The development of cold-induced thermogenesis and the structure of brown adipocyte mitochondria in genetically-obese (*ob/ob*) mice

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1. The onset of cold-induced thermogenesis was studied in a strain of mice which produced among their offspring genetically-obese (*ob/ob*) individuals. A thermogenic response was present in a majority by day 5 after birth.

2. The thermogenic response to cold was measured on days 5, 10 or 15 after birth, and the animals reared and the onset of obesity noted. The correlation between the subsequent development of obesity and a poor thermogenic response in early life was low.

3. A poor thermogenic response at day 15 was associated with the presence in brown adipocytes of mitochondria with disordered internal structures.

4. At day 42 both non-obese and obviously-obese mice showed a similar thermogenic response to moderate cold exposure.

5. It would seem that in this strain of mice disordered internal mitochondrial structure in brown adipose tissue is associated with a poor thermogenic response to cold, but not invariably with the subsequent onset of obesity.

Many years ago it was shown that genetically-obese (*ob/ob*) mice had poor thermoregulatory control (Davies & Mayer, 1954). More recently it has been suggested that this is due to a defect in the thermogenic capacity of their brown adipose tissue (Hogan & Himms-Hagen, 1980). In most species of mammals, brown adipose tissue has been shown to be most active in the immediate post-natal period (Alexander, 1975) and so if there were a defect in brown-adipose-tissue thermogenesis, it should be most evident in early life. By measuring the rectal temperature during cold exposure of 17-d-old mice, Trayhurn *et al.* (1977) found they could predict which mice were going to become obese. However, they did not measure the rate of thermogenesis directly.

The aim of the present study was to document the onset of thermogenesis in strains of mice which produce *ob/ob* offspring and to explore further the possibility that their poor thermoregulatory control was due to a defect in brown-adipose-tissue thermogenesis.

METHODS

Five heterozygote (+/*ob*) pairs of a strain of mice which produce genetically-obese (*ob/ob*) offspring were used to establish the colony. The young mice in each litter were marked and weighed regularly from the first to the sixth week of life. Only nestling animals who thrived were studied. The colony was housed in a room with an air temperature of $21 \pm 2°$ and a 12 h dark–12 h light cycle. The mice were fed on a rat and mice breeding diet.

In the first series of experiments (A) the rate of oxygen consumption of pups from eighteen litters was measured individually in a 'comfortable' and a cold environment on days 2, 5, 10 or 15 after birth. Occasionally a pup was challenged on more than one of these days. The animals were then reared and those becoming obese identified.

In a second series of experiments (B) the response to cold exposure was examined on day 15 only in the offspring of eight pairs who had previously produced young who had become obese. Five of the young which had poor thermogenic responses, and sixteen which had good thermogenic responses, were killed and their cervical brown adipose tissue removed

and studied under the electron microscope. The remaining young were reared and those becoming obese noted.

In a third series of experiments (C) the metabolic rate at a series of temperatures (35, 30, 25 and 15°) was measured in 42-d-old mice to define more precisely their thermogenic response to cold. In this study obviously-obese mice were selected and their responses compared with those of lean litter-mates of the same sex. At the conclusion of the experiment twelve of the animals (six obese and six lean) were killed, as much adipose tissue as possible was removed by dissection, and the animals reweighed to obtain some indication of the non-adipose-tissue body-weight.

In a fourth series of experiments (D) the rate of oxygen consumption of eighteen healthy 15-d-old mice from a non-obese CS1 strain was measured at 33 and 17°.

In the four series of experiments the rate of O_2 consumption was measured continuously in a specially-designed chamber, the details of which have been described previously (Elphick *et al.* 1981). In the system, the air temperature and surrounding surface temperatures were the same. The relative humidity was kept close to 100% by placing a moist filter paper under the perforated animal platform. The O_2 consumption is reported as ml O_2 at standard temperature and pressure. The objective of the investigation was to demonstrate the onset of cold-induced thermogenesis, and not to examine the metabolic changes with hypothermia or to measure the maximal metabolic rate; thus temperatures were used that were expected to be within the animals' range of thermal control. They were based on ambient temperatures which we had found previously, with CS1 strain mice of similar age and size (Vinter *et al.* 1982), to be within the range occurring in the thermoneutral environment, and a cold challenge that might stimulate at least a twofold increase in metabolic rate; i.e., day 2, 37 and 31°; day 5, 35 and 31°; day 10, 35 and 27°; day 15, 33 and 17°. The ambient conditions were held until the rate of O_2 consumption stabilized for at least 30 min.

