

Expanding the body mass range: associations between BMR and tissue morphology in wild type and mutant dwarf mice (*David mice*)

Carola W. Meyer · Juliane Neubronner · Jan Rozman · Gabi Stumm ·
Andreas Osanger · Claudia Stoeger · Martin Augustin · Johannes Grosse ·
Martin Klingenspor · Gerhard Heldmaier

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Abstract We sought to identify associations of basal metabolic rate (BMR) with morphological traits in laboratory mice. In order to expand the body mass (BM) range at the intra-strain level, and to minimize relevant genetic variation, we used male and female wild type mice (C3HeB/FeJ) and previously unpublished ENU-induced dwarf mutant littermates (*David mice*), covering a body mass range from 13.5 g through 32.3 g. BMR was measured at 30°C, mice were killed by means of CO₂ overdose, and body composition (fat

mass and lean mass) was subsequently analyzed by dual X-ray absorptiometry (DEXA), after which mice were dissected into 12 (males) and 10 (females) components, respectively. Across the 44 individuals, 43% of the variation in the basal rates of metabolism was associated with BM. The latter explained 47% to 98% of the variability in morphology of the different tissues. Our results demonstrate that sex is a major determinant of body composition and BMR in mice: when adjusted for BM, females contained many larger organs, more fat mass, and less lean mass compared to males. This could be associated with a higher mass adjusted BMR in females. Once the dominant effects of sex and BM on BMR and tissue mass were removed, and after accounting for multiple comparisons, no further significant association between individual variation in BMR and tissue mass emerged.

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C. W. Meyer (✉) · J. Neubronner · J. Rozman ·
M. Klingenspor · G. Heldmaier
Department of Biology, Animal Physiology,
Philipps-Universität Marburg,
Karl-von-Frisch Strasse 8, 35043 Marburg, Germany
e-mail: meyer@staff.uni-marburg.de

G. Stumm · A. Osanger · C. Stoeger · M. Augustin · J. Grosse
Ingenium Pharmaceuticals AG, Fraunhoferstrasse 13,
82152 Martinsried/München, Germany

Present Address:
M. Augustin
Kendle GmbH & Co. GMI KG,
Stefan-George-Ring 6, 81929 München, Germany

Present Address:
J. Grosse
Paradigm Therapeutics Ltd.,
162 Cambridge Science Park, Cambridge,
CB4 0GP, England

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Abbreviations

BAT Brown adipose tissue
BM Body mass
BMR Basal metabolic rate
CET Central European time
DEE Daily energy expenditure
ENU Ethyl-nitroso-urea
eWAT Epididymal white adipose tissue
GLM General linear modeling
iBAT Inter-scapular brown adipose tissue
MR Metabolic rate
RMR Resting metabolic rate
WAT White adipose tissue
WT Wildtype

Introduction

In endotherms, basal metabolic rate (BMR) comprises a substantial part of daily energy expenditure (DEE, also referred to as field metabolic rate, FMR, when measured on free living animals). BMR, or standard metabolic rate (SMR), by definition, refers to resting heat production or resting metabolic rate (RMR) of non-sleeping, non-growing or non-reproducing, post-absorptive and normothermic individuals measured at thermoneutrality (Kleiber 1961). Approximately 30–40% of an animals DEE can be attributed to basal maintenance costs (Speakman et al. 2003) and investigating BMR is of current interest because it could provide novel targets for manipulations in overall energy expenditure.

Meta-analyses of data derived from field studies have revealed associations of BMR with taxonomy (Hayssen and Lacy 1985), food habits (McNab 1986), life history traits (Harvey et al. 1991) and climate (Lovegrove 2000, 2003) at the inter-specific level. At the intra-specific level, one hypothesis suggests that gut morphology plays an important role in sustaining high BMR (Daan et al. 1990; Hammond and Diamond 1997; Ksiazek et al. 2004). To unravel potential associations between the alimentary system and basal rates of metabolism at the intra-specific level, investigating domesticated animals may be advantageous over wild species because it is assumed that a substantial amount of confounding phenotypic variability is minimized. Based on such considerations, Konarzewski and Diamond (1995) chose to compare different strains of laboratory mice and found that strains with high BMR tend to have higher organ masses, specifically dry liver and kidney mass. However, only about 10% of total BMR variability was due to organ mass differences within strains. Another mouse study (Johnson and Speakman 2001) investigated lactating mice (MF1 outbred) but failed to detect any correlations between RMR at thermoneutrality and residual organ masses. On the other hand, Selman et al. (2001) used two phenotypes of mice specifically selected for high and low food intake and found that liver was the most significant morphological trait linked to changes in RMR measured at thermoneutrality, consistent with an earlier study which associated total alimentary system (including liver) with changes in BMR (Speakman and McQueenie 1996).

