Number of Mice per Cage Influences Uncoupling Protein Content of Brown Adipose Tissue (43461)

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Abstract. **The effect of housing density of mice on the thermogenic state and capacity of their brown adipose tissue was studied. Mice were housed one, two, or six per cage at 28°C for 15 days. Increased housing density suppressed the thermogenic capacity of brown adipose tissue (decreased the total amount of uncoupling protein) and decreased the thermogenic state of brown adipose tissue mitochondria (decreased GDP binding). A density of six mice per cage had a greater effect than a density of two mice per cage. The size of brown adipose tissue (wet weight and protein content), the content of mitochondria in it (cytochrome oxidase content), and the total activity of thyroxine 5′-deiodinase were not altered by housing density. We conclude that even at a temperature close to thermoneutrality (29–33°C for the mouse), the occurrence of social thermoregulation (huddling) reduces the requirement for brown adipose tissue thermogenesis and results in a reduction in its thermogenic capacity. It is clearly of importance that the design of studies of mouse brown adipose tissue take into account not only the temperature at which the mice are housed, but also the number of mice housed per cage.** [P.S.E.B.M. 1992, Vol 200]

Brown adipose tissue has the function of thermogenesis. This function is controlled by the sympathetic nervous system, primarily in relation to environmental temperature but also in relation to diet (1–3). Not only can thermogenesis in brown adipose tissue (BAT) be rapidly switched on and off by increases and decreases in secretion of norepinephrine from the sympathetic nerves that innervate it, but the capacity of the BAT for thermogenesis can increase or decrease according to the extent of chronic stimulation. This capacity is determined by the content of mitochondria and by the concentration of uncoupling protein (UCP) in these mitochondria. UCP is unique to BAT mitochondria and its stimulated function allows the reversible uncoupling of oxidative phosphorylation from electron transport when the BAT is stimulated by norepinephrine, the basis for the thermogenic function of the tissue (see Refs. 2 and 4 for review). UCP has a binding site for purine nucleotides and the level of binding by isolated mitochondria serves as a measure of both the amount of this protein and the thermogenic state of the mitochondria (1, 5).

In commonly used laboratory rodents, such as mice, the concentration of UCP in BAT mitochondria is directly related to the environmental temperature to which the animals are adapted (6). Mice, for which thermoneutrality is rather high (29–33°C) (7), are already halfway to being fully cold acclimated at a commonly used animal facility temperature of 22°C, as judged from the high concentration of UCP in their BAT mitochondria (6), and their overall energy expenditure is almost doubled at this temperature (7). However, in this species, in which social thermoregulation commonly occurs (8), the environment includes not only the ambient temperature, but also the presence of other mice in the same cage. The reduction in cold stress permitted by behavioral means, i.e., by huddling, allows group-housed mice to thermoregulate as one larger animal with a smaller surface area, and hence lower heat loss, than that of the total number of mice. Thus, the mass of BAT increases as environmental

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temperature decreases in singly housed mice, but this increase is very much dampened when more than one mouse is present in a cage (8, 9). Biochemical studies of BAT have shown that the level of GDP binding to BAT mitochondria is much greater in mice housed singly at 23°C than in mice housed three or six per cage at this same temperature; in the absence of any major difference in UCP concentration in these same mitochondria, this finding is interpreted as an indication of greater thermogenic activity of BAT in singly housed mice (10). Since the total content of cytochrome oxidase in BAT was also larger in the singly housed mice than in the group housed mice, it was concluded that the total mitochondrial thermogenic capacity was also reduced by the group housing (10). Even in mice housed at 4°C, the cold-induced growth of BAT, as measured by its increased protein and cytochrome oxidase contents, and the increase in thermogenic activity, as indicated by increased mitochondrial GDP binding, were attenuated in group-housed mice, although, again, no clear effect of caging on the cold-induced increase in UCP concentration in BAT mitochondria was observed (10).

