

On Raising Energy Expenditure in *ob/ob* Mice

Jay C. Erickson *et al.* (1) describe the energy intake and energy expenditure of mutant obese mice (*ob/ob*) in which the gene for neuropeptide Y (NPY) is disrupted. They conclude that deficiency of NPY in the *ob/ob* mouse reduces the elevated food intake and increases the low energy expenditure, partially ameliorating the obesity. Another report, by Mary Ann Pelleymounter *et al.* (2), describes energy intake and energy expenditure of *ob/ob* mice treated with OB protein or saline. They conclude that treatment with OB protein (leptin) reduces food intake and increases energy expenditure, ameliorating the obesity.

In both studies, food intake is presented in units of grams per mouse. Food intake of *ob/ob* mice is indeed reduced both by disruption of the gene for NPY and by treatment with OB protein. However, data for energy expenditure are expressed as milliliters of oxygen consumed per kilogram of body weight per hour. Why divide energy expenditure by body weight? The *ob/ob* mouse contains much more metabolically inert body fat than the lean mouse. The NPY^{-/-} *ob/ob* mouse contains less body fat than the *ob/ob* mouse, but still more than the lean mouse. Likewise, the OB-treated *ob/ob* mouse contains less body fat than the saline-treated *ob/ob* mouse. If, from the data in the report, resting oxygen consumption is expressed as total milliliters of oxygen consumed per mouse per hour, a different outcome is seen. Total oxygen consumption in *ob/ob* mice and in NPY^{-/-} *ob/ob* mice is similar and in both cases higher than in lean mice. The apparent increase caused by removal of NPY disappears. Likewise, total oxygen consumption in saline-treated *ob/ob* mice and OB-treated *ob/ob* mice is similar. The apparent increase caused by the OB treatment disappears.

In both studies, and quite correctly, energy intake was not expressed in terms of body weight; had it been so expressed, this would have produced the nonsensical result that *ob/ob* mice eat much less than lean mice. Energy expenditure should not be expressed in this way either. I suggest that both research groups recalculate their results (1, 2) to express them as total oxygen consumed per mouse per hour and reconsider their conclusions.

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REFERENCES

1. J. C. Erickson, G. Hollopeter, R. D. Palmiter, *Science* **274**, 1704 (1996).
2. M. A. Pelleymounter *et al.*, *ibid.* **269**, 540 (1995).

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Response: The comment by Himms-Hagen reveals the complexity of using basal oxygen consumption rates to compare the metabolic activity of animals that differ greatly in body size, body composition, and other characteristics. If oxygen consumption is calculated on a per-whole-animal basis, rather than on a per-weight basis, then there is no significant difference between NPY-deficient *ob/ob* mice (71.3 ± 2.9 ml per hour per mouse) and control *ob/ob* mice (71.5 ± 3.1 ml per hour per mouse); similarly, when calculated in this manner, leptin treatment does not raise the oxygen consumption rate of *ob/ob* mice (see the response by Pelleymounter *et al.*). However, these results do not necessarily mean that the leaner phenotypes produced by NPY deficiency and leptin treatment are unrelated to changes in metabolic activity.

A problem in interpreting these data is that the measurements were obtained when significant differences in physical characteristics—such as body weight and body adiposity, which themselves affect oxygen consumption—existed between the groups of mice being compared. For example, the larger size of the control *ob/ob* mice necessitates that they devote greater metabolic activity to cardiac output, body support, and other physiological processes influenced by body size. The difficulty in comparing whole-animal oxygen consumption rates of animals varying profoundly in size and composition is exemplified by studies showing that adult *ob/ob* mice consume as much oxygen as or more than normal lean mice (1, 2). On the basis of this information, one might mistakenly conclude that the obesity of *ob/ob* mice develops despite a seemingly normal or faster-than-normal metabolic rate, an assertion that directly conflicts with studies demonstrating that a metabolic component contributes to the onset of obesity in these mice (2–5). We therefore expressed oxygen consumption on a per-weight basis to more accurately reflect metabolic efficiency (6), a strategy used by other investigators (2, 7, 8).

Regardless of one's interpretation of the oxygen consumption measurements, NPY deficiency and leptin treatment both resulted in increases in body temperature and physical activity of *ob/ob* mice (6,9), effects that would tend to promote increased energy expenditure and hence to reduce adiposity. Further studies are needed to define the complicated effects of leptin treatment and NPY deficiency on the metabolism of *ob/ob* mice. Examination of whole-animal oxygen consumption rates before drastic changes in

body weight and composition occur as well as pair-feeding studies could provide essential information.

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REFERENCES

1. J. Mayer, R. E. Russell, M. W. Bates, M. M. Dickie, *Endocrinology* **50**, 318 (1952).
2. G. A. Boissoneault, M. J. Hornshuh, J. W. Simons, D. R. Romsos, G. A. Leveille, *Proc. Soc. Exper. Biol. Med.* **157**, 402 (1978).
3. C. Chlouverakis, *Experientia* **26**, 1262 (1970).
4. L. C. Alonso and T. H. Maren, *Am. J. Physiol.* **183**, 284 (1955).
5. D. L. Coleman, *Int. J. Obesity* **9**, 69 (1985).
6. J. C. Erickson, G. Hollopeter, R. D. Palmiter, *Science* **274**, 1704 (1996).
7. M. L. Kaplan and G. A. Leveille, *Am. J. Physiol.* **227**, 912 (1974).
8. M. L. Kaplan, *Int. J. Obesity* **5**, 51 (1981).
9. M. A. Pelleymounter *et al.*, *Science* **269**, 540 (1995).