One disadvantage of studying correlations at the intra-specific or intra-strain level in mice is the relatively narrow body mass range provided. In order to expand the intra-strain body mass range, we investigated a previously unpublished recessive mouse

mutant phenotype, *David*, which has been identified in an ethyl-nitroso-urea (ENU) mutagenesis screen due to its abnormally small size (S1). Whereas adult wild type (WT; genetic background: C3HeB/FeJ) mice weigh ~ 28 g at an age of 3 months, *David* littermates have a body mass of only ~18 g. Based on current knowledge of the mutagenic action of ENU (Noveroske et al. 2000), *David* phenotypes are expected to carry a single point mutation at a yet to be identified locus. Preliminary analysis mapped the putative point mutation to chromosome 4 (S2). In our study, we sought to identify the association of BMR with morphological traits in wild type and *David* phenotypes. By doing so, we aimed to test whether BMR reflects metabolic costs of maintenance of energetically expensive organs.

Materials and methods

Mice and maintenance

Mice were phenotyped by weighing at the age of 6 weeks (day-42), when the threshold body mass for classifying *David* phenotypes was ≤ 18.4 g in males and ≤ 15.0 g in females (calculated from Fuchs et al. 2000). Mating *David* phenotypes to WT mice (genetic background: C3HeB/FeJ) produced WT and dwarf (*David*) phenotypes in the expected Mendelian ratios. All WT phenotypes used in our metabolic study were heterozygous for the putative mutation, as assessed from offspring phenotype frequencies in breeding pairs.

Mice were kept in a non-specific pathogen free animal facility on a 12 h–12 h light–dark cycle (lights on: 6:00 central European time (CET)) at an ambient temperature of $23 \pm 2^\circ\text{C}$ (relative humidity: 40–50%) and fed standard breeding chow *ad libitum* (Altromin 1314, Lage, Germany). They were maintained in groups of 3–5 individuals of the same sex.

Basal metabolic rate measurements

Measurements of RMR in WT mice and *David* phenotypes had revealed (data not shown) that RMR was lowest at ambient temperatures ranging from 28 to 32°C in both groups, which corresponds to previous determinations of thermoneutrality in different strains of mice (Hart 1971; Heldmaier 1974; Meyer et al. 2004). We therefore chose 30°C as the ambient temperature for measuring MR, which we call BMR when determined at rest.

Measurements of metabolic rate were carried out in an open system using an electrochemical O_2 analyzer

(S-3A, Ametek). The basic set-up has been published elsewhere (Heldmaier and Ruf 1992). In each trial, up to three mice and one reference channel were sequentially read for 60 s, i.e. individual readings were taken every 4 min. Oxygen-consumption was then converted to metabolic rate (= MR) according to the following equation: $MR(\text{ml O}_2 \text{ h}^{-1}) = \Delta \text{Vol\%O}_2 \times \text{flow} [\text{l h}^{-1}] \times 10$, at a flow rate of $\sim 30\text{--}35 \text{ l/h}$. Starting at 8:00 CET (=2 h after “lights on”), mice were exposed to 30°C for 4–5 h. Basal metabolic rate was determined between 10:30 and 12:30 CET from mean O_2 -consumption of four subsequent measurement points (equivalent to a period of 16 min) when mice were resting and had lowest metabolic rates (coefficient of variation across the mean of four subsequent measurement points was $\leq 6\%$).

Body composition and tissue morphology

After termination of the metabolic measurements, mice were weighed ($\pm 0.1 \text{ g}$) and euthanized with CO_2 . Body fat content ($\pm 0.1 \text{ g}$) was then determined by dual energy X-ray absorptiometry (DEXA, PIXImus2, GE Lunar, Version 1.46.007). The GE Lunar Piximus2 DEXA scanner gives accurate information on differences in body composition in mice (Nagy and Clair 2000; Brommage 2003; Johnston et al. 2005). We previously validated this method against Soxhlet (SOX) fat extraction in mice and also found a strong correlation ($r^2 > 0.95$) between fatDEXA and fatSOX (unpublished data). Lean mass was calculated by subtracting fat mass from body mass.

After transection of the Inferior vena cava, organs (liver, kidney, lungs, brain, spleen, heart, stomach, intestine, epididymal white adipose tissue (eWAT), inter-scapular brown adipose tissue (iBAT) and testes were dissected and weighed ($\pm 0.001 \text{ g}$). Prior to weighing, stomach and intestine contents were removed. Carcass mass equals lean mass minus summed organ masses (excluding eWAT and testes, since uteri and gonadal WAT were not dissected in females). All research procedures were approved by the German animal welfare authorities.

