The role of thermoregulatory thermogenesis in the development of obesity in genetically-obese (*ob/ob*) mice pair-fed with lean siblings

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1. In order to quantitatively assess the energetic significance of reduced thermoregulatory thermogenesis in the accumulation of excess body fat in genetically-obese (*ob/ob*) mice experiments have been conducted at four environmental temperatures (17, 23, 28 and 33°) in which young growing obese animals were pair-fed for 10 d to the *ad lib.* intake of lean siblings.

2. The food intake of the lean mice increased with decreasing environmental temperature: at 17° the intake was 86% higher than that at 33°.

3. The estimated gain in carcass energy of lean mice rose with increasing temperature, from 82 kJ at 17° to 150 kJ at 33°. The energy gain of the pair-fed obese mice was higher than that of the lean at all temperatures but showed a slight decrease with increasing temperature, from 231 kJ at 17° to 191 kJ at 33°.

4. Environmental temperature affected the 'excess' energy gain of the obese mice. At 17° the obese mice deposited 182% more energy than the lean but this difference decreased progressively with increasing temperature to 127, 62 and 27% more energy at 23, 28 and 33° respectively.

5. At all environmental temperatures the pair-fed obese mice deposited considerably less protein than their lean controls. The deposition ranged from 32% (at 17°) to 56% (at 28°) of that of the lean mice.

6. It is concluded that environmental temperature plays a major role in determining the excess energy gain of *ob/ob* mice receiving the same amount of food as lean controls, and that the low energy expenditure and consequent high metabolic efficiency of this mutant is due primarily to reduced thermoregulatory thermogenesis.

The genetically-obese (*ob/ob*) mouse has been widely investigated in order to gain insight into the causes of obesity in man. Obesity in this mutant is only partly caused by an excessive consumption of food, since if the energy intake of *ob/ob* mice is reduced to below that of lean mice, or normalized by pair-feeding to the *ad lib.* intake of lean siblings, the deposition of energy is still abnormally high (Alonso & Maren, 1955; Chlouverakis, 1970; Welton *et al.* 1973; Dubuc, 1976; Woodward *et al.* 1977). In order to account for an excess energy gain on a normal food intake one or more components of the energy expenditure of the obese mouse must be reduced. At thermoneutrality (33°) the resting metabolic rate of adult *ob/ob* and lean mice, when expressed in absolute terms (ml oxygen/h per animal), is the same, whereas at lower temperatures the metabolic rate of the obese is 20% below that of the lean (Trayhurn & James, 1978). This indicates that at normal environmental temperatures (20–25°) *ob/ob* mice expend less energy than lean animals on the thermoregulatory thermogenesis needed to maintain body temperature. A reduction in thermoregulatory thermogenesis, which is a major part of the total energy expenditure of mice maintained at 20–25°, may therefore be the primary reason for the low energy expenditure of *ob/ob* mice.

In order to obtain further evidence for this view, and to assess its quantitative significance, we have conducted a series of energy-balance experiments. These are not subject to the major criticism of the metabolic rate measurements, namely that short-term studies (< 1 h) of restrained animals do not necessarily accurately reflect 24 h energy expenditure. The

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experiments have been designed to measure the 'excess' energy retention of obese animals pair-fed to the *ad lib.* food intake of lean siblings, both at temperatures where substantial thermoregulatory thermogenesis is required and at thermoneutrality, where there is no demand for thermoregulatory heat.

Part of this study has been presented elsewhere in preliminary form (Thurlby *et al.* 1978).

EXPERIMENTAL

Animals

The animals used in these experiments were from the colony of *ob*/*ob* mice established in our laboratory in 1974 and were all males. They were derived from the 'Aston' strain where C57 BL/6J mice were originally out-crossed in order to transfer the '*ob*' gene to strains with a high growth rate and large litter size.

Breeding was carried out between animals which were heterozygous for the '*ob*' gene. Litters were raised in a room at 23°, and weaned when aged 21 d.