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Response: Himms-Hagen has an excellent point. The first thing that we would like to point out, however, is that the y axis on the graph in figure 2A in our 1995 report [(1), p. 541] had an omission. Oxygen consumption was actually expressed and calculated as milliliters per kilogram to the 0.7 power per hour [$\text{ml kg}^{-0.7} \text{hr}^{-1}$], that is, as a power function, not as a mass function. Power functions are used as a mass-independent method of expressing the average volume of oxygen (vO_2) consumed, reflecting surface area rather than mass (2). One assumes, with power functions, that surface area should directly reflect metabolizable tissue. However, when there is as large a discrepancy in mass as there is between the *ob/ob* mouse and its lean littermate (even at 5 to 6 weeks of age), the power function is no longer independent of mass. When our data were analyzed in units of milliliters per mouse (as suggested by Himms-Hagen), there were no significant differences in vO_2 between *ob/ob* and lean mice, which is consistent with earlier data expressed in the same manner (3). Needless to say, the leptin effect also disappeared.

Whether one could now conclude that the *ob/ob* does not have a metabolic defect or that leptin or NPY deficiencies do not affect energy utilization is, however, far from clear. As pointed out in the response by Erickson *et al.*, a normal resting metabolism in the *ob/ob* mouse is not consistent with other aspects of its phenotype, that is, abnormally low body temperature, reduced locomotor activity, and considerably greater feed efficiency (food intake divided by body

weight, as suggested by Himms-Hagen). These inconsistencies could suggest that some normalization is still necessary in order to compare lean animals with the *ob/ob* mouse. The *ob/ob* mouse, after all, has a much larger surface area and more

Table 1. Effects of leptin (10 mg/kg) after 3 weeks of administration on O_2 consumption in (+/+), (*ob*/+), and (*ob*/*ob*) mice. vO_2 is expressed as a function of FFM (sum of carcass water and lean mass) $\pm$ SE. Oxygen consumption corresponds to the average volume of O_2 (vO_2) consumed during 15 1-minute sampling periods. Measurements were taken in an airtight chamber with an O_2 flow rate of 0.75 L/min, with the use of the Oxymax system (Columbus Instruments, Columbus, Ohio).

Treatment	vO_2 (ml/kg FFM/hour)	
	PBS	Leptin
(+ / +)	*4381.9 $\pm$ 96.8	4581.5 $\pm$ 171.1
(<i>ob</i> / +)	*4787.6 $\pm$ 109.5	4961.5 $\pm$ 113.1
(<i>ob</i> / <i>ob</i>)	3678.0 $\pm$ 177.8	†4678.5 $\pm$ 169.9

**ps* < 0.0005 to 0.0001 in comparison to *ob/ob* mice;
†*pf* < 0.0001 in comparison to PBS treatment.

carcass water than a lean counterpart along with having more fat than "metabolically active" tissue. If vO_2 is expressed as a function of fat-free mass (water and carcass lean mass; FFM), then the *ob/ob* mouse retains its hypometabolic nature and leptin raises vO_2 to that found in lean mice (Table 1). Studies showing reduced vO_2 in *ob/ob* mice have either used a comparison of weight-matched non-*ob/ob* mice with *ob/ob* mice or have normalized the data against mass or surface area (4, 5). Unfortunately, any normalization of vO_2 (using ratios) is still somewhat controversial because the regression line for vO_2 versus fat-free mass or mass does not have a zero intercept, violating one of the assumptions necessary for using ratio data (6). Therefore, we agree with Erikson *et al.* that the only meaningful comparison of vO_2 in *ob/ob* mice may be in very young, weight-matched *ob/ob* versus non-*ob/ob* mice, as has been done by Oh and Kaplan. (5).

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REFERENCES AND NOTES

1. M. Pelleymounter *et al.*, *Science* **269**, 540 (1995).
2. A. Heusner, *Am. J. Physiol.* **246**, R839 (1984).
3. P. Trayhurn and W. James, *Pflügers Arch.* **373**, 189 (1978); S. Hogan and J. Himms-Hagen, *J. Am. J. Physiol.* **238**, E301 (1980); V. Drescher, H.-L. Chen, D. Romsos, *J. Nutr.* **124**, 524 (1994).
4. J. C. Erickson, G. Holloper, R. D. Palmiter, *Science* **274**, 1704 (1996); M. Houlden and J. Andrews, *Ann. Nutr. Metab.* **32**, 200 (1988); M. Kaplan, *Int. J. Obesity* **5**, 51 (1981); D. Richard, S. Chapdelaine, Y. Deshaies, M.-C. Pepin, N. Barden, *Am. J. Physiol.* **265**, R146 (1993).
5. S. Oh and M. Kaplan, *Proc. Soc. Exper. Biol. Med.* **207**, 260 (1994).
6. J. Roscoe, *Fundamental Research Statistics for the Behavioral Sciences* (Holt, Rinehart & Winston, New York, ed. 2, 1969), pp. 18–19.
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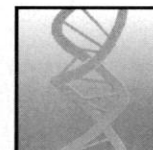
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