

Effect of environmental temperature and food intake on the distribution of fat in growing hairless mice

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1. The fat content of the skin and of the skin-free carcass was measured in young, growing hairless mice about 4–8 weeks old kept at either 22° (cool environment, i.e. below their critical temperature) or at 33° (warm environment; i.e. within their thermoneutral range). The food intake of groups of the mice reared at each temperature was restricted to between 77 and 89% of that of a litter-mate fed *ad lib.*

2. In all the mice, whether fed *ad lib.* or on a restricted intake, those reared in the warm environment contained about 1.5 times as much fat as those reared in the cool environment. At both temperatures and feeding levels, approximately 25% of the total body fat was present in the skin.

3. Each mouse reared at 33° on an *ad lib.* regimen reached about the same plateau weight in the same period as its litter-mate reared at 22°. However its food intake during this period of growth was only about half that of the mouse reared at the lower temperature, so its food conversion ratio (dry food intake:body-weight gain) was twice as efficient.

4. It is concluded that neither the growth rate nor the distribution of fat within the body of the growing mouse can be influenced by rearing the animals at these different environmental temperatures. Rearing at the higher temperature, however, both decreases food intake and also promotes deposition of more fat within the body.

The body composition of the growing mammal can be altered by the amount of food which it eats and by the environmental temperature in which it lives. If its environmental temperature is within its thermoneutral zone (the range of ambient temperature within which metabolic rate is minimal), a large proportion of the energy of its food can be stored as protein and fat. If its environmental temperature is much below its lower critical temperature, its metabolic rate is raised, heat loss from its surface is increased, and much of the energy of its food may be used in maintenance. In such conditions, less fat and protein would be stored even if the animal were fed *ad lib.* In some species, subcutaneous fat has an insulatory function, and it is possible that fat stored by an animal reared in a cool environment may be distributed among the available body depots in a different way from that deposited by an animal reared in thermoneutral conditions. The experiments to be described were undertaken to determine whether it is possible to modify the proportion of fat deposited in the subcutaneous fat depots of a young growing mammal by manipulating the environmental temperature or the feeding level. Hairless mice (a genetic-recessive strain of albino mice in which the adults lack hair) were used for these experiments. As experimental animals mice have the advantage of a short period of growth and ease of handling for carcass analysis, and the hairless strain is particularly sensitive to environmental temperature. However, mice have the disadvantage common to all small mammals that there is only a narrow limit within which the level of food intake can be altered and still permit growth.

METHODS

Animals. Hairless mice were bred by mating a hairless (hh) sire with a dam heterozygous (Hh) for the hairless gene; as the gene is recessive the dam had the normal albino hair covering and on average half the animals of each of her litters were hairless. Eight breeding pairs were set up simultaneously, and the hairless offspring were used as they became available, at about 28 d of age, approximately 1 week after weaning. As far as possible, in any one experiment male litter-mates were used, and at the start of each experiment the animals were matched for body-weight, which was between 15 and 22 g.

Housing and management. The mice were housed individually, on grids in boxes which were changed twice/week. They were weighed daily and their food intake was measured by offering daily known weights of food and separating and weighing any uneaten food left in the box at each change. The food, a commercial pelleted diet (Breeding Diet; Oxoid Ltd, Wade Road, Basingstoke, Hants.) contained 100 g moisture/kg. The energy value of the dried food was 17 kJ/g.

Two environmental temperatures were used. The lower temperature, that of the boxes in which all stock mice were born and reared, was maintained at 22° by fan-heaters under thermostatic control, and by a fan-extractor-ventilator to the outside. The warm environment was a well-insulated room maintained at 33° by fan-heaters. These temperatures were selected as being much lower than, and slightly higher than the lower critical temperature of hairless mice (Mount, 1971). Each mouse lived at one or other of these temperatures for about 3 weeks, and was killed when its weight had reached a plateau. This value, for a mouse fed *ad lib.*, was about 27 g. The mice were thus being observed during a period of rapid growth in which food intake could be measured and altered experimentally.

