An enzymatic defect in the obese (*ob/ob*) mouse: Loss of thyroid-induced sodium- and potassium-dependent adenosinetriphosphatase

(obesity/genetic/hyperthyroid/thermogenesis)

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ABSTRACT Genetically obese (*ob/ob*) mice, mice that became obese after treatment with gold thioglucose, and lean animals were studied in the euthyroid state, after induction of hypothyroidism, and after treatment with triiodothyronine. The activity of glycerol 3-phosphate dehydrogenase [*sn*-glycerol-3-phosphate:(acceptor) oxidoreductase; EC 1.1.99.5] was reduced in the livers from hypothyroid animals and was increased by treatment with triiodothyronine in all groups. The activity of the ouabain-suppressible sodium- and potassium-dependent ATPase (ATP phosphohydrolase; EC 3.6.1.3) was increased by triiodothyronine and reduced by hypothyroidism in the lean and gold thioglucose-treated obese animals. In the obese (*ob/ob*) mice, on the other hand, treatment with triiodothyronine did not increase the activity of this enzyme, which remained at the level found in hypothyroid animals. This enzymatic activity was reduced in both liver and kidney. Adenylate cyclase [ATP pyrophosphate-lyase (cyclizing); EC 4.6.1.1] activity in liver membranes, however, was similar in all three groups of mice. This enzyme complex was activated by glucagon and was unaffected by treatment with thyroid hormones. The lack of a thyroid-dependent ouabain-suppressible ($\text{Na}^+ + \text{K}^+$)-ATPase in the tissues of the obese (*ob/ob*) mouse could explain most, if not all, of the abnormalities that have been described in this animal.

Those genetic diseases that are transmitted as autosomal Mendelian recessive traits are frequently manifested biochemically as the loss in function of a single enzyme. This one gene/one enzyme hypothesis has proven highly productive in the study of clinical disease (1). The present paper has used this concept to explore a genetically transmitted form of obesity for an enzymatic defect that might account for the myriad of alterations in this disease state (2, 3).

Animals carrying the *ob/ob* gene can first be detected around 10–15 days of age, either by their reduced oxygen consumption (4, 5) or by their decreased body temperature (6, 7). Body fat content, fat cell size, and serum insulin are also increased prior to weaning (2, 8–10), after which the *ob/ob* mice rapidly deposit excess body fat. It has recently been proposed that the obesity in the *ob/ob* mouse might reflect a defect in some major energy-consuming process (6). Three pieces of evidence support this concept: (i) the animals are hypometabolic prior to weaning (4–6); (ii) they are unable to regulate body temperature on exposure to the cold (6, 7, 11–14); and (iii) they remain in positive energy balance despite pair-feeding with food intake of lean littermates (14–17).

Edelman and his colleagues (18, 19) have convincingly demonstrated the important role of a thyroid-induced ($\text{Na}^+ + \text{K}^+$)-adenosine triphosphatase (ATP phosphohydrolase; EC 3.6.1.3) in the regulation of energy balance. A deficiency of such a thyroid hormone-stimulated thermogenic process might

provide a biochemical basis for the defect in the *ob/ob* mouse. Certain processes that are normally regulated by thyroid hormones, such as the increase in body temperature (7, 11) and stimulation of epinephrine-induced lipolysis (20, 21), appear to be resistant to the action of this hormone in the *ob/ob* mouse. On the other hand, processes such as induction of glycerol-3-phosphate dehydrogenase [*sn*-glycerol-3-phosphate:(acceptor) oxidoreductase; EC 1.1.99.5] (7, 20) and maturation of brain (22) show a normal response to thyroid hormone. This paper reports experiments on the regulation of the ($\text{Na}^+ + \text{K}^+$)-ATPase by thyroid hormones in the obese (*ob/ob*) mouse.

METHODS

The female C57B1/6J-*ob/ob* mice and their respective lean littermates (+/?) or known homozygous lean (+/+) animals were purchased from the Jackson Laboratories, Bar Harbor, ME. The *ob/ob* mice were 12 weeks old at the start of the experiment. A control nongenetic obesity was also produced by the intraperitoneal injection of 0.8 mg of gold thioglucose per kg (Sigma Chemical Co., St. Louis, MO) into 2-month-old lean mice that had not eaten for 24 hr. These animals were used 6 weeks later. All animals were housed in a temperature-controlled room (28°) with a 14-hr and 10-hr dark-light cycle.