Statistical analysis

Statistical analysis was performed using SPSS Version 12.0 on \log_{10} transformed data. Distributions of all variables were tested for normality using the Kolmogorov–Smirnov-test. For comparison of crude means between males and females of each genotype, *t* test was performed. After verifying that variances in all group were homogenous (Levene test) we used general linear

modeling (GLM) to account for differences in body mass and to explore the potentially different effects of sex and phenotype on tissue morphology and BMR. Each model incorporated sex (dummy coded 1 for males and 0 for females) and phenotype (dummy coded 1 for WT and 0 for David mice) as cofactors and tissue mass as a covariate. Once the shared variation due to tissue mass, sex and phenotype was removed, residual tissue masses were then correlated with residual BMR. In tables and figures r^2 indicates squared Pearson correlation coefficients. The level of statistical significance was set to 5% ($P < 0.05$). To account for Type-1 errors (FDR; false discovery rate) in multiple tests, we calculated *Q*-values (Storey 2003) from *P*-values using the program QVALUE run on R (The R foundation for Statistical Computing).

Results

Growth curves

The genetic defect associated with the *David* mutation lead to pronounced dwarfism evident from day seven onwards (Fig. 1). Body mass in mice which were younger than 7 days was not determined, but in litters of new-born some individuals were already noticeable due to their exceptionally small size. Up to day-35, coinciding with termination of the peri-pupertal growth spurt in mice (Silver 1995), postnatal growth was further reduced in *David* phenotypes. After this time point the growth rates (g/day) became indistinguishable between WT and *David* phenotypes, and during the remainder of their lives *David* phenotypes did not catch up with size. At the age of 10–12 weeks, naso–anal body length in *David* phenotypes was 83–87 mm ($n = 9$) and 95–102 mm ($n = 6$) in wild type mice. Body mass and length in mice heterozygous for the mutation was indistinguishable from WT mice, supporting that dwarfism associated with the *David* mutation was a fully recessive trait. *David* phenotypes were fertile and, with the exception of their smaller size, they showed no gross behavioral or morphological abnormalities.

Body composition and organ masses

Male and female WT phenotypes were significantly heavier than males and females from the *David* phenotypes, had higher lean and carcass mass and contained more fat (all $P < 0.001$, Table 1). Table 1 also presents crude mean masses of each dissected tissue for mice of both sexes and phenotypes. Even though within

Fig. 1 Growth curves of male and female WT (wild type) and *David* phenotypes ($n = 9$ – 16) from day-7 to day-70 \pm 1. Based on phenotype offspring frequencies the WT mice are genotypically (+/+) or (+/-)

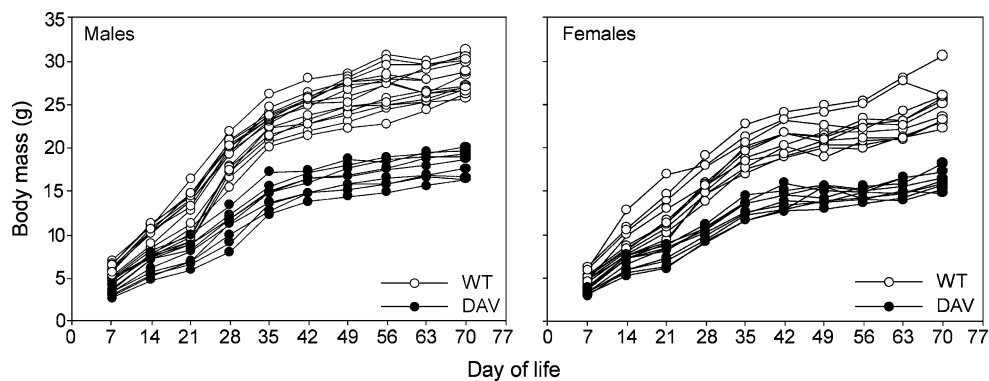


Table 1 Gross aspects of body mass, basal metabolic rate (BMR) and mean tissue masses (\pm SD) dissected from male and female wild type and *David* littermates

	WT phenotype		<i>David</i> phenotype	
	Males $N = 10$	Females $N = 10$	Males $N = 13$	Females $N = 11$
Body mass (g)	28.1 \pm 2.7	*** 24.1 \pm 3.1	18.1 \pm 1.3	*** 15.2 \pm 1.2
BMR (ml O ₂ min ⁻¹)	33.59 \pm 3.91	0.08 36.74 \pm 3.63	24.70 \pm 3.81	* 27.58 \pm 2.64
Lean (g)	22.81 \pm 1.70	*** 19.15 \pm 1.70	15.33 \pm 1.10	*** 13.05 \pm 0.95
Fat (g)	5.26 \pm 1.30	4.92 \pm 1.47	2.75 \pm 0.44	*** 2.15 \pm 0.29
Carcass (g)	18.09 \pm 1.37	*** 15.01 \pm 1.35	12.09 \pm 0.92	*** 9.97 \pm 0.77
Brain (g)	0.471 \pm 0.012	0.475 \pm 0.012	0.381 \pm 0.013	* 0.391 \pm 0.010
Heart (g)	0.127 \pm 0.015	** 0.108 \pm 0.010	0.088 \pm 0.006	** 0.080 \pm 0.006
iBAT (g)	0.079 \pm 0.016	* 0.061 \pm 0.016	0.047 \pm 0.008	0.042 \pm 0.006
Intestine (g)	1.006 \pm 0.077	0.998 \pm 0.108	0.709 \pm 0.057	0.732 \pm 0.058
Kidneys (g)	0.513 \pm 0.063	*** 0.342 \pm 0.040	0.329 \pm 0.053	*** 0.223 \pm 0.018
Liver (g)	1.476 \pm 0.150	** 1.243 \pm 0.155	1.020 \pm 0.082	** 0.911 \pm 0.073
Lungs (g)	0.155 \pm 0.008	0.151 \pm 0.010	0.117 \pm 0.007	0.121 \pm 0.014
Spleen (g)	0.074 \pm 0.008	*** 0.094 \pm 0.013	0.050 \pm 0.005	*** 0.065 \pm 0.010
Stomach (g)	0.127 \pm 0.011	0.133 \pm 0.016	0.080 \pm 0.009	0.085 \pm 0.009
eWAT (g)	0.657 \pm 0.240	0.223 \pm 0.061	0.223 \pm 0.061	
Testis (g)	0.133 \pm 0.009	0.093 \pm 0.020	0.093 \pm 0.020	

