quantified the Na,K-ATPase concentration in skeletal muscle by measuring the [^{3}H]ouabain-binding-site concentration in muscle samples (Nørgaard *et al.*

1983). Unlike the methods based on measurements of enzyme activities in subcellular fractions (Jones & Besch, 1984), this assay determines the total concentration of Na,K-ATPase (Clausen, 1986; Kjeldsen, 1986).

METHODS

Animals and treatment

All experiments were carried out using fed female Wistar rats which were 4–12 weeks old when started on special diets. The animals had free access to water and were kept in a constant temperature (23°) environment with constant humidity and day length.

Semi-starvation was induced in mature rats (12 weeks old) by maintaining them on 7, 8·5 or 10 g standard diet/d for the 1st, 2nd or 3rd week of treatment respectively. This was equivalent to 96, 117 and 138 kJ (23, 28 and 33 kcal)/d, i.e. one-third to half their normal daily energy intake. This treatment caused a reduction in body-weight of up to 40%. After 1 week of free access to food the semi-starved rats gained weight to 89% of that of the control level (Fig. 2, p. 523). Semi-starvation was induced in young rats (4 weeks old) by giving them 4 g diet/d, equivalent to 54 kJ (13 kcal)/d, for 1 week. The normal daily food intake of 4-week-old rats increased from 6 to 11 g standard diet/d during the 5th week of life. Thus, they received between 67 and 37% of the usual food intake. This diet caused a reduction in body-weight of up to 58%. Complete starvation was induced in mature rats (12 weeks old) receiving no food for 5 d. This produced a loss in body-weight of 25%. All animals had free access to tap water. Thyroid status was evaluated by determination of total plasma 3,5,3′-triiodothyronine (T_3) and thyroxin (T_4) hormones by radioimmunoassay as well as by measurements of body temperature.

K deficiency was induced by maintaining mature rats (12 weeks old) on a diet containing 2 mmol K and 152 mmol Na/kg dry weight, and distilled water. Control rats were kept on a standard diet containing 262 mmol K and 87 mmol Na/kg dry weight, and tap-water. Metal frames were positioned in the bottom of the cages of the starved as well as the K-depleted rats to prevent the animals from having access to urine and faeces. K depletion for up to 2 weeks caused no decrease in body-weight. It has previously been shown that K depletion resulting in a 48% decrease in [^{3}H]ouabain-binding-site concentration in skeletal muscle occurs without changes in total T_3 and T_4 concentrations (Kjeldsen *et al.* 1986*a*). As it has been shown that variations in muscle activity affect the concentration of [^{3}H]ouabain-binding sites (Kjeldsen *et al.* 1986*b*) the effect of placing grids instead of wood chips in the bottom of the cages was tested. This was done by measuring [^{3}H]ouabain binding in soleus and extensor digitorum longus (edl) muscles of 12-week-old rats who had been maintained on wood chips or grids for 1 week. This gave mean values (pmol/g wet weight) of 253 (SE 9) (*n* 5) and 247 (SE 8) (*n* 5) (not significant) in soleus and of 364 (SE 30) (*n* 5) and 353 (SE 26) (*n* 5) (not significant) in edl (values corrected for unspecific uptake and retention of [^{3}H]ouabain and radiopurity of [^{3}H]ouabain, see p. 521). Thus, placing grids in the bottom of the cages for 1 week did not cause a change in muscle activity that could be detected in the [^{3}H]ouabain-binding-site concentration.

The combined effect of semi-starvation and K deficiency was studied in mature rats (12 weeks old) maintained on 7 g diet deficient in K/d (96 kJ (23 kcal)/d) for 2 weeks. This reduced body-weight by 23%. Semi-starved control rats received 7 g standard diet/d (96 kJ (23 kcal)/d) for a similar period of time and a reduction in body-weight of 25% was seen.

[^{3}H]ouabain binding to muscle samples

Muscle samples were prepared for vanadate-facilitated [^{3}H]ouabain binding. This method has been demonstrated to give the same concentration of [^{3}H]ouabain-binding sites as