Two procedures were used to regulate thyroid status of the mice. In the first procedure, lean and obese gold thioglucose-treated mice were injected subcutaneously for 20 days with 0.5 mg of methimazole daily in two equal doses, while eating a low iodine diet. For the final 11 days, the animals were subdivided so that they received either triiodothyronine (T_3) (20 $\mu\text{g}/\text{kg}$ per day in two equal doses) or an equal volume of saline. In the second procedure, thyroid status of *ob/ob*, gold thioglucose-treated obese and lean mice was manipulated by placing the animals on a low iodine diet and giving them 1% potassium perchlorate in their drinking water for a total of 14 days. After 7 days of this treatment, the animals in each group were subdivided to receive either T_3 (20 $\mu\text{g}/\text{kg}$ per day) in a single daily dose or an equal volume of saline.

($\text{Na}^+ + \text{K}^+$)-ATPase was measured on liver homogenates by the method of Ismail-Beigi and Edelman (23). Kidney membranes from *ob/ob* and lean mice were prepared by the method of Lo *et al.* (24) except that whole kidneys were pooled rather than separated into medulla and cortex. The L-fraction was partially purified through the first step by treatment with sodium iodide as described (25). Liver was homogenized with six strokes of a Teflon-glass homogenizer in 10 volumes of 0.25 sucrose M 1.25 mM ethyleneglycol bis(β -aminoethyl ether)- N,N' -tetraacetate/1.0 mM Tris at pH 7.0, and further diluted

Abbreviation: T_3 , triiodothyronine.

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1:3 with the same medium. Aliquots were incubated in triplicate in a final volume of 1 ml containing (all values mM) MgCl_2 (5.0)/ NaCl (120)/ KCl (12.5)/Tris buffer, pH 7.4 (27)/ $\text{Na}_2\text{S}_2\text{O}_8$ (5.0)/ATP (disodium or Tris salt) (5.0) for 5 min at 37°. (Na^+ + K^+)-ATPase was assayed as the difference between the phosphate concentration in this medium and in medium containing ouabain (1 mM) or in which all Na^+ and K^+ were replaced by sucrose. Similar values were normally obtained by both methods. Reactions were stopped by the addition of trichloroacetic acid (final concentration 5 mM). Phosphate was assayed by the method of Fiske and Subbarow (26). As an additional marker, the liver glycerol-3-phosphate dehydrogenase was measured on crude liver homogenates (1/20 wt/vol) in 0.15 M KCl by the method of Richert *et al.* (27). Protein was determined by the method of Lowry *et al.* (28).

Oxygen consumption was measured in a Gilson differential respirometer. The outer well contained Krebs-Ringer phosphate buffer (pH 7.4) with one-third the recommended calcium, or a medium in which the sodium had been replaced by sucrose (19). The center well contained 10% NaOH and a filter-paper wick for improving CO_2 absorption. Animals were killed by cervical dislocation and the abdominal muscle was freed from the skin and separated at the mid-line. The muscles were cut below the ribs, to the psoas muscle posteriorly and at the iliac crest inferiorly, and then placed in cold buffer. Each muscle was divided into two pieces; one was incubated in the sodium-containing medium and the other in the sodium-free medium. One pair of mice was studied at a time in such a way that all possible combinations of obese and lean mice with and without T_3 treatment (20 $\mu\text{g}/\text{kg}$ per day) for 7 days were studied together. Data are expressed as μl of oxygen uptake per mg of dry tissue per hr.

Stimulation of hepatic adenylate cyclase [ATP pyrophosphate-lyase (cyclizing); EC 4.6.1.1] in the presence and absence of glucagon was measured on the 2000 $\times g$ pellet of liver homogenate (29). The assay components were 2 mM ATP, 4 mM MgCl_2 , 15 mM creatine phosphate, 5 mM theophylline, 20 μg of creatine phosphokinase per tube, 40 mM Tris-HCl (pH 7.3), and approximately 150 μg of membrane protein a final volume of 150 μl . After incubation at 37° for 15 min, the reaction was stopped by boiling. The samples were suspended in 50 mM acetate buffer, (pH 4.0) and cyclic-AMP was measured by the protein binding assay of Gilman (30) from which the protein kinase inhibitor was omitted.

