The Development of Obesity in Genetically Diabetic-Obese (db/db) Mice Pair-fed with Lean Siblings

The Importance of Thermoregulatory Thermogenesis

P. Trayhurn and L. Fuller

Dunn Nutrition Laboratory, University of Cambridge and Medical Research Council, Cambridge, England

Summary. The importance of reduced thermoregulatory thermogenesis as a mechanism for the high metabolic efficiency of the diabetic-obese (db/db) mouse has been investigated. Young db/db mutants were pair-fed to the ad libitum food intake of lean siblings for two weeks at two different environmental temperatures, 23 and 33 °C. At 23 °C, a temperature at which there is a substantial demand for thermoregulatory thermogenesis, the diabetic-obese animals deposited 51% more total energy and 75% more fat than the lean mice. At 33 °C (thermoneutrality) where there is no requirement for thermoregulatory heat, the mutants deposited 25% more fat than lean animals, but there was no significant difference in the total energy gain of the two groups. Pair-feeding resulted in a reduced protein deposition at both temperatures in the diabetic-obese animals compared to the lean. It is concluded that the high metabolic efficiency of the diabetic-obese mutant, like that of the obese (ob/ob) mouse, is caused by a low energy expenditure on thermoregulatory thermogenesis.

Key words: Obesity, diabetes, mouse, thermoregulation, thermogenesis, pair-feeding, energy, fat, protein.

Since its discovery some 15 years ago [13] the diabetic-obese (db/db) mouse has been increasingly used in experimental studies on diabetes and obesity [2, 4, 11]. These disorders in this animal result from a mutation which is recessively inherited. Hyperinsulinaemia is detectable in db/db mice from 10 days of age [6] and an increased body fat content has been reported at 12 days [14]. Obesity as such is not apparent visually, however, until the animals are aged approximately 4 weeks. Recent work has indicated that obesity in the db/db mouse is the result of two

distinct factors – hyperphagia and an elevated metabolic efficiency [4, 7]. The high efficiency has been demonstrated in studies where young diabetic-obese mice were pair-fed to the ad libitum food intake of lean siblings [4, 7]. The presence of both an elevated efficiency and hyperphagia in obesity has also been recorded in other commonly studied obese mutants, such as the obese (ob/ob) mouse [1, 3, 9, 18] and the fatty (fa/fa) rat [24].

In the present communication we report the results of pair-feeding experiments on the db/db mouse which were conducted at two different environmental temperatures; 23 °C, a temperature where there is a substantial demand for thermoregulatory thermogenesis and 33 °C (thermoneutrality) where there is no requirement for thermoregulatory heat. The aim of the study was to determine whether the low energy expenditure of the db/db mouse, which is the underlying reason for its high efficiency, is due to reduced thermoregulatory thermogenesis. This is the explanation for the high efficiency of the ob/ob mouse [18, 21, 22] and measurements of body temperature [20, 23], and metabolic rate [20] have suggested that a similar mechanism may also operate in the db/db mutant. The diabetic mouse maintains a low body temperature, and its resting metabolic rate (per animal) at temperatures below the thermoneutral zone is lower than in normal mice, while at thermoneutrality there is no difference [20].

Materials and Methods

Animals

The mice used in these experiments were females from a colony established in our laboratory from breeding stock obtained in 1977 from the Jackson Laboratory, Bar Harbor, Maine, USA. The db gene was on a C57BL/ks background in repulsion to the misty coat gene (m). Animals homozygous for diabetes-obesity were obese

and black, heterozygotes were thin and black, while homozygous normal animals were thin and grey.

The db/db mice were bred from heterozygotes, and were weaned at 21 days of age. The colony was housed in a temperature controlled room ($23 \pm 1^\circ\text{C}$), with a 12 h light/12 h dark cycle, the light period operating from 0800–2000 h.