Pair-feeding

The animals were selected at an age of 23–26 d, when their body-weight was between 13 and 20 g, and the obese animals were just identifiable visually. The genotype of the lean mice was unknown (*ob*/+ or +/+). Each lean mouse was selected so that its initial weight was as close as possible to that of its obese litter-mate. The mice were caged separately in wire-mesh cages suspended 10 mm above absorbent paper so that food spillage could be collected and measured. The cages were placed in a ventilated, temperature-controlled (±1°) cabinet of volume 780 l. The cabinets were maintained with a 12 h light–12 h dark cycle, the light period beginning at 06.00 hours.

The lean mice were allowed to feed *ad lib.* The diet used was Spiller's–Spratt's Rodent Breeding Diet No. 1 (Spratt's Patent Ltd, Barking, Essex) which contains (g/kg): protein 213, fat 34, and has an energy density of 17·4 kJ/g. The food intake of the lean mice was determined each day, and this quantity of food was then given to the obese litter-mate for the succeeding day. Faeces were collected in order to determine the digestible energy (DE) intake. In an effort to minimize any effects of differences in meal pattern between the lean and obese animals, the latter were given their food in two 'meals' each day. The first meal, which amounted to one-third of the total daily ration, was given between 09·00 and 10·00 hours, and the remaining two-thirds was given at 18.00 hours. Each mouse was weighed daily between 09.00 and 10.00 hours.

Pair-feeding studies were conducted at four environmental temperatures; 17, 23, 28 and 33°. The obese mice, when allowed free access to food at 17°, were found to eat approximately the same quantity as lean mice. The 'pair-fed' group for this temperature was therefore taken from a large group of animals fed *ad lib.*, but selecting only those pairs where the difference in food intake was small. The pair-feeding was conducted for 10 d at each temperature. This period was sufficient to allow an increase in the weight of the lean mice of at least two-thirds. A longer period was not used so that any secondary effects of obesity itself, such as marked hyperinsulinaemia, would be minimized.

In order to estimate the energy gained during the 10 d study the carcasses of ten lean and ten obese male mice, aged 23–26 d, were analysed for nitrogen and total energy in the same way as for the experimental group (see p. 379). Regression equations were then obtained for both nitrogen and total energy *v.* body-weight, and they were used to predict the initial composition of the pair-fed animals at their starting weight.

Carcass analysis

Mice were killed by inhalation of diethyl ether vapour and then stored at −20° until required for analysis.

The gut contents were removed and the carcasses cut into small pieces with scissors before being autoclaved at 10^4 kg/m^2 for 30 min (Lofti *et al.* 1976). The carcasses were then homogenized in 50 ml water with a high-speed mixer (Silverson Machines Ltd., Chesham, Bucks). The homogenates were freeze-dried to constant weight and stored in a desiccator to await further analysis.

The gross energy content of the carcasses and faeces was measured with a Gallenkamp Adiabatic Calorimeter (Model CB-100), which had been calibrated using dry benzoic acid standards (Gallenkamp and Co. Ltd, London). The N content was determined by a micro-Kjeldahl method. Nitrogenous compounds were converted to ammonium sulphate by boiling 500 mg samples of the dried carcasses with 10 ml concentrated sulphuric acid in the presence of 4 ml hydrogen peroxide (300 g/l) as an oxidizing agent and with selenium (5 g/l sulphuric acid) as a catalyst. Digestion was complete after 2 h at 370 °. The ammonium sulphate was determined colorimetrically with a Technicon AutoAnalyzer (Weber, 1973). Protein content was then calculated as $N \times 6{\cdot}25$.

Statistical analyses

The statistical significance of differences between lean and obese animals was assessed by Student's paired *t* test.

RESULTS

Initial composition

The energy and protein contents of both the lean and the obese mice, at 23–26 d of age, were found to be closely correlated with body-weight. The regression equations and the correlation coefficients were:

energy content of lean (kJ) = $9{\cdot}5\ W - 26{\cdot}7$ (r 0·913),
energy content of obese (kJ) = $15{\cdot}8\ W - 76{\cdot}5$ (r 0·924),
protein content of lean (mg) = $139\ W + 186$ (r 0·992),
protein content of obese (mg) = $111\ W + 319$ (r 0·959),
where W is body-weight (g).