obtained using intact muscle fibres (Nørgaard *et al.* 1983). After decapitation, gastrocnemius, soleus, edl and diaphragm muscles were dissected out and the fibres cut transversely into samples in the wet-weight range 2–8 mg in order to ensure ready access of vanadate to the inside of the plasma membrane. A buffer containing 10 mM-Tris chloride, 3 mM-magnesium sulphate, 1 mM-Tris vanadate and 250 mM-sucrose was used. pH was adjusted to 7·3 using Tris chloride. To ensure agitation of muscle samples in the buffer, the buffer was continuously gassed with air. Specimens were prewashed twice for 10 min each time at 0°. In the standard experiments, muscle samples were then incubated for two periods of 60 min at 37° in buffer containing [^{3}H]ouabain (2·08 μCi/ml) and unlabelled ouabain at a total concentration of 1 μM. Following the incubation with [^{3}H]ouabain, the samples were washed four times, each for 30 min at 0°, in unlabelled buffer so as to remove [^{3}H]ouabain activity from the extracellular space. The samples were then blotted, weighed and taken for liquid scintillation counting of the ^{3}H activity. The amount of ^{3}H activity retained after the cold wash was calculated and expressed as the relative uptake of the incubation-medium [^{3}H]ouabain activity into the muscle samples (ml incubation-medium [^{3}H]ouabain activity taken up/g tissue wet weight, expressed as a percentage). For each experimental condition a set of samples was incubated with the addition of 1 mM-ouabain, on the basis of which the unspecific [^{3}H]ouabain uptake and retention were determined. The total concentration of [^{3}H]ouabain-binding sites was calculated by first subtracting the unspecific [^{3}H]ouabain uptake and retention from the [^{3}H]ouabain uptake and retention after incubation with 1 μM-ouabain and converting the relative uptake (%) to [^{3}H]ouabain-binding sites occupied (pmol/g wet weight). The total [^{3}H]ouabain-binding-site concentration was then calculated by multiplying by three correction factors to allow for (1) radiopurity of the [^{3}H]ouabain isotope (1·05; see below) and (2) loss of specifically-bound [^{3}H]ouabain during washout in the cold (1·21). This was assessed in separate experiments with washouts for twelve 30 min periods at 0°. To determine the affinity constants for [^{3}H]ouabain-receptor–ligand interaction, muscle samples were incubated for four 60 min periods in buffer containing [^{3}H]ouabain (0·20–2·08 μCi/ml) and ouabain added to final concentrations of 0·01–5 μM. This showed that incomplete saturation associated with measurements at 1 μM-ouabain can be corrected for by multiplying by 1·07 (third correction factor, see Fig. 4, p. 527). It has earlier been shown in soleus muscle samples that equilibrium is achieved after incubation for two 60 min periods and four 60 min periods at ouabain concentrations of 1 μM and 0·01 μM respectively (Kjeldsen, 1986).

Na and K contents

Immediately after decapitation, tissue samples were taken from the soleus and gastrocnemius muscles, homogenized in 0·3 M-trichloroacetic acid and centrifuged. The Na and K contents of these extracts were determined using a Radiometer FLM 3 flame photometer with lithium as internal standard.

Chemicals

All chemicals were of analytical grade. Ouabain was obtained from Sigma Co., St Louis, MO, and [^{3}H]ouabain (20·9 Ci/mmol) from New England Nuclear Corporation, Boston, MA. The purity of [^{3}H]ouabain was checked by the Na,K-ATPase extraction method (Hansen & Skou, 1973). The radiopurity was found to be 95%, and this was corrected for in the calculation of specific [^{3}H]ouabain binding. The K-deficient diet was obtained from Altromin Co., Lage, West Germany.

Statistics

All results are given as mean values with their standard errors. The statistical significance of any difference was assessed using the two-tailed t test for non-paired observations. Any P value above 0·05 was designated as not significant.

RESULTS

Semi-starvation of 12-week-old rats for 1–3 weeks induced a decrease in [^{3}H]ouabain-binding-site concentration in soleus muscles of 19–25%. These reductions were not significantly different. Most of the decrease appeared within the 1st week of semi-starvation, thereafter the [^{3}H]ouabain-binding-site concentration showed nearly the same relative reduction with age in controls and semi-starved rats. Thus, semi-starvation caused an initial decrease in [^{3}H]ouabain-binding-site concentration followed by a new steady-state with the normal age-dependent decrease in the [^{3}H]ouabain-binding-site concentration (Fig. 1). It should be noted that the decrease in [^{3}H]ouabain-binding-site concentration in the 1st week of semi-starvation coincided with the major drop in body-weight (Fig. 2). The water content determined after incubations of soleus muscle samples from 12-week-old rats semi-starved for a further 1 week and controls was 77·7 (SE 0·5)% (n 4) and 75·6 (SE 0·6)% (n 4) respectively ($P < 0·05$). This marginal relative increase in water content of 2·8% in soleus muscle samples with semi-starvation cannot account for any substantial part of the decrease in [^{3}H]ouabain-binding-site concentration.

When 12-week-old rats semi-starved for 2 weeks were given free access to food the [^{3}H]ouabain-binding-site concentration returned to the normal range within 3 d (Fig. 1). The body-weight, however, had only reached 76% of the control value (Fig. 2).

