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Phenotypic flexibility of traits related to energy acquisition in mice divergently selected for basal metabolic rate (BMR)

Aneta Książek*, Jan Czerniecki and Marek Konarzewski
Institute of Biology, University of Białystok, Świerkowa 20b, 15-950 Białystok, Poland
*Author for correspondence (e-mail: anetak@uwb.edu.pl)

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SUMMARY

Theoretical considerations suggest that one of the main factors determining phenotypic flexibility of the digestive system is the size (mass) of internal organs. To test this, we used mice from two lines selected for high and low levels of basal metabolic rate (BMR). Mice with higher BMRs also have larger internal organs and higher daily food consumption (C) under non-stressful conditions. We exposed animals from both lines to a sudden cold exposure by transferring them (without prior acclimation) from an ambient temperature of 23°C to 5°C. Cold exposure elicited a twofold increase in C and a 25% reduction of apparent digestive efficiency. For the same body mass-corrected C, small intestine, kidneys, heart and liver of cold-exposed low-BMR mice were smaller than those of the high-BMR line. Therefore, the internal organs of low-BMR animals were burdened with substantially higher metabolic loads (defined as C or digestible food intake per total mass of a particular organ). The mass-specific activity of citrate synthase (CS) in the liver and kidneys (but not heart) was also lower in the low-BMR mice. The magnitude of phenotypic flexibility of internal organ size and CS activity was strictly proportional to the organ mass (in the case of kidneys and liver, also mass-specific CS activity) prior to an increased energy demand. Thus, phenotypic flexibility had additive rather than multiplicative dynamics. Our results also suggest that variation in BMR positively correlates with the magnitude of an immediate spare capacity that fuels the initial response of internal organs to a sudden metabolic stress.

Key words: phenotypic flexibility, immediate spare capacity, organ size, organ mass, metabolic load, citrate synthase, artificial selection.

INTRODUCTION

Phenotypic flexibility is the ability of organisms to reversibly modulate traits in response to environmental challenge (Roff, 2002; Piersma and Drent, 2003). In recent years, the magnitude of this modulation has been extensively quantified at different levels of biological organization, from enzymes (Sabat and Bozinovic, 2000; Weiss et al., 1998) to tissues (Villarin et al., 2003), organs and whole animals (Kristan and Hammond, 2006). Several studies have demonstrated that processes related to energy acquisition and expenditure are among the most sensitive to changes in environmental conditions (Hammond and Kristan, 2000; Konarzewski and Diamond, 1994; McWilliams and Karasov, 2002; Naya et al., 2005; Toloza et al., 1991; Tracy and Diamond, 2005). The digestive system is the 'interface' between animal and environment for energy acquisition and, for this reason, its phenotypic flexibility has attracted particular attention (Karasov and McWilliams, 2005). Although such studies have identified several major physiological processes underlying phenotypic flexibility in digestive performance, there is little consensus on their relative significance in accommodating changing energy and nutrient requirements [Secor and Diamond *vs* Starck (Secor and Diamond, 1998; Starck, 1999a)].

Theoretical considerations suggest that the phenotypic response to environmental challenge involves two components, as reviewed by Karasov and McWilliams (Karasov and McWilliams, 2005). First, an initial increase of energy acquisition is realized from the immediate spare capacity of the digestive system (Diamond and Hammond, 1992; Karasov and McWilliams, 2005), which is built into the digestive organs but not utilized under routine physiological loads. The immediate spare capacity handles increased energy demands prior to the second phase of the response – upregulation of physiological processes underlying energy acquisition [e.g. increased organ mass, transporter density, etc. (Karasov and McWilliams, 2005)].

Although this theoretical framework is physiologically reasonable, it does not reveal the specific proximate mechanisms determining the magnitude of phenotypic flexibility. It is also unclear how the benefits of maintaining an immediate spare capacity for quick response to increased demands trades off with the metabolic costs of maintaining that capacity in routine conditions. Nevertheless, if Karasov and McWilliams' model (Karasov and McWilliams, 2005) is correct, then one of the main factors determining the magnitude of immediate spare capacity and phenotypic flexibility should be the size (mass) of the digestive organs immediately prior to metabolic stress. Here, we propose two hypotheses relating the initial organ size to their phenotypic flexibility. First, we hypothesize that phenotypic flexibility of internal organs has additive rather than multiplicative dynamics. If so, we predict that animals having relatively larger digestive organs will respond to a sudden demand by increasing the magnitude of energy intake and upregulation of the functions of the internal organs, which will be strictly proportional to the initial organ size. This proportionality should particularly hold in the first, most-challenging phase of the upregulation of the functions related to energy acquisition, which is handled by an immediate spare capacity. If, however, the dynamics of phenotypic flexibility is multiplicative, one can predict two scenarios: first, the phenotypic response of animals having initially larger organs should be more than

proportional, which will allow them to achieve a new physiological steady state faster and at higher levels. Second, animals having smaller organs under routine conditions may respond with proportionately greater phenotypic flexibility, which will allow them to match energy acquisition rates of animals with initially larger organs.