Pair-feeding

Mice were selected at an age of 3.5 weeks. At this time the obesity of the mutants was just detectable visually, and the body weight of both the mutants and the controls was between 8.0 and 10.5 g. The control lean mice were heterozygotes, and they were selected so that their weight was as close as possible to that of their diabetic-obese littermate. Each mouse was housed individually in a metal cage with a wiremesh floor. The cages were suspended 1 cm above absorbent paper so that food spillage could be readily collected and measured. The cages were placed in a ventilated, temperature-controlled cabinet of volume 780 l. The cabinets were maintained at either 23 or 33 °C. A 12 h light: 12 h dark cycle was operated in the cabinets in synchrony with the cycle of the main colony.

The lean mice were allowed to feed ad libitum, the diet used being Spillers-Spratts Rodent Breeding Diet 1 (Spratts Patent, Barking, Essex, U. K.). This diet contains 21.3% protein and 3.4% fat and 17.4 kJ/g. Tap water was freely available to all animals. The food intake of each lean mouse was determined daily between 0900 and 1030 h, with a correction being made for spillage. This quantity of food was then given on the following day to the diabetic-obese partner.

In an attempt to reduce any effects of 'meal-feeding' the daily ration for the db/db animals was given in two meals (see Discussion). The first meal was presented at approximately 1000 h, and this amounted to one-third of the total ration. The remaining two-thirds was given at approximately 1800 h. Faeces were collected throughout the experimental period so that the digestible energy intake could be determined.

Pair-feeding was conducted for 14 days, a period which allowed an almost two-fold increase in the weight of the lean animals. A longer period was not used because of the need to minimise any secondary effects of the diabetic-obese state.

Carcass Analysis

Mice were killed by inhalation of diethyl ether, and stored at -20°C until required for analysis.

The gut contents were removed from the carcasses which were then cut into small pieces and autoclaved at a pressure of 10^4 kg/m^2 for 30 min [15]. The softened carcasses were homogenised in a small volume of water using a highspeed mixer (Silverson Machines, Chesham, Bucks, U. K.), and freeze-dried to constant weight.

The gross energy of the dried carcasses and the faeces was determined using a Gallenkamp Adiabatic Calorimeter (Model CB-100) which had been calibrated with dry benzoic acid (Gallenkamp, London, U. K.). Carcass nitrogen content was determined by a micro-Kjeldahl procedure using a Technicon Auto-Analyzer [18], and the protein content was derived by multiplying the nitrogen values $\times 6.25$. The accuracy of the nitrogen determination was approximately 1%. Body fat was not measured in this study since with the smaller animals there was insufficient material remaining after the energy and nitrogen determinations. An estimate of fat content was made, however, by subtracting the energy as protein from the total carcass energy – assuming that this represented body fat. Values of 22.4 kJ/g and 39.2 kJ/g were taken for the energy content of protein and fat, respectively [8].

Carcass analysis was also performed on a group of 10 lean and 10 diabetic-obese mice of the same weight as the initial weight of the pair-fed animals.

Statistical Analysis

The statistical significance of differences between groups was assessed using Student's unpaired 't' test.

Results

Initial Body Composition

In order to determine the effects of pair-feeding on the body composition of the diabetic-obese mice it is necessary to have an estimate of their 'initial' composition. This was obtained by taking a reference group of lean and diabetic-obese animals which were similar in age and weight range to the experimental group at the beginning of the pair-feeding period. From the body composition of the reference animals a series of regression equations were obtained for each genotype of total energy, protein and estimated fat against weight. The equations, and the correlation coefficients observed for each of these parameters were:

$$\begin{aligned} \text{energy content of lean (kJ)} &= 12.3 W - 53.0 \quad (r = 0.859). \\ \text{energy content of diabetic-obese (kJ)} &= 19.4 W - 69.9 \quad (r = 0.904). \\ \text{protein content of lean (g)} &= 0.177 W - 0.226 \quad (r = 0.932). \\ \text{protein content of diabetic-obese (g)} &= 0.058 W + 0.805 \quad (r = 0.691). \\ \text{fat content of lean (g)} &= 0.213 W - 1.232 \quad (r = 0.743). \\ \text{fat content of diabetic-obese (g)} &= 0.463 W - 2.250 \quad (r = 0.874). \end{aligned}$$