To investigate whether the decrease in [^{3}H]ouabain binding with semi-starvation was general in the skeletal muscle pool, the [^{3}H]ouabain-binding-site concentration was also measured in the edl, diaphragm and gastrocnemius muscles (Fig. 3). For these muscles the relative decrease was 15, 18 and 17% respectively, after 2 weeks of semi-starvation. Again, these reductions were not significantly different. As the four different muscles studied contain varying proportions of slow-twitch and fast-twitch fibres, they would seem to be representative of the entire skeletal muscle pool, indicating that a general reduction of the skeletal muscle [^{3}H]ouabain-binding-site concentration of 15–24% occurs when mature rats are semi-starved for 2 weeks.

In order to determine whether the decrease in skeletal muscle [^{3}H]ouabain-binding-site concentration was associated with changes in the Na and K contents, these values were determined in soleus and gastrocnemius muscles (Table 1). The general picture is a tendency to minor decreases in K content and increases in Na content with semi-starvation.

As could be expected from the decrease in body-weight with semi-starvation (Fig. 2), a decrease in muscle mass could be observed. Following semi-starvation for 1 week the wet weight of the soleus muscle decreased by 16 and 48% in adult and 4-week-old rats respectively (Table 2). Thus, with semi-starvation the total number of [^{3}H]ouabain-binding sites in soleus muscle decreased due to a reduction in muscle mass as well as a decrease in [^{3}H]ouabain-binding-site concentration. Following semi-starvation for 1 week the total number of [^{3}H]ouabain-binding sites decreased by 32 and 58% for adult and 4-week-old rats respectively. When adult rats had been semi-starved for 2 weeks and were then refed for 3 d the [^{3}H]ouabain-binding-site concentration was within the normal range. The muscle wet weight was still reduced by 17%. The total number of [^{3}H]ouabain-binding sites in soleus muscles was accordingly still reduced by 21%.

As the effect of semi-starvation on [^{3}H]ouabain binding could be the result of differences

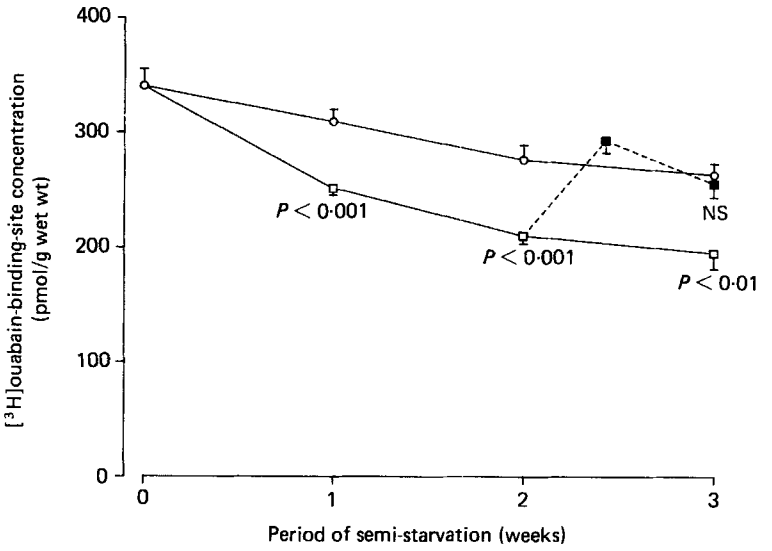

Fig. 1. Effect of semi-starvation on [^{3}H]ouabain-binding-site concentration in rat soleus muscle. Rats (12 weeks old) were semi-starved on a standard diet at 96, 117 and 138 kJ (23, 28 and 33 kcal)/d for the 1st, 2nd and 3rd week of treatment respectively. The animals had free access to drinking-water. After the 2nd week of treatment, one group was allowed free access to the diet. [^{3}H]ouabain-binding-site concentration was determined using the standard assay. After correction for unspecific uptake and retention the results were expressed as pmol/g wet weight and corrected for radiopurity of the isotope and for the loss of specifically bound [^{3}H]ouabain during the washout at 0° as well as for measuring at 1 μM-ouabain (see p. 521). Values are means, with their standard errors represented by vertical bars, for three to fourteen animals. Statistical significance levels for differences between controls (○) and semi-starved (□) and controls and semi-starved refed (■) rats are given. NS, not significant.