The above hypotheses also embrace two possible alternative relationships between metabolic costs of maintaining internal organs, their immediate spare capacities and the magnitude of phenotypic flexibility. If basal metabolic rate (BMR) subsumes these costs under routine conditions, as suggested by some studies (e.g. Konarzewski and Diamond, 1995), then one can expect that the magnitude of phenotypic flexibility of internal organs expressed due to sudden demand will be positively correlated with the level of BMR preceding this demand. Alternatively, however, the ability of animals having small internal organs (and low BMR) to match the energy acquisition rates of animals having larger organs would point to the independence of BMR (measured under routine conditions) and phenotypic flexibility expressed under metabolic stress.

To test the above hypotheses, we used mice from two lines selected for high and low BMR (Książek et al., 2004). There are two reasons why mice of these lines are good models for studying relationships between digestive organ size, immediate spare capacities and phenotypic flexibility. First, apart from a 30% difference in BMR, these lines distinctly differ in the relative sizes of the small intestine, liver, kidneys and heart (Książek et al., 2004). Second, under warm thermal conditions (23°C), low-BMR mice have 10% lower energy intake than mice from the high-BMR line. Accordingly, we expected that because of their smaller organ sizes, and hence immediate reserve capacities, low-BMR mice would respond to a sudden metabolic demand with a smaller increase in energy assimilation rate (relative to organ size) than the high-BMR line. More specifically, we predict that metabolic loads (defined as food intake or digestible food intake per mass of a particular organ) incurred by a sudden increase in energy demand should be smaller for relatively larger internal organs.

To produce a sudden metabolic challenge, we transferred animals, without prior acclimation, from an ambient temperature of 23°C to 5°C. As thermoregulatory heat production needs to be much higher at 5°C, we expected a corresponding immediate increase in daily energy expenditures and hence daily energy assimilation rate. We then analyzed the between-line differences in changes of energy assimilation rate in relation to changes in masses of internal organs most significantly contributing to BMR – small intestine, liver, kidneys and heart (Konarzewski and Diamond, 1995). However, changes in organ mass may result from an inexpensive and functionally inconsequential tissue rebuilding (i.e. an increase of hydration) rather than changes in mass-specific metabolic capacity (Starck, 1999b). Therefore, we also quantified the activity of citrate synthase (CS), a mitochondrial enzyme, which is a good indicator of the aerobic metabolic activity of tissues (Choi et al., 1993; Hammond et al., 2000; Houle-Leroy et al., 2000; Janssens et al., 2000; Schaarschmidt and Jürss, 2003; Tripathi and Verma, 2004).

MATERIALS AND METHODS

Animals and their maintenance

The two genetic lines with different BMR were developed from outbred Swiss–Webster laboratory mice (*Mus musculus*). Briefly, we measured the BMR of 12-week-old mice for 3 h in an open-circuit respirometry system (for details, see Książek et al., 2004). Males and females characterized by highest and lowest body mass-corrected BMR were chosen as progenitors of subsequent generations of high (H-BMR) and low (L-BMR) lines, respectively. Multiple generations of similar selection have resulted in significant, genetically based differentiation of the two lines in BMR, visceral organ masses and daily food intake (Książek et al., 2004). In this study, we used males from generation 25. The experiment was conducted on 108 mice (16–18 weeks old, 30–45 g body mass), following measurements of BMR as part of the selection procedure. Subjects were randomly chosen from the pool of animals not qualified as progenitors. BMR differed considerably between high- and low-BMR individuals entering the experiment [$65.3\pm0.5\,\mathrm{ml\,O_2\,h^{-1}}$ *vs* $51.8\pm0.6\,\mathrm{ml\,O_2\,h^{-1}}$; analysis of covariance (ANCOVA) with body mass as a covariate, $F_{1,105}=405.43$, $P<0.0001$].

A week before the onset of the experiment, all animals were placed in individual plastic cages with an elevated bottom but no bedding material, on a 12 h:12 h light schedule and ambient temperature of 23°C, which is a housing temperature in our colony. Mice had free access to food (murine laboratory chow, Labofeed, Poland) and water.