*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$ for significant differences between males and females of each phenotype (t test)

phenotypes males were significantly larger than females, they did not always display larger organ masses. Notably, spleen mass was significantly higher in females of both groups, whereas intestine, lung and stomach mass were not significantly different between sexes (Table 1). Across all $n = 44$ individuals, organ masses were strongly correlated with BM (Fig. 2; Table 2).

GLM explained 84–99% of the variability in individual organ masses (Table 3). When adjusted for body mass, pronounced effects of sex on the masses of most organs emerged, but now females had higher (stomach, intestine, spleen, lung, brain, iBAT) or lower (kidney) masses than males of the same body mass. Mass adjusted, females in both groups of mice also contained more fat, less lean mass and less carcass mass, respectively, compared to males. Significant phenotype effects were only observed in iBAT, brain, and to a lesser extent in carcass and liver masses, which were either lighter (carcass) or heavier (liver, iBAT, brain) in *David* phenotypes. In addition, significant 2-way

interactions of (phenotype \times sex) emerged in heart, liver, iBAT and lean mass. These interactions were variable with respect to the direction of the effect and P -values do not reach statistical significance when accounting for type-1 errors. Taken together, once variation in BM was controlled for, the effect of sex on the variation in individual organ masses was much stronger than the effect of phenotype.

BMR and body mass

BMR was significantly higher in males and females from WT compared to *David* phenotypes (all $P < 0.001$; Table 1). BMR was numerically higher in females, although this finding did not reach significance in WT phenotypes.

Within phenotypes and sex, the relationship between BMR and BM was significant in WT females ($P < 0.01$), but not in any of the other groups. Across all individuals there were significant independent

Fig. 2 Associations between body mass (BM) and selected tissue masses from wild type (empty symbols) and *David* (filled symbols) mice at $n = 10\text{--}13$ individuals per group. Triangles indicate males, circles indicate females. All correlations (see Table 2) were significant at $P < 0.001$ ($n = 44$)

