Interaction between organ mass and citrate synthase activity as an indicator of tissue maximal oxidative capacity in breeding European Starlings: implications for metabolic rate and organ mass relationships

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Summary

1. Numerous studies have suggested that basal metabolic rate (BMR) is related to variation in mass of specific 'energy-expensive' organs such as heart and kidney.

2. However, there are inconsistencies among studies with regard to those organs that show positive relationships with BMR, potentially because such studies assume constant organ metabolic intensity or capacity (e.g. oxidative activity).

3. This paper investigates metabolic adjustments (citrate synthase activity) in four key organs (pectoral muscle, heart, kidney and liver) in free-living reproductive female European Starlings (*Sturnus vulgaris*) throughout the breeding season to determine if the cost of egg production results in readjustments in organ mass-specific enzyme activity.

4. Citrate synthase (CS) activity varied in relation to breeding stage and/or year, but this variation was not consistent among different organs. For some organs, total enzymatic activity was directly related to changes in organ mass in relation to breeding stage. For others, increased organ mass was associated with a decrease in mass-specific CS activity, resulting in no net change in total organ oxidative capacity among breeding stages.

5. Even though the liver is actively involved in yolk precursor production during egg formation, no evidence was found for up-regulation of mass-specific oxidative enzyme activity in this organ.

6. Metabolic adjustments at the organ level may occur independently from organ mass changes and this confounding effect may be responsible for part of the inconsistencies found between previous studies in terms of organs mass–RMR relationships.

Key-words: Body composition, BMR, egg production, phenotypic flexibility

Functional Ecology (2005) **19**, 119–128

Introduction

Integrated measures of metabolism, such as basal or resting metabolic rate (BMR and RMR, respectively) or daily energy expenditure (DEE), are often used to describe physiological states or as an indicator of the amount of effort animals put into specific activities (Peterson, Nagy & Diamond 1990; Hammond & Diamond 1997; Piersma 2002). It is generally accepted that BMR – defined as the overall energy consumption of an animal at rest, in a postabsorptive state and at thermoneutrality – reflects the overall maintenance energy cost of complex and often expensive physiological machinery (Piersma 2002). This implies that increased sustained energy demand which results in up-regulation of physiological systems and organ mass will, in turn, induce higher levels of BMR (Hammond & Diamond 1997; Piersma 2002). This theory has led to an increasing number of studies investigating the basis for variation in BMR and RMR through intercorrelations between these variables and the mass of specific organs (see Piersma 2002 for a review). However, the results of these studies are often inconsistent in terms of which organs relate to metabolic rate: differences can occur within single species when comparing different physiological states or between species for a given physiological state (Vézina & Williams 2003). This confusion is perhaps not surprising since internal organ mass variation in birds and mammals has also been interpreted as a response to changes in ecological factors

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such as ambient temperature, reproduction, diet or geographical location (Geluso & Hayes 1999; Hilton *et al*. 2000; Dekinga *et al*. 2001; Hammond, Szewczak & Krol 2001; Vézina & Williams 2002). Phenotypic flexibility, defined as reversible transformations in behaviour, physiology or morphology in response to changes in ecological conditions (Piersma & Drent 2003), may therefore confuse this type of analysis.

However, conflicting findings may also result from the underlying assumptions associated with organ mass–BMR relationships (which would be equally applicable to physiological and ecological studies). Explaining variations in BMR simply through changes in organ mass makes assumptions about constant organ metabolic intensity, that is, that the amount of energy used per unit tissue mass does not vary. However, it is clear that tissue-specific metabolism, and thus tissue energy consumption, might be adjusted with little or no change in tissue mass. As an example, the activity of citrate synthase (CS) – an enzyme commonly used as an index of tissue maximal oxidative capacity (Emmett & Hochachka 1981; Hochachka, Emmett & Suarez 1988) – was reported to be 42% higher in the leg muscle of dominant compared with subordinate male Red Junglefowl, with no differences in overall muscle mass (Hammond *et al*. 2000). Similarly, in Blue-Winged Teal (*Anas discors*) at the end of the moulting period, there is a rapid increase in pectoral muscle CS activity that is of greater magnitude than the mass gain in this organ (Saunders & Klemm 1994). Changes in metabolic intensity coupled with rapid and reversible changes in organ mass in response to variations in ecological conditions (Piersma & Lindström 1997; Piersma & Drent 2003) might therefore occur simultaneously within an organism perhaps even in a compensating manner among different organs. The effect of organ metabolic flexibility can therefore obscure the outcome of ecological studies relating whole organism metabolic rate to variation in organ mass. This important confounding factor has been highlighted many times in animals (Weber & Piersma 1996; Kvist & Lindström 2001; Piersma 2002) including humans (Sparti *et al*. 1997; Hsu *et al*. 2003) and clearly calls for examination of variation of metabolic intensity at the organ level.