The residual standard deviation about the regression line for carcass energy was ±10·9 kJ for the lean and ±17·3 kJ for the obese. Similarly, for the protein content the residual deviation was ±47 mg protein for the lean and ±87 mg protein for the obese. These errors are small in relation to the gain of these two components seen during 10 d of growth.

Food intake and weight changes

The DE intake of the lean mice fed *ad lib.* over the 10 d growth period is shown in Table 1. The intake was found to be lowest at thermoneutrality (33°) and to increase progressively as the environmental temperature decreased. At 17° the energy intake was 86% higher than at 33°. The digestibility of the diet was found to be the same for lean and obese mice at all temperatures. The obese animals were successfully pair-fed to the *ad lib.* intake of their lean litter-mates; at each temperature the total energy intake of the two groups differed by no more than 0·8%. On pair-feeding, the obese mice gained significantly less weight than the lean mice at all temperatures (Table 1).

Table 1. *The deposition of energy and protein in* ob/ob *mice pair-fed to the* ad-lib. *food intake of lean siblings at different environmental temperatures*

(The results are mean values with their standard errors for eight lean and eight obese mice at each temperature)

Temperature (°) . . .		17		23		28		33	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Food intake: DE (kJ)									
Lean		929	24	710	20	645	21	500	15
Obese		928	24	707	20	643	17	496	15
	P	NS		NS		NS		NS	
Initial wt (g)									
Lean		16·7	0·6	15·7	0·9	14·5	0·5	14·7	0·6
Obese		18·1	0·3	16·3	1·0	15·4	0·5	15·2	0·7
	P	< 0·02		NS		NS		NS	
Final wt (g)									
Lean		27·7	0·8	26·8	1·1	28·9	0·5	27·8	0·6
Obese		27·1	1·0	24·8	0·9	25·6	0·5	25·0	0·6
	P	NS		< 0·01		< 0·001		< 0·001	
Gain in wt (g)									
Lean		11·0	0·8	11·1	0·8	14·5	0·4	13·1	0·7
Obese		9·0	0·9	8·4	0·5	10·2	0·5	9·9	0·7
	P	< 0·02		< 0·01		< 0·001		< 0·001	
Carcass protein (g)*:									
Initial									
Lean		2·51	0·09	2·37	0·13	2·19	0·07	2·23	0·09
Obese		2·33	0·04	2·14	0·11	2·03	0·06	2·01	0·08
	P	< 0·05		< 0·05		NS		< 0·001	
Final									
Lean		4·65	0·14	4·44	0·17	4·66	0·10	4·17	0·11
Obese		3·02	0·13	3·04	0·11	3·41	0·09	3·04	0·11
	P	< 0·001		< 0·001		< 0·001		< 0·001	
Gain									
Lean		2·14	0·12	2·07	0·11	2·47	0·07	1·94	0·11
Obese		0·69	0·12	0·90	0·08	1·38	0·07	1·03	0·09
	P	< 0·001		< 0·001		< 0·001		< 0·001	
Carcass energy (kJ)*:									
Initial									
Lean		131	6	122	9	110	5	112	6
Obese		209	5	181	15	166	8	163	10
	P	< 0·001		< 0·001		< 0·001		< 0·001	
Final									
Lean		213	9	214	11	238	8	262	10
Obese		440	18	390	17	375	10	354	14
	P	< 0·001		< 0·001		< 0·001		< 0·001	
Gain									
Lean		82	8	92	9	129	6	150	8
Obese		231	16	209	6	209	10	191	12
	P	< 0·001		< 0·001		< 0·001		< 0·001	
Energy density of gain (kJ/g)									
Lean		7·5	0·6	8·3	0·5	8·9	0·3	11·5	0·6
Obese		26·0	0·8	25·3	1·4	20·6	0·8	19·6	0·8
	P	< 0·001		< 0·001		< 0·001		< 0·001	

NS, not significant ($P > 0·05$).
DE, digestible energy.
* Estimated from initial body-weight.