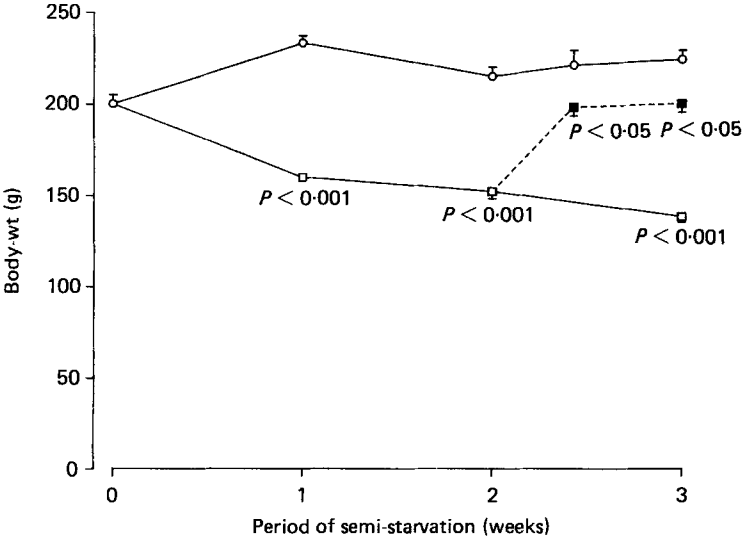

Fig. 2. Effects of semi-starvation and refeeding on body-weight. Rats (the same as those in Fig. 1) were semi-starved on a standard diet at 96, 117 and 138 kJ (23, 28 and 33 kcal)/d for the 1st, 2nd and 3rd week of treatment respectively. The animals had free access to drinking-water. After the 2nd week of treatment, one group was allowed free access to the diet. Values are means, with their standard errors represented by vertical bars, for three to fourteen animals. Statistical significance levels for differences between controls (○) and semi-starved (□), and controls and semi-starved refed (■) rats are given.

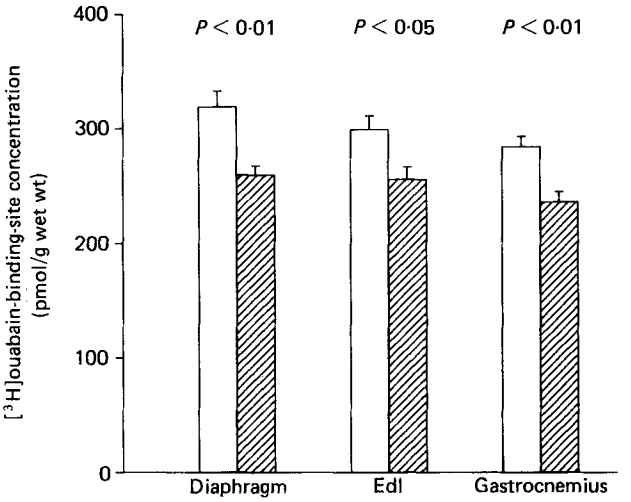

Fig. 3. Effect of 2 weeks of semi-starvation on [³H]ouabain-binding-site concentration in diaphragm, extensor digitorum longus (edl) and gastrocnemius muscles. Rats (12 weeks old) were semi-starved for 2 weeks on a standard diet at 96 and 117 kJ (23 and 28 kcal)/d for the 1st and 2nd week of treatment respectively. The animals had free access to drinking-water. [³H]ouabain-binding-site concentration was determined in muscle samples using the standard assay and corrected for unspecific uptake and retention and for radiopurity of the isotope. Values are means, with their standard errors represented by vertical bars, for six animals. Statistical significance levels for differences between control (□) and semi-starved (▨) rats are given.

in affinity of the Na,K-ATPase, the binding of [³H]ouabain to soleus muscle samples from 12-week-old rats semi-starved for 3 weeks and controls was tested over a range of ouabain concentrations (0·01–5 μM; Fig. 4). From Fig. 4(*a*) it can be seen that the uptake of [³H]ouabain shows saturation in samples from control as well as from semi-starved rats. The marginal increase in [³H]ouabain binding in samples from control rats when the concentration of ouabain in the incubation medium was increased from 1 to 5 μM was not significant. When presented as a Scatchard-type plot (Fig. 4(*b*)) it appeared that the maximal specific binding of [³H]ouabain (EO_{max}) for soleus muscle samples from control and semi-starved rats was 295 and 237 pmol/g wet weight respectively. Thus, in this experiment 3 weeks of semi-starvation in mature rats was associated with a decrease in [³H]ouabain-binding-site concentration of 20%. This is in agreement with the decrease of 25% shown in Fig. 1 for similar animals. It should be noted that the EO_{max} values given in Fig. 4(*b*) are based on values which are fully corrected for experimental errors associated with [³H]ouabain binding (see p. 521). Thus, these values represent the total [³H]ouabain-binding-site concentration in the soleus muscle of 12-week-old rats semi-starved for a further 3 weeks and their controls. The apparent dissociation constant was 0·06 and 0·07 μM for preparations from control and semi-starved rats respectively. Thus, incomplete saturation associated with standard measurements incubating at an ouabain concentration of 1 μM was corrected for by multiplying by 1·07.