This paper investigates metabolic adjustments at the organ level throughout reproduction in European Starlings by comparing individuals at the non-breeding, one-egg and chick-rearing stages, using the enzyme citrate synthase as an indicator of maximal tissue aerobic capacity (Blomstrand, Radegran & Saltin 1997; Wibom, Hagenfeldt & von Dobeln 2002). Production of eggs in starlings induces a 22% increase in mass-corrected RMR compared to prebreeding values (Vézina & Williams 2002). However, the single organ significantly related to laying-RMR, the oviduct, explains only 18% of its variation (Vézina & Williams 2003) and the organs specifically related to RMR in starlings differ depending on whether the individuals are at the non-breeding, one-egg or chick-rearing stage (Vézina & Williams 2003). Thus

in this system, it is possible that the metabolic intensity of some organs is adjusted to sustain the demands of egg production, confounding the relationship between metabolic rate and organ mass. Using birds for which we previously measured RMR, we examine the changes in oxidative capacity of the pectoral muscle, heart, kidney and liver. Pectoral muscle contributes substantially to energy expenditure during exercise (Emmet & Hochachka 1981; Hochachka *et al*. 1988) and represents a significant proportion of overall body mass (19% in non-breeding starlings; F. Vézina unpublished data). Heart and kidneys are thought to be among the most metabolically active tissues and are correlated with BMR interspecifically (Krebs 1950; Schmidt-Nielsen 1984; Daan, Masman & Groenewold 1990). Finally, we include the liver in our analysis because of its important contribution to the egg formation process through yolk precursor production. Despite a high level of hepatic activity directed toward yolk precursor production (Gruber 1972; Griffin & Hermier 1988), liver mass is not related to RMR in egg-laying starlings (Vézina & Williams 2003). Thus we investigate whether egg formation results in mass-independent changes in metabolic activity of organs known for their high aerobic energy use.

Materials and methods

FIELD SITE AND COLLECTION OF BIRDS

Field work was carried out at the Pacific Agri-Food Research Center (PARC) in Agassiz, BC, Canada (49°14′ N, 121°46′ W) under a Simon Fraser University animal care permit (499B), following the guidelines of the Canadian Council on Animal Care. Each year, about 175 nestboxes were checked daily to determine dates of clutch initiation and clutch completion and the laying sequence of eggs. Females caught at the one-egg and chick-rearing stage were taken from their nest-boxes during night-time (generally between 20.00 h and 24.00 h); during late chick-rearing, provisioning females were trap-caught (always within an hour before sunset). Non-breeding females were mist-netted at a barn used as a roosting site. Data presented in this paper were collected in 1999 and 2000 as part of a wider study on the metabolic costs of egg production. Data on RMR and organ mass variation in breeding starlings have been extensively described in previous papers (Vézina & Williams 2002, 2003) and in this paper we restricted our analysis to birds for which we had CS activity and body composition data for the four organs as indicated in the introduction.

REPRODUCTIVE STAGES

For both years, we measured organ CS activity in birds caught at the end of the wintering period (non-breeding, NB; *n* = 19 in 1999 and *n* = 19 in 2000), and the one-egg stage of laying (LY − 1; *n* = 20 in 1999 except for kidney because of the loss of a sample and

n = 23 in 2000 except in heart because of the loss of three samples; LY − 1 sample also includes four individuals caught after laying the second egg and one caught after laying the fourth egg, no difference in CS activity compared with LY − 1 individuals for all organs $P > 0·1$ in all cases). We measured birds at the one-egg stage of laying since this represents peak investments in egg production including fully developed reproductive organs, yolky follicle hierarchy and elevated levels of yolk precursors (Challenger *et al*. 2001; Vézina & Williams 2003). At this point, all birds have laid one egg, the second egg of the clutch is in the oviduct and all the remaining follicles are sequentially developing in the ovary. We obtained data for chick-rearing (CK) birds only in 1999 (n = 20; owing to a liquid nitrogen leak and loss of year 2000 samples). However preliminary analysis of the NB and LY − 1 groups revealed no significant year effect in CS activity in all organs; we therefore consider our 1999 CK data as adequately representing this group for both years.

RESTING METABOLIC RATE, BODY COMPOSITION ANALYSIS AND YOLK PRECURSOR ASSAY

Laying birds have to be considered in an 'active physiological state' because egg production process induces elevated levels of energy consumption (Vézina & Williams 2002). Therefore we consider the term resting metabolic rate most appropriate in the present case and use this term throughout rather than BMR. We measured RMR of all individuals the night following capture, using an open flow respirometry set-up, as described in Vézina & Williams (2002). After RMR measurements, birds were killed by exsanguination under anaesthesia (ketamine : xylasine at doses of 20 mg kg^{-1} and 4 mg kg^{-1}, respectively), and were dissected. Tissues samples (*c*. 300–700 mg) from the pectoral muscle, liver, heart (left and right ventricle) and kidneys were dissected out in this specific order, weighed in a cryovial and deep-frozen in liquid nitrogen within 10 min of the bird's death. We then completed the dissection of these organs to obtain total fresh mass. We also recorded the fresh mass of the reproductive organs (oviduct, follicles and follicle-free ovary) as well as non-reproductive organs (see Vézina & Williams 2003). Tissue samples were stored at −80 °C. Plasma vitellogenin (VTG) and very low density lipoprotein (VLDL) levels were measured as previously reported in Vézina & Williams (2003) and Vézina *et al*. (2003) using vitellogenin zinc and triglyceride kits (Mitchell & Carlisle 1991; Zinc kit, Wako Chemicals, Richmond, VA; interassay CV = 16·3%; Triglyceride E kit, Wako Chemicals; interassay CV = 15·3%).