RESULTS

The activity of (Na^+ + K^+)-ATPase in liver homogenates of untreated obese (*ob/ob*) mice was significantly lower than in lean or gold thioglucose-treated obese mice (Table 1). The glycerol-3-phosphate dehydrogenase, activity which was measured in the *ob/ob* and lean animals, was not significantly different, however. The activity of the (Na^+ + K^+)-ATPase was clearly influenced by the thyroid status of both lean and gold thioglucose-treated obese mice (Table 2). In these two groups, the (Na^+ + K^+)-ATPase showed a 2- to 3-fold stimulation in mice treated with T_3 whereas (Mg^{2+})-ATPase was unaffected by this treatment. The thyroid status of the animals was indicated by the low level of glycerol-3-phosphate dehydrogenase activity in the methimazole-treated animals (hypothyroid) and its enhancement after T_3 therapy. The higher activity of glycerol-3-phosphate dehydrogenase in the animals subjected to the second protocol for inducing hypothyroidism indicated that the hypothyroidism was probably less marked (Table 3), but the rise in activity of this enzyme in T_3 -treated mice indicates that hyperthyroidism had been produced. The (Na^+ +

Table 1. Enzyme activities in liver from euthyroid mice*

Mice	(Na^+ + K^+)-ATPase†	P	(Mg^{2+})-ATPase†	P	Glycerol-3-phosphate dehydrogenase‡
Lean	1.78 ± 0.34 (5)		3.36 ± 0.46 (5)		12.7 ± 1.8 (4)
Obese	0.60 ± 0.16 (5)	<0.05	2.66 ± 0.43 (5)	NS§	13.7 ± 7.0 (4)
Gold thio-glucose	1.36 ± 0.23 (4)	<0.05	4.05 ± 0.21 (4)	<0.05	

* The activity of total and ouabain-dependent hydrolysis of ATPase was measured in homogenates of liver from euthyroid animals. Oxygen uptake in the presence of cyanide, phenazinemethosulfate, and glycerol 3-phosphate was also measured. Values are mean ± SEM (number of animals is in parentheses).

† Values are μmol of P_i /mg of protein per hr.

‡ Values are μl of O_2 /mg of protein per hr.

§ NS, not significant.

K^+)-ATPase activity of both the lean and the fat, gold thioglucose-treated mice, varied with thyroid status, falling in the hypothyroid animals to values similar to those in the *ob/ob* mice, and being enhanced by T_3 treatment. In contrast, the (Na^+ + K^+)-ATPase activity in liver of *ob/ob* mice showed no significant rise after treatment with T_3 . The effect of T_3 was, however, clearly observed on glycerol-3-phosphate dehydrogenase activity of *ob/ob* mice, the activity being significantly greater than in lean T_3 -treated mice and almost 2-fold greater than in euthyroid *ob/ob* mice (Table 3). Once again, (Mg^{2+})-ATPase was unaffected by changes in thyroid status.

The activity of the (Na^+ + K^+)-ATPase in kidney membranes was also reduced in the obese mice (Table 4). Treatment of the membrane fraction with sodium iodide increased the enzymatic activity per mg of protein in both groups, but activity from the *ob/ob* mouse was still lower than in the lean mice.

The effects of treatment with T_3 on oxygen uptake by muscle incubated with and without sodium in the incubation medium is shown in Table 5. In control experiments (not shown), there were no significant differences in oxygen uptake between right and left abdominal muscles in eight normal mice. Treatment of the lean mice with T_3 (20 $\mu\text{g}/\text{kg}$ per day) significantly in-

Table 2. Effect of thyroid status on (Na^+ + K^+)-ATPase, (Mg^{2+})-ATPase, and glycerol-3-phosphate dehydrogenase activities of liver in lean and gold thioglucose-treated mice*

Mice	T_3 treatment	(Na^+ + K^+)-ATPase†	(Mg^{2+})-ATPase†	Glycerol-3-phosphate dehydrogenase‡
Lean				
(+/?)	0	0.53 ± 0.18(5)	3.28 ± 0.48(5)	6.0 ± 0.74(4)
	+	1.96 ± 0.40(4)	3.26 ± 0.26(4)	18.3 ± 1.6 (4)
		$P < 0.01$		$P < 0.01$
Gold thioglucose				
(+/+)	0	0.50 ± 0.24(5)	3.49 ± 0.44(5)	8.9 ± 1.53(4)
	+	1.36 ± 0.25(5)	3.26 ± 0.32(5)	16.0 ± 1.3 (4)
		$P < 0.05$		$P < 0.02$

* Values are mean ± SEM (number of animals is in parentheses).

† Values are μmol of P_i /mg of protein per hr.

‡ Values are μl of O_2 /mg of protein per hr.