Tissues for the electron-microscopic studies were taken from the interscapular brown adipose tissue from at least three sites in each animal. The tissues were fixed in glutaraldehyde (30 g/l) and osmium, dehydrated in alcohol, embedded in araldite and stained with lead citrate.

RESULTS

On random mating, thirteen litters from the eighteen litters in experimental series A did not produce obese offspring. The rates of O_2 consumption of eighty-eight mice from these thirteen litters on days 2, 5, 10 or 15 after birth are shown in Fig. 1. As none of the young became obese it is probable that they are the product of a $+/ob$, $+/+$ or a $+/+$, $+/+$ mating, although there is obviously a possibility that one or two of the litters were from a heterozygote pair $ob/+$, $ob/+$ who did not produce an ob/ob offspring. In this strain of mice a predictable response to cold exposure did not develop until day 10 and, at all ages studied, the scatter of the responses was wide.

The rates of O_2 consumption at day 15 of thirty-five mice from the five litters in which some subsequently became obese are shown in Fig. 2. Fig. 2 also gives the rates for the offspring of pairs who had previously produced young who became obese (presumed $+/ob$, $+/ob$ mating, experimental series B). The responses of mice taken for electron-microscopic study of their brown adipose tissue are also shown.

At 42 d of age the obese mice were obvious from inspection. Ten obese mice, mean body-weight (g) 48·7 (SE 1·5), range 39–56, and ten lean litter-mates, mean body-weight (g) 29·5 (SE 1·0), range 24–34, were exposed to successively cooler temperatures (Fig. 3, experimental series C). The minimal metabolic rate and the rates at the cool temperatures were significantly lower expressed per kg body-weight in the obese (ob/ob) mice compared

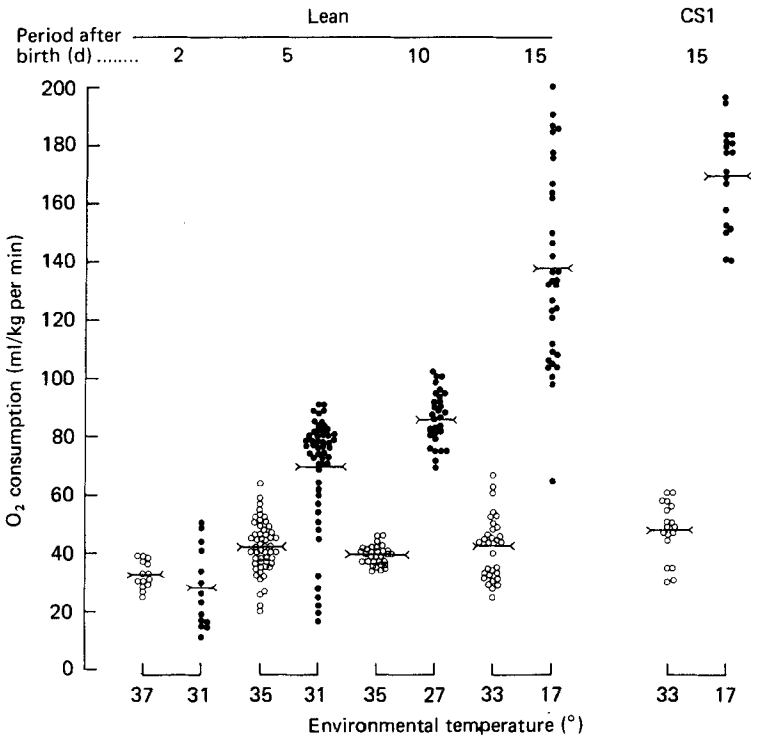

Fig. 1. The rates of oxygen consumption (ml/kg per min) of the mice from the thirteen litters of experimental series A which produced only lean offspring. Points represent individual rates at a warm (○) and a cool (●) environmental temperature and group means are represented by horizontal bars. Rates are also shown for day 15 of a normal (CS1) strain of mice; the mean differed significantly ($P < 0{\cdot}001$) from that of day-15 lean mice.