As hypothyroidism has been found to be associated with a decrease in [³H]ouabain-binding-site concentration in rat skeletal muscle by up to 60% (Kjeldsen *et al.* 1986), it was of interest to determine whether semi-starvation was associated with a decrease in thyroid hormone levels and body temperature. In control rats values for total T_3 and T_4 (nmol/l) were 1·68 (SE 0·09) (*n* 5) and 34·52 (SE 1·72) (*n* 5) respectively. In 12-week-old rats semi-starved for 3 weeks the corresponding values (nmol/l) were 0·92 (SE 0·08) (*n* 4)

Table 1. *Effects of semi-starvation and refeeding on total sodium and potassium contents in rat soleus and gastrocnemius muscles*

(Rats (12 weeks old) were semi-starved for 2 weeks on a standard diet at 96 and 117 kJ (23 and 28 kcal)/d for the 1st and 2nd week of treatment respectively. One group was refed by giving them free access to the standard diet for 3 d. The animals had free access to drinking-water. Values are means with their standard errors; no. of animals in parentheses)

	Soleus						Gastrocnemius					
	K (μmol/g wet wt)			Na (μmol/g wet wt)			K (μmol/g wet wt)			Na (μmol/g wet wt)		
Age and treatment	Mean	SE	Statistical significance: $P <$	Mean	SE	Statistical significance: $P <$	Mean	SE	Statistical significance: $P <$	Mean	SE	Statistical significance: $P <$
14-week-old controls	85	3 (5)	NS	33	2 (5)	NS	108	5 (6)	NS	28	1 (6)	0·05
14-week-old semi-starved 2 weeks	82	5 (7)	NS	38	2 (8)	0·05	104	4 (8)	NS	33	1 (7)	0·01
14-week- and 3-d-old, semi-starved 2 weeks and refed 3 d	85	3 (3)		31	2 (3)		101	3 (3)		24	2 (3)	

NS, not significant.

Table 2. *Effects of semi-starvation and refeeding on muscle wet weight, [^{3}H]ouabain-binding-site concentration and the total number of [^{3}H]ouabain-binding sites in rat soleus muscle*

(Rats (12 weeks old) were semi-starved on a standard diet at 96 and 117 kJ (23 and 28 kcal)/d for the 1st and 2nd week of treatment respectively. One group was refed by giving them free access to the standard diet for 3 d. Rats (4 weeks old) were semi-starved for 1 week by giving them the standard diet at 96 kJ (23 kcal)/d. The animals had free access to drinking-water. The [^{3}H]ouabain-binding-site concentration was determined and calculated as described in the methods section. The total number of [^{3}H]ouabain-binding sites was calculated by multiplying the [^{3}H]ouabain-binding-site concentration by the muscle wet weight. Values are means with their standard errors; no. of animals in parentheses)

	Muscle wet wt (mg)			[^{3}H]ouabain-binding-site concentration (pmol/g wet wt)			
Age and treatment	Mean	SE	Statistical significance: $P <$	Mean	SE	Statistical significance: $P <$	No. of [^{3}H]ouabain-binding-sites (pmol/muscle)
5 weeks old:							
Control	40	2 (6)		470	13 (6)		19
Semi-starved 1 week	21	1 (6)	0·001	379	15 (6)	0·001	8
13 weeks old:							
Control	90	2 (6)		309	11 (6)		28
Semi-starved 1 week	76	3 (6)	0·01	251	6 (6)	0·001	19
14 weeks and 3-d old:							
Control	96	6 (3)		303	1 (6)		29
Semi-starved 2 weeks, refed 3 d	80	8 (3)	NS	293	11 (6)	NS	23

NS, not significant.