Measurement of water and fat content. Each mouse, when it had been killed by chloroform inhalation, was weighed. The skin, with its adherent subcutaneous fat, was removed as completely as possible from trunk, limbs and head, and was weighed, as was also the skin-free carcass. Loss of weight during the process of skinning was less than 1%. Skin and skin-free carcass were dried to constant weight on tared petri-dishes in an oven at 85–90°. The skin and carcass were then placed in Soxhlet thimbles (28 × 80 mm) over tared flasks in a water-bath and extracted by a mixture of chloroform-methanol (2:1, v/v) until the solvent around the thimble was free of colour (about 8 h). The bulk of the solvent was distilled from the fat and the remaining traces of solvent were removed in a ventilated incubator until the flask and its contents were of constant weight. The weight of fat in the flask was obtained, and checked by loss of weight from the thimble. The initial body fat was calculated for each mouse as being 8% of its initial body-weight, this value being the mean value derived from ten measurements made on hairless mice of body-weight between 14 and 22 g.

Design of experiment. Four mice of about the same age and weighing approximately 18 g were taken. Two were placed in individual boxes in the room at 33°, the other two at 22°. Of each pair, one was fed *ad lib.* and its food intake was recorded. The other was fed a restricted amount of food such that its intake was between 77 and 89% of its *ad*

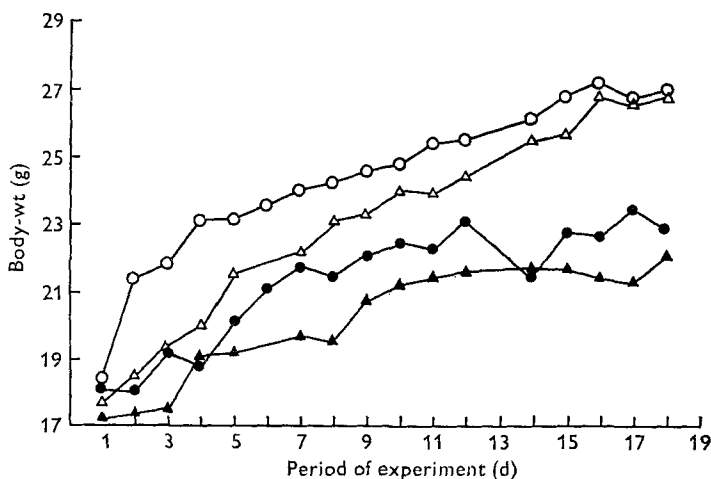


Fig. 1. Growth rate of four mice, two housed at 33° and two housed at 22°. At each temperature, one mouse of each pair was fed *ad lib.*; the food intake of the other was restricted to approximately 83% of the *ad lib.* intake. ○—○, 33°, *ad lib.*; ●—●, 33°, restricted; △—△, 22°, *ad lib.*; ▲—▲, 22°, restricted.

lib.-fed litter-mate. The duration of each experiment was not less than 2 and not more than 4 weeks, and the experiment was replicated six times as mice became available. Fig. 1 illustrates the growth rate of four mice used in an experiment. A few more observations were made at each of the two temperatures, using mice in the same range of body-weight, fed *ad lib.*

RESULTS

Table 1 shows the mean percentage of fat in the carcass and the proportion of fat in the skin of hairless mice reared at the two environmental temperatures. The mice were fed either *ad lib.* or at a restricted feeding level (between 77 and 89% of *ad lib.* intake), from about 7 d after weaning until the body-weight reached a plateau at about 55 d of age.

The mice fed *ad lib.* reached about the same plateau weight, about 27 g, in the same time at the two temperatures. At this body-weight, the mice reared in the warm environment had on average about 1.5 times as much fat in the body as those reared in the cool environment. The distribution of the fat did not differ. In both groups of mice, about 25% of the total body fat was present in the skin.