Obesity in mice is generally associated with a reduced thermogenic activity and a lower content of UCP in BAT, as for example, in the genetically obese *ob/ob* mouse (11–13). In our studies of obese and lean mice, we have usually housed them in groups of similar mice at 26–28°C. The findings cited above of Jennings *et al.* (10), who studied the effect of housing density on BAT of mice housed at 23°C, most probably apply to partially cold-acclimated mice. While it seemed unlikely that housing conditions would influence the measurements of BAT thermogenic capacity and activity at the higher environmental temperature of 28°C, just below thermoneutrality for the mouse and associated with very little increase in overall energy expenditure (7), it seemed important to us for the design of future experiments, by us and by others, to know whether housing density could influence BAT thermogenic state and capacity in mice housed at 28°C. Therefore, the objective of the present experiments was to find out to what extent the number of mice housed per cage might influence the thermogenic activity and capacity of BAT when these mice are housed at 28°C.

Materials and Methods

Female C57BL/6 mice were obtained from Charles River, Inc., St-Constant, Québec, Canada, at 5 weeks of age. They were initially housed in plastic cages (27.5 × 22.5 × 16 cm) with wood chip bedding (approximately 0.5 cm deep) in groups of four at 28°C, with free access to food (Purina Rat Chow) and water and with lights on from 0730 to 1930 hr for 1 week after their arrival. They were then weighed and separated into groups of six mice per cage in six cages, two mice per cage in another six cages, and one mouse per cage in another six cages. Mean weights of groups at this time were not significantly different. They were maintained for an additional 15 days under the same conditions.

Mice were weighed and then sacrificed by decapitation, and their blood was collected. Their rectal temperature was measured within 30 sec with a probe inserted to a depth of 2 cm (mouse rectal probe RET-3; Bailey Instruments, Saddle Brook, NJ). All mice in one cage were sacrificed on any one day. Interscapular and subscapular BAT were then removed, cleaned of adhering white adipose tissue and muscle, and weighed. BAT was homogenized, and samples of homogenate were removed and frozen for later assay of protein, UCP, and cytochrome oxidase and thyroxine 5′-deiodinase activities. Mitochondria were isolated from the remaining homogenate and used for the measurement of GDP binding and protein. Protein was measured by a modified Lowry method, UCP by radioimmunoassay using mouse UCP as a standard and a rabbit anti-hamster UCP antiserum, thyroxine 5′-deiodinase by rate of $^{125}I^-$ release from [^{125}I]thyroxine, and specific GDP binding by binding of [^{3}H]GDP in the presence and absence of 1 m*M* ADP, all as described before (12). Cytochrome oxidase was measured by a spectrophotometric method using Lubrol-activated samples of BAT homogenate (14). Serum 3,5,3′-triiodothyronine (T_3) and thyroxine were measured by radioimmunoassay using specific antisera (gift of Dr. P. R. Larsen) and mouse thyroid-hormone-free serum for preparation of standard curves, as described previously (12). Blood was centrifuged and serum was stored at −20°C for later determination of T_3 and thyroxine concentration by radioimmunoassay.

Data were analyzed by completely randomized design one-way analysis of variance using a SAS computed statistics program and Scheffé's post hoc test to assess differences between all possible means. Means were considered significantly different when $P < 0.05$.

Results

The most important finding in these experiments is the pronounced effect of the number of mice per cage on two indices of BAT thermogenesis. Mitochondrial GDP binding was progressively reduced by increasing the number of mice per cage (Fig. 1). The homogenate concentration of UCP (Fig. 2) and total UCP (Table I) were both progressively reduced by increasing the number of mice per cage.

The number of mice per cage did not alter their body weight gain during the period of study or their rectal temperature at the time they were sacrificed (Table I). Serum thyroid hormones, BAT weight, homogenate protein, specific and total cytochrome oxidase activity of homogenate, and specific and total