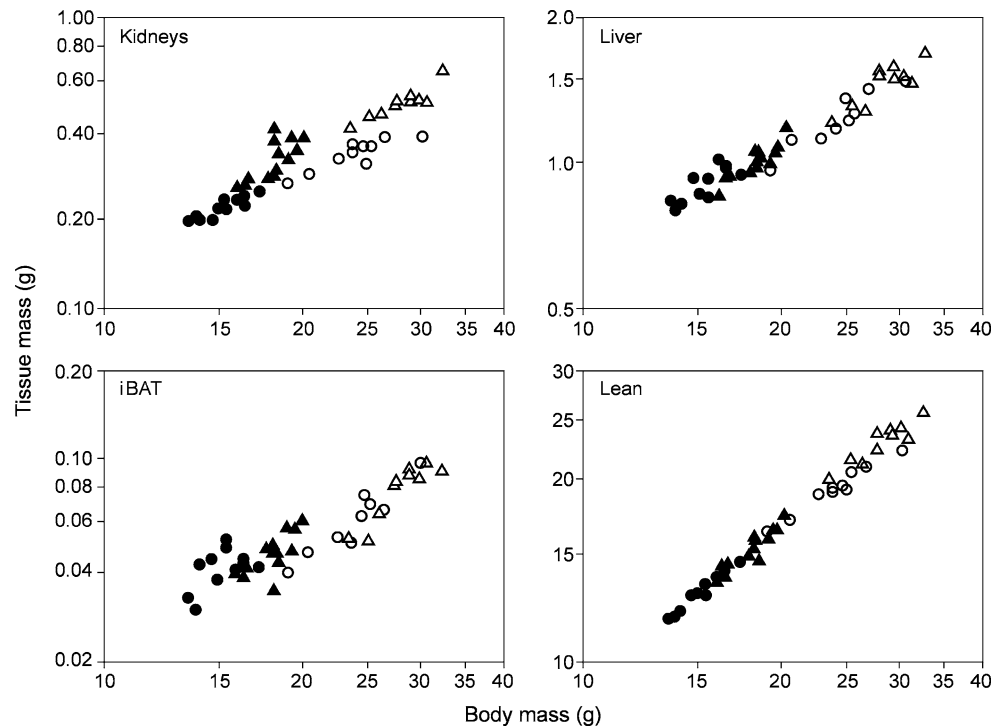


Table 2 Associations between tissue mass and body mass (BM) or basal metabolic rate (BMR; $\text{ml O}_2 \text{ h}^{-1}$) across $n = 44$ male and female wild type and *David* phenotypes. All correlations were positive at $P < 0.001$ (except for kidney mass vs. BMR, $P < 0.01$)

	Log BM	Log BMR
	r^2	r^2
Lean (g)	0.981	0.369
Fat (g)	0.908	0.456
Carcass (g)	0.976	0.370
Brain (g)	0.739	0.623
Heart (g)	0.910	0.387
iBAT (g)	0.801	0.395
Intestine (g)	0.794	0.578
Kidneys (g)	0.824	0.146
Liver (g)	0.941	0.421
Lungs (g)	0.721	0.458
Spleen (g)	0.608	0.358
Stomach (g)	0.761	0.640
eWAT (g)	0.905	0.647 ^a
Testis (g)	0.474	0.389 ^a

^a Males only ($n = 23$)

effects of phenotype ($P < 0.001$), sex ($P = 0.05$), and BM ($P < 0.001$), on BMR. BM explained 43% of the variability in BMR (Figs. 3, 4). Neither a three way interaction term [body mass \times sex \times phenotype] nor a 2-way interaction of [body mass \times sex] or [sex \times phenotype] were significant, but the interaction [phenotype \times body mass] was highly significant ($P < 0.001$) at an r^2 of 0.60. The latter most likely reflects the finding that sexual dimorphism in size and thus BMR was larger in WT as compared to *David* mice.

When we used GLM to account for the influence of sex, phenotype, and BM on BMR, sex ($P < 0.001$) and BM ($P = 0.023$) emerged as significant parameters. The equation (Eq. 1):

$\text{Log BMR (ml O}_2 \text{ h}^{-1}) = 0.867 + 0.454 \times \text{logBM} + 0.070 \text{ 'sex'} - 0.049 \text{ 'phenotype'} + 0.015 \text{ 'sex} \times \text{phenotype'}$ explained 70.5% of individual variation in BMR ($F_4 = 23.32$, $P < 0.001$). Mass adjusted (20.32 g), BMR of females was $33.11 \text{ ml O}_2 \text{ h}^{-1}$ (95%CI: $31.40\text{--}34.83 \text{ ml O}_2 \text{ h}^{-1}$) and thus significantly higher ($P < 0.001$) than BMR of males ($26.73 \text{ ml O}_2 \text{ h}^{-1}$ (95%CI: $25.47\text{--}28.05 \text{ ml O}_2 \text{ h}^{-1}$)).

BMR and tissue morphology

The correlations between each tissue mass and BMR (Fig. 3 and Table 2) were highly significant, albeit weaker than those obtained for BM versus tissue mass. To further explore the associations between BMR and tissue morphology, we removed the phenotype, sex and BM effects on BMR and organ masses by using the parameters from the respective linear models (Table 3 and Eq. 1). Table 4 illustrates the resulting associations between BMR and tissue morphology once the shared variation due to BM, sex and phenotype was removed. We found a correlation between iBAT mass and BMR, all other tissue correlations with BMR were not significant. After correction for FDR, the association between residuals of iBAT mass with BMR did not remain significant ($Q = 0.312$).

Table 3 GLM regression coefficients for the effects of body mass (BM), phenotype (wild type or *David*) and sex on organ masses in mice (log tissue mass = intercept + slope log BM + 'phenotype' + 'sex' + 'phenotype × sex'), and body mass-adjusted tissue mass estimates for each phenotype of male and female mice. BM for all calculations was log 20.32 g. Parameter estimates are based on $n = 10$ WT males, $n = 10$ WT females, $n = 13$ male *David* and $n = 11$ female *David* mice