A restricted food intake was not easy to contrive, because when hairless mice were fed at 70–75% of their *ad lib.* intake they failed to thrive, lost weight and died. The restriction had therefore to be less severe, and was arranged so that the mouse on the restricted intake continued to grow, but more slowly than its littermate fed *ad lib.*; the food intake of 'restricted' mice was recorded, and was found to be between 77 and 89% of the amount of food eaten by their *ad lib.*-fed litter-mates. The plateau body-weight obtained was, as expected, less than that of *ad lib.*-fed mice reared at each temperature. However, as for *ad lib.*-fed mice, those on restricted intake reared in a warm environment had on average a higher percentage of the body-weight as fat,

Table 1. *Mean body-weight at slaughter, carcass fat and skin fat of young growing hairless mice reared at two environmental temperatures and two feeding levels*

(Mean values with their standard errors, no. of mice in parentheses)

Feeding level	Body-wt (g)	Fat (% body-wt)	Skin fat (% total fat)
Environmental temperature: 22°			
<i>Ad lib.</i>	26.2 ± 0.7 (13)	9.4 ± 0.6 (13)	25.9 ± 1.6 (13)
Restricted (77-89 % of <i>ad lib.</i> intake)	22.5 ± 0.4 (6)	6.9 ± 0.8 (NS) (6)	21.6 ± 1.9 (6)
Environmental temperature: 33°			
<i>Ad lib.</i>	25.9 ± 0.6 (11)	13.5 ± 1.2* (11)	26.1 ± 1.4 (11)
Restricted (77-89 % of <i>ad lib.</i> intake)	23.2 ± 0.8 (6)	12.6 ± 0.8** (6)	27.1 ± 1.8 (6)

NS, not significantly different from value for *ad lib.*-fed group.

Mean values significantly different from corresponding values at 22°: * $P = 0.02$, ** $0.001 > P > 0.01$.

Table 2. *Food intake, mean daily weight increase, calculated fat deposition and food conversion ratio (dry food intake:weight gain) of young growing hairless mice reared at two environmental temperatures and two feeding levels*

(Mean values with their standard errors for thirteen mice/group at 22° and eleven mice/group at 33°)

Feeding level	Food intake (g/d)	Wt gain (mg/d)	Food conversion ratio	Fat deposition (mg/d)
Environmental temperature: 22°				
<i>Ad lib.</i>	5.5 ± 0.15	403 ± 51	17 ± 3	48 ± 10
Restricted (77-89 % of <i>ad lib.</i> intake)	4.7 ± 0.21	246 ± 53	22 ± 5	24 ± 15
Environmental temperature: 33°				
<i>Ad lib.</i>	3.1 ± 0.07	394 ± 37	8 ± 0.7*	105 ± 24
Restricted (77-89 % of <i>ad lib.</i> intake)	2.7 ± 0.12	253 ± 44	12 ± 2 (NS)	65 ± 5

NS, not significantly different from value for mice reared at 22°.

* Mean value significantly different from corresponding value at 22°: $P = 0.02$.

and the subcutaneous fat constituted a slightly higher proportion of the total carcass fat, but the difference was not significant.

The ability of the mice to convert food into body tissue can be compared at the two temperatures. The lower the food conversion ratio (dry food intake: weight gain) the more efficient is the conversion of food to body tissue. The spontaneous food intake of the mice reared at 33° was just over half that of the mice reared at 22°. The gain in body-weight was similar at the two temperatures, so clearly the food conversion ratio at the lower temperature was about twice (i.e. half as efficient) that at the higher temperature. If the food intake was restricted so that the mice gained weight more slowly, the food conversion ratio was much more variable; although the mean value for the group reared in the warm environment was about twice that for the group

reared in the cool environment (Table 2), there was considerable variability within the group, and the difference was not statistically significant. Table 2 also shows the calculated increase in body fat during the experiment.

DISCUSSION

The results (Table 1) show that the total body fat of the hairless mice could be modified by altering the environmental temperature, though not by reducing food intake within the limits tolerated by the animals. The finding of the comparatively large amount of fat in the mice reared in the warm is emphasized by the results given in Table 2. For the mice at 33°, the calculated daily gain of fat is about one-quarter of the observed daily gain of body-weight, whereas for the mice at 22° the fat deposition is only about one-tenth of the weight gain.

