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Na^+,K^+-ATPase Enzyme Units in Skeletal Muscle and Liver of 14-Day-Old Lean and Obese (*ob/ob*) Mice (40526)[1]

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A reduction in oxygen consumption of genetically obese (*ob/ob*) mice has been detected as early as 5 days after birth (1). While preweaned obese mice consume less oxygen than lean littermates (2–4), their energy intakes are similar (5). Consequently, the obese mice are able to retain more energy than lean littermates and this is reflected in an increased body fat content of the obese mice as early as 7 days of age (1). Reduced energy expenditure is thus an important contributing factor to the development of obesity in these mice. The underlying biochemical mechanism responsible for the improved energy efficiency in obese mice, however, has not been identified.

Energy required for Na^+,K^+-ATPase, the enzymatic equivalent of the sodium pump, is thought to represent a significant component of maintenance energy expenditure of mammals (6, 7). Observations that 3- to 6-week-old obese mice have a 40% lower maintenance energy requirement than lean littermates (8) and that 4- to 8-week-old obese mice have 35 to 50% fewer Na^+,K^+-ATPase enzyme units in skeletal muscle than lean littermates (9, 10) are consistent with the hypothesis that Na^+,K^+-ATPase is involved in the etiology of obesity in these obese mice. However, it would be important to establish if changes in Na^+,K^+-ATPase occur early and therefore possibly contribute to the development of the obesity, or are merely a secondary consequence of the obesity.

In this report we identified 14-day-old obese and lean littermates by their reduced oxygen consumption and then measured the number of Na^+,K^+-ATPase enzyme units in skeletal muscle and liver preparations. Body fat content of the mice was also determined.

Methods. Fourteen-day-old male obese (*ob/ob*) and lean (*ob/+* or +/+) littermate mice were obtained from litters of heterozygote breeding pairs.[2] Breeding mice were fed a stock diet[3] *ad libitum*. Ambient temperature was 25 ± 2°. On Days 12, 13, and 14 oxygen consumption of individual pups was measured at 30° as previously described (1) and expressed as an average rate for the 3 days. At 14 days of age, lean and obese male littermates were selected, on the basis of differences in oxygen consumption, and were killed by cervical dislocation. The vena cava was immediately incised. After perfusing each mouse with 10 ml of cold saline solution via the left ventricle of the heart to remove blood, the forelimbs and hindlimbs and liver were quickly removed. The skeletal muscle, stripped of all visible fat, was removed from the bone. Food residue was removed from the stomach and the carcasses (except liver, and forelimb and hindlimb skeletal muscle) were homogenized in an equal weight of water. Carcass fat was extracted with chloroform/methanol (3:2 v/v) and quantitated gravimetrically.

Particulate fractions from combined forelimb and hindlimb muscle preparations and from liver were prepared as described (9, 10) and were assayed for the number of Na^+,K^+-ATPase enzyme units (EC 3.6.1.3), using the [^{3}H]ouabain-binding method. [^{3}H]Ouabain binding to Na^+,K^+-ATPase follows classical Michaelis–Menten kinetics (11). The amount of specific [^{3}H]ouabain bound to Na^+,K^+-

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ATPase in the presence of a fixed concentration of labeled ouabain, the apparent dissociation constant for [^{3}H]ouabain binding to Na^+,K^+-ATPase, and the high-affinity [^{3}H]ouabain-binding site concentration in skeletal muscle and liver were determined as previously described (9, 10, 11). Binding of [^{3}H]ouabain to low-affinity, nonspecific, nonsaturable sites was determined in the presence of the 4 m*M* nonlabeled ouabain.

The data were analyzed by the paired *t* test (12) and were presented as mean ± SEM.

Results. Body weight, body fat content, and oxygen consumption of the lean and obese mice are presented in Table I. Obese mice weighed more and contained more fat than their lean littermates. The lower oxygen consumption of obese mice is readily apparent at this age. These results are in agreement with a previous report (1) and indicate that alterations in energy metabolism occur early in the life of the obese mouse.