ENZYME ASSAYS

Sub-samples (*c*. 40–90 mg) of pectoral muscle, heart, kidneys and liver where weighed frozen (±0·0001 g),

minced on ice and homogenized in 9 volumes of ice-cold buffer (20 mM Na$_2$HPO$_4$, 0·5 mM EDTA, 0·2% BSA (defatted), 50% glycerol, 0·1% Triton X-100, pH 7·4). Homogenization was carried out on ice at moderate speed using a VirTiShear (Virtis, Gardiner, NY) Tempest homogenizer (3 × 10 s, 30 s rest) and further sonicated (model Virtis 60,Virtis; 3 × 10 s, 30 s rest). This homogenization buffer allows samples to be stored for an extended period of time without loss of enzyme activity (Mommsen & Hochachka 1994). Citrate synthase (EC 4·1·3·7) maximal activity was measured in a 1 ml reaction volume in a Perkin Elmer UV/visible spectrophotometer (model Lambda 2, Perkin Elmer, Wellesley, MA) using glass cuvettes. The reaction temperature was maintained at 39 °C by a cuvette holder connected to a Lauda K −2/R circulating water bath (Brinkmann Instruments, Westbury, NY). The reaction was initiated by adding oxaloacetate (0·5 mM, omitted for control) to a solution of Tris (50 mM pH 8·4), acetyl CoA (0·15 mM), DTNB (0·15 mM) and 10 µl crude homogenate (re-dilution 1:20 muscle, 1:50 heart, 1:10 liver and 1:10 kidney). Enzyme activity is presented as international units (µmoles of substrate converted to product per minute) per gram of wet tissue, per gram of protein (Bradford protein assay using BSA standard; Bio-Rad, Hercules, CA) and as overall organ CS activity calculated based on organ wet mass.

STATISTICAL ANALYSIS

The data on organ mass and organ enzymatic activity were analysed using ANCOVA models with year and breeding stage as independent variables and non-reproductive body mass (NRBM) as a covariate. We used non-reproductive body mass – which is the fresh body mass minus the mass of the reproductive organs, yolky follicles and oviductal egg – in order to control for the extra weight in LY − 1 females (see Vézina & Williams 2003). This allows for the comparison of birds within the same range of body masses. In order to identify specific differences between breeding stages, we then performed post-hoc multiple comparisons with Bonferroni correction (Rice 1989; *P*-value corrected to 0·016). To examine potential relationships between yolk precursor plasma levels and liver CS activity in LY − 1 birds, we used an unpaired *t*-test to confirm the absence of a year effect in the restricted data set, and then correlations to evaluate the relationship between the variables. To investigate covariance between organ mass and the mass-specific enzymatic activity within groups, we used ANCOVA models relating protein-corrected mass-specific CS activity to fresh organ mass controlling for year. Finally, ANCOVA models were used to study the correlations between RMR and CS activity in all organs controlling for year and NRBM effects. Data are presented as mean ± SE or least squares means ± SE when controlling for year or NRBM.

Results

VARIATION IN ORGAN MASS AND CS ACTIVITY WITH BREEDING STAGE

Pectoral muscle

Fresh pectoral muscle varied in mass between breeding stages ($F_{2,107} = 44\cdot2$, $P < 0\cdot0001$) and this effect was independent of year (no significant year–breeding stage interaction term). However, year and NRBM were significantly related to muscle mass (year: $F_{1,107} = 8\cdot4$, $P < 0\cdot005$; NRBM: $F_{1,107} = 139\cdot5$, $P < 0\cdot0001$; interaction year – NRBM: $F_{1,107} = 9\cdot3$, $P < 0\cdot005$). Controlling for these variables, the breeding stage difference translated into non-breeding individuals having a 7·5% heavier pectoral muscle compared with the average for LY – 1 and CK groups (post-hoc comparison $P < 0\cdot0001$ in both cases; Fig. 1a). Mass-specific CS activity was also affected by breeding stages ($F_{2,98} = 10\cdot2$, $P < 0\cdot0001$; no significant effect of year, NRBM or interaction; see Fig. 1b). Post-hoc comparison revealed a significant difference between NB and LY – 1 individuals ($P < 0\cdot0005$) with the latter showing 10·8% lower CS activity. However, this stage effect was lost when reporting CS activity per gram of tissue protein ($P = 0\cdot2$, no significant effect of year, NRBM or interaction; Fig. 1b). Protein-corrected CS activity was independent of fresh muscle mass for all breeding stages when controlling for year ($P = 0\cdot3$ in all cases). Total pectoral muscle CS activity varied with stage ($F_{2\cdot97} = 22\cdot7$, $P < 0\cdot0001$; no significant year effect) and was affected by NRBM ($F_{1\cdot97} = 18\cdot8$, $P < 0\cdot0001$, no significant interaction). Controlling for the latter, NB birds had total muscle enzymatic activity 14·4% higher than mean activity level for LY – 1 and CK birds (post-hoc comparison $P < 0\cdot0001$ in both cases; Fig. 1c). There was a significant interaction between year and breeding stage in pectoral muscle protein content ($F_{1\cdot96} = 5\cdot9$, $P < 0\cdot05$). However, analysing both years separately revealed that muscle protein content was independent of breeding stage ($P = 0\cdot1$ in all cases).