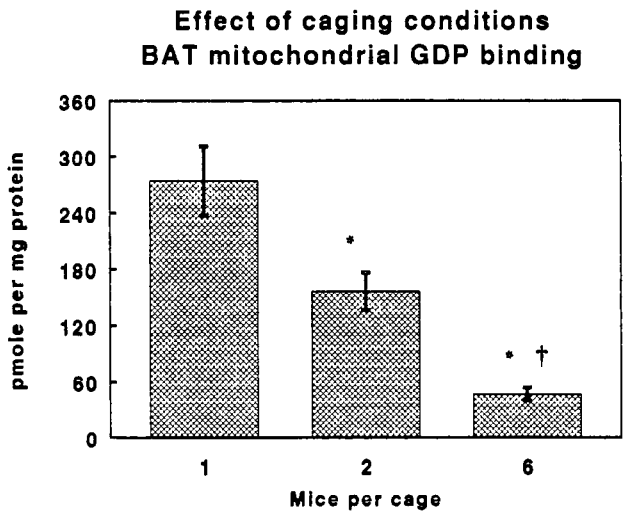

Figure 1. Binding of GDP to isolated BAT mitochondria in relation to the number of mice housed per cage. Values are the means ± SE for the numbers of mice listed in Table I. The asterisk denotes a significant difference from the value for one mouse per cage. The dagger (†) denotes a significant difference from the value for two mice per cage.

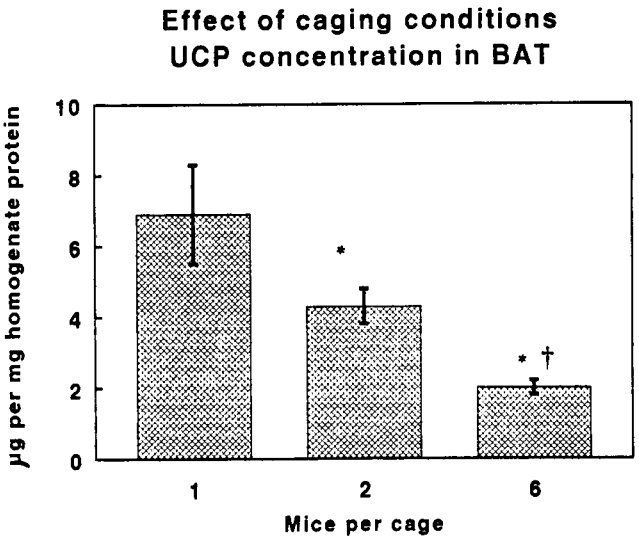

Figure 2. Concentration of UCP in homogenates of BAT in relation to the number of mice housed per cage. Values are the means ± SE for the numbers of mice listed in Table I. The asterisk denotes a significant difference from the value for one mouse per cage. The dagger (†) denotes a significant difference from the value for two mice per cage.

thyroxine 5′-deiodinase activity of homogenate were likewise not significantly affected by the number of mice per cage. There was a trend for an increase in BAT weight when more mice were present in the cage, which, in the absence of any difference in protein content, probably means an increase in lipid content and a decrease in thyroxine 5′-deiodinase activity when more mice were present in the cage.

Discussion

The principal conclusion from these results is that even at a housing temperature of 28°C, the thermogenic activity of BAT, as indicated by mitochondrial GDP binding, and the thermogenic capacity of BAT, as indicated by its UCP content, are both greater when mice are housed singly than when they are housed in pairs or in sixes. They are greater also when mice are housed in pairs than when they are housed in sixes. No other differences in BAT or in energy balance or thermoregulation of the mice were detected from the measurements made. In the absence of measurements of food intake or 24-hr energy expenditure, we are unable to determine whether the mice housed singly did indeed have a greater rate of thermogenesis, but this seems highly likely.

The differences observed in the singly housed mice would be consistent with the greater "cold stress," i.e., greater heat loss at 28°C, experienced by these mice in comparison with the group-housed mice. Both mitochondrial GDP binding and total amount of UCP are increased by exposure and acclimation of mice to cold and are related to the temperature of acclimation (6, 11, 12). However, the differences seen in BAT of the singly housed mice do not include other changes seen in BAT of cold-exposed or -acclimated mice, such as an increase in cytochrome oxidase content (6, 11) and an increase in thyroxine 5′-deiodinase activity and in the serum level of T_3 (12, 15). Although the lack of increase in thyroxine 5′-deiodinase activity might be attributed to the return of this activity to normal after long-term acclimation to cold, the level of T_3 would be expected to remain elevated (12, 15), and no elevation of serum T_3 was seen in the singly housed mice. These differences between the effect of single housing at 28°C and cold acclimation might be attributable to the relatively mild cold stress experienced by mice at a temperature of 28°C.