Results of the [^{3}H]ouabain-binding studies performed on skeletal muscle from 14-day-old lean and obese mice are summarized in Table II. Total forelimb and hindlimb muscle weights and particulate protein content were not influenced by phenotype. Nonspecific binding of [^{3}H]ouabain to muscle preparations was not altered by phenotype. The specific [^{3}H]ouabain binding to muscle from obese mice was 26% lower than that from lean mice (Table II). Obese and lean mice exhibited similar K_d values of skeletal muscle Na^+,K^+-ATPase for [^{3}H]ouabain binding, as we also noted previously with older mice (9, 10). Thus, the lower [^{3}H]ouabain-binding capacity in muscle of obese mice, coupled with no change in binding affinity, indicates that obese mice have a lower number of [^{3}H]ouabain-binding sites than lean mice. The total [^{3}H]ouabain-binding site number in the mixture of forelimb and hindlimb muscles from obese mice was 28% lower than in lean littermates.

TABLE I. BODY WEIGHT, BODY FAT CONTENT, AND OXYGEN CONSUMPTION OF 14-DAY-OLD LEAN AND OBESE MICE[a]

Parameter	Lean	Obese
Body weight (g)	7.0 ± 0.3	7.8 ± 0.3*
Body fat (%)	8.3 ± 0.7	14.2 ± 1.1*
Oxygen consumption (ml/g/hr)	4.06 ± 0.07	2.90 ± 0.08*

[a] Mean ± SEM for six pairs of lean and obese mice. The asterisk indicates a significant difference ($P < 0.05$) between lean and obese mice.

Liver weights and particulate protein content and nonspecific binding of [^{3}H]ouabain to liver preparations of 14-day-old obese mice were similar to respective values in lean littermates (Table II). In contrast to the lower specific [^{3}H]ouabain binding in skeletal muscle of obese mice, phenotype did not influence specific [^{3}H]ouabain binding in liver preparations. As in skeletal muscle, K_d values for specific [^{3}H]ouabain binding to Na^+,K^+-ATPase in the livers were not altered by phenotype. The number of [^{3}H]ouabain-

TABLE II. COMBINED FORELIMB AND HINDLIMB MUSCLE WEIGHTS, LIVER WEIGHTS, AND [^{3}H]OUABAIN BINDING TO MUSCLE AND LIVER PREPARATIONS OF 14-DAY-OLD LEAN AND OBESE MICE[a]

	Forelimb and hindlimb muscles		Liver	
	Lean	Obese	Lean	Obese
Total tissue weight (g)	0.63 ± 0.07	0.66 ± 0.04	0.28 ± 0.02	0.32 ± 0.01
Particulate protein (mg/g tissue)	56 ± 2	55 ± 1	44 ± 3	45 ± 2
Nonspecific [^{3}H]ouabain binding[b] (pmole/mg protein)	2.4 ± 0.1	2.4 ± 0.1	1.76 ± 0.10	1.68 ± 0.09
Specific [^{3}H]ouabain binding[c] (pmole/mg protein)	2.7 ± 0.08	2.0 ± 0.10*	0.26 ± 0.03	0.25 ± 0.05
K_d value[d] (μM)	0.039 ± 0.003	0.038 ± 0.003	14.1 ± 1.3	13.4 ± 2.0
[^{3}H]Ouabain-binding site concentration[a]				
(pmole/mg protein)	3.0 ± 0.09	2.2 ± 0.1*	9.8 ± 1.1	8.9 ± 1.5
(pmole/total tissue)	109 ± 12	79 ± 7*	115 ± 7	119 ± 13

[a] Mean ± SEM for six pairs of mice. The asterisk indicates a significant difference ($P < 0.05$) between lean and obese mice.

[b] [^{3}H]Ouabain (0.4 μM) binding observed in the presence of excess nonlabeled ouabain (4 m*M*).

[c] [^{3}H]Ouabain (0.4 μM) binding minus [^{3}H]ouabain (0.4 μM) binding observed in the presence of excess nonlabeled ouabain (4 m*M*).

[d] Calculated as described elsewhere (11).

binding sites per total liver in the 14-day-old obese mice was similar to the values obtained in their lean counterparts.