Heart

Fresh heart mass varied with breeding stage and year (year–breeding stage interaction term; $F_{2,102} = 11\cdot8$, $P < 0\cdot0001$; Fig. 2a). We therefore investigated changes in heart mass separately for both years. Controlling for NRBM, heart mass was 16·3% and 9·4% heavier in NB birds compared with LY – 1 and CK individuals, respectively, in 1999 ($F_{2\cdot55} = 24\cdot5$, $P < 0\cdot001$; Post-hoc comparison $P < 0\cdot005$ in all cases). In 2000, heart mass was independent of breeding stage or NRBM ($P = 0\cdot1$, no significant interaction). CS activity calculated as both mass-specific or corrected for protein content was not affected by year, NRBM or breeding stage ($P > 0\cdot5$ in all cases, no significant interaction; Fig. 2b) and protein-corrected CS activity was independent of fresh heart mass within all breeding stages when controlling
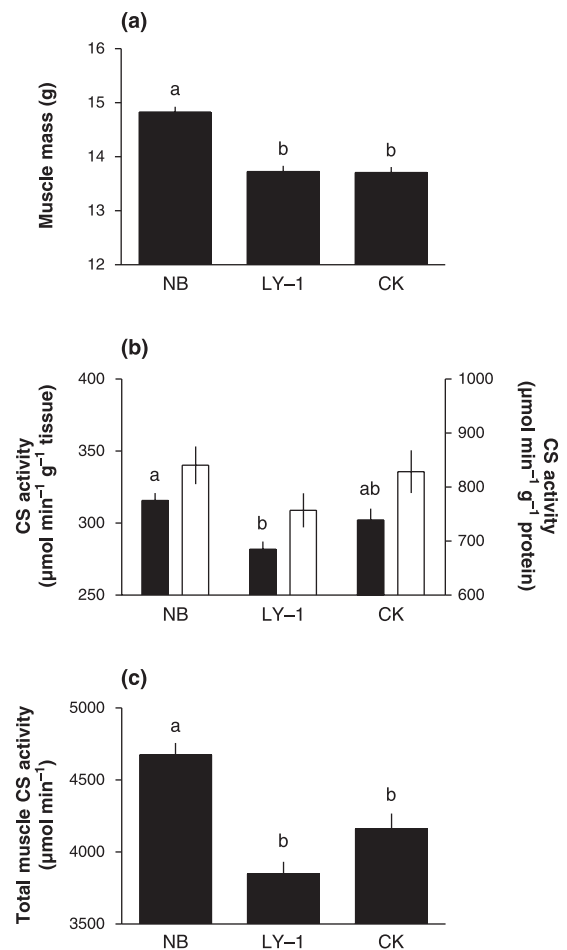

Fig. 1. Variations in (a) fresh mass, (b) mass-specific CS activity and (c) total CS activity for the pectoral muscle in European Starlings. Different letters represent significant differences between breeding stages, no letters indicate no differences. Muscle mass is presented as least square means controlling for the effect of year and non-reproductive body mass. Total muscle CS activity is presented as least square means controlling for the effect of NRBM. NB = non-breeding, LY – 1 = one-egg, CK = chick-rearing. Black bars in (b) are for activity per gram of wet tissue and white bars are for activity per gram of protein.

for year ($P = 0\cdot2$ in all cases). Total heart CS activity was not affected by year or breeding stage when controlling for the effect of NRBM ($P = 0\cdot06$; Fig. 2c). Heart protein content did not change significantly with either year or breeding stages ($P = 0\cdot07$ in both cases).