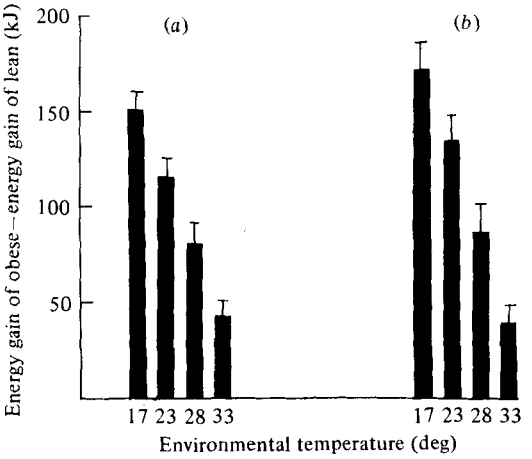

Fig. 1. The effect of environmental temperature (°) on the 'excess' energy gain (kJ) of young *ob/ob* mice pair-fed to the *ad-lib.* food intake of lean siblings. (*a*), Observed; (*b*), adjusted for the presumed heat lost in growth. The results are mean values with their standard errors, represented by vertical bars, for eight mice at each temperature.

Protein deposition

The initial content of protein was lower for the obese animals than the lean animals (Table 1). The protein deposited by the obese during the 10 d growth period was considerably less than that of the lean at all four temperatures, ranging from 32% of the lean at 17° to 56% at 28°. At 28° the protein deposition of both the lean and the obese animals was at a maximum.

Energy gain

The estimated carcass energy of the obese mice at the beginning of the study was between 46 and 60% greater than that of the lean (Table 1). At each temperature the gain in energy of the obese was considerably greater, despite their lower weight gain. The lean mice accumulated more energy as the environmental temperature increased; at 33° it was 86% greater than at 17°. The obese mice, however, showed little change in energy gain with temperature, although there was a trend for them to deposit more energy as the temperature decreased; the energy deposited by the obese at 17° was 21% greater than that at 33°.

The 'differences in energy gain' between the lean and pair-fed obese animals reflect the low energy expenditure and consequent high metabolic efficiency of the obese mutant. These differences were highly dependent on the environmental temperature. At 17° the obese mice deposited 182% more energy than the lean, but the difference declined as the temperature rose, so that at 23° it was 127%, at 28° 62%, and at thermoneutrality the difference had fallen to only 27%. Fig 1 (*a*) shows the relationship between environmental temperature and the absolute 'excess' energy gain of the obese mice, i.e. the energy gain of obese minus the energy gain of the lean. As the temperature increased the excess energy gain of the obese animals fell from a value of 149 kJ at 17° to 41 kJ at 33°. Thus 73% of the excess gain seen at 17° was removed when the experiment was conducted at thermoneutrality. The energy density of the weight gain of the obese mice was always greater than that of the lean. It increased at higher temperatures in the lean mice but decreased in the obese. The theoretical limits for energy gain are approximately 5 kJ/g for a gain consisting entirely of well-hydrated lean tissue and 33 kJ/g when the gain is pure adipose tissue consisting (g/kg) of 150 water and less than 30 protein.

DISCUSSION

Various studies have indicated that the obesity of *ob/ob* mice develops or is maintained on a normal, or even a much reduced, intake of food (Alonso & Maren, 1955; Chlouverakis, 1970; Welton *et al.* 1973; Dubuc, 1976). Energy balance experiments have recently been conducted to quantify the elevated metabolic efficiency of *ob/ob* mice (Woodward *et al.* 1977). In these experiments obese mice aged 4 weeks were pair-fed to the *ad lib.* intake of lean litter-mates for a period of 6 weeks. The gross efficiency (energy deposition ÷ energy intake) was found to be considerably higher in the obese mice (0·317) than in the lean (0·138). This high efficiency has been subsequently shown to result from a decrease in maintenance requirements (Woodward, 1978).