Experimental procedures

Two days prior to cold exposure, 13 randomly chosen animals from H-BMR line and 14 L-BMR animals were subjected to 48 h-long feeding trials to determine food consumption (C) and digestive efficiency (D) at 23°C. Upon completion of the trial the animals were killed by cervical dislocation to determine body composition. The remaining animals were randomly assigned to three groups and suddenly transferred from an ambient temperature of 23°C to 5°C. Each group consisted of 14 mice from L-BMR line and 13 mice from H-BMR line and differed in the duration of cold exposure. The first group was maintained at 5°C for two days, the second group for four days and the third group for six days, which is the time required to reach a new steady state of body mass, C and D (Toloza et al., 1991).

In each experimental group, we measured body mass, C and D. Following termination of cold exposure, animals were killed to determine internal organ masses and CS activity. All procedures were accepted by the Local Ethical Committee in Białystok (permit no. 2005/26).

Food consumption, digestibility and morphometrics

Food consumption (C) was calculated individually for each mouse as the mass of food disappearing from the food dispenser that day minus spilled, uneaten food [orts (Konarzewski and Diamond, 1994)]. Orts and faeces were separated from each other, dried in an oven at 70°C and weighed to the nearest 0.001 g. D was calculated as the difference between C and faecal output, divided by C (Drożdż, 1968). The retention time of digesta may exceed 24 h (Toloza et al., 1991), so C and D were analyzed in two-day periods. Digestible food intake (DFI) was approximated as the product of C and D.

Animals were killed and weighed to the nearest 0.1 g. Small intestine, liver, kidneys and heart were excised, cleared of blood or digesta and adherent fat and weighed to an accuracy of 0.001 g.

Activity of citrate synthase

CS (EC 4.1.3.7) catalyzes condensation of acetyl coenzyme A and oxaloacetate to form citrate in the citric acid cycle (Krebs's cycle) and thus provides a good index of oxidative metabolism in tissues (Choi et al., 1993; Janssens et al., 2000).

After dissection, organs (liver, kidneys and heart) were immediately frozen in liquid nitrogen and then stored at –80°C. Frozen samples were homogenized in ice-cold Tris-HCl buffer

(100 mmol l^{-1}, pH 7.5) using a Miccra D-1 tissue homogenizer (CARL ROTH GmbH, Karlsruhe, Germany). Heart samples of 0.07–0.1 g were homogenized 1:20 (w/v) whereas kidney and liver samples of 0.2–0.4 g were homogenized 1:5 (w/v). Temperature was maintained at 4°C during homogenization. Homogenates were centrifuged at 15,000 ***g*** for 15 min at 4°C, and supernatants were stored at –80°C until used in assays. CS activity was estimated in 10 µl of samples of assay mixture containing 0.4 ml of 200 mmol l^{-1} Tris-HCl (pH 8.0) buffer, 0.1 ml of 1 mmol l^{-1} DTNB (5,5′-dithio-bis-2-nitrobenzoic acid), 0.1 ml of 3 mmol l^{-1} acetyl coenzyme A and 0.29 ml of de-ionized water. We pre-incubated the sample for 3 min at 25°C and initiated the reaction by adding 0.1 ml oxaloacetate. The progress and products of the reaction were monitored for 1 min and analyzed using a temperature-controlled spectrophotometer (Beckman DU 640, Fullerton, CA, USA) (Houle-Leroy et al., 2000; Schaarschmidt and Jürss, 2003) at extinction of 412 nm. All enzyme assays were run in duplicate. Specific activity of CS was expressed in international units (µmol substrate transformed to product min^{-1}) per gram of tissue wet mass (Houle-Leroy et al., 2000).

Statistics

Differences in body mass between experimental groups were tested by means of two-way analysis of variance (ANOVA) with line-type (L-BMR or H-BMR) and group affiliation (control group exposed to 23°C and two-, four- and six-day cold exposure to 5°C) as the main effects. To analyze differences in C, D, organ masses and the activity of CS, we applied similarly structured analysis of covariance (ANCOVA)/ANOVA with body mass (minus foodstuffs contained in the digestive tube) as a covariate.