with the lean (presumably *ob*/+ and +/+) mice. When the metabolic rates were expressed as ml O_2/animal per min the reverse was the case. In six obese and six lean mice the fat was dissected away; if the rates of O_2 consumption are expressed per kg body-weight excluding dissectable fat the rates at all temperatures were very similar. The mean rates at 20°, for example, were 85 ml O_2/kg per min (SD 16) for the lean mice and 86 ml O_2/kg per min (SD 28) for the obese mice. At 15° the mean rates were 119 ml O_2/kg per min (SD 18) and 101 ml O_2/kg per min (SD 15) for the lean and obese groups respectively. The mean body-weight for the six obese was 44·7 g and the dissectable fat was 14·3 g, the mean body-weight of the six lean mice was 26·4 g and the dissectable fat was 2·6 g.

Plate 1 shows the structure of the mitochondria from three day-15 mice. Plate 1 (*a*) is from a CS1 mouse which had had a large increase in metabolic rate on cold exposure. The mitochondria contained densely-packed ordered cristae. Plate 1 (*c*) is from a mouse in experimental series B which had had a poor response, the mitochondria are pleomorphic and the cristae are disordered and have loops, branches and free ends. An intermediate situation is shown in Plate 1 (*b*) taken from another mouse in experimental series B which had had a good response to cold exposure. In an attempt to quantify this phenomenon, we studied three micrographs taken randomly from each animal illustrated in Fig. 2, experimental series B, and estimated the percentage of mitochondria present which contained cristae with free ends, branches or loops. Fig. 4 gives the percentage of these

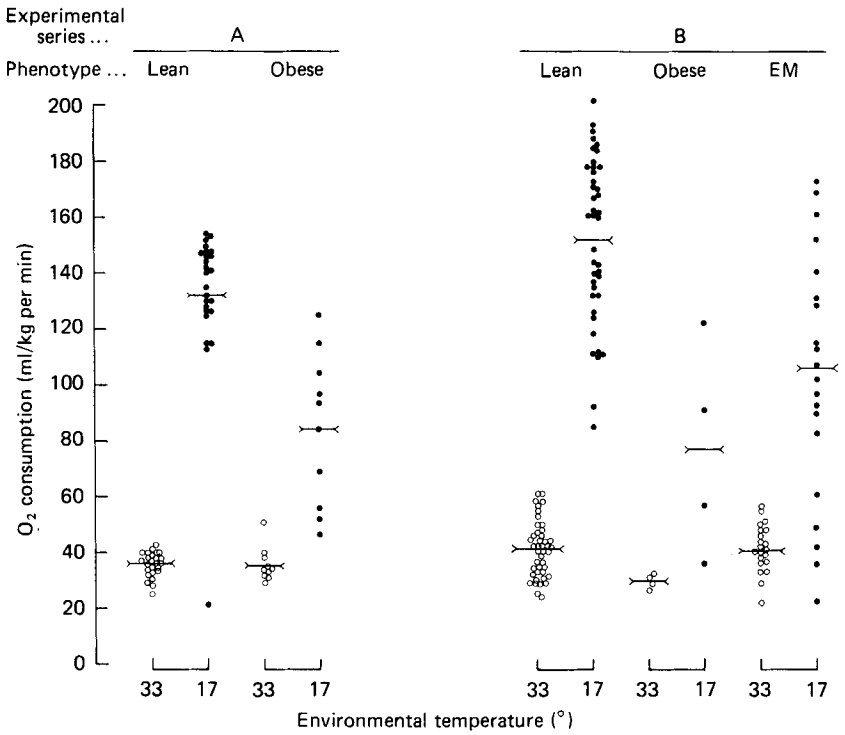

Fig. 2. The rates of oxygen consumption (ml/kg per min) of mice at day 15 from the five litters of experimental series A which produced obese offspring, and mice from the eight litters of experimental series B. Points represent individual rates at a warm (○) and a cool (●) environmental temperature and group means are represented by horizontal bars. EM, responses of mice taken for electron-microscopic study of their brown adipose tissue.