Pair-feeding at 23 °C

The results of the pair-feeding experiment conducted at an environmental temperature of 23 °C are shown in Table 1. The starting weight of the lean and diabetic-obese animals was similar. There was no significant difference in the weight gain during pair-feeding, both groups showing an almost two-fold increase. The gross energy intake of the lean and diabetic-obese animals was the same, as was the digestibility of the dietary energy and therefore the digestible energy intake.

The initial energy content of the diabetic-obese mice was 75% higher than that of the lean. During pair-feeding the diabetic-obese gained 50% more energy than the lean, and their gross efficiency was elevated by a similar amount. The energy density of the weight gain was considerably lower in the lean animals than in the diabetic-obese. The mutant animals had an estimated initial fat content which was

Table 1. Weight, energy, protein and fat gains of diabetic-obese mice, pair-fed to the ad libitum food intake of lean siblings at 23 °C

	Lean	Diabetic-obese	Diabetic-obese lean
Initial wt (g)	9.85 ± 0.51	9.76 ± 0.43	0.991
Final wt (g)	18.55 ± 0.39	17.57 ± 0.39	0.947
Weight gain (g)	8.67 ± 0.37	7.82 ± 0.56	0.902
Food intake (kJ)	834 ± 15	827 ± 16	0.992
Digestible energy intake (kJ)	703 ± 13	697 ± 13	0.991
Initial energy content (kJ)	68 ± 6	119 ± 8 ^a	1.757
Final energy content (kJ)	182 ± 7	291 ± 7 ^a	1.601
Energy gain (kJ)	114 ± 7	171 ± 8 ^a	1.507
Energy density of gain (kJ/g)	13.1 ± 0.4	22.2 ± 0.7 ^a	1.695
Initial protein (g)	1.51 ± 0.06	1.36 ± 0.03	0.901
Final protein (g)	2.68 ± 0.06	1.93 ± 0.02 ^a	0.720
Protein gain (g)	1.16 ± 0.06	0.57 ± 0.03 ^a	0.491
Initial fat (g)	0.87 ± 0.11	2.39 ± 0.16 ^a	2.747
Final fat (g)	3.11 ± 0.17	6.31 ± 0.17 ^a	2.029
Fat gain (g)	2.24 ± 0.17	3.92 ± 0.22 ^a	1.750
Gross efficiency (%) ^b	16.2 ± 0.9	24.5 ± 1.1 ^a	1.512

The results are mean values ± SEM for 8 lean and 8 diabetic-obese animals. ^ap < 0.001

The initial energy, protein and fat contents were estimated from the analyses obtained on the reference group of animals

^bGross efficiency = energy gain divided by digestible energy intake

Table 2. Weight, energy, protein and fat gains of diabetic-obese mice pair-fed to the ad libitum food intake of lean siblings at 33 °C

	Lean	Diabetic-obese	Diabetic-obese lean
Initial wt (g)	8.93 ± 0.27	9.01 ± 0.40	1.009
Final wt (g)	16.34 ± 0.49	15.32 ± 0.63 ^b	0.938
Weight gain (g)	7.41 ± 0.33	6.31 ± 0.44 ^b	0.852
Food intake (kJ)	480 ± 19	479 ± 18	0.998
Digestible energy intake (kJ)	408 ± 18	404 ± 15	0.990
Initial energy content (kJ)	57 ± 3	105 ± 8 ^c	1.855
Final energy content (kJ)	170 ± 8	231 ± 11 ^c	1.359
Energy gain (kJ)	114 ± 6	127 ± 7	1.113
Energy density of gain (kJ/g)	15.3 ± 0.3	20.2 ± 0.6 ^c	1.320
Initial protein (g)	1.35 ± 0.06	1.33 ± 0.03	0.985
Final protein (g)	2.24 ± 0.06	1.76 ± 0.06 ^c	0.786
Protein gain (g)	0.89 ± 0.03	0.43 ± 0.03 ^c	0.483
Initial fat (g)	0.67 ± 0.06	1.92 ± 0.19 ^c	2.866
Final fat (g)	3.07 ± 0.17	4.90 ± 0.27 ^c	1.596
Fat gain (g)	2.40 ± 0.15	2.99 ± 0.20 ^c	1.246
Gross efficiency (%) ^d	27.9 ± 1.0	31.4 ± 1.6 ^a	1.125