Kidney

As for the heart, differences in kidney fresh mass between breeding stage was dependent on year (year–breeding stage interaction term; $F_{2,105} = 7\cdot3$, $P < 0\cdot05$; see Fig. 3a). In 1999, when controlling for the effect of NRBM, LY – 1 kidney mass was 11·2% heavier than in NB birds, while CK individuals were intermediate (post-hoc comparison $P < 0\cdot0005$, no significant interaction). In 2000, however, kidney mass was significantly different at all stages and heaviest in LY – 1 individuals with a maximal mass difference of 22·8% between this
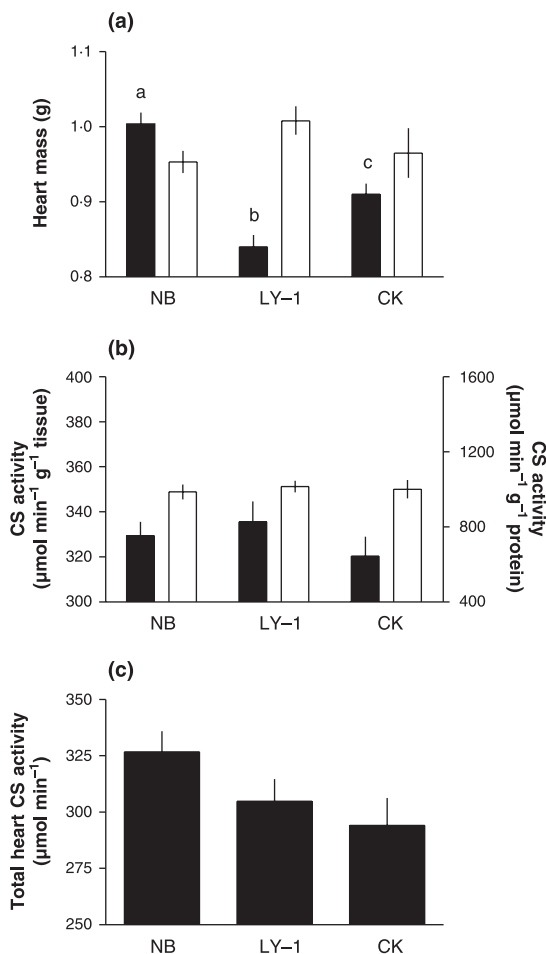
Fig. 2. Variations in (a) fresh mass, (b) mass-specific CS activity and (c) total CS activity for the heart in European Starlings. Different letters represent significant differences between breeding stages, no letters indicate no differences. Heart mass for year 1999 is presented as least square means controlling for non-reproductive body mass (see text). Total heart CS activity is presented as least square means controlling for the effect of NRBM. NB = non-breeding, LY − 1 = one-egg, CK = chick-rearing. Black bars in (a) are for year 1999 and white bars indicate year 2000. Black bars in (b) are for activity per gram of wet tissue while white bars indicate activity per gram of protein.

Fig. 3. Variations in (a) fresh mass, (b) mass-specific CS activity and (c) total CS activity for the kidney in European Starlings. Different letters and numbers represent significant differences between breeding stages, no letters or numbers indicates no significant differences. Kidney mass for year 1999 is presented as least square means controlling for non-reproductive body mass (see text). NB = non-breeding, LY − 1 = one-egg, CK = chick-rearing. Black bars in (a) are for year 1999 and white bars indicate year 2000. Black bars in (b) are for activity per gram of wet tissue while white bars indicate activity per gram of protein.

group and NB birds (post-hoc comparison $P < 0.01$ in all cases, no significant effect of NRBM or interaction). Mass-specific CS activity showed a reversed pattern with the highest level of activity being recorded in NB individuals and lowest in LY − 1 (18·8% difference; post-hoc comparison $P < 0.0001$; overall model $F_{2,97} = 10.55$, $P < 0.0001$, no effect of year, NRBM or interaction, Fig. 3b). When performing this analysis on protein-corrected CS activity, the breeding stage effect was still present ($F_{2,96} = 10.05$, $P < 0.0001$, no effect of year, NRBM or interaction) and showed the same pattern (post-hoc comparison $P < 0.01$; Fig. 3b). Protein-corrected CS activity was independent of fresh kidney mass in NB and CK birds when controlling for year ($P = 0.2$ in both cases). However, there was a positive but weak relationship between the two variables in LY − 1 individuals ($r = 0.3$, $n = 41$, $P < 0.05$; no
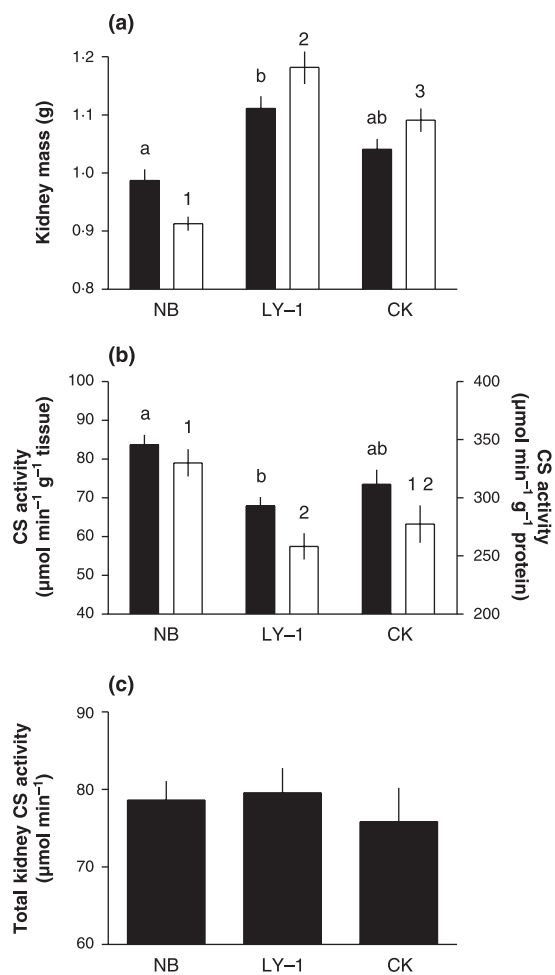
year effect), indicating that increasing kidney mass was associated with higher CS activity per unit mass protein than would be predicted from the simple increase in the overall mass of the organ. Total kidney enzyme activity was not affected by year, NRBM or breeding stage ($P = 0.7$, no significant interaction; Fig. 3c) as the increased kidney mass in LY − 1 and CK birds was counterbalanced by a reduced CS activity. Protein content in the kidney did not change significantly with either year or breeding stages ($P = 0.4$ in both cases).