Table 3. Effect of thyroid status on (Na⁺ + K⁺)-ATPase, (Mg²⁺)-ATPase, and glycerol-3-phosphate dehydrogenase of liver in lean, *ob/ob*, and gold thioglucose-obese mice*

Mice	T ₃ treatment	(Na ⁺ + K ⁺)-ATPase [†]	P	(Mg ²⁺)-ATPase [†]	Glycerol-3-phosphate dehydrogenase [‡]	P
Lean (C57Bl/6J) (+/?)	0	0.89 ± 0.17(5)		4.74 ± 0.47(5)	8.70 ± 2.0(3)	
	+	1.57 ± 0.29(6)	<0.05	4.11 ± 0.39(6)	15.50 ± 0.91(3)	<0.05
Obese (C57Bl/6J). (<i>ob/ob</i>)	0	0.83 ± 0.17(6)		4.44 ± 0.30(6)	11.67 ± 0.73(3)	
	+	0.96 ± 0.24(5)	NS [§]	5.27 ± 0.48(5)	24.80 ± 0.74(3)	<0.001
Gold thioglucose (C57Bl/6J)	0	0.30 ± 0.14(4)		3.77 ± 0.17(4)		
	+	1.27 ± 0.15(4)	<0.01	3.52 ± 0.21(4)		

* Values are mean ± SEM (number of animals is in parentheses).

† Values are μmol of P_i/mg of protein per hr.‡ Values are μl of O₂/mg of protein per hr.

§ NS, not significant.

creased oxygen consumption of the abdominal muscles. In the absence of sodium (Table 5), there was also a significant increase in oxygen uptake ($P < 0.05$). In the obese (*ob/ob*) mice, on the other hand, treatment with T₃ in doses known to increase circulating levels of T₃ to values similar to those of the lean mice (11) produced no significant increase in oxygen consumption. There was likewise no significant effect of treating obese mice with T₃ on the uptake of oxygen by abdominal muscle incubated in the absence of sodium.

The activity of adenylate cyclase was clearly stimulated by the presence of 1 or 10 nM glucagon. Of importance for the present study is the fact that the basal activity and response to glucagon was similar in both types of obese mice and was uninfluenced by hypothyroidism or treatment with T₃ (Table 6).

DISCUSSION

These results show that the activity of hepatic and renal (Na⁺ + K⁺)-ATPase in untreated obese (*ob/ob*) mice is lower than in lean controls. Treatment with T₃ failed to increase the sodium-dependent oxygen consumption or the activity of the (Na⁺ + K⁺)-ATPase in the liver from *ob/ob* mice but produced the expected rise in lean animals. In contrast, the activity of both the glycerol-3-phosphate dehydrogenase and adenylate cyclase had similar values in hypothyroid and in T₃-treated lean and

ob/ob mice. We thus propose that a defect in the T₃-induced (Na⁺ + K⁺)-ATPase may be the basis for the obesity in *ob/ob* mice. This enzymatic difference cannot simply result from obesity *per se* since the activity of the (Na⁺ + K⁺)-ATPase was normal in the liver of obese gold-thioglucose-treated mice and exhibited the normal rise after treatment with thyroid hormone.

A defective (Na⁺ + K⁺)-ATPase system could explain many of the metabolic changes that have been reported in the *ob/ob* mouse (see reviews, refs. 2, 3, and 31). Edelman and colleagues have shown that thyroid stimulation of the (Na⁺ + K⁺)-ATPase is the basis of the thyroid enhancement of oxygen consumption by a number of tissues (18, 19). Deficiency of this enzyme could thus be the basis of the defective thermogenesis that results in a lower body temperature (6, 7, 11), increased susceptibility to cold (11, 13), and the increased efficiency of food utilization in these animals (14–17). Although it is possible to increase the body temperature of *ob/ob* mice to normal by treatment with high doses of thyroid hormone for prolonged periods (7, 11), comparable doses of this hormone increase the temperature of normal mice to supranormal levels. The partial but impaired thermic response of *ob/ob* mice presumably results from the

Table 5. Effect of triiodothyronine on oxygen consumption of abdominal wall muscle of lean and obese (*ob/ob*) mice*

Group	Sodium in medium	T ₃ treatment	Oxygen uptake, μl O ₂ /mg tissue dry wt per hr	P
Lean (+/?)	0	0	0.51 ± 0.05	P <
	+	0	1.06 ± 0.06	0.01
	0	+	0.69 ± 0.06	P <
	+	+	1.37 ± 0.09	0.01
Obese (<i>ob/ob</i>)	0	0	0.46 ± 0.05	P <
	+	0	1.23 ± 0.06	0.01
	0	+	0.56 ± 0.06	P <
	+	+	1.32 ± 0.18	0.01

* Mean ± SEM. Number of animals was 6 in all cases.

