SUPPLEMENTAL MATERIALS

Supplemental Methods

Considerations Relating to Allometric scaling of metabolic rate

In traditional allometric scaling of MR (1-4), the logarithm (log) of MR is regressed on log(TBM), resulting in an expression of the form: *predicted arithmetic mean* of log(MR) = c + $b$log(TBM). Exponentiation yields a power equation of the form: *predicted geometric mean* of MR = aTBM^b where a is the scaling coefficient and b is the scaling exponent. Normalizing MR by forming the ratio MR / [TBM^b] yields the constant a such that the value of the ratio does not systematically vary with variation in [TBM^b]. This approach, which dates back some 120 years to the work of Max Rubner (1), remains the subject of considerable contemporary interest and debate (e.g., (5) (4) (6)) and has faced interpretational difficulty concerning the "meaning" of TBM^b. R.E. Keesey used allometric analysis that assumed a scaling exponent of 0.75 to provide evidence that MR is modulated in accordance with set-point regulation of body fat stores (7). The 0.75 scaling exponent was popularized by Max Kleiber (2) and much later "derived" in a paper (5) based on disputed assumptions involving fractal geometry, energy minimization (8) (9) and the very notion that the empirical database supports the 0.75 value (10). However, it is now well established that there is no universally-applicable within- or between-species values for the scaling exponent, and this exponent must be identified based on ones data (3, 6). Moreover, the traditional approach to allometric analysis has been challenged on grounds of parameter estimate-biasing stemming from the least squares optimization of logarithmic MR values that fail to satisfy the equal variance assumption of traditional regression, and the fact that back transformation (exponentiation to the arithmetic scale) results in a model that estimates the geometric rather than the arithmetic mean of MR (11). Finally, recent work challenges the very notion that the relationship between TBM and MR conforms to a pure power equation (12). Taken together, these considerations challenge the use of normalization strategies based on allometric scaling when comparing MR either across species or within species, especially when groups being compared differ substantially in body composition (e.g., comparing lean and obese mice).

About the Project by genotype (P x G) interaction. Project and genotype were modeled as factors. There were 4 levels of project with each level corresponding to a unique cohort of mice. Within each mouse cohort there were two levels of genotype, WT and mutant, and the mutants harbored a gene mutation that was unique to the cohort. Accordingly, defining the P x G interaction resulted in 8 groups, e.g., P1 WT, P1 mutants; p2 WT, p2 mutants, and so forth. Thus, the regression models for energy expenditure identified a coefficient corresponding to the effect on EE of membership within each level of project and genotype. Inspection of Supplemental Tables 1 and 2 will make this clear.

Supplemental Results

Supplemental Table 1. Full regression model for 24-h average energy expenditure (cal/min) for main regression analysis. Multiple $R^2 = 0.90$.

Independent variable	Coefficient*	SE	P
Intercept	0.156	0.694	0.822
LBM (g)	0.269	0.0395	0.00001
FM (g)	0.144	0.0232	0.00001
Sex (0=female, 1=male)	-0.547	0.2022	0.007
Diet (0=chow, 1=HFD)	1.311	0.291	0.00001
Activity (counts/min)	0.011	0.0056	0.058
Project=1 * Genotype=Mutant	2.041	0.228	0.00001
Project=1 * Genotype=Wildtype	1.206	0.207	0.00001
Project=2 * Genotype= Mutant	1.809	0.2983	0.00001
Project=2 * Genotype=Wildtype	1.877	0.2399	0.00001
Project=3 * Genotype= Mutant	0.621	0.4892	0.204
Project=3 * Genotype=Wildtype	1.344	0.323	0.00001
Project=4 * Genotype= Mutant	0.974	0.2292	0.00001
Project=4 * Genotype=Wildtype	0 (ref. group)		

* Estimated change in mean energy expenditure per unit change in the independent variable
SE: standard error of the coefficient estimate

Supplemental Table 2. Full regression model for minimum light cycle energy expenditure (cal/min) for main regression analysis. Muliple R2 = 0.89.