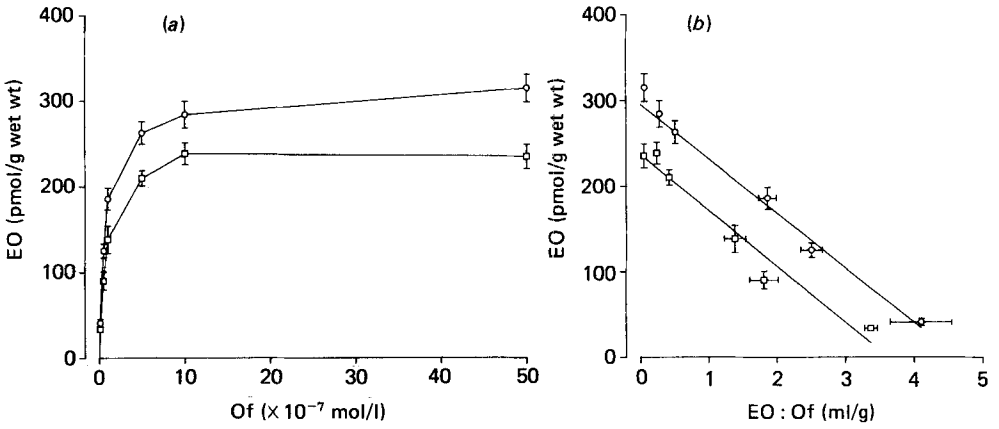

Fig. 4. The effects of semi-starvation and the concentration of ouabain on the binding of [^{3}H]ouabain in rat soleus muscle samples. Rats (12 weeks old) were semi-starved for 3 weeks on a standard diet at 96, 117 and 138 kJ (23, 28 and 33 kcal)/d for the 1st, 2nd and 3rd week of treatment respectively. The animals had free access to drinking-water. [^{3}H]ouabain binding was determined using the standard assay except that muscle samples were incubated at 0·01–5 μM-ouabain for four periods of 60 min at 37°. (*a*) 'Bound' (EO) [^{3}H]ouabain as a function of the concentration of ouabain in the incubation medium (Of). (*b*) 'Bound' (EO) *v.* 'bound:free' (EO:Of) [^{3}H]ouabain. After correction for unspecific uptake and retention of [^{3}H]ouabain, the values were corrected for radiopurity of the isotope and for the loss of specifically bound [^{3}H]ouabain during the washout at 0°. The isotherms of the Scatchard-type plot have been constructed using linear regression analysis and the calculated values for the maximum [^{3}H]ouabain-binding-site concentration (EO_{max}) as well as the apparent dissociation constants for receptor–ligand interaction (K_D) were 295 pmol/g wet weight and 0·06 μM for controls (○) and 237 pmol/g wet weight and 0·07 μM for semi-starved (□) respectively. Values are means, with two standard errors represented by vertical bars, for five animals.

($P \leqslant 0{\cdot}001$) and 16·20 (SE 1·85) (n 5) ($P < 0{\cdot}001$). Body temperature was 38·5 (SE 0·2)° (n 5) and 37·1 (SE 0·1)° (n 5) ($P < 0{\cdot}001$) in the controls and the 3-week semi-starved rats respectively.

To study the effect of more severe energy deficiency on skeletal muscle Na and K contents and [^{3}H]ouabain-binding, 12-week-old rats were completely deprived of food for 5 d. As for semi-starvation, complete starvation caused a tendency to a decrease in K content. The [^{3}H]ouabain-binding-site concentration decreased by 25% ($P < 0{\cdot}001$). This is a somewhat faster decrease than that observed during semi-starvation, where a decrease of 19% was seen after 7 d (Fig. 1).

In order to compare the effect of combined semi-starvation and K depletion on skeletal-muscle [^{3}H]ouabain-binding-site concentration and Na and K contents, mature rats were semi-starved on a K-deficient diet for 2 weeks (Tables 3 and 4). This treatment caused a decrease in the [^{3}H]ouabain-binding-site concentration in soleus muscle of 33%. In comparison, the same degree of semi-starvation on the control diet caused a decrease of 25% and, when rats had free access to the K-deficient diet for 2 weeks, the [^{3}H]ouabain-binding-site concentration decreased by 38% (Table 3). Whereas semi-starvation is associated with a decrease in body-weight as well as in soleus muscle mass, no reduction was seen following 2 weeks on the K-deficient diet (Table 3). Thus, when mature rats were K-depleted for 2 weeks the relative reductions in [^{3}H]ouabain-binding-site concentration and the total number of [^{3}H]ouabain-binding sites in the entire soleus muscle were equal. On the other hand, when animals were semi-starved the reduction in [^{3}H]ouabain-binding-site concentration was less than the reduction in the total number of [^{3}H]ouabain-binding sites.

Table 3. *Effects of semi-starvation and potassium-depletion on body-weight, soleus weight and the concentration and the total number of [^{3}H]ouabain-binding sites in rat soleus muscle*

(Rats (12 weeks old) were semi-starved for 2 weeks on a standard or K-deficient diet at 96 kJ (23 kcal)/d. The animals had free access to drinking-water. The [^{3}H]ouabain-binding-site concentration was determined and calculated as described in the methods section. The total number of [^{3}H]ouabain-binding sites was calculated by multiplying the concentration of [^{3}H]ouabain-binding sites by muscle wet weight. Values are means with their standard errors for five animals in each group)

	Body-wt (g)			Soleus wt (mg)			[^{3}H]ouabain-binding-site concentration (pmol/g wet wt)			
	Mean	SE	Statistical significance: $P <$	Mean	SE	Statistical significance: $P <$	Mean	SE	Statistical significance: $P <$	No. of [^{3}H]ouabain-binding sites (pmol/muscle)
Control	218	2		82	2		298	11		24
Semi-starved 2 weeks	163	1	0·001	74	1	0·01	224	10	0·001	17
K depleted 2 weeks	220	5	NS	82	2	NS	184	10	0·001	15
Semi-starved on K-deficient diet, 2 weeks	168	1	0·001	76	2	0·05	201	11	0·001	15

NS, not significant.