The results of this study differ in several respects from those of Jennings *et al.* (10), who studied the effect of housing density on BAT of mice living at 23°C. First, whereas we found an increase in total UCP content of BAT, which, in the absence of any change in cytochrome oxidase content, can be interpreted as an increase in the concentration of this protein in mitochondria, they found no marked effect of caging density on mitochondrial UCP concentration. Second, whereas we found no change in cytochrome oxidase content, interpreted as no change in total mitochondrial mass, they found an increase in cytochrome oxidase content, together with unchanged concentration of UCP in the mitochondria. Thus, although we both saw an increase in thermogenic capacity, in our experiments it seemed to be due to altered composition of an unchanged number of mitochondria, whereas in their experiments it was due to an increase in the number of mitochondria without any change in composition. We did both see

Table I. Effect of Number of Mice per Cage on Body Weight Gain, Rectal Temperature, Serum Thyroid Hormones, and Various Measures of BAT Mass and Thermogenic Capacity[a]

	Mice per cage		
	1 ($n = 6$)	2 ($n = 12$)	6 ($n = 36$)
Body wt change (g)	3.2 ± 0.4	3.0 ± 0.2	3.4 ± 0.1
Rectal temperature (°C)	36.7 ± 0.3	37.2 ± 0.2	37.1 ± 0.1
Serum T_3 (ng/dl)	64.8 ± 10.7	54.0 ± 3.4	64.6 ± 2.2
Serum Thyroxine (μg/dl)	3.08 ± 0.18	3.24 ± 0.14	3.47 ± 0.16
BAT			
Wt (mg)	112.0 ± 8.2	126.6 ± 4.8	136.2 ± 5.0
Protein (mg)	9.9 ± 0.65	10.1 ± 0.27	8.8 ± 0.29
UCP (μg)	67 ± 12	44 ± 5[b]	17 ± 1[b,c]
Thyroxine 5′ deiodinase (fmol/hr)	854 ± 148	774 ± 75	533 ± 59
Cytochrome oxidase (nmol/min)	16.4 ± 3.2	23.5 ± 7.4	21.7 ± 5.0

[a] Values are means ± SE for the number of mice shown in the second row.
[b] Value is significantly different from the value for one mouse per cage.
[c] Value is significantly different from the value for two mice per cage.

an increase in mitochondrial GDP binding, although we could see a difference between mice housed two per cage and singly housed mice and they could not. Also, the housing density did not alter body weight gain of the mice in our experiments, whereas housing singly actually increased body weight gain in their experiments. The reason for these differences in outcome in two studies in which the major difference was the temperature at which the mice were housed is not clear. The lability of GDP binding in BAT of mice is noteworthy; the level was low in animals sacrificed while in their home cage in groups of three at 26°C, and was higher when they were transferred to a new and warm environment for 15 min, a phenomenon even more marked when the mice were food-restricted and torpid (regulating at a low body temperature) in the home cage and raised their body temperature rapidly after the transfer (16). It is possible that a difference in the handling of the mice might account for some of the differences in measurement of GDP binding.

The activity of mice was not assessed in the present experiments. It is possible that the presence of a large number of mice per cage might increase their activity. This would be expected to result in suppression of BAT thermogenesis (17, 18). Also, the temperature within the cage might have been slightly higher when more mice were present, and this could also account for suppression of BAT thermogenesis.

The extreme sensitivity of biochemical indices of BAT function to environmental temperature is well known, and careful control of the temperature of animal facilities is of vital importance in studies of BAT to reduce variability in measurements and to ensure reproducibility from one location to another. To this must be added the number of mice per cage, which, even at the relatively high temperature of 28°C, can bring about long-term changes in the amount of UCP in the BAT, together with changes in mitochondrial GDP binding. The design of experiments in which BAT of mice is to be studied, therefore, must include a consideration of the appropriate number of mice per cage and must ensure that this number does not change in the course of the experiment.

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