Discussion. We used [^{3}H]ouabain binding as an index of the number of Na^+,K^+-ATPase enzyme units present in skeletal muscle and liver of the mice. Previous reports (13, 14) have shown that the amount of ouabain bound to receptor sites correlates with the number of Na^+,K^+-ATPase enzyme units. The 14-day-old obese mice had 28% fewer Na^+,K^+-ATPase units in skeletal muscle than did lean mice. This represents one of the earliest reported developmental differences in an enzyme system between obese (*ob/ob*) and lean mice. Later in development the difference in number of Na^+,K^+-ATPase enzyme units (per mg skeletal muscle protein) between lean and obese mice is even greater (9, 10). Further, obese mice accumulate less skeletal muscle than lean mice between 3 and 8 weeks of age (15). Consequently, 8-week-old obese mice have less than 50% as many hindlimb skeletal muscle Na^+,K^+-ATPase enzyme units as observed in their lean counterparts (9, 10).

In contrast to observations in skeletal muscle, the number of Na^+,K^+-ATPase enzyme units in livers of the 14-day-old obese mice was not reduced. But, later in development differences are observed. Four- and eight-week-old obese mice have 19 and 54% fewer Na^+,K^+-ATPase enzyme units per milligram of liver protein, respectively, than their lean counterparts (10). Thus, the onset of observable alterations in Na^+,K^+-ATPase enzyme units in livers of obese mice occurs well after the initial appearance of obesity. Increases in liver weight of obese mice during development, however, offset the lower number of Na^+,K^+-ATPase enzyme units per milligram of protein. Consequently, adult obese mice do not have fewer Na^+,K^+-ATPase enzyme units per total liver than their lean counterparts (10). The number of Na^+,K^+-ATPase enzyme units in kidneys of the 14-day-old mice was not evaluated, but we previously noted that the number of Na^+,K^+-ATPase enzyme units in kidneys of older obese mice was similar to the numbers observed in their lean counterparts (10).

Obese (*ob/ob*) mice have been suggested to be hypothyroid (16, 17), but the levels of Na^+,K^+-ATPase in three thyroid hormone responsive tissues (skeletal muscle, liver, and kidney) of the obese mice suggest that additional factors are also involved in the regulation of Na^+,K^+-ATPase in obese mice. For example, the number of Na^+,K^+-ATPase units in skeletal muscle of obese mice is lower than in their lean counterparts before weaning, but the enzyme is not changed in the livers of the obese mice until later in development (10). The kidney enzyme appears less responsive to phenotype than does the enzyme in the other two organs (10). The number of Na^+,K^+-ATPase enzyme units in each of the three organs of obese mice, however, increased to a greater extent after thyroid hormone injection than did the enzyme in these tissues of lean mice (10). Thus, the hyperresponsiveness of Na^+,K^+-ATPase to thyroid hormone administration in obese mice is similar to the effects of thyroid hormone administration on the enzyme in hypothyroid rats (18). Additional studies are needed to clarify the role of thyroid hormones in the regulation of Na^+,K^+-ATPase enzyme units in tissues of obese mice.

Adult obese mice are insulin resistant, but tissue sensitivity to insulin changes during development of obesity (19). For example, adipose tissue of young obese (*ob/ob*) mice is supersensitive to insulin, whereas later it becomes resistant (19). Insulin resistance in muscle may appear much earlier in development of obesity than in liver or adipose tissue (20). Insulin has also been shown to increase K^+ uptake and Na^+,K^+-ATPase in muscle preparations (21). Thus, the possibility that the development of insulin resistance and the lower number of Na^+,K^+-ATPase enzyme units in skeletal muscle of obese mice are interrelated warrants further study.

In conclusion, the lower number of Na^+,K^+-ATPase enzyme units in skeletal muscle of 14-day-old obese mice represents one of the earliest reported enzymatic alterations in these mice. The extent to which this alteration contributes to the lower energy expenditure and subsequent increased energy storage in these mice remains to be established.

Summary. The number of Na^+,K^+-ATPase enzyme (EC 3.6.1.3) units in skeletal muscle and liver of 14-day-old lean and obese (*ob/*

ob) mice was assayed from the concentration of high-affinity [^{3}H]ouabain-binding sites in the tissue preparations. The number of [^{3}H]ouabain-binding sites in forelimb and hindlimb muscles of obese mice was 28% lower than in lean littermates. In contrast, livers of lean and obese mice possessed equal numbers of [^{3}H]ouabain-binding sites. The lower number of Na$^+$,K$^+$-ATPase enzyme units observed in skeletal muscle of the 14-day-old obese mice represents one of the earliest reported developmental alterations in an enzyme system in these mice and may be associated with the lower energy expenditure of young obese mice.

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