Liver

Fresh liver mass changed between breeding stages and the difference varied between years (year–breeding stage interaction term; $F_{2,106} = 10.0$, $P < 0.0001$; Fig. 4a). In 1999, when controlling for the effect of NRBM,
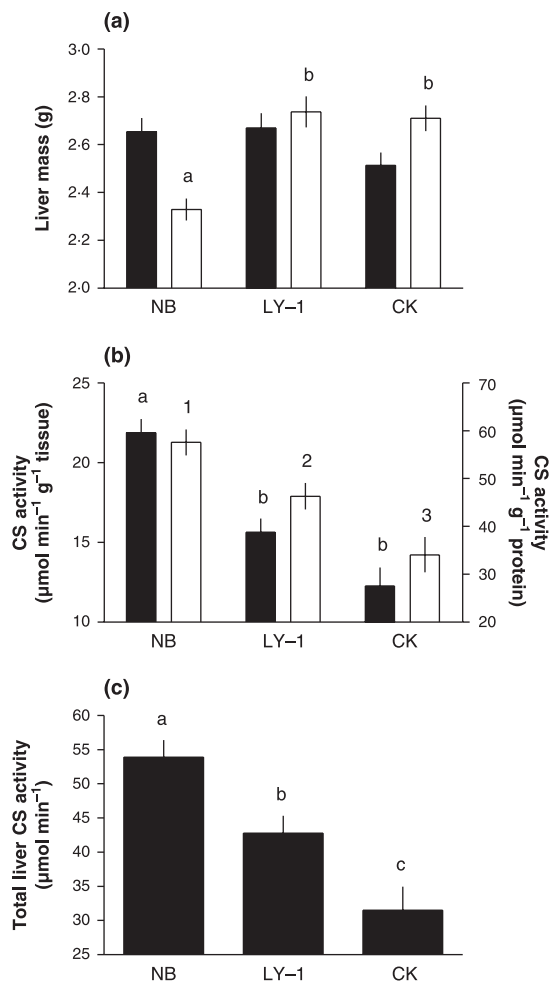
Fig. 4. Variations in (a) fresh mass, (b) mass-specific CS activity and (c) total CS activity for the liver in European Starlings. Different letters and numbers represent significant differences between breeding stages, no letters or numbers indicates no significant differences. Liver mass for year 1999 is presented as least square means controlling for non-reproductive body mass (see text). CS activity per gram or wet tissue or protein as well as total liver enzymatic activity are presented as least square means controlling for an interaction between breeding stage and non-reproductive body mass. NB = non-breeding, LY – 1 = one-egg, CK = chick-rearing. Black bars in (a) are for year 1999 and white bars indicate year 2000. Black bars in (b) are for activity per gram of wet tissue while white bars indicate activity per gram of protein.

liver mass was independent of breeding stage ($P = 0.09$, no significant interaction term) but in 2000, LY – 1 and CK individuals had comparable liver masses that were heavier than that of NB birds (14·5% difference, post-hoc comparison $P < 0.0001$ in both cases; overall model $F_{2,51} = 15.3$, $P < 0.0001$, no NRBM effect or interaction). Mass – specific and protein-specific CS activity were dependent on breeding stage (tissue: $F_{2,95} = 4.9$, $P < 0.01$, protein $F_{2,95} = 4.0$, $P < 0.05$, no year effect in both cases). We also found a significant breeding stage by NRBM interaction (tissue: $F_{2,95} = 5.8$, $P < 0.005$, protein $F_{2,95} = 4.2$, $P < 0.05$) for both measurements. Post-hoc multiple comparison on least square means revealed that mass-specific CS activity

decreased by 33·2% from the NB stage to LY – 1 and CK ($P < 0.0001$ in both cases, Fig. 4b). The same pattern was found for protein-corrected activity, but in this case differences were significant for all groups ($P < 0.01$ in all cases, Fig. 4b). This translated into a 40·8% difference between NB and CK birds. Within breeding stage, protein-corrected CS activity was independent of liver mass in NB and LY – 1 individuals ($P = 0.1$ in both cases), but the variables were positively correlated in CK ($r = 0.6$, $n = 20$, $P < 0.005$). Total liver CS activity was also affected by breeding stage ($F_{2,95} = 3.8$, $P < 0.05$) and an interaction between stage and NRBM ($F_{2,95} = 4.1$, $P < 0.05$). Post-hoc multiple comparison on least square means revealed a significant difference between all stages ($P < 0.01$ in all cases) with a maximal difference of 41·5% in total liver activity between NB and CK individuals (Fig. 4c). Liver protein content was also significantly affected by breeding stage ($F_{2,98} = 3.7$, $P < 0.05$; no significant year effect of interaction term), but this effect was weak as post-hoc multiple comparison failed to find any significant differences ($P = 0.02$ in all cases).