The results are mean values ± SEM for 8 lean and 8 diabetic-obese animals. ^ap < 0.05, ^bp < 0.01, ^cp < 0.001

The initial energy, protein and fat contents were estimated from the analyses obtained on the reference group of animals

^dGross efficiency = energy gain divided by digestible energy intake

nearly three times greater than lean mice, and during the experimental period their gain in fat was 75% higher than in the lean.

The initial body protein content was slightly, but not significantly, higher in the lean animals than the mutants, but pair-feeding greatly inhibited protein deposition in the latter.

Pair-feeding at 33 °C

The results obtained when pair-feeding was conducted at 33 °C are shown in Table 2. The quantitative differences seen between lean and diabetic-obese animals in the initial energy, protein and fat contents were similar to those observed in the experiment per-

formed at 23 °C. Successful pair-feeding was again achieved. The gross energy intake and the digestible energy intake were the same in the two groups. The digestibility of the diet was the same at 33 °C as at 23 °C. Environmental temperature did affect the gross energy intake, however, for at 33 °C it was only 58% of that observed at 23 °C.

Pair-feeding at 33 °C resulted in a higher fat gain (25%) in the diabetic-obese mice than in the lean, but the gain in protein was lower by one-half. The net effect of these changes was that overall the total energy gain of the diabetic-obese mutants was not significantly different from that of the normal animals.

Discussion

The pair-feeding experiment conducted at 23 °C – a normal temperature for housing mice – clearly shows that the diabetic-obese (db/db) mutant is more efficient than its lean siblings in that a greater proportion of dietary energy is retained (Table 1). This higher efficiency reflects the greater deposition of body fat in the diabetic-obese animal. The conclusion that obesity can develop in the db/db mouse in the absence of hyperphagia is in agreement with previous results [4, 7].

In the present experiments, unlike the previous studies, the total energy gain of the lean and diabetic-obese animals was measured directly. At 23 °C the mutants deposited 51% more energy than the normal mice. This is much lower than the 320–430% increase which can be calculated from the experiments of Cox and Powley [7] on the db/db mutant (assuming energy values of 39.2 kJ/g for fat and 20.4 kJ/g for 'fat-free solids'). The environmental temperature in the two studies was the same, but there are three other possible explanations for the different findings. In the present experiments pair-feeding was conducted for two weeks during the rapid phase of growth, whereas Cox and Powley [7] used a period of six weeks, and it is evident from the growth curves which they presented that during the second half of the experiment the lean controls were effectively at weight maintenance. Thus the lean animals must have deposited little or no energy for three weeks while energy deposition was still occurring in the diabetic-obese. The second explanation relates to their use of mice which were homozygous-normal (with respect to the db genotype) as lean controls while we used heterozygotes; there is some evidence that heterozygotes may have a higher efficiency than the homozygous-normal animals [5]. Heterozygotes were used as the lean controls in the present experi-

Fig. 1. The effect of environmental temperature (°C) on the 'excess' energy gain of diabetic-obese mice pair-fed to the *ad libitum* food intake of lean siblings. (a) observed (b) adjusted for the presumed heat lost in growth; the adjustment is based on the assumption that the energy costs of fat and protein deposition are the same for lean and diabetic-obese animals, and that each kJ fat deposited costs 0.36 kJ and each kJ protein 1.25 kJ (see [18])

ments because of the difficulty in obtaining sex-matched homozygous-normal and diabetic-obese animals from the same litter. The third explanation is that Cox and Powley [7] used the db gene on a C57BL/6J background while the animals used in our experiments were the C57BL/ks strain. Since the diabetic syndrome induced by the db mutation is more severe on the latter background [4] the energy lost as glucose in the urine is likely to be greater than with the C57BL/6J strain. In consequence, with the C57BL/ks mice less of the digestible energy intake may be available to deposit as fat.