	Tissue mass estimates (means ± SEM)										
	Intercept	BM	Phenotype	Sex	Pheno x Sex	r^2	<i>P</i>	WT males	WT females	David males	David females
Lean (g)	0.225	0.759 ^{WT}	-0.016	-0.029 ^M	0.071	0.993	<0.001	16.518 ± 0.191	15.451 ± 0.107	15.920 ± 0.110	15.274 ± 0.177
Fat (g)	-2.068	1.920 ^{WT}	0.090	0.097 ^F	-0.057	0.933	<0.001	2.778 ± 0.206	3.474 ± 0.172	3.418 ± 0.136	3.748 ± 0.259
Carcass (g)	0.111	0.792 ^{WT}	-0.024 ^{WT}	-0.028 ^M	0.004	0.986	<0.001	14.032 ± 0.261	13.156 ± 0.152	13.278 ± 0.123	12.564 ± 0.204
Brain (g)	-0.560	0.161 ^{WT}	-0.062 ^D	0.015 ^F	0.009	0.953	<0.001	0.447 ± 0.007	0.463 ± 0.004	0.388 ± 0.004	0.410 ± 0.006
Heart (g)	-1.927	0.711 ^{WT}	-0.020	-0.023	0.034	0.920	<0.001	0.101 ± 0.004	0.096 ± 0.002	0.096 ± 0.002	0.099 ± 0.003
iBAT	-3.660	1.760 ^{WT}	0.120 ^D	0.007 ^F	0.071	0.865	<0.001	0.044 ± 0.003	0.045 ± 0.002	0.058 ± 0.002	0.069 ± 0.005
Intestine (g)	-1.068	0.739 ^{WT}	-0.011	0.046 ^F	0.024	0.921	<0.001	0.792 ± 0.028	0.881 ± 0.021	0.772 ± 0.014	0.907 ± 0.030
Kidney (g)	-1.838	1.068 ^{WT}	0.008	-0.103 ^M	0.018	0.947	<0.001	0.362 ± 0.017	0.286 ± 0.009	0.369 ± 0.009	0.304 ± 0.014
Liver (g)	-1.171	0.925 ^{WT}	0.077 ^D	-0.012	0.033	0.955	<0.001	1.094 ± 0.031	1.064 ± 0.020	1.138 ± 0.018	1.194 ± 0.033
Lung (g)	-1.512	0.484 ^{WT}	-0.029	0.023 ^F	0.026	0.837	<0.001	0.132 ± 0.005	0.139 ± 0.004	0.124 ± 0.003	0.138 ± 0.005
Spleen (g)	-2.442	0.904 ^{WT}	0.002	0.166 ^F	0.016	0.876	<0.001	0.055 ± 0.004	0.081 ± 0.003	0.055 ± 0.002	0.084 ± 0.005
Stomach (g)	-2.193	0.985 ^{WT}	-0.032	0.080 ^F	0.014	0.939	<0.001	0.125 ± 0.005	0.150 ± 0.004	0.116 ± 0.003	0.144 ± 0.006

Against the column labeled BM and phenotype, WT indicates that wild type and D indicates that *David* phenotypes had significantly larger organs (all $P < 0.001$) once the other effects had been controlled for. Against the column labeled sex, M indicates that males and F indicates that females had significantly larger organ masses once the other effects had been controlled for. Parameters highlighted in italics indicate that *P*-values did not reach significance after accounting for Type-1 errors

Discussion

Much of the variation in the rates of metabolism in mammals is associated with body mass, an observation that holds from the inter-specific to the intra-strain level (Kleiber 1961; Hemmingsen 1960; McNab 1992; Gillooly et al. 2001; Krol et al. 2003; Labocha et al. 2004). However, animals of similar body mass may differ substantially in the rates of metabolism expected from the inter-specific relationship linking BM and BMR (Hemmingsen 1960; Hayssen and Lacy 1985; McNab 1986; Glazier 2005). Numerous studies have since aimed to understand the nature and variability of metabolism both at the tissue (Field et al. 1939; Krebs 1950; Porter 2001), and to a much larger extent at the organism level (Hayssen and Lacy 1985; McNab 1986; Elgar and Harvey 1987; Lovegrove 2000, 2003; Speakman et al. 2003; White and Seymour 2003; Munoz-Garcia and Williams 2005). The observation that in mammals DEE (FMR) is positively associated with BMR has fuelled the hypothesis that high BMRs were functionally linked to larger maximal sustainable metabolic rates (Drent and Daan 1980; Ricklefs et al. 1996). The former may be interpreted as an increment in maintaining the (elevated) size of the alimentary tract in order to cope with energetically demanding situations (see Speakman et al. 2004, 2005 for review).

To-date, intra-specific studies associating alterations in BMR with variability in organ masses have yielded conflicting results (Daan et al. 1989; Koteja 1996; Meerlo et al. 1997; Geluso and Hayes 1999; Johnson and Speakman 2001; Selman et al. 2001; Nespolo et al. 2002). WT and *David* littermates provided us with a novel model to study the association of tissue-organ morphology with BMR: 1. Genetic variability is constrained to the size phenotype at the inbred strain level in mice maintained under standardized laboratory conditions. 2. Incorporating *David* phenotypes into the analysis expanded the intra-strain body mass range to the lower end by almost 30% (13.5 g–32.3 g). Because it is generally assumed that BMR is a function of BM (but see Schmid et al. 2000), expanding the body mass range provides an advantage for regression analysis at the intra-specific level.