Table 4. *Effects of semi-starvation and potassium-depletion on total sodium and K contents in rat soleus muscle*

(Muscle samples for the determination of Na and K were prepared from the same animals as those used for the experiments in Table 3. Rats (12 weeks old) were semi-starved for 2 weeks on a normal or K-deficient diet at 96 kJ (23 kcal)/d. The animals had free access to drinking-water. Values are means with their standard errors for five animals in each group)

	Na content (μmol/g wet wt)			K content (μmol/g wet wt)		
	Mean	SE	Statistical significance: $P <$	Mean	SE	Statistical significance: $P <$
Control	30	1		89	1	
Semi-starved, 2 weeks	32	1	NS	81	2	0·01
K-depleted, 2 weeks	40	1	0·001	70	1	0·001
Semi-starved on K-deficient diet, 2 weeks	33	1	NS	75	2	0·001

NS, not significant.

Another difference between semi-starvation and K-depletion was that whereas semi-starvation was only associated with a minor decrease in the K:Na ratio in soleus muscles, this value was reduced by 41% during K depletion for 2 weeks (Table 4). During semi-starvation on the K-deficient diet the K:Na ratio in soleus muscles was only reduced by 24%. Together with the observations on [^{3}H]ouabain-binding-site concentration and number this points to the possibility that combined semi-starvation and K-depletion is less severe than K-depletion alone with respect to skeletal-muscle Na and K contents and [^{3}H]ouabain-binding-site concentration. However, combined semi-starvation and K depletion causes a reduction in muscle mass which was not seen following K depletion alone.

DISCUSSION

Semi-starvation causes a decrease in body-weight and muscle mass. If the [^{3}H]ouabain-binding-site concentration in the muscles were kept constant this would in itself produce a reduced number of [^{3}H]ouabain-binding sites in the total pool of skeletal muscles. The present study shows that in addition to these effects, semi-starvation is associated with a decrease in the [^{3}H]ouabain-binding-site concentration in the remaining muscle tissue. This accordingly causes a further decrease in the number of [^{3}H]ouabain-binding sites in the muscle pool than can be predicted from the loss in body-weight or muscle mass. The decrease in [^{3}H]ouabain-binding-site concentration was found in slow-twitch as well as fast-twitch fibres indicating that it is general in the skeletal muscle pool. Thus, from our results it can be estimated that following 1 week of semi-starvation, the total pool of [^{3}H]ouabain-binding sites in skeletal muscle is reduced by up to 58%.

We have earlier shown that hypothyroidism is associated with a reduced [^{3}H]ouabain-binding-site concentration in skeletal muscle (Kjeldsen *et al.* 1984, 1985, 1986*a*). Thus, in rats, a reduction of 50% in T_3 and 92% in T_4 was associated with a decrease of 49% in [^{3}H]ouabain-binding-site concentration (Kjeldsen *et al.* 1984). In human subjects a reduction of 33% in T_3 and 70% in T_4 was associated with a reduction of 50% in [^{3}H]ouabain-binding sites (Kjeldsen *et al.* 1985). In the present study, semi-starvation was associated with a reduction of 45% in T_3 and 53% in T_4 and of 25% in skeletal muscle [^{3}H]ouabain-binding-site concentration. This indicates that the reduction in thyroid hormone levels with semi-starvation can be of importance in the decrease in [^{3}H]ouabain-binding-site concentration. Semi-starvation does not cause the reduction in [^{3}H]ouabain-binding-site concentration with hypothyroidism as body-weight does not decrease in hypothyroid adult rats (Kjeldsen *et al.* 1986*a*).

A previously described cause of reduced [^{3}H]ouabain-binding-site concentration in skeletal muscle is K depletion (Nørgaard *et al.* 1981; Kjeldsen *et al.* 1984). A striking difference between the effect of semi-starvation and K depletion is, however, that whereas the former causes major reduction in body mass as well as muscle mass, the latter only leads to moderate changes. Furthermore, during K depletion the relative decrease in skeletal-muscle [^{3}H]ouabain-binding-site concentration is of the same order of magnitude as the relative decrease in K content (Kjeldsen *et al.* 1984). During semi-starvation, however, the decrease in [^{3}H]ouabain-binding-site concentration is pronounced whereas there is only a tendency to a decrease in K content. This points to the possibility that during starvation there is a major reduction in [^{3}H]ouabain-binding-site concentration due to the reduced T_3 and T_4 levels and a minor reduction caused by the diminished K content. Conversely, as shown in previous studies (Kjeldsen *et al.* 1984), the decrease in [^{3}H]ouabain-binding-site concentration induced by K depletion as such is not associated with any significant reduction in plasma T_3 and T_4 and therefore is likely to develop by a different mechanism. In the K-depleted animal, the synthesis of Na,K-ATPase in muscle cells is closely correlated

with, and possibly the result of, the selective K loss from these cells (Clausen & Kjeldsen, 1986).

