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Activity-induced thermogenesis in lean and genetically obese *(ob/ob)* mice

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Summary. Motor activity was approximately 60% lower in genetically obese than in lean mice, during three consecutive hours at thermal neutrality. It is suggested that this must have contributed to the lower heat production measured in the obese mice and that activity-induced thermogenesis contributes significantly to differences in energy expenditure between the genotypes, at least in the short-term.
Key words. Activity; genetics; obesity; thermogenesis.

In previous investigations on energy expenditure and obesity, considerable attention has been focussed on possible differences in the various components of resting metabolism both in man[1-3] and in rodents[4,5]. This emphasis is in accord with the fact that a large proportion of energy utilized by the body is spent on resting metabolism. However, variations in resting metabolism tend to be small compared with the influence of activity on metabolic rate[6-9], and yet the role of spontaneous activity has been relatively neglected and examined in only a few studies of obesity[3]. Differences in activity could be of importance not only in obese subjects but also in lean subjects during over-feeding, since they might help to explain any apparent discrepancy between actual and predicted weight-gain during over-feeding[10]. In the present investigation, the contribution made by motor activity to energy expenditure has been studied in lean and genetically obese *(ob/ob)* mice living undisturbed at thermal neutrality.

Materials and methods. Mature mice of the strain C57BL/6-*ob*/01a were investigated. Mean b.wt ± SEM were 35 ± 1.8 g for the lean (n = 12) and 54 ± 2.5 g for the obese mice (n = 9). The animals were housed separately at 28 ± 1 °C on a 12 h light : 12 h dark cycle (light = 07.30–19.30 h) and food (CRM, Labsure, Christopher Hill Group Ltd, RHM) and water were available ad libitum.

Simultaneous measurements of energy expenditure and motor activity were made on each animal over a 3-h period. Energy expenditure was measured using an open-circuit indirect calorimeter housed in a temperature-controlled room at 28 ± 0.1 °C[11]. The conditions were as similar as possible to the animal's usual living conditions: the clear perspex chamber was similar in size to the box in which it usually lived and the animal's own bedding of wood shavings was used. The calorimeter was continuously ventilated at 750 ml/min and a change-over box directed ingoing or outgoing air to a paramagnetic oxygen analyser (Type OA184, Taylor Servomex Ltd, Crowborough, Sussex). The principle of the system was similar to that used in this laboratory for human subjects[12]. Animals were accustomed to the procedure by being placed in the chamber on three earlier occasions, each of several hours. They remained undisturbed during the measurements and food and water were available ad libitum.

Mean values of oxygen consumption were obtained for every 1-h period of measurement and converted to STP. Errors associated with measurement of oxygen consumption alone can be minimized by converting it to heat production[13,14]. Several suggestions for the conversion factor have been made[15-18] and the average value of 20.5 J/ml O_2 was used in the present investigation.

Motor activity was monitored while the subject occupied the respiration chamber by two independent methods, one visual and the other using a modified Doppler-type burglar alarm[19]. The visual method used a closed-circuit television system and a score was given for each 5-min period, the technique being based on the activity-diary used for man[20]. Activity was allocated to one of five categories and given a score (shown in parentheses): no activity (0) e. g. lying quietly with no movement; minor activity (1) e.g. sitting, with a little movement; feeding or drinking (1);

Motor activity (visual score/h) and heat production (J/$g^{0.67}$ per h) of lean (n = 12) and obese (n = 9) mice during three consecutive hours at 28 °C (mean values ± SEM)

Measurement and hour of observation	Lean	Obese
Motor activity		
Hour 1	219 ± 15.1	108 ± 11.5
Hour 2	185 ± 13.2	56 ± 13.9
Hour 3	140 ± 14.4	44 ± 11.1
Heat production		
Hour 1	238 ± 9.8	146 ± 7.0
Hour 2	206 ± 8.8	120 ± 8.0
Hour 3	172 ± 7.8	101 ± 6.8

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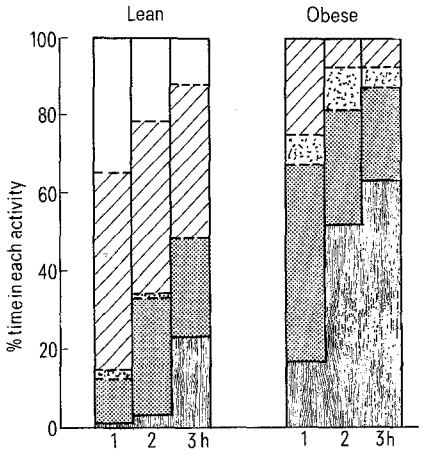