Restricting the analysis to the LY – 1 group allowed for investigation of potential liver metabolic adjustments for yolk precursor production within this group of birds. There was no year effect on fresh liver mass or liver CS activity presented as mass-specific, protein corrected or total (unpaired *t*-test $P > 0.3$ in all cases) within LY – 1 birds. Combining both years, no significant correlations were found between VTG or VLDL plasma levels and organ mass-specific, protein corrected or total liver CS activity ($P > 0.6$ in all cases).

CITRATE SYNTHASE AND RMR

When controlling for the effect of year and non-reproductive body mass, total organ CS activity was independent of RMR for all organs in all groups ($P = 0.2$ in all cases). Pooling the NB, LY – 1 and CK data and including breeding stage in the model did not change this result ($P = 0.3$ in all cases). Investigating a possible relationship between RMR and the summed total CS activity for all organs revealed a significant interaction between year and total CS activity when controlling for breeding stage and NRBM ($F_{1,66} = 4.3$, $P < 0.05$). However, splitting the analysis by year revealed no significant correlations between summed CS activity and RMR when controlling for stage and NRBM ($P > 0.2$ in both years). Redoing this analysis within breeding stage and controlling for year and NRBM did not reveal significant relationships between RMR and summed organ CS activity either ($P > 0.2$ in all cases).

Discussion

This study shows that organ maximal oxidative capacity is not a fixed phenotypic trait. Indeed, CS activity measured as either mass-specific, protein-corrected or total can vary more or less independently from variation

in organ mass. For example, an increase in kidney mass was associated with a decrease in mass-specific CS activity, resulting in no significant change among breeding stages in total organ oxidative capacity. Similarly, in the liver, this effect resulted in an inverse relationship between total CS and organ mass in 1 of 2 years. The organ mass-independent variation in enzyme activity reported here is not simply an effect of variation in tissue protein content acting on the amount of enzyme present in the organ. Indeed, we found stable levels of proteins per unit mass across breeding stages in all organs. Despite a liver actively involved in yolk precursor production and significant changes in organ mass between breeding stages and years (see Vézina & Williams 2003), the oxidative capacity of the organs measured in this study do not appear to be adjusted to the demand of egg production. Furthermore, egg production does not induce up-regulation of the liver maximal oxidative capacity compared with the non-breeding and chick-rearing physiological stages as CS activity actually decreases from non-breeding to chick-rearing.

VARIATIONS IN METABOLIC INTENSITY IN PECTORAL MUSCLE, HEART AND KIDNEY

Total pectoral muscle CS activity was 14% higher in non-breeding birds relative to the average for one-egg and chick-rearing stages, but it appears that this difference was mainly driven by the decrease in muscle mass from NB to CK stages. We did find a significant decrease in mass-specific CS activity from non-breeding to laying but the difference was lost when controlling for the homogenate protein content, suggesting that the change in CS activity per gram of wet tissue had more to do with metabolically inactive cell content than with enzymatic activity per se. This general effect of organ mass affecting total oxidative capacity was also shown in the kidney and to a lesser extent in the heart. In these particular cases, a year effect was detected in organ mass change between stages but not in the enzymatic activity calculated as mass or protein specific. When converted to total organ CS activity, the year interaction was lost. The change in organ mass between breeding stages had an obvious effect in the kidney by buffering the decrease in mass-specific CS activity. In the heart, although not significant, a trend for a decreasing total heart CS activity was probably driven by the strong differences in heart mass between breeding stages in 1999.

Vézina & Williams (2003) reported a correlation between RMR and pectoral muscle mass in non-breeding individuals using a larger sample size from the same starling population. It was suggested that the relationship may have to do with shivering thermogenesis needs as these birds were caught at the end of their wintering period. However, the constant level of protein-specific CS activity across breeding stages reported here suggests that shivering thermoregulation does not involve up-regulation of the pectoral muscle oxidative capacity. Accordingly, CS activity was shown to be constant in the pectoral muscle of both summer- and winter-acclimatized American Goldfinches (*Carduelis tristis*; Marsh & Dawson 1982) and House Finches (*Carpodacus mexicanus*; Carey *et al.* 1988). Higher mass-specific levels of muscle CS activity have been documented in trained rats (Garrido, Guzman & Ordiozola 1996; Matsuo *et al.* 1999) and active lizards (John-Alder & Joos 1991) and also in migrating birds where it has been attributed to the high energy consumption of the muscle during flight (Lundgren & Kiessling 1985, 1986; Lundgren 1988). Our protein-specific CS activity data would therefore suggest that the loco-motor activity level in our birds at the three breeding stages was similar in terms of muscle aerobic demands. Alternatively, breeding starlings may change the total flight duration per day while maintaining a constant muscle power output that could then result in change in muscle mass but constant CS activity.