Muscles from animals semi-starved on the K-deficient diet showed a less pronounced decrease in [^{3}H]ouabain-binding-site concentration and K content than those obtained from rats which had only been K-depleted. This is probably due to the release of K from the wasting of tissue during semi-starvation. This causes a smaller reduction in total body K and thus a smaller decrease in the K content of skeletal muscles. Accordingly the reduction in [^{3}H]ouabain-binding-site concentration is less pronounced when comparing combined K depletion and semi-starvation with the effect of K depletion. Thus, the loss of tissue during starvation prevents an additive effect of K depletion and semi-starvation from being observed.

The reduction in skeletal muscle Na,K-ATPase concentration due to starvation, determined in the present study by measuring [^{3}H]ouabain binding to intact skeletal muscle samples, was less than that reported in the literature using Na,K-ATPase activity determinations on purified skeletal muscle membranes (Swann, 1984). In the present study mature rats showed a decrease of 25% in [^{3}H]ouabain-binding-site concentration after 5 d on total fast, whereas a decrease of 50% after 3 d has been reported (Swann, 1984). This difference can be ascribed to recovery problems associated with Na,K-ATPase determinations in skeletal muscle. Thus, it has been pointed out that measurements on purified membrane fractions only determine a minor proportion of the total Na,K-ATPase in skeletal muscle (Jones & Besh, 1984; Clausen, 1986; Kjeldsen, 1986). Unfortunately no information is given in the study of Swann (1984) which allows the calculation of the recovery of skeletal muscle Na,K-ATPase. Since it is not known whether the enzyme activity obtained after purification is representative of the total population of Na,K-ATPase molecules, a quantitative evaluation of changes in the concentration of Na,K-ATPase in muscle cannot, at present, be based on measurements on membrane fractions. That this problem is of major importance with respect to the study of the concentration of Na,K-ATPase in skeletal muscles during different nutritional states is emphasized by the observation that the Na,K-ATPase activity in membrane fractions obtained from hind-limb muscles of obese mice was less than half the value observed in lean mice (Lin *et al.* 1978), whereas no significant difference was observed when the Na,K-ATPase concentration was assessed using [^{3}H]ouabain binding to intact muscles (Clausen & Hansen, 1982).

The changes in the concentration and total number of skeletal-muscle [^{3}H]ouabain-binding sites with semi-starvation and complete starvation have various implications. Together with its direct importance for muscle cell function it influences digitalis distribution and K homeostasis.

The skeletal muscle [^{3}H]ouabain-binding-site concentration has been shown to be of importance for the distribution of digitalis glycosides (Kjeldsen *et al.* 1985). During semi-starvation the decrease in muscle mass causes a decrease in the distribution volume. This can be corrected for by the administration of digitalis glycosides according to the reduced body-weight. However, since the concentration of digitalis receptors in the remaining skeletal muscle mass also decreases, the binding capacity is further decreased during semi-starvation than would be expected from the reduction in body-weight. Thus, when a normal dose of digitalis glycosides per kg body-weight is given to starved subjects they can be expected to achieve a higher plasma digitalis glycoside level. Accordingly, more digitalis glycoside will be available for binding to the heart.

Due to their large volume and high concentration of Na,K pumps, the skeletal muscles play a central role in the clearance of K from plasma and extracellular fluid. During exercise an increase in plasma K resulting from a net loss of K from the working muscles takes place (Hazeyama & Sparks, 1979; Hirche *et al.* 1980; Hermansen *et al.* 1984). Following training,

this increase is reduced (Tibes *et al.* 1974, 1976), possibly because the concentration of Na,K-ATPase in skeletal muscle is increased (Knochel *et al.* 1985; Kjeldsen *et al.* 1986*b*). Conversely, the present results show that semi-starvation may reduce the total pool of Na,K pumps in the skeletal muscles by up to 58%, leading to a severe impairment of the capacity to clear K from the plasma during exercise or the ingestion of K. Therefore, energy deficiency may be associated with an increased risk of the development of hyperkalaemia with its cardiotoxic effects. Oral and, in particular, intravenous administration of K to undernourished subjects should be carried out taking this into consideration.

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