Figure 1. Percentage of time spent in each of five levels of activity in 12 lean and 9 obese adult mice during three consecutive hours at 28 °C. Animals lived in a respiration chamber (266 mm long, 217 mm wide, 175 mm high) sited inside a temperature-controlled room. Observations were made continuously during the three hours for every 5-min period, with a closed-circuit television system, and spontaneous motor activity was categorized as follows: ▒, no activity; ▒, minor activity; ⁝⁝, feeding or drinking; \\\, moderate activity; □, major activity. (See text for details).

moderate activity (2) e.g. walking; major activity (3) e.g. running. It was thus possible to give a total visual score for each hour of observation and also to determine the proportion of time spent in each level of activity. Two independent observers obtained close agreement using this visual method for scoring activity, and this in turn agreed well with the Doppler meter. Over short periods, the visual method gives more detailed information about motor activity and the results using this method are therefore presented here.

Measurements were made once on each animal during three consecutive hours between 09.00 and 14.00 h. Hourly values for motor activity and heat production were calculated, with the latter being expressed both on a whole animal basis and per unit metabolic body size (g b.wt to the power two-thirds). Statistical significance of differences between lean and obese mice was evaluated using Student's t-test.

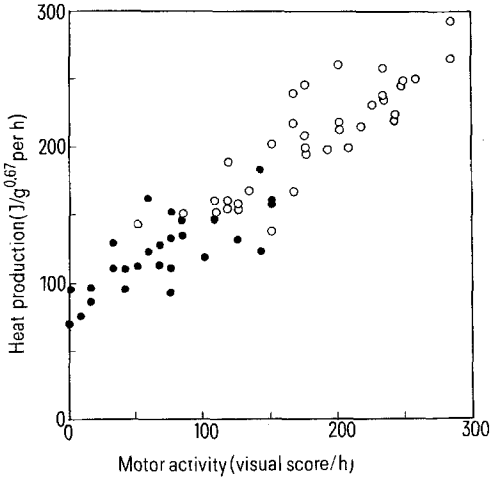

Figure 2. Relation between motor activity and metabolic rate for 12 lean (○) and 9 obese (●) adult mice while living for three consecutive hours at 28 °C in a respiration chamber.

Results and discussion. Differences in motor activity between the genotypes were striking and figure 1 shows the percentage of time spent in each of the five levels of activity during the three consecutive hours of observation. The lean animals were considerably more active than the obese and even during the third hour some of the lean were still active whereas the obese tended to show little or no movement. The obese never indulged in major activity and thus spent a greater proportion of their time in the lower levels of activity, including feeding. The table shows that for both genotypes, activity and heat production decreased with time, indicating that the initial high level of activity probably included a substantial component of reactive activity. The time for which reactive activity due to a novel environment persists is a matter of discussion[21,22], but in the present investigation it had diminished considerably by the second hour, particularly in the obese. It is unlikely that the results indicated only differences in reactive activity, rather than in true spontaneous activity, since the animals were not reared in isolation and they were accustomed to the experimental procedure which used a chamber very similar to their normal living quarters.

The table shows that not only was activity significantly greater in the lean than the obese mice ($p < 0.001$) but so also was energy expenditure per unit metabolic body size ($p < 0.001$). For the three hours of measurement, motor activity was less in the obese than the lean by 51, 70 and 69%, while heat production (J/g$^{0.67}$ per h) was less by 39, 42 and 41%. This suggested that at least some of the difference in energy expenditure was related to activity. Even on a total b.wt basis, energy expenditure was greater in the lean than the obese mice. Mean values ± SEM (J/animal per h) for the lean were 2533 ± 115, 2182 ± 81 and 1820 ± 70 for the 3 h, compared with 2054 ± 76, 1673 ± 93 and 1414 ± 91 for the obese animals.

The relation between activity and heat production is shown for all the animals in figure 2. This demonstrates a good linear relation ($r = 0.93$, $p < 0.001$) between the two variables and thus acts also as a validation of the method for scoring activity. It shows the same linear relation between motor activity and heat production as that found in studies of lean and obese human subjects using the Doppler method for recording spontaneous activity[23]. The slope of the line for each genotype gives an indication of the extra energy involved for a given increase in activity, and if the separate regression lines of motor activity against heat production are extrapolated back to the y-axis, estimates of metabolic rate at zero activity can be obtained. However, it should be remembered that the animals were allowed to eat ad libitum during the measurements. Therefore, the extrapolated values of heat production at zero activity cannot be equated with 'basal' metabolic rate, since the obese ate more than the lean mice during the period of measurement and this would have elevated the regression line for the obese to a greater extent than it would for the lean[24]. Under the present conditions, figure 2 shows that there was NS difference in either the slope or the intercept between the lean and obese mice. By contrast, the position of the data points on the line was different for the two groups, with none of the obese having an activity score of more than 150/h.