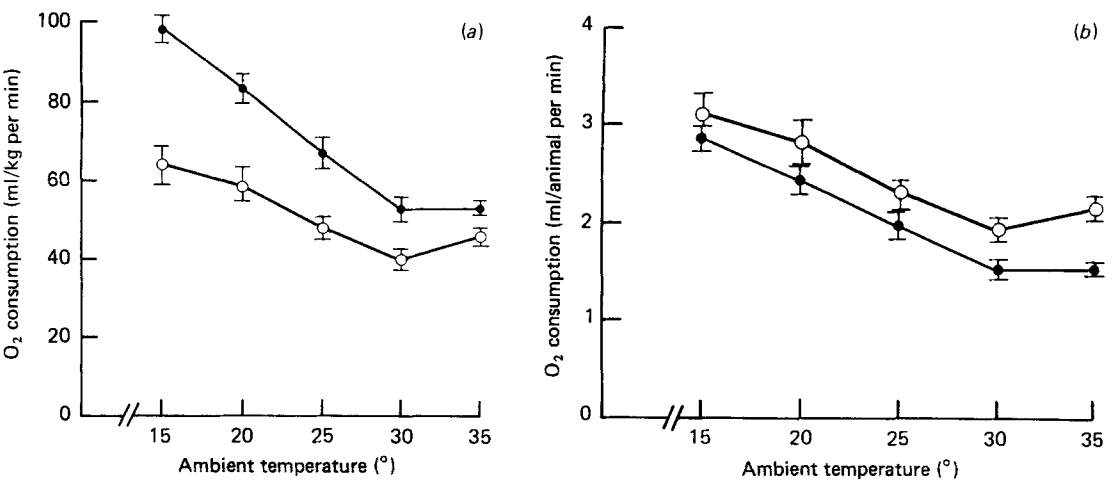

Fig. 3. The rates of oxygen consumption (ml/min) of obese (○) and lean (●) mice aged 42 d at various ambient temperatures, expressed as (*a*) per kg body-weight and (*b*) per animal. Points are mean values with their standard errors represented by vertical bars.

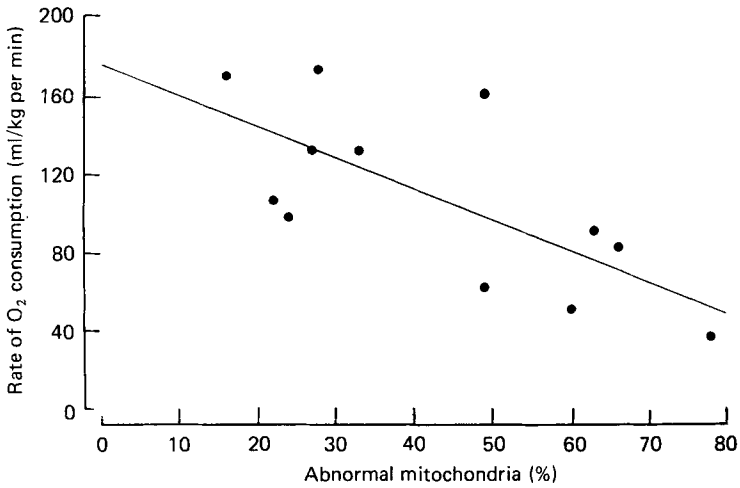

Fig. 4. The relationship between metabolic rate (oxygen consumption; ml/kg per min) at 17° and the percentage of 'abnormal' mitochondria from twelve day-15 mice from experimental series B. *r* 0·72.

'abnormal' mitochondria as seen in the three micrographs *v* the metabolic rate of the mouse at an environmental temperature of 17°.

DISCUSSION

Poor thermoregulatory control in *ob/ob* mice was initially demonstrated by subjecting the obese adults to a severe cold challenge. It is not surprising that mice reared in animal-house conditions become hypothermic when suddenly exposed to 4°. The point of interest was that lean litter-mates did not. Some considered the poor response was due to abnormal central control (Macdonald & Stock, 1979), others to a limited capacity for thermogenesis (Hogen & Himms-Hagen, 1980; Batt & Hambi, 1982). The latter led to the postulate that it was a defect in brown-adipose-tissue thermogenesis which led to the subsequent obesity.