Because measurements of BMR are very labor intensive, we were not able to produce replicate lines in our selection experiment. The lack of replication confounds the interpretation of the between-line differences because the possible effect of genetic drift cannot be effectively controlled (Garland, 2003; Henderson, 1989; Henderson, 1997; Konarzewski et al., 2005). To remedy this problem, we relied not only on ANCOVA/ANOVA models described above but we also analyzed between-line differences according to the guidelines suggested by Henderson (Henderson, 1989; Henderson, 1997). Briefly, we first calculated within-line means of analyzed traits, (computed from family means) using the residual values for the respective ANCOVA models. We then calculated the respective within-line s.d. (hereafter called phenotypic s.d., interpreted as the products of the square root of the heritabilities and genetic s.d.) (Henderson, 1997). Finally, we calculated standardized between-line difference (d) as the difference between within-line residual mean values divided by mean within-line phenotypic s.d. and tested them against the lower boundary of 95% confidence interval of d (95% CI), which estimates the expected separation of a given trait, assuming the effect of genetic drift. We computed 95% CI using eqn 16 from Henderson (Henderson, 1997):

$$\sigma_{(d)} \cong 2\sqrt{(h^2 F + 1/n)}. \quad (1)$$

where h^2 is the narrow sense heritability of a given trait, F is the coefficient of inbreeding and n is the number of families subjected to the selection. F (Falconer and Mackay, 1996) in generation F25 of our selected lines was 0.37 (A.K., unpublished) and the number of families equalled 20.

Individual *a priori* pair-wise differences between experimental groups (within-lines) were tested by t-statistics corresponding to the two-sided P-values, adjusting the conventional level of significance by applying a Bonferroni correction. To do this, we divided α=0.05 by 6, i.e. the number of inter-group tests performed. All tests were carried out using SAS 9.1.3 statistical package (SAS Institute, Cary, NC, USA).

RESULTS

Body mass, food consumption and digestibility

Body mass in all experimental groups was similar at the conclusion of the experiments ($F_{3,100}$=0.42, P=0.7). There were also no differences in the mass of carcasses [calculated as the total body mass minus the mass of internal organs and digesta ($F_{3,97}$=0.77, P=0.5)], which we used as a proxy for possible changes in carcass fat content.

Cold exposure elicited a twofold increase in C, with most of this increment realized during the first two days of the experiment (Fig. 1A). Throughout the whole experiment, C of H-BMR mice was considerably higher than in L-BMR mice (Table 1; Fig. 1A). However, the scope of cold-elicited increase in C was similar in both lines, as indicated by lack of significant interactions between line-type and experimental group ($F_{3,99}$=2.01, P=0.1).

The increase in C was associated with 25% reduction of D (Fig. 1B). This reduction was significantly larger in L-BMR than H-BMR mice, as indicated by significant interaction between line-type and group affiliation ($F_{3,100}$=4.89, P=0.003). When differences in D were analyzed separately for the first two days of cold exposure and the remaining part of experiment the interaction remained significant only for the former period ($F_{1,53}$=8,76, P=0.004 and $F_{2,77}$=0.99, P=0.4, respectively).

Digestible food intake (C×D) was significantly higher in H-BMR line and was affected by experimental group (Table 1) but there were no significant interactions.

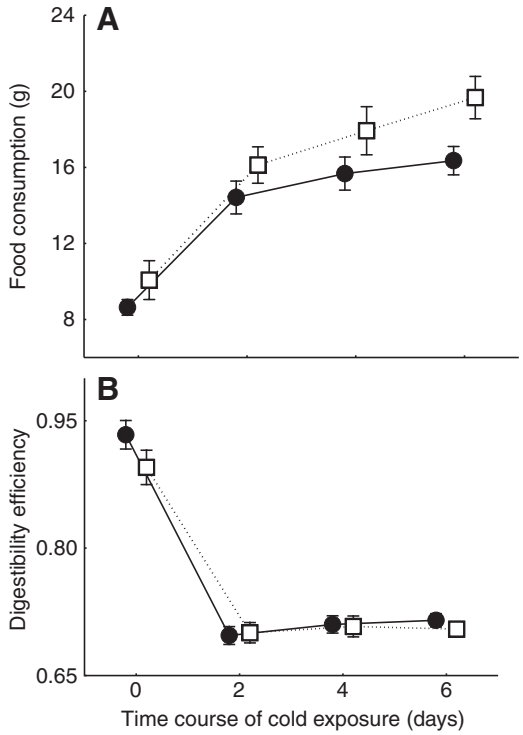

Fig. 1. Food consumption (A) and apparent digestive efficiency (B) in control mice maintained at 23°C (denoted as 0) and exposed to 5°C for two, four and six days, respectively. Values are least-square means from ANCOVA/ANOVA (± s.e.m.). In this and subsequent graphs, closed circles and solid lines indicate low-basal metabolic rate mice (L-BMR) whereas open squares and broken lines indicate high-BMR (H-BMR) mice.