The present results thus indicate that over short periods when there is a component of reactive activity the energy cost of movement is not an insignificant part of the difference in metabolic rate between the two genotypes. One possibility is that 28 °C was slightly below the critical temperature for some of the mice but the effect of this must have been small since at zero activity there was no significant difference in the estimated rate of heat production. The mean rate of heat production observed in the lean mice was about three times the estimated heat production at zero activity and it is unlikely that such a high metabolic rate could be sustained for more than a short period. The differences between the two groups might be related to differences in either the amplitude or the phase angle of a circadian rhythm in activity. Studies are now in progress to determine the

contribution made by motor activity to 24 h energy expenditure in the two groups in order to assess whether spontaneous activity contributes to the development and maintenance of obesity in the *ob/ob* mouse. Additional studies could also help to resolve the current discussion and controversy on 'cafeteria-feeding' and 'diet-induced thermogenesis'[25,26].

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Molt-induced muscle atrophy decreases the zinc content of the pectoralis of the Giant Canada Goose *(Branta canadensis maxima)*

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Summary. During molt-induced atrophy of the pectoralis muscle of the Giant Canada Goose *(Branta canadensis maxima)*, the zinc content of the muscle was significantly reduced ($p \leq 0.0139$), though the concentration of zinc per unit weight of muscle appeared higher ($p \leq 0.0232$). Zinc lost from the muscle during molt could be utilized for growth of the new flight feathers.
Key words. Zinc; muscle; molt; geese.

Zinc is a ubiquitous element in the tissues of both plants and animals[2,3]. More than 200 different enzymes require zinc for maximum catalytic activity[4]. Zinc is important in a wide variety of functions, and is especially essential for tissue growth[4-6]. Most (90%) of the zinc in the human body is located in the musculoskeletal system, most of which is in the muscles[2,3]. While the function of zinc in muscle is largely unknown[2,7], other than being a component of certain enzymes, it is known to prolong contraction[8], increase oxygen affinity of myoglobin[3], assist in glycolysis[9], and is necessary for the growth of muscle and the differentiation of muscle fiber types[10]. Red muscle and slow muscle contain proportionately more zinc than do white muscle and fast muscle[7,10], respectively.

Disuse of a muscle can result in a reduction in muscle mass and the concentration of many enzymes[11,12]. Little is known about zinc in muscle under these conditions. While the concentration of zinc in muscle does not change in response to a deficiency of zinc in the diet[7], it is believed that zinc is lost from muscle during injury induced atrophy[13].

A natural model of disuse atrophy occurs in the pectoralis muscle of ducks and geese during their annual feather molt. These birds are flightless during this period[14], and they exhibit a concomitant decrease in the mass of their major flight muscles while they are growing new flight feathers[15-17]. We have recently examined both the concentration (amount/unit of muscle) and content (total amount in entire muscle) of iron in the pectoralis muscle of a wild population of molting geese[18]. The purpose of the present study was to examine the effect of this molt-induced disuse atrophy on the concentration and content of zinc in the pectoralis of the same population.

Materials and methods. Adult male Giant Canada Geese *(Branta canadensis maxima)* were collected from a nonmigratory feral population on the Toronto Islands (43°37'N, 79°20'W) during molt (June 1982), postmolt (October 1982) and premolt (March 1983) periods. Molting birds were obtained during the annual goose 'round up' conducted on the Toronto Islands by the Canadian Wildlife Service, Ontario Ministry of Natural Resources and Toronto Parks Department. Postmolt and premolt birds were captured through the use of a cannon net. Birds were euthanized by an i.p. injection of a euthanasia solution (T-61, Hoechst Inc.).

Ingesta-free body weights were determined, and the right pectoralis muscle *(M. pectoralis)* was excised from each bird. Each muscle was then oven-dried, ground and lipid extracted in order to obtain the corresponding lean dry weight[18]. Tissue samples were prepared for analysis by flame atomic absorption spectroscopy[19]. Zinc concentration was analyzed in a Varian 1275 AA spectrophotometer at a wavelength of 213.9 nm, with a slit of 1 cm at 5 amperes. The standards used were 0, 12.5, 50 and 100 ppm zinc. A statistical analysis was conducted using the software of Statistical Analysis System[20]. The variation between periods for each factor was tested with a one-way analysis of variance (ANOVA), and the differences between cell means were assessed by the method of least squares means[21].

Results. The results (table) indicate that while there is no significant difference in body weights between premolt, molt and postmolt periods, molting birds have significantly lighter pectoralis muscles. Zinc concentration and zinc content are, respectively, significantly higher and significantly lower in the pectoralis of molting birds as compared to premolt and postmolt