Table 4. Activity of (Na⁺ + K⁺)-ATPase in kidney of obese and lean mice*

	(Na ⁺ + K ⁺)-ATPase [†]		(Mg ²⁺)-ATPase [†]	
Preparation	Lean	<i>ob/ob</i>	Lean	<i>ob/ob</i>
L-Fraction	24	13	69	84
NaI-treated	204	79	71	31

* Kidneys from 10 mice were pooled and membrane fraction (L-fraction) was prepared from the whole kidney as described by Lo *et al.* (24). Sodium iodide treatment increased the specific activity of the (Na⁺ + K⁺)-ATPase in both groups, but the difference between *ob/ob* and lean animals was still present.

† Values are μmol of P_i/mg of protein per hr.

Table 6. Adenylate cyclase activity* in liver membranes from lean and obese mice incubated in the presence and absence of glucagon

Mice	T ₃ treatment	Basal activity	Activity in presence of glucagon		
			0.1 nM	1 nM	10 nM
Lean					
(C57Bl/6J +/-)	0	0.30 ± 0.04(7)	0.44 ± 0.07(5)	0.69 ± 0.10(7)	2.02 ± 0.42(5)
	+	0.31 ± 0.06(7)	0.37 ± 0.07(5)	0.60 ± 0.07(7)	1.33 ± 0.29(5)
Obese					
(C57Bl/6J <i>ob/ob</i>)	0	0.23 ± 0.07(5)	0.29 ± 0.10(5)	0.68 ± 0.20(5)	1.35 ± 0.26(5)
	+	0.29 ± 0.09(4)	0.40 ± 0.11(4)	1.00 ± 0.23(4)	2.08 ± 0.37(4)
Gold thioglucose					
(C57Bl/6J +/-)	0	0.15 ± 0.02(4)	0.30 ± 0.08(2)	0.49 ± 0.10(4)	0.83 ± 0.44(2)
	+	0.21 ± 0.03(4)	0.15 ± 0.02(2)	0.45 ± 0.07(4)	0.74 ± 0.12(2)

* Values are nmol of cyclic AMP/mg of protein per 15 min, mean ± SEM (number of animals is in parentheses).

induction of other thermoregulatory processes, such as the glycerol-3-phosphate cycle.

The reuptake of norepinephrine from synapses is a ouabain-suppressible process thought to involve a (Na⁺ + K⁺)-ATPase (32). The impairment of such a system in *ob/ob* mice could provide a basis for the various hypothalamic dysfunctions in the *ob/ob* mice (2), including the hyperphagia and altered reproductive function (33, 34). A change in transmembrane ionic balance for Na⁺ and K⁺ could also alter many other metabolic processes. Two defects that may possibly be explained by this mechanism are the diminished uptake of 2-deoxy-D-glucose by muscle (35) and the loss of vasopressin inhibition of fatty acid synthesis (36).

A major defect in the *ob/ob* mouse is the hypersecretion of insulin (2, 3). The pancreatic islets contain an active (Na⁺ + K⁺)-ATPase which may be diminished during glucose-induced insulin secretion (37). A low (Na⁺ + K⁺)-ATPase activity in pancreatic islets might thus be the basis of the hyperinsulinemia in these animals.

The precise defect in the thyroid-dependent ATPase system of *ob/ob* mice awaits further clarification. It is clearly not a generalized defect in thyroid-induced processes since thyroid-sensitive parameters such as glycerol-3-phosphate dehydrogenase (7, 21), brain maturation (22), and suppression of methimazole-induced goiters (11) respond normally to thyroid in these animals. It is more likely that the (Na⁺ + K⁺)-ATPase induced by thyroid hormones is structurally abnormal and has little or no activity.

The (Na⁺ + K⁺)-ATPase has been fractionated into two major proteins (38), one with a mass of 100,000 daltons and a glycoprotein of 40,000 daltons. It was previously proposed from membrane-binding studies (39) that *ob/ob* mice had a generalized defect in membrane glycoproteins. It is thus possible that the defect in the (Na⁺ + K⁺)-ATPase is an enzymic example of a more generalized deficiency in glycoprotein metabolism in the *ob/ob* mice. Whatever the mechanism underlying the loss of ATPase activity in *ob/ob* mice, this would appear to be the first example of a specific enzyme defect underlying an impairment in thermogenesis that results in obesity.

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