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A MITOCHONDRIAL DEFECT IN BROWN ADIPOSE TISSUE OF THE OBESE (ob/ob) MOUSE: REDUCED BINDING OF PURINE NUCLEOTIDES AND A FAILURE TO RESPOND TO COLD BY AN INCREASE IN BINDING

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SUMMARY: Atractyloside-insensitive binding of purine nucleotides is reduced in brown adipose tissue mitochondria of the obese (ob/ob) mouse. Exposure of the ob/ob mouse to 4°C does not induce the usual increase in binding. Atractyloside-insensitive binding of purine nucleotides is believed to be a measure of the heat-producing proton conductance pathway in brown adipose tissue mitochondria. It is, therefore, suggested that the impaired thermogenesis of the ob/ob mouse is due to a defect in this pathway in the mitochondria of the brown adipose tissue, the major thermogenic tissues in rodents. The greater metabolic efficiency which would result from a reduced operation of this pathway might be the basis for the obesity in the ob/ob mouse.

INTRODUCTION: The genetically obese mouse (ob/ob) normally has a lower metabolic rate (1) and a lower body temperature (2) than the lean counterpart, and is unable to survive for more than a few hours when exposed to cold (3) due to failure of normal thermogenesis (4). Evidence that this defect is not due to the obesity is provided by the observations of the reduced metabolic rate (5,6) and the impaired calorigenic response to cooling (7) in weanling and even preweanling mice, before the development of the obesity. Indeed, it is probable that reduced thermogenesis is the cause of the obesity (5-8).

Although the thyroid function of obese mice is relatively normal (2,9-10), the possibility of some selective defect in tissue response to thyroid hormone (9-11) cannot be entirely excluded. However, prolonged treatment of ob/ob mice with thyroid hormone does increase heat production and reduce the obesity (1-2,9-11).

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Edelman's hypothesis for the action of thyroid hormone (12) has influenced a number of studies of the impaired mechanism of thermogenesis in ob/ob mice. This hypothesis emphasises the major controlling role of the Na^+ pump in determining the basal metabolic rate and proposes that thyroid hormone acts by increasing the amount and/or activity of the Na^+K^+ ATPase in the plasma membrane of its target tissues (12). The finding of a reduced amount of Na^+K^+ ATPase in skeletal muscle (21), liver and kidney (11) of ob/ob mice has led to the suggestion that both the obesity and the cold-sensitivity of these animals may be due to a defect in the Na^+ pump, leading to a greater metabolic efficiency (11,21) and to a less effective action of thyroid hormone on the pump (11). The hypothesis has recently been extended to explain cold-induced nonshivering thermogenesis (13-18), a process characterized not by a raised basal metabolic rate but by a rapid catecholamine-induced increase in metabolic rate (see 19-20 for review). Catecholamine-induced thermogenesis is known to be defective in the ob/ob mouse (8).

Recent studies on catecholamine-induced nonshivering thermogenesis in cold-acclimated rats have shown that, contrary to previous beliefs (see 19-20), a major proportion of nonshivering thermogenesis occurs in the brown adipose tissue (22,23) rather than muscle, liver and other organs. Since this major thermogenic tissue has hitherto been neglected in studies of altered thermogenesis in the ob/ob mouse, the work described in this preliminary report was undertaken to find out whether the normal amount of brown adipose tissue was present and whether the mitochondria of this tissue have the normal characteristics of brown adipose tissue mitochondria. The characteristic chosen for initial study was the atractyloside-insensitive binding of purine nucleotides. The reason for this choice is that brown adipose tissue mitochondria possess a unique proton conductance pathway (24-26) which allows very high rates of respiration and the pathway is inhibited by purine nucleotides (GDP and ADP) (24-26), which appear to act by binding to a 32 000 polypeptide on the outer surface of the inner mitochondrial membrane (27). Both the extent of binding

(atractyloside-insensitive) of purine nucleotides and the amount of the 32 000 polypeptide can be correlated with the capacity of the animal for nonshivering thermogenesis (27,28). For example, in the rat, cold-exposure causes a rapid increase in purine nucleotide binding which is apparent within one hour and reaches a maximum after 3 - 7 days (28); the cold-acclimated rat also has a higher proportion of the 32 000 polypeptide present in the membranes of the brown adipose tissue mitochondria (28-30).

METHODS: C_{57}BL/6J ob/ob mice and their normal lean controls (+/+ or ob/+) were obtained at 8-10 weeks of age from the Jackson Memorial Laboratories, Bar Harbor, ME. They were kept in groups in plastic cages at 28° with a 12 h lighting cycle. In the experiment in which mice were exposed to cold they were placed for 3 hours at either 28° or 4° in individual metal cages containing a thin layer of wood chip bedding and food and water. Mice were killed by cervical dislocation and the interscapular brown adipose tissue removed. The tissue in the obese mice was difficult to identify because it was itself full of fat and completely immersed in large masses of white fat; however, its faint brown colour permitted its separation from the surrounding white adipose tissue.

