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THE Na^+-K^+-PUMP, ENERGY METABOLISM, AND OBESITY

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SUMMARY: In intact soleus and extensor digitorum longus muscles obtained from lean and obese mice, the number of [^{3}H]-ouabain binding sites showed no significant difference. In the same muscles obtained from obese mice, the Na^+-K^+-pump mediated [^{42}K]-uptake was respectively 39 and 33% larger than in those of lean littermates. This together with the earlier observation that intact muscles require at most 6% of their basal energy production for active Na^+-K^+-transport indicates that this process is of no quantitative importance for development of obesity.

INTRODUCTION

It has repeatedly been proposed that the basal metabolic rate of the intact organism is to a significant extent determined by the energy requirement of the Na^+-K^+-pump (1-8). In keeping with this idea, it was suggested that the development of obesity might be related to a reduced rate of active Na^+-K^+-transport. Thus, particulate fractions obtained from the hind limb muscles of obese mice were found to contain up to 36% fewer [^{3}H]-ouabain binding sites than those prepared from lean littermates (9-11). Furthermore, in erythrocytes obtained from obese individuals, the rate of active Na^+-K^+-transport as well as the number of Na^+-K^+-pump units were reduced (12). In contrast, others have recently demonstrated that obesity in mice is entirely due to lower energy expenditure in the brown adipose tissue (13).

The putative role of the Na^+-K^+-pump in overall energy consumption depends critically upon a quantitative analysis of the rate of active Na^+-K^+-transport and its energy requirement.

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Based on measurements of active Na^+-K^+-transport, O_2-consumption and heat production in isolated intact rat and mouse skeletal muscle, it has been calculated that the energy requirement of this process amounts to between 2 and 6% of the total energy expenditure of the tissue (14-16).

A method developed for the measurement of [^{3}H]-ouabain binding to intact muscles has been found to give very reproducible values that were appreciably higher than those obtained using a particulate fraction of membrane (17-18). Since this method has proven to allow the detection of changes induced by thyroid hormones (19), denervation (20) and K^+-depletion (21), it seemed of interest to compare the total number of ouabain binding sites in representative hind limb muscles obtained from lean and obese mice. These measurements were combined with determinations of [^{42}K]-uptake as well as the Na^+-K^+-contents of gastrocnemius muscles.

METHODS

All experiments were performed using 9-20 week old fed female or male obese (ob/ob) and lean (ob/+ or +/+) littermate mice (C57 BL/6J). The animals were fed a stock diet ad libitum and were maintained at constant humidity and temperature (23°C). Following decapitation, intact soleus and extensor digitorum longus (E.D.L.) muscles were dissected out as previously described (15,22) and equilibrated at 30°C in Krebs-Ringer bicarbonate buffer under continuous bubbling with a mixture of 95% O_2 and 5% CO_2. The specific displaceable binding of [^{3}H]-ouabain was determined as described in detail elsewhere (17-18) as well as in the legend to table 1. The measurements were throughout performed by comparing pairs of muscles obtained from obese mice and their lean littermates. The purity of [^{3}H]-ouabain (New England Nuclear) was checked as described elsewhere (23). [^{42}]-uptake was measured by incubating the muscles for 10 or 20 min in buffer containing 0.4 μCi/ml of [^{42}K] (Danish Atomic Energy Commission Isotope Laboratory, Risø, Denmark) without or with ouabain (10^{-3}M). The ouabain-suppressible component of [^{42}K]-uptake was determined as the difference between the values obtained without or with the glycoside. In order to ensure complete inhibition of the active Na^+-K^+-transport, the muscles exposed to ouabain were preequilibrated for 15 min with buffer containing 10^{-3}M of the glycoside (for details, see ref. No 24). From the male animals, segments of the epididymal fat pads were taken for measurements of [^{42}K]-uptake. These experiments were performed as described for the muscles with the sole difference that 1% of dialyzed bovine serum albumin was added to the buffer and that incubation took place at 37°C. Samples

Table 1: SPECIFIC DISPLACEABLE [^{3}H]-OUABAIN BINDING IN SOLEUS AND E.D.L. MUSCLES FROM OBESE MICE AND THEIR LEAN LITTERMATES.

The total number of [^{3}H]-ouabain binding sites was determined by incubating intact muscles for 120 min in K^+-free Krebs-Ringer bicarbonate buffer containing 5 mM D-glucose and from 1 to $5x10^{-6}$M [^{3}H]-ouabain (1.6 µCi per ml). Following 3x20 min wash in ice-cold buffer, the amount of [^{3}H]-activity retained by the muscles was determined and expressed as pmol per g wet weight using the specific activity of the incubation medium. All values were corrected for non-specific retention of [^{3}H]-activity measured by incubating the contralateral muscles with [^{3}H]-ouabain (1.6 µCi/ml) and 10^{-3}M unlabelled ouabain (for details, see ref. No 17-18). The results are given as mean ± S.E. of the number of observations indicated in the parentheses.

Muscle	Lean controls	Obese littermates	p
Soleus	667 ± 36 (15)	657 ± 30 (16)	>0.80
E.D.L.	479 ± 32 (16)	526 ± 19 (16)	>0.20

of the gastrocnemius muscle were homogenized in 5% trichloroacetic acid. Following centrifugation, the Na^+-K^+-contents of the clear supernatant was determined using an FLM flame photometer with internal Li^+-standard (Radiometer, Copenhagen).