In our study none of the specific associations between alimentary tract morphology and BMR emerged. Once the effect of BM, phenotype and sex were controlled for, iBAT was the only tissue dissected which correlated with variability in BMR. This finding is of interest, since brown adipose tissue (BAT) is the primary site of non-shivering thermogenesis (NST) in small mammals (<10 kg) and neonates (Foster and Frydman 1978; Heldmaier and Buchberger 1985).

Fig. 3 Associations between selected tissue masses and BMR (basal metabolic rate) from wild type (*empty symbols*) and *David* (*filled symbols*) mice at $n = 10\text{--}13$ individuals per group. *Triangles* indicate males, *circles* indicate females. All correlations (see Table 2) except for paired kidney mass ($P < 0.01$) were significant at $P < 0.001$ ($n = 44$)

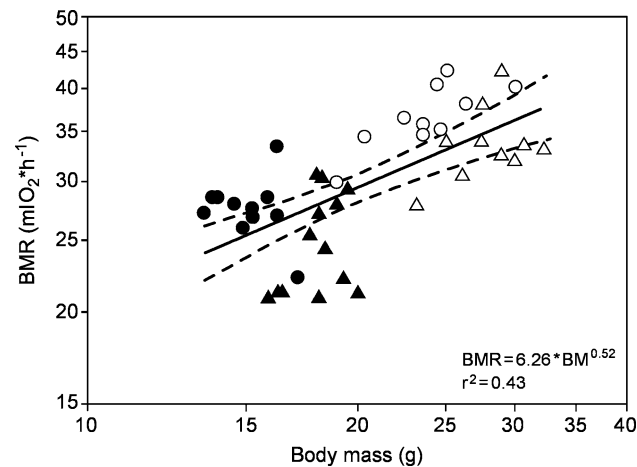
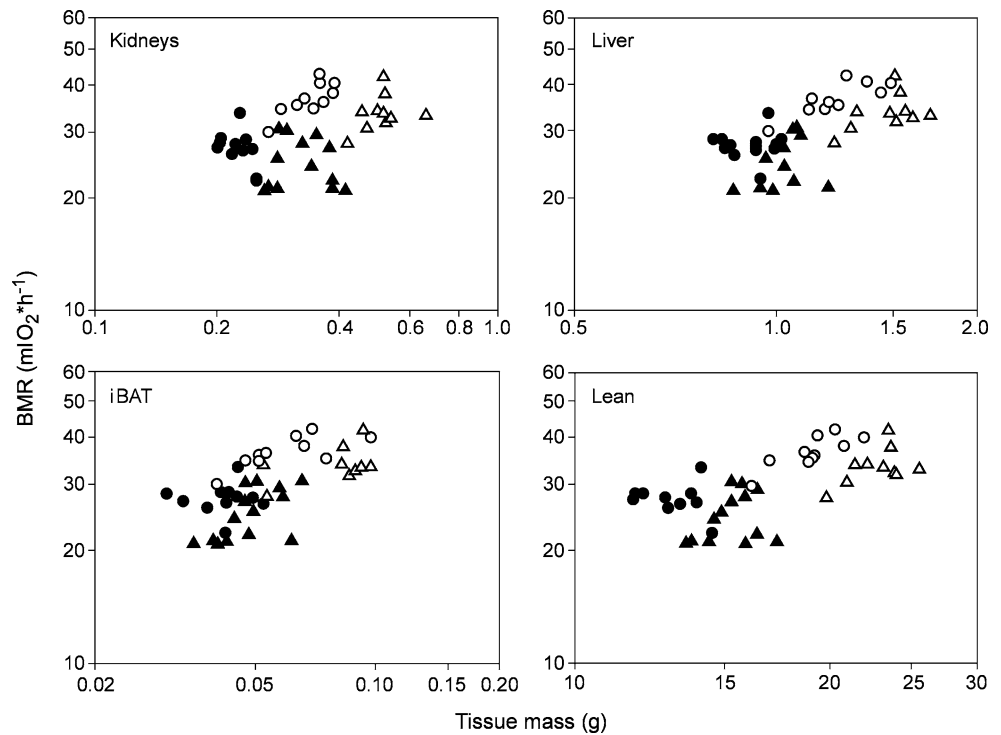


Fig. 4 Association between body mass (BM) and basal metabolic rate (BMR) in wild type (*empty symbols*) and *David* (*filled symbols*) phenotypes at $n = 10\text{--}13$ mice per group. *Triangles* indicate males, *circles* indicate females. The regression line and the corresponding equation illustrates the estimated linear relationship between BM and BMR across the $n = 44$ individuals used in the study. The *hatched curves* indicate upper and lower 95% CI [slope]