Thermoregulatory heat production in brown adipose tissue is most evident in the majority of mammals in the early days or weeks of life. In one strain of mice, it was found to develop within the first 2 d of life and probably reaches a peak by the end of the second week (Vinter *et al.* 1982; J. Vinter, D. Hull and M. C. Elphick, unpublished results). In adults, alternative mechanisms, like shivering and controlled activity, are more evident. In adolescent rats, cafeteria feeding was shown by some (Stock & Rothwell, 1979) to stimulate diet-induced thermogenesis in brown adipose tissue. The significance of these findings is under debate (Hervey & Tobin, 1983; Rothwell & Stock, 1983). Overfeeding and prolonged cold exposure appear to induce similar changes in the structure of brown adipose tissue. If the mouse becomes obese because of a defect in the thermogenic activity of brown adipose tissue, then it would show a poor thermogenic response to cold early in life. Trayhurn *et al.* (1977) found that they could predict which mice would become obese by the fall in rectal temperature on cold exposure at 17 d of age before the mice were obviously obese. This, however, did not establish necessarily that it was the thermogenic capacity of the brown adipocyte which was at fault.

In the present study we found that the lean members of the strain of mice which produced obese offspring had a more delayed and variable development of thermogenesis on cold exposure compared with the CS1 strain (Vinter *et al.* 1982). On day 15 their metabolic rate

was on average just below 140 ml O_2/kg per min compared with that of 165 ml O_2/kg per min in CS1 strain mice and the scatter of response was much wider.

If a poor response to cold exposure at 17° is defined as a metabolic rate of under 80 ml O_2/kg per min, then out of seven mice with a poor response in the present study, six became obese. However, of fourteen animals who became obese, only six had a poor thermogenic response on day 15. From this it may be concluded that although a poor thermogenic response to cold on day 15 tended to be associated with the subsequent onset of obesity, the two were not inevitably linked.

In view of these findings we studied the rates of O_2 consumption in obese adult mice and their litter-mates in more detail. The results showed that they do not have a deficiency in their thermogenic response to cold exposure and this therefore is not the reason for their poor thermoregulating control. The observations were made in individual animals in a closed chamber; the animals could move freely but not far.

We cannot, from the present study, say whether all, some or none of the thermoregulatory heat production in day-42 mice took place in brown adipose tissue. Even so, our findings do add support to the conclusion reached by Batt & Hambi (1982) that the defective thermoregulating control of obese mice is related more to their relative immobility and inactivity than their genetic make-up. They found that by pair-feeding with lean litter-mates the *ob*/*ob* mice did not become obese and were not then subject to hypothermia.

We are still left with the phenomenon that in this strain of mice many young have a poor thermogenic response to cold. In this investigation we found that this correlated with the structure of their brown adipose tissue mitochondria. Suter (1969*a*, *b*), in her studies of brown adipocytes during development and during cold adaptation in rats, describes a sequence of changes. In the newborn rat, or the 'warm'-adapted adult, the mitochondria in brown adipocytes are relatively small with ordered mitochondria. With growth after birth or cold adaptation (in both situations thermogenesis in brown adipose tissue might be expected to be increasing) the mitochondria become larger and the cristae more numerous but are still well ordered. Then when cold adaptation is stopped or as the rats reach a certain age, the mitochondria become pleomorphic, some very large, others small, and they take on various forms. The cristae become fewer and more-widely spaced, they form loops, branch and have loose ends. A similar sequence has been described during arousal from hibernation (Grodums, 1977). It is not difficult to imagine that these pleomorphic mitochondria are less effective at thermogenesis.

Preliminary studies on the CS1 strain of mice suggest that in this species, as with the rat, the mitochondria in brown adipose tissue go through a sequence of enlargement and then disorder. It is this reaction to an extreme degree that appears to be occurring in mice with a poor thermogenic response to cold at day 15. It might be anticipated that such animals, living their lives within a laboratory cage, allowing their body temperatures to fluctuate with room temperatures and having good access to food, will tend to be obese. It is not necessary to postulate that they have a defect in diet-induced thermogenesis.

What is perhaps of greater interest is the underlying mechanism which leads to the apparent dissolution of the thermogenic capacity in brown adipose tissue which appears to occur prematurely and to a marked extent in some of the strain of mice which produce obese offspring.

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EXPLANATION OF PLATE

Plate 1. Electron micrographs of mitochondria from (*a*) a CS1 strain mouse aged 15 d and from a 15-d-old obese strain mouse from experimental series B with (*b*) a good thermogenic response and (*c*) a poor thermogenic response to cold exposure.

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