Mammals and birds, the homeothermic vertebrates, are the only animals whose bodies contain appreciable fat stores (Vague & Fenasse, 1965). These stores provide a source of energy available in conditions of decreased energy intake, such as starvation, or of increased metabolic demand such as low environmental temperature. In certain mammals such as seals (Scholander, Hock, Walters & Irving, 1950) and man (Keatinge, 1960) and possibly in semi-aquatic birds such as ducks (Evans, 1972) the subcutaneous fat depot may have an additional biological function as an insulation against loss of body heat in the cold.

In one mammalian species, the pig, the total body fat and its relation to environmental temperature and food intake has been thoroughly studied (Holme & Coey, 1967; Fuller & Boyne, 1971; Holmes, 1971). Fuller & Boyne (1971) found little difference in total carcass fat between pigs reared at 5, 13 and 23°, when differences in food intake were considered. They concluded that while 'the slow growth achieved by reduction in food intake is associated with a decreased fatness, that produced by cold is not'. In relation to total body fat, an important difference between the metabolism of mice and that of pigs or ducks is due to their size. The *ad lib.* food intake of a growing mouse appears to be only about 1.25 times the maintenance intake required to keep it at a constant weight, whereas for a growing pig, the *ad lib.* intake, some four times the maintenance level, offers much more possibility of storage of energy as fat. As much as 42% of the carcass weight of an adult pig (above 90 kg carcass weight or 118 kg live body weight) may be fat (Cuthbertson & Pomeroy, 1962). Even in 90 kg pigs reared on restricted food intake in the cold, some 26% of the ingesta-free carcass weight was fat (Fuller & Boyne, 1971), a proportion greatly in excess of the mere 15% in the body of the fattest mice of these experiments, those fed *ad lib.* in the thermoneutral range. It is clear that the difference in size and consequential metabolic demands between small mammals such as mice and large ones such as pigs precludes similarity of proportion of total fat. Furthermore, this difference in metabolic demand would preclude the performance of 'pair-fed' experiments on mice at 33° and 22°; a mouse could not survive at 22° on the *ad lib.* food intake of a mouse at 33° which would be only half its own *ad lib.* intake (Table 2).

Whereas total body fat of the mice could be modified by environmental temperature, it is clear from the results (Table 1) that the partition of fat between subcutaneous and

deep body sites could not be so modified: in all the conditions of these experiments, between 22 and 26 of the total body fat was present in the subcutaneous fat depot. The amounts of fat in the subcutaneous site as compared with that elsewhere in the body has not been as fully investigated as has total body fat. A few observations have been made on the effects of undernutrition on the distribution of the body fat. In undernourished sheep, subcutaneous fat seems to be depleted more extensively than fat elsewhere in the body. This effect occurs both in pregnant ewes feeding during winter on the reduced herbage of hill pastures (Russel, Gunn & Doney, 1968), and also in experimentally undernourished 1-year-old wethers made to lose 25% of their live body-weight (Little & Sandland, 1975). In 10-week-old ducks, on the other hand, in which between 50 and 60% of the total fat was subcutaneous, fat was mobilized more or less uniformly from all depots during fasting (Evans, 1972). However, the distribution of fat during the nutritionally contrived loss of weight of an adult animal may be quite different from that achieved by the nutritionally contrived retardation of growth of a young growing animal, such as the mice described in this paper. As to the effect of temperature on fat distribution, relevant work in any species is scanty. D. L. Ingram (personal communication) and Weaver & Ingram (1969) found that the back-fat depth of a pig reared at 35° was greater than that of a litter-mate reared to the same weight at 5°. Holmes (1971) reported that pigs reared in a hot environment (33°) had a greater back-fat depth than those reared at 22–24°, and raised the question of a possible redistribution of fat within the body in consequence of the temperature difference. Although in the mice described in this work the effect of ambient temperature on fat distribution was trivial, an experiment on a larger species such as the pig, in which subcutaneous fat may have an insulating as well as a storage role, might give a different outcome.

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