Independent variable	Coefficient*	SE	P
Intercept	0.653	0.568	0.251
LBM (g)	0.144	0.0301	0.00001
FM (g)	0.143	0.0214	0.00001
Sex (0=female, 1=male)	-0.816	0.1890	0.00002
Diet (0=chow, 1=HFD)	1.327	0.2569	0.00001
Project=1 * Genotype= Mutant	1.407	0.2321	0.00001
Project=1 * Genotype=Wildtype	0.504	0.2066	0.015
Project=2 * Genotype= Mutant	1.146	0.2582	0.00001
Project=2 * Genotype=Wildtype	1.377	0.2134	0.00001
Project=3 * Genotype= Mutant	0.673	0.4031	0.095
Project=3 * Genotype=Wildtype	0.880	0.3030	0.004
Project=4 * Genotype= Mutant	1.191	0.2001	0.00001
Project=4 * Genotype=Wildtype	0 (ref. group)		

* Estimated change in mean energy expenditure per unit change in the independent variable
SE: standard error of the coefficient estimate

Supplemental Discussion

Sample size considerations in multiple regression

Factors that may hinder widespread use of multiple regression to normalize MR in murine research include both the need for biostatistical expertise and the concern (13) that the sample size required for this type of analysis may exceed what is typically employed for such studies (e.g., 6-10/group). With the caveat that investigators are well advised to obtain power and sample size analyses from a qualified biostatistician during study planning, we anticipate that minimum group sizes for identifying a reliable body size-adjusted effect of an independent variable on energy expenditure will be 6-10 mice/group when regression methods are employed, given that measurements of energy expenditure and body mass variables are of sufficient quality. Reliable identification of independent variables with subtle effects will require larger sample sizes. The sample size can be reduced by employing a study design in which two or more repeated measurements of energy expenditure are obtained per animal, and using statistical methods accommodate longitudinal data (such as generalized estimating equations (14)). We anticipate that biostatistical advice pertaining to these issues will be made available as a resource to investigators via the NIH-funded Mouse Metabolic Phenotyping Center program.

Supplemental References

1. Rubner M. Uber den einfluss der korpergrosse auf stoff- un kraftwechsel. Z Fur Biol 1883; 19:535-562
2. Kleiber M. Body size and metabolic rate. Physiol Rev 1947; 27:511-541
3. Lighton JRB. *Measuring Metabolic Rates: A Manual for Scientists*. New York, NY, Oxford Univ. Press, 2008.
4. White CR, Seymour RS. Allometric scaling of mammalian metabolism. J Exp Biol 2005; 208:1611-1619
5. West GB, Brown JH, Enquist BJ. A general model for the origin of allometric scaling laws in biology. Science 1997; 276:122-126
6. White CR, Cassey P, Blackburn TM. Allometric exponents do not support a universal metabolic allometry. Ecology 2007; 88:315-323
7. Keesey RE, Corbett SW. Adjustments in daily energy expenditure to caloric restriction and weight loss by adult obese and lean Zucker rats. Int J Obes 1990; 14:1079-1084
8. Painter PR. The fractal geometry of nutrient exchange surfaces does not provide an explanation for 3/4-power metabolic scaling. Theor Biol Med Model 2005; 2:30
9. Chaui-Berlinck JG. A critical understanding of the fractal model of metabolic scaling. J Exp Biol 2006; 209:3045-3054
10. White CR, Seymour RS. Mammalian basal metabolic rate is proportional to body mass2/3. Proc Natl Acad Sci U S A 2003; 100:4046-4049
11. Packard GC, Birchard GF. Traditional allometric analysis fails to provide a valid predictive model for mammalian metabolic rates. J Exp Biol 2008; 211:3581-3587
12. Kolokotrones T, Savage V, Deeds E, Fontana W. Curvature in metabolic scaling. Nature 2010; 464:753-756
13. Butler AA, Kozak LP. A recurring problem with the analysis of energy expenditure in genetic models expressing lean and obese phenotypes. Diabetes 2010; 59:323-329
14. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika 1986; 73:13-22