Table 1. Summary of ANCOVA/ANOVA results, standardized between-line differences (*d*) and lower boundary of their 95% confidence interval (CI) for anatomical and physiological traits

	Line-type				Experimental group		Body mass	
	$F_{d.f.}$	*P*	*d*	95% CI	$F_{d.f.}$	*P*	$F_{d.f.}$	*P*
C	$96.91_{1,99}$	<0.0001	2.05	1.12[1]	$227.32_{3,99}$	<0.0001	$35.76_{1,99}$	<0.0001
DFI	$98.06_{1,99}$	<0.0001	–	–	$114.64_{3,99}$	<0.0001	$44.2_{1,99}$	<0.0001
Small intestine	$135.1_{1,99}$	<0.0001	1.88	0.89[2]	$10.5_{3,99}$	<0.0001	$17.8_{1,99}$	<0.0001
Liver	$65.3_{1,102}$	<0.0001	1.82	0.89[2]	$1.0_{3,102}$	0.4	$149.2_{1,102}$	<0.0001
Kidneys	$55.3_{1,99}$	<0.0001	0.94	0.89[2]	$6.7_{3,99}$	0.0004	$78.5_{1,99}$	<0.0001
Heart	$15.4_{1,101}$	0.0002	0.73	0.89[2]	$3.1_{3,101}$	0.03	$70.8_{1,101}$	<0.0001
CS of liver	$7.9_{1,103}$	0.006	0.56	0.56[3]	$21.9_{3,103}$	<0.0001	–	–
CS of kidneys	$6.6_{1,100}$	0.01	0.67	0.56[3]	$6.6_{3,100}$	0.0004	–	–
CS of heart	$9.0_{1,102}$	0.003	0.71	0.56[3]	$1.9_{3,102}$	0.1	–	–

[1]The value of 95% CI calculated for heritability of food intake h^2=0.78 (Bachmanov et al., 2002).
[2]The value of 95% CI calculated for heritability of organ masses h^2=0.4 (Schlager, 1968).
[3]The value of 95% CI calculated for heritability of activity of CS h^2=0.08 (Garland et al., 1990).
Line-type and experimental group were main factors whereas body mass was a covariate. C, food consumption; DFI, digestible food intake.

Changes in mass of internal organs and metabolic loads

An increase in C elicited by exposure to 5°C resulted in sizable between-line differences in metabolic loads [defined as C or DFI (g 2 days^{-1}) per mass of a particular organ] on all internal organs. We demonstrated this effect in two ways. First, ANCOVA with line-type and group affiliation as fixed effects and body mass as a covariate revealed a highly significant effect of line-type on the masses of all examined organs (Table 1). We then visualized metabolic loads as least-square means from the above ANCOVA plotted against the respective mean values of C (small intestine) or digestible food intake (DFI, other organs) of each experimental group (Fig. 2). By using C or DFI, we attempted to take into account the fact that intestines are responsible for most of the processing and absorption of consumed food. Fig. 2 shows that for the same C or DFI, the masses of small intestine, kidneys, heart and liver of L-BMR mice were considerably lower than those of H-BMR individuals. The smaller organs of L-BMR mice were burdened with larger metabolic loads. This conclusion is also strongly supported by a highly significant effect of line-type (P<0.001) on all examined organs in an ANCOVA with line-type as a fixed effect and body mass and C or DFI as covariates. In this analysis, we did not include group as a fixed effect because of its collinearity with C and DFI.

An increase in C elicited by cold exposure was associated with increases (indicated by a significant group effect, Table 1) of the mass of the small intestine (Fig. 2A), kidneys (Fig. 2B) and heart (Fig. 2C), albeit the differences in the latter were not significant after Bonferroni correction. Cold exposure did not affect liver mass