Tissues were weighed and then homogenized in a medium containing 0.25M sucrose, 0.2 mM EDTA (potassium salt) and 1 mM HEPES (N-2-hydroxyethyl piperazine-N'-ethanesulfonic acid) pH 7.2 and made to a volume of 10 ml. Protein was estimated by a modified Lowry method (31) after precipitation with 12.5% trichloroacetic acid. Samples of homogenate containing 2.5 mg protein were activated with lubrol (3 mg/mg protein) and diluted to 5 ml before the use of 50 µl (25 µg protein) in the assay of cytochrome oxidase by a polarographic method as described previously (32). For isolation of mitochondria, tissues from 4-5 mice were pooled, homogenized and mitochondria isolated as described by Slinde, Flatmark and Pedersen (33). The yield of mitochondria was similar in the lean and obese mice. Mitochondria were finally resuspended in 0.25 M sucrose at a concentration of 8-10 mg protein per ml. Purine nucleotide binding sites were measured by the binding of [^{3}H]GDP or [^{3}H]ADP in the presence of atractyloside, essentially as described by Nicholls (25) with the modification described before (28).

RESULTS AND DISCUSSION: The total amount of interscapular brown adipose tissue appears to be normal in the ob/ob mouse, as judged from its normal total protein content (Table 1). The total amount of mitochondria in the tissue is also normal, as shown by the total cytochrome oxidase content (Table 1). The tissue is grossly swollen with fat, obvious from visual inspection and from the more than three-fold increase in wet weight in the ob/ob animals (Table 1). The normal amount of protein and cytochrome oxidase in the interscapular brown adipose tissue of the ob/ob mouse indicates that the defect in thermogenesis cannot be ascribed to a lack of this tissue or its mitochondria. This is in

Table 1: Weight, protein and cytochrome oxidase content of interscapular brown adipose tissue of lean and ob/ob mice.

	Lean mice (n = 12)	ob/ob mice (n = 9)
Body weight (g)	30.6 ± 0.48	54.6 ± 1.73[a]
Interscapular brown adipose tissue		
wet weight (mg)	129.6 ± 4.8	458.2 ± 33.5[a]
Protein (mg)	9.63 ± 0.37	8.82 ± 1.0
Cytochrome oxidase (μg atoms O_2/min)	4.39 ± 0.40	4.65 ± 0.47

[a] Significantly different from lean animals, $P < 0.05$ or less.

contrast to the dystrophic hamster in which a reduced amount of brown adipose tissue can account for the reduced calorigenic response to catecholamines (34).

The mitochondria of the interscapular brown adipose tissue are, however, not normal. The atractyloside-insensitive binding of GDP is reduced by 50% in obese mice maintained at 28° (Table 2). Moreover, the large increase of 45% in the binding of GDP which is induced by cold-exposure of the lean mouse for only 3 hours was completely absent in the obese mouse (Table 2). Likewise binding of ADP was increased in the cold-exposed lean mice but not in the cold-exposed obese mice. The reduction in atractyloside-sensitive purine nucleotide binding suggests a reduced amount of the heat-producing proton conductance pathway, with which this binding is normally correlated (24-26) and thus a reduced capacity for thermogenesis. Moreover, the failure of the binding to increase in the normal way when the ob/ob mouse is exposed to cold indicates some defect in the control of the amount of the pathway.

A relative lack of the specific proton conductance pathway in brown

Table 2: Atractyloside-insensitive binding of purine nucleotides (GDP and ADP) to brown adipose tissue mitochondria of lean and ob/ob mice exposed to either 28° or to 4° for 3 hours.

	LEAN MICE		OBESE MICE	
	28°	4°	28°	4°
Body weight (g)	22.5 ± 0.2	21.0[b] ± 0.3	49.4[a] ± 0.6	49.4[a] ± 0.6
Interscapular brown adipose tissue (mg)	95.0 ± 2.0	60.0[b] ± 8.0	404[a] ± 21	443[a] ± 28
Mitochondria				
Binding of GDP pmoles/mg protein	83.4 ± 12.7	120.7[b] ± 5.1	41.2[a] ± 6.8	50.5[a] ± 3.4
Binding of ADP pmoles/mg protein	58.8 ± 8.5	93.0[b] ± 5.6	42.8 ± 6.2	31.6[a] ± 11.2

Values are means ± standard errors of three experiments. Weights of mice and tissues are means for 12-13 animals used in the three experiments.

[a]Significantly different from lean animals at the same temperature, $P < 0.05$ or less.

[b]Significantly different from similar animals at 28°C, $P < 0.05$ or less.

adipose tissue mitochondria and/or a defect in the control of the amount of this pathway could explain why the ob/ob mouse has an impaired response to cold (3-4,7-8) and an impaired calorigenic response to noradrenaline (8). It could also account for the obesity since a reduced operation of this pathway at temperatures below thermoneutral for the mouse would result in less wasting of substrates for heat production and thus a greater metabolic efficiency. Thermoneutral temperature for the mouse appears to be rather high (8,35-36) and well above the temperature at which mice are usually kept in the animal house.

It appears that the obesity and the hyperglycemic-insulin-resistant state which accompanies it are secondary to a thermogenic defect which is apparent even in the preweanling ob/ob mouse (6-7). It is proposed that the thermogenic defect resides in the mitochondria of the brown adipose tissue and

that other changes in the animals are secondary to the altered thermoregulation imposed by the defect.

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