The pair-feeding system employed in the present study allowed the diabetic-obese animals the opportunity to "meal-feed". An attempt was made to minimise this by presenting the food in two meals each day, since the effects of meal-feeding in raising the efficiency of animals are well recognised [10]. However, Cox and Powley [7] found that by giving db/db mice their food in one meal per day, efficiency was *reduced* rather than increased in comparison to animals which did not meal-feed. Meal-feeding is therefore unlikely to be a significant factor in the elevated efficiency reported here for db/db mice.

It is noteworthy that in the present study the energy gain of the lean mice fed *ad libitum* was identical at 23° and 33 °C. This observation is in contrast to the results obtained in previous studies where energy gain was observed to be greater in mice housed at thermoneutrality than at normal temperatures [17, 18]. Strain differences are likely to account for this discrepancy.

The primary aim of the present study was to determine whether a reduced energy expenditure on thermoregulatory thermogenesis (in practise non-shivering thermogenesis) was an important factor in the high efficiency of the db/db mouse. Both body temperature [20, 23] and metabolic rate measurements [20] had suggested that this was likely to be the case. In order to make a quantitative nutritional assessment of the importance of reduced thermoregulatory thermogenesis to energy balance pair-feeding experiments were conducted at thermoneutrality (33 °C) where, contrary to studies at lower temperatures, there is no requirement for thermoregulatory heat. At thermoneutrality, the energy gain of the diabetic-obese mice was only slightly, but not significantly, greater than that of the lean animals to which they were paired (Table 2).

The effect of environmental temperature (23° and 33 °C) on the difference in energy gain between lean and diabetic-obese animals (i. e. energy gain of mutants minus energy gain of controls) is shown in Figure 1. By conducting the pair-feeding at thermoneutrality, and therefore removing what is a major component of energy expenditure at normal temperatures, 78% of the extra energy gained by the mutants over the lean animals at 23 °C is removed (Fig. 1a). This value rises to 87% when the differing energy costs of growth are taken into account (Fig. 1b); this adjustment has been made since on the one hand the greater fat deposition of the diabetic-obese animals involves an increased energy expenditure, while on the other hand their lower protein deposition results in a reduced expenditure.

It is interesting to note that at both 23° and 33 °C the pair-fed diabetic-obese mice deposit only half as much protein as their lean siblings (Tables 1 and 2). Thus, even at thermoneutrality, there is a positive discrimination against the deposition of protein in favour of fat storage. The reasons for such a discrimination, which also occurs in the ob/ob mutant [18], are not clear. Although protein turnover has been implicated in the reduced thermogenesis of the ob/ob mouse [16], it now appears that almost all of the decreased energy expenditure on thermogenesis in this mutant can be attributed to the mitochondrial proton conductance pathway in brown adipose tissue [12, 19] – and this is likely to also apply to the diabetic-obese mouse.

In conclusion, the present experiments together with the earlier metabolic rate measurements [20], clearly indicate that the low energy expenditure and consequent high metabolic efficiency of the diabetic-obese mutant is due to reduced thermoregulatory thermogenesis. This is also the mechanism by which the obese (ob/ob) mouse achieves its high efficiency

[18, 21, 22]. This similarity has prompted the suggestion that a reduction in thermoregulatory, or non-shivering, thermogenesis is the general mechanism through which the “thrifty” genotype associated with diabetes-obesity may operate [20].

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Dr. P. Trayhurn
Dunn Nutrition Laboratory
University of Cambridge and Medical Research Council
Milton Road
Cambridge CB4 1XJ
England