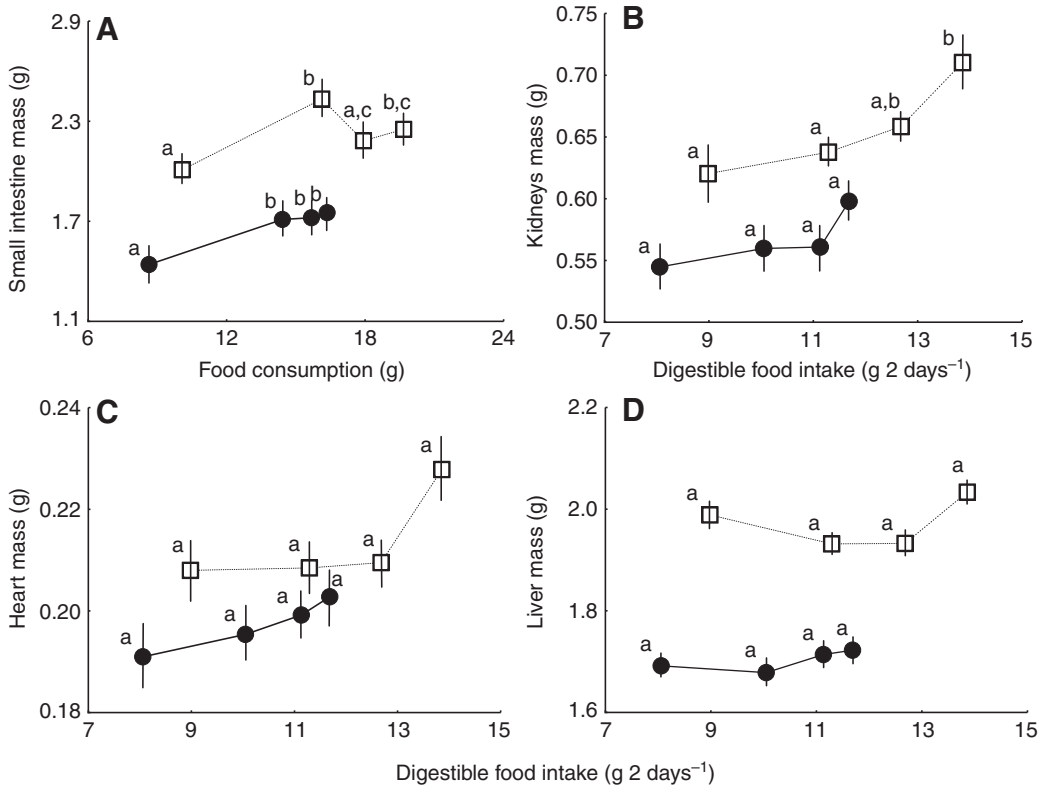

Fig. 2. Metabolic load exerted on small intestine (A), kidneys (B), heart (C) and liver mass (D) following two-, four- and six-day-exposure to 5°C. To demonstrate the magnitude of these loads, the mass of internal organs is plotted against food consumption (small intestine) and digestible food intake (the remaining organs). Here and in Fig. 3 position of points from left to right reflects changes in mass between subsequent two-day periods of the experiment. Values are least-square means from ANCOVA (± s.e.m.) presented in Table 1. Different superscripted characters represent significant differences between groups (within lines) tested by pair-wise *t*-tests, after applying a Bonferroni correction. For further details, see Fig. 1.

(Fig. 2D). In all of these comparisons, the interaction between line-type and group was not significant. It was also non-significant in a comparison of animals maintained at 23°C and those killed just after two days of cold exposure. Thus, the scope of changes in organ masses was similar in both lines and strictly proportional to the between-line differences at 23°C.

Activity of CS

Cold exposure elicited an increase in mass-specific CS activity in liver and kidneys of mice of both lines (Table 1; Fig. 3A,B), although throughout the experiment it was higher in H-BMR mice. By contrast, the activity of CS in heart was not affected by cold exposure (Table 1; Fig. 3C) whereas the effect of line-type was significant, with higher CS activity in L-BMR mice. In all of these comparisons, the interaction between line-type and group was not significant.

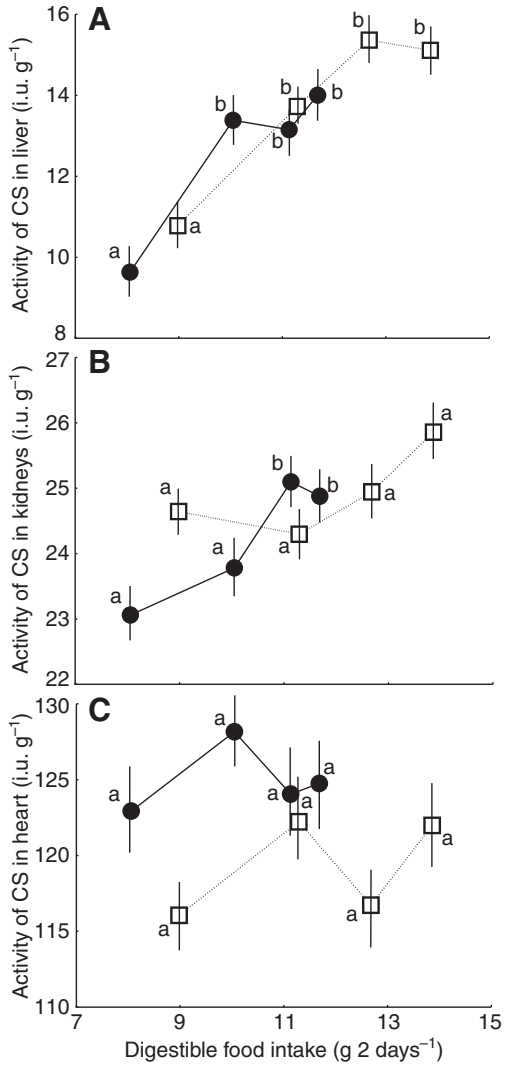

Fig. 3. Activity of citrate synthase (CS) in liver (A), kidneys (B) and heart mass (C) following two-, four- and six-day-exposure to 5°C. Specific activity of CS is expressed in international units defined as μmol substrate transformed to product min^{-1} g^{-1} of organ wet mass. Values are least-square means from ANOVA (± s.e.m.) presented in Table 1. For further details, see Figs 1 and 2.