Clearly some component(s) of the energy expenditure of obese mice is reduced in order to account for the excess accumulation of energy seen with a normal intake of food. The resting metabolic rate of adult *ob/ob* mice is lower than that of lean litter-mates when measurements are made at temperatures below the thermoneutral zone, and this results from a specific decrease in thermoregulatory thermogenesis (Trayhurn & James, 1978). At temperatures normally used to house laboratory mice the reduction in the resting metabolic rate was found to be approximately 20%. However, these short-term (30–60 min) O_2 consumption measurements may not necessarily reflect 24 h energy expenditure and the present study was therefore undertaken to establish the quantitative role of reduced thermoregulatory thermogenesis in the observed differences in metabolic efficiency between lean and obese mice.

Metabolic efficiency was determined by measuring the gain in carcass energy of obese mice pair-fed to the *ad lib.* intake of lean siblings. This was carried out at four environmental temperatures, the lowest of which was 17°. The two intermediate temperatures, 23 and 28°, are typical of those used for housing laboratory mice and it is at these temperatures that the metabolic efficiency differences have previously been found. The highest temperature, 33°, was chosen since it falls within the thermoneutral zone of these mice (Trayhurn & James, 1978). An important feature of the design of these experiments was the use of mice which were as young as possible. This was achieved by taking animals a few days after weaning, and using a short growth period of 10 d. This procedure was employed to minimize one of the major problems in the study of obesity: the separation of primary factors from the many secondary abnormalities which develop in older animals once obesity is established.

The results obtained demonstrate that the excess energy gain of the growing *ob/ob* mouse was dependent on environmental temperature. At 'normal' temperatures the excess gain was large, at 116 and 80 kJ for 23 and 28° respectively. At 17°, when lean mice are expending a considerable amount of energy on thermoregulatory thermogenesis, the obese mice showed an even greater ability to be more efficient than the lean. At this temperature the excess energy gain was 149 kJ. Conversely, at thermoneutrality where there is no energy expenditure on thermoregulatory thermogenesis the excess gain of the obese was low at only 42 kJ.

The net deposition of body protein was also measured in the present experiments, and this allows the separation of the total energy gain into two components: energy gain as protein and (by difference) the energy gain as non-protein matter, which may be taken to represent the gain in fat. These values are shown in Table 2. At all temperatures the obese mice deposited more energy as fat and less energy as protein when compared with lean controls. In addition, the relative deposition of energy and protein varied considerably even within phenotype at the different temperatures. This was especially true for the lean mice, where the protein energy gain as a percentage of the total energy gain ranged from 59% (at 17°) to 29% (at 33°). For the obese animals the range was from 7% (at 17°) to 15% (at 28°). The

Table 2. *The energy cost of growth of lean and obese mice pair-fed for* 10 *d at different environmental temperatures*

(The results are mean values with their standard errors for eight mice per group)

Temperature (°) . . .	17		23		28		33	
	Mean	SE	Mean	SE	Mean	SE	Maen	SE
Gain in energy as: Protein (kJ)								
Lean	48	3	47	3	55	2	43	3
Obese	16	3	20	2	31	1	23	3
Fat (kJ)								
Lean	34	7	46	9	73	6	106	6
Obese	215	14	188	5	178	10	168	12
Heat lost in: Protein deposition (kJ)*								
Lean	60	3	58	3	69	2	54	3
Obese	19	3	25	2	39	2	29	3
Fat deposition (kJ)†								
Lean	13	2	17	3	26	2	38	2
Obese	78	5	68	2	64	4	60	4
Total heat lost in growth (kJ)								
Lean	73	4	75	5	96	3	92	5
Obese	97	8	93	3	103	4	89	5
Contribution to excess energy gain of the obese (kJ)	−24	4	−18	5	−7	4	3	3
Excess energy gain of the obese (kJ)	149	11	116	11	80	12	42	8
Differences in components of energy expenditure other than the heat lost in growth (kJ)	173	15	134	15	87	16	39	10

* 1·25 × kJ protein deposited. † 0·36 × kJ fat deposited.

absolute gain in energy as fat was found to be much higher in lean animals when they were kept at thermoneutrality than at normal temperatures. The same effect has been reported by Stanier (1977) for hairless mice.