RESULTS

[^{3}H]-ouabain binding was measured using 10^{-6}, $2x10^{-6}$ and $5x10^{-6}$M [^{3}H]-ouabain, i.e. concentrations which can be assumed to allow more than 90% saturation of all available ouabain binding sites (Na^+-K^+-ATPase units). Since there was no significant difference between the values obtained at these 3 concentrations, the results were pooled. Furthermore, the number of [^{3}H]-ouabain binding sites showed no detectable variation with age (9-20 weeks) or sex.

Whether obtained from obese or lean mice, neither soleus nor E.D.L. muscles showed any significant difference with respect to the total amount of [^{3}H]-ouabain bound per gram tissue wet weight. The results are in good agreement with those earlier obtained using muscles from NMRI mice (19-20) (table 1).

On the other hand, the ouabain-suppressible component of [^{42}K]-uptake in both soleus and E.D.L. was significantly larger in muscles obtained from obese mice (table 2). In the epididymal

Table 2: OUABAIN-SUPPRESSIBLE [^{42}K]-UPTAKE AND Na^+-K^+-CONTENTS IN MUSCLES AND ADIPOSE TISSUE FROM OBESE MICE AND THEIR LEAN LITTERMATES.

[^{42}K]-uptake was measured by incubating soleus or E.D.L. muscles for 10 or 20 min in Krebs-Ringer bicarbonate buffer containing 0.4 µCi/ml of [^{42}K] without or with ouabain (10^{-3}M). The difference between the amount of [^{42}K] taken up in the two groups of muscles (obtained from the same animals) was expressed as nmol/g wet wt. per min (see ref. No 24). The ouabain-supressible [^{42}K]-uptake in segments of the epididymal fat pad was determined in a similar way, using a buffer containing 1% albumin. The results are given as mean ± S.E. of the number of observations indicated in the parentheses. The Na^+-K^+-contents of gastrocnemius muscles were determined by flame photometry of trichloroacetic acid extracts of samples excised immediately after decapitation of the animals.

Tissue	Lean controls	Obese Littermates	p
	Ouabain-suppressible [^{42}K]-uptake (nmol/g wet wt.)		
Soleus	306 ± 31 (10)	424 ± 27 (13)	<0.01
E.D.L.	341 ± 19 (10)	455 ± 40 (13)	<0.05
Adipose tissue	28 ± 3 (6)	27 ± 4 (6)	>0.70
	Na^+-contents µmol/g wet wt.)		
Gastrocnemius	22.0 ± 0.5 (10)	23.1 ± 0.9 (10)	>0.30
	K^+-contents (µmol/g wet wt.)		
Gastrocnemius	108.5 ± 0.9 (10)	109.7 ± 1.7 (10)	>0.50

adipose tissue segments, the ouabain-suppressible [^{42}K]-uptake was the same in the two groups of animals. In agreement with earlier observations, the [^{42}K]-uptake in adipose tissue was appreciably smaller than in muscle (17-18). As can be seen in table 2, the Na^+-K^+-contents of the gastrocnemius muscle was virtually the same in the obese and lean mice.

DISCUSSION

The present study shows that soleus and E.D.L. muscles, when incubated under conditions allowing more than 90% saturation with [^{3}H]-ouabain (17), bind between 500 and 700 pmol of [^{3}H]-ouabain per g tissue wet weight. This is 4-5 times more than the values obtained with particulate fractions obtained

from homogenates of hind limb muscles (9-11), indicating that the use of intact muscles allow a more complete estimate of the total number of Na^+-K^+-pumps. A similar discrepancy between results obtained with intact muscles and particulate fractions of homogenates has been demonstrated in two earlier studies (20, 25). When all available specific ouabain binding sites can be quantified, there seems to be no significant difference between muscles from obese and lean mice. It should be noted that our animals were kept at almost the same temperature (23°C) as the 25°C reported to give rise to the maximum difference in [^{3}H]-ouabain binding per g muscle (11).

Furthermore, the Na^+-K^+-contents of the gastrocnemius muscle was the same in the two groups of animals, indicating that the obesity was not associated with any major change in the Na^+-K^+-homeostasis of skeletal muscle. Surprisingly, the ouabain-suppressible component of [^{42}]-uptake was significantly larger in the muscles obtained from obese animals. This together with the observation that in adipose tissue, [^{42}K]-uptake was virtually the same in the two groups of mice argues against obesity being associated with any decrease in energy demand for active Na^+-K^+-transport.

Measurements of heat production have shown that blocking the active Na^+-K^+-transport with ouabain gave a decrease of 5-7%, both in rat (15) and mouse (16,19) soleus muscles. Conversely, catecholamines at a concentration which doubles the rate of active Na^+-K^+-transport, increased heat production by only 5% in mouse soleus (16). Although the rate of active Na^+-K^+-transport varied in proportion to the thyroid status, the ouabain-suppressible fraction of heat production was not significantly larger in muscles obtained from hyperthyroid mice than in those from hypothyroid animals (19). Taken together,

these studies and the present results indicate that obesity is unlikely to be the outcome of a reduction in the amount of energy consumed by the Na^+-K^+-pump.

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