Effects of selection

When expressed as multiples of the phenotypic s.d. (*d*), the between-line differences in C, the masses of all internal organs as well as the activity of CS in liver, kidneys and heart were equal to or higher than the boundary of the 95% CI estimating the stochastic effect of genetic drift (Table 1). They therefore most probably reflect genetic correlation with the primary, selected trait – BMR.

DISCUSSION

A considerable increase in energy intake, elicited by a sudden metabolic challenge, results in an upregulation of the function of internal organs (Toloza et al., 1991). The mechanistic basis of the upregulation was intensively studied particularly in the context of changing food availability (Secor and Diamond, 1998; Starck and Beese, 2001; Starck and Beese, 2002). However, factors determining the magnitude of this upregulation and its relation to the immediate spare capacities are still poorly known (Starck, 2005). Here, we asked if lines of mice with sizable and genetically determined differences in BMR and the masses of major internal organs differ in their ability to immediately increase energy throughput when faced with a sudden cold exposure.

Like Toloza and colleagues (Toloza et al., 1991), we found that, compared with mice housed at 23°C, mice of both lines exposed to a sudden cold exposure were able to double their C within the first 48 h (Fig. 1A). However, in both lines, the increase in C was accompanied by a 25% decrease in D (Fig. 1B). This was not observed in similar studies (Naya et al., 2005; Toloza et al., 1991), in which the animals were exposed to milder temperatures. Furthermore, Toloza and colleagues (Toloza et al., 1991) maintained their mice on an easily digestible high-sucrose diet whereas we fed our animals a laboratory chow containing 5% cellulose. It is also important to note that to identify the contribution of the immediate spare capacity, one needs to use experimental conditions that force animals to utilize that capacity in full but not force them into negative energy balance. For example, moderate increases in energetic demands trigger changes in digesta distribution within the gut without simultaneous upregulation of organ function (Naya et al., 2005). However, sudden exposure of our mice of both lines to ambient temperatures lower than 5°C not only causes a reduction in D but also in body mass (M.K., unpublished). We therefore argue that the decrease in D observed during first two days of cold exposure and the simultaneous lack of reduction of body mass indicate an exhaustion of immediate spare capacity, which was not associated with the utilization of energy reserves stored, e.g. in fat.

The immediate decrease of D triggered by cold exposure was greater in L-BMR than H-BMR mice, which was indicated by a significant line-type × experimental group interaction (Fig. 1B). This was the first manifestation of the low ability of L-BMR mice to respond to sudden cold exposure through the utilization of an immediate spare capacity of internal organs. This supposition is supported by a comparison of the between-line differences in masses of internal organs. For the same cold-elicited food intake (or DFI), animals from the L-BMR line had significantly smaller small intestine, kidneys, heart and liver than mice from the H-BMR line (Fig. 2A–D). We conclude that, even though L-BMR mice upregulated organ function, they were burdened with larger metabolic loads than those of H-BMR mice. However, the lack of interaction between line-type and experimental group indicates that, in relation to values at 23°C, the scope of the upregulation at 5°C did not differ between lines.

These findings also shed light on the relationship between organ masses prior to a sudden metabolic challenge and immediate spare

capacity. Similar scopes of increase of DFI in the two lines, particularly during the first two days of cold exposure, suggest that the functional elements of the tissues forming the immediate spare capacity of a given organ under non-stressful conditions are strictly proportional to its total mass. Evaluation of the costs of maintenance of immediate reserve capacities of internal organs and their phenotypic flexibility is one of the important and still unresolved problems of physiological ecology (Starck, 2005). Although we did not attempt their precise evaluation, we demonstrated that these costs most probably correlate with variation in BMR – the primary trait under selection in our experiment. We have reported elsewhere that at 23°C the between-line differences in the mass of studied organs are genetically correlated with BMR (Książek et al., 2004). In the present study, we have shown that these mass differences are maintained throughout the course of a sudden and acute cold exposure that elicited substantial size and CS activity increases in most of the studied organs (Fig. 2A–D; Fig. 3) and that the differences are greater than those expected under genetic drift (Table 1). Thus, we conclude that the absolute magnitude of the phenotypic flexibility is, in significant part, genetically correlated with BMR.