When the food intake of *ob/ob* mice is restricted to below their normally elevated *ad lib.* level they show an impairment in the growth of lean body mass and protein (Alonso & Maren, 1955; Dubuc, 1976). This was also found in the present experiments, where the restriction of intake to that of lean litter-mates was associated with low protein deposition. Groups of similar obese mice allowed to feed *ad lib.* deposit considerably more protein than these pair-fed groups, although it is always less than that of lean controls (P. L. Thurlby, unpublished results).

One factor which influences the amount of energy available for gain is the amount that is expended on the processes involved in growth. The two energetically-important components of growth are protein and fat deposition. However, the energy expenditure associated with the deposition of energy in these two forms is dissimilar. For each 1 kJ protein deposited 1·25 kJ are lost as heat whereas for each 1 kJ of fat deposited only 0·36 kJ are lost (Pullar & Webster, 1977). It follows that any difference in the relative proportion of protein and fat comprising carcass energy gain will alter the over-all energy cost of growth. Since the ratio, protein:fat deposition is much lower in *ob/ob* mice than in lean mice, and since environmental temperature influences this value in both phenotypes it is important to calculate the energy cost of growth for each experimental group to determine to what extent these differences account for the observed differences in metabolic efficiency. The calculations are shown in Table 2, and assume that the energy cost of fat and protein deposition is the same for lean and obese mice. This assumption has been made previously by Pullar & Webster

(1977) for Zucker or fatty rats. Although the protein deposition is lower in the obese mice the calculated energy loss resulting from the higher fat deposition more than compensates for this. The total energy expenditure associated with growth is higher in the obese than in the lean at every temperature except 33°, where there is little difference. The obese mice are therefore not more efficient because they use less energy for growth. Indeed, the greater cost of growth in the obese implies that the other components of their energy expenditure must be even less than that suggested by the energy gain on pair-feeding (Table 2 and Fig. 1 *b*).

At thermoneutrality the obese still gain more energy than the lean. Several components of energy expenditure may be reduced to account for this. First, the basal metabolic rate (BMR) may be lower since the pair-fed obese mice are both smaller than the lean and show a deficit in protein content (and therefore lean body mass). BMR and lean body mass are closely correlated, at least in man (see Garrow, 1978) and this appears to hold even in obesity (James *et al.* 1978). Secondly, dietary-induced thermogenesis may be lower in the obese mice because of altered meal patterns. Meal patterns are known to affect metabolic efficiency with 'meal feeding' being more efficient than 'nibbling' (Fabry, 1969). In the present experiments a feeding schedule was employed which may only have partially eliminated the opportunity of the obese mice to indulge in meal feeding. Thirdly, physical activity may also have been lower in the obese mice. Morrison (1968) found that for rats kept at thermoneutrality approximately 25% of the total energy expenditure was related to exercise. In the present experiments the total energy expenditure was approximately 350 kJ (DE intake—energy gain) for the lean. If the physical activity of the obese was half that of the lean then this could provide a sizeable contribution to the residual gain.

In conclusion we would suggest that the results presented in this paper together with the earlier metabolic rate measurements (Trayhurn & James, 1978) leave little doubt as to the primary cause of the elevated metabolic efficiency of *ob/ob* mice. At temperatures below thermoneutrality the amount of energy expended on thermoregulatory thermogenesis is less in the obese animals than in the lean, and this allows an excess of carcass energy to be deposited even on a normal intake of food. Changes in the energy cost of growth associated with an altered fat:protein deposition do not contribute to the elevated efficiency. At thermoneutrality the *ob/ob* mice are still slightly more efficient than the lean and reductions in the BMR, dietary-induced thermogenesis and activity may all be responsible. Nevertheless, the major factor accounting for the high metabolic efficiency of the obese mouse is the subnormal rate of thermoregulatory thermogenesis.

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