Upon cold exposure, proliferation of this tissue, mitochondrial biogenesis and increased expression of UCP1 (uncoupling-protein 1) will facilitate endogenous heat production and thus ensure maintenance of stable body temperature (Klingenspor 2003; Cannon and Nedergaard 2004; Kanzleiter et al. 2005). Mice living at 22–24°C (i.e. normal maintenance conditions) are moderately cold exposed, and an association

Table 4 Relationship between the residual of log (organ mass) and the residual of log (basal metabolic) rate across $n = 44$ male and female wild type and *David* phenotypes. After accounting for false discovery rates (FDR) the positive association between iBAT mass and BMR residuals did not remain significant

	r^2	P
Lean (g)	0.035	0.227
Fat (g)	0.000	0.954
Carcass (g)	0.000	0.985
Brain (g)	0.000	0.909
Heart (g)	0.000	0.956
iBAT	0.105	0.032
Intestine (g)	0.002	0.770
Kidney (g)	0.071	0.079
Liver (g)	0.062	0.104
Lung (g)	0.062	0.104
Spleen (g)	0.008	0.552
Stomach (g)	0.003	0.736

between iBAT tissue mass and BMR may reflect individual variability in BAT proliferation known to occur at this temperature (Heldmaier 1974). However, after accounting for FDR, the association between iBAT and BMR residuals did not remain significant. Since iBAT represents approximately 50% of total BAT-tissue in mice (Heldmaier 1975), it may be hypothesized that a (stronger) association with BMR variability might have emerged had we dissected all BAT depots. In summary, no specific association between BMR and any of the tissues dissected was found. Since each tissue mass contributes to body mass, there is a strong

multicollinearity between all possible predictors of variability in BM and BMR. Thus, it may well be that no specific tissue contributes significantly to BMR, because most of the variation is already explained by BM.

Our morphological results are in line with the expectation that sex is a major determinant of body composition in mice (Wiedmer et al. 2004): females contained more fat mass (i.e. fat represents a higher fraction of body mass, and fat mass was increased in females, when body mass adjusted; Table 3) and less lean mass. Unexpectedly, although female mice were smaller than males, body mass adjusted internal organ masses (with the exception of kidney mass) were greater (Table 3). The differential body composition between sexes was accompanied with a higher mass-adjusted BMR in females, which has interesting implications for the interplay of morphology and BMR: assuming that fat mass is metabolically relatively inert (Klaus 2004) the higher (mass adjusted) BMR of females could be attributed to the relatively larger size of their internal organs. In this respect, our results fuel the hypothesis that changes in organ size rather than changes in their mass-specific metabolic rates are primary contributors to BMR, as has been proposed by Ksiazek et al. (2004) for visceral organs.

From where else could the (unexplained) variability in BMR have arisen? Mice investigated in our study were not fasted. Even though postabsorptivity is one requirement for BMR, it introduces practical problems when measuring small rodents such as mice because they will increase their activity upon starvation and thus render determination of “true” (postabsorptive) BMR (according to Kleiber 1961) almost impossible. A slightly less rigorous definition, RMRt (resting metabolic rate at thermoneutrality) was introduced in order to account for this practical problem in, i.e. animals need not be postabsorptive (reviewed by Speakman et al. 2004). Since feeding in mice predominantly occurs during the dark phase, we assume that mice in our study had potentially not fed for at least 4 h, a period probably sufficient to exclude significant elevations in metabolic rate due to specific dynamic action of food. However, it may also be speculated that inter-individual variability observed in our study reflects individual temporal differences in feeding/food processing, and differences in individual determination of RMRt/BMR in relation to last meal timing.

Our study is the first to investigate the novel ENU-induced phenotype of *David* mice, and the underlying mutation is presently unknown. The phenotypic observations from our study suggest that with respect to overall morphology, and scaling, *David* phenotypes

resemble smaller WT mice, without any specific changes in body composition. It may be envisioned that the ENU-induced mutation in *David* mice leads to impaired development and linear growth, possibly via the GH-IGF-1 (growth hormone-insulin-like growth-factor-1) axis (Butler and Le Roith 2001). However, neither GH or IGF-1 or the corresponding receptors are located on Chromosome 4. GH deficiency or-resistance in mice and humans (e.g. Laron syndrome; Laron 2002) is furthermore associated with obesity and lowered body temperature (Hull and Harvey 1999; Bartke et al. 2001; Meyer et al. 2004), but *David* phenotypes do not become obese, and rectal probing of WT and *David* phenotypes did not reveal any significant differences in body temperature (data not shown). Thus, these phenotypic characteristics indicate that disturbance of the GH axis is not the primary cause for dwarfism in *David* mice.

Taken together our study suggests that while no specific metabolically active organ contributes to BMR variability, relatively larger internal organs may contribute to higher BMR by virtue of their size. *David* is a novel ENU-induced mutant, and the present study further suggests that whole body resting metabolism and morphometry are proportionally smaller in these dwarf mice.

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