We believe that this finding adds an important component to the functional understanding and interpretation of BMR. The advantages of high BMR are mainly discussed in the context of the evolution of endothermy and are thought to be an unavoidable result of an ability to sustain high metabolism (e.g. Bennett and Ruben, 1979; Koteja, 2002). Our results suggest that high BMR confers another advantage: when faced with sudden physiological challenges, immediate spare capacities of individuals with high BMR enable them to consume and process substantially more food than those having low BMR. Metabolic loads exerted on organs of low-BMR mice were significantly larger than those observed in the H-BMR line. So, in absolute terms, both lines of mice did not respond in a similar way to the same metabolic challenge; other things being equal it is reasonable to conclude that low-BMR mice were closer to entering negative energy balance. Thus, even though high BMR is linked with high C (Książek et al., 2004), this energetic cost may turn into a selective advantage when animals are faced with a sudden and unpredictable deterioration of environmental conditions.

The question remains whether the observed increase in organ masses reflects metabolically costly upregulation of function or an inexpensive cellular hypertrophy (Starck et al., 2004). For example, postprandial growth of the small intestine of colubrid snakes does not involve the production of new tissues (Starck and Beese, 2001; Starck and Beese, 2002). However, an increase of the mammalian gut capacity to absorb nutrients is largely determined by the rate of energetically costly cellular replacement (Starck, 2005), with new cells capable of producing more digestive enzymes (Karasov and Hume, 1997). Some studies have not found a correlation between organ masses (e.g. liver, small intestines and kidneys) and BMR (Chappell et al., 2007; Russell and Chappell, 2007; Selman et al., 2001; Speakman et al., 2004). This inconsistency can be attributed to the variability of mass-specific organ oxidative capacity (Vezina and Williams, 2005) or by contributions of other tissues (e.g. avian pectoral muscles) to BMR (Chappell et al., 1999). The changes of CS activity reported here suggest that an increase of kidney mass was accompanied by a considerable increase of mass-specific metabolic intensity (Fig. 2B; Fig. 3B). However, cold-elicited increase in CS activity but not mass may be important in the liver, the largest internal organ (Fig. 2D; Fig. 3A). This corroborates the results of earlier studies showing that upregulation of liver oxidative capacity plays an important role in the response to cold exposure (Villarin et al., 2003) and significantly contributes to metabolic rates (Vezina and Williams, 2005).

The effect of sudden cold exposure on the masses of internal organs and their respective CS activities provides interesting information on their relative contribution to phenotypic flexibility in relation to selection on BMR. The response of secondary traits, such as organ mass, to selection depends on the strength of genetic correlation between the primary (selected) and secondary trait(s), which is partly determined by the respective heritabilities (Falconer and Mackay, 1996). Narrow sense heritabilities (h^2) of internal organ masses are relatively high and reach 0.4 (Schlager, 1968). This explains a substantial, indirect response of internal organ masses to selection on BMR. Heritabilities of the activities of the underlying oxidative capacity of enzymes are thought to be much lower (Garland et al., 1990). If so, the between-line differences in energy assimilation observed in our study should be mainly determined by changes in the frequency of genes related to cell hyperplasia, which should not directly affect the enzymatic activities. This is in contrast to the magnitude of changes in CS activity in liver and kidneys elicited by cold exposure, which were far greater than differences due to line-type (Figs 2 and 3). This suggests that the upregulation of the activity of oxidative enzymes in those organs is only weakly related to genetically determined differences in BMR. The organ-specific nature of the relationship between enzymatic activity, BMR and cold acclimation is also shown by higher CS activity in the heart of L-BMR mice and the absence of cold-elicited changes. Phenotypic plasticity *per se* is heritable (Scheiner, 2002). Our results suggest that the scope of reversible upregulation (phenotypic flexibility) of the mass-specific oxidative capacity of internal organs can respond to artificial and, perhaps also, natural selection.

In summary, we demonstrated that the phenotypic flexibility of internal organs is largely determined by their size/mass and (in the case of kidneys and liver) mass-specific CS activity prior to an increased energy demand, which supports the corollary of Karasov and McWilliams' model (Karasov and McWilliams, 2005). Indirectly, our results suggest that variation in BMR, by definition measured under non-stressful conditions, most probably correlates with the magnitude of the immediate spare capacities that handle the initial phase of the upregulation of the function of internal organs, when challenged with increased energy demands. Finally, the lack of line-type × experimental group interactions points to the additive rather than multiplicative dynamics of the phenotypic flexibility of internal organs.

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