Mass or protein-specific heart CS activity did not change between breeding stages and there was no significant differences in total heart oxidative capacity even though the mass of this organ varied significantly among breeding stages in 1999. The physiological changes associated with the passage from non-breeding to egg-producing and to chick-rearing stages therefore does not seems to require large metabolic adjustments in the heart in starlings. We found a much different response in the kidney, where kidney mass in egg-laying and chick-rearing birds increased compared with non-breeding individuals (Vézina & Williams 2003; this study). However, a lower mass-specific maximal CS activity meant that total kidney oxidative capacity did not change between breeding stages. The reported phenomenon is not simply a dilution effect due to a larger proportion of water or metabolically inactive content in the tissue since results on mass-specific CS activity corrected for protein content showed the same pattern. Furthermore, the change in kidney mass reported by Vézina & Williams (2003) were based on lean dry mass. Vézina & Williams (2003) suggested that the gain in kidney mass may be a response to a gradual increase in the protein content of the diet during the transition from winter to summer as the birds may eat an increasing proportion of insects through time (Feare 1984). However, to our knowledge, there is no information on the effect of diet on oxidative capacity of kidney in birds. It seems plausible that there is a need for a seasonally associated structural reorganization in this organ and this may occur more or less independently from the cell metabolic adjustment, leading to a larger organ with a slower pace.

EFFECT OF EGG PRODUCTION ON ORGAN OXIDATIVE CAPACITY

Vézina & Williams (2003) suggested that mass-specific metabolism of non-reproductive organs could be adjusted independently of organ mass in order to sustain

metabolic demands of reproduction. Our present study clearly showed that metabolic adjustments can happen within organs independently of their mass. However, we found no support for adjustments in non-reproductive organs in relation to egg production, as pectoral muscle, heart and kidney did not show significant reduction or increase in total CS activity during egg production. We were interested in liver oxidative capacity because of its role in biosynthesis of yolk precursors during egg formation (Williams 1998; Challenger *et al*. 2001; Vézina & Williams 2003). Vézina *et al*. (2003) suggested that the increased biosynthesis activity of the liver, and thus its potentially elevated energy consumption, may be counterbalanced by simultaneous down-regulation of other systems or organs leading to reallocation of energy, with little effect on RMR. We found no support for this hypothesis in this study. If liver activity is up-regulated in terms of aerobic capacity for the process of yolk precursor production, we would have expected a peak in the liver's CS maximal activity at the one-egg stage compared with non-breeding or chick-rearing individuals. Surprisingly, we found a marked decrease in the enzyme activity over the three breeding stages. This translated into a 42% decrease in total liver oxidative capacity even while there was a 15% increase in the organ mass in LY − 1 and CK individuals in one year. Clearly, yolk precursor production in wild starlings does not necessitate an up-regulation of the liver's aerobic machinery compared with the other physiological stages. There are no studies that we are aware of that reported liver CS activity in relation to breeding.

IMPLICATIONS FOR BMR *vs* ORGAN MASS RELATIONSHIPS

Organs such as the heart and kidneys that were proposed to explain part of the interspecific variation in BMR in chick-rearing birds (Daan *et al*. 1990) typically represent less than 1% of overall body mass in birds and mammals (Schmidt-Nielsen 1984; Daan *et al*. 1990). Early tissue slice respiration experiments (Krebs 1950) suggested that these tissues were some of the most metabolically active within the body (although these experiments should be viewed with caution, see chapter 8 in Schmidt-Nielsen 1984). Therefore, a small variation in the mass of these organs is likely to have an impact on overall resting energy consumption compared with the large proportion of inactive or low metabolic rate tissues (water, plumage, fat, bone, skin, etc.; Schimidt-Nielsen 1984; Daan *et al*. 1990). Why then, are we not finding a common pattern among studies in terms of which organ correlate with BMR?

Organ mass–BMR relationships do not take variation in organ metabolic intensity into account. Our study clearly demonstrates that, for a given species, changes in organ mass among physiological stages may also be accompanied by more or less independent variations in organ maximal oxidative capacity. These changes may even occur in opposite directions resulting in no variation in total organ capacity (kidney in the present study) or a completely inverse pattern (1999 liver in this study). Because CS activity represents the maximal oxidative capacity of the tissue, correlation with metabolism, if any, should naturally be expected for maximal metabolic rate (Vo_2max) rather than BMR or RMR (Emmett & Hochachka 1981). However, it is thought that supporting a high level of maximal metabolism requires more cellular metabolic machinery (including more enzymes; Rolfe & Brown 1997) resulting in elevated levels of energy consumption at rest, mostly because of protein turnover (12–25% of BMR in mammals; Rolfe & Brown 1997). Therefore, if BMR reflects the 'physiological maintenance cost', one would assume that maintaining a high level of enzyme activity needed for increased levels of sustained performance should also be associated with higher basal or resting metabolic rate. We have shown that, for a given physiological stage, organ mass does not affect CS activity per unit mass protein (except in two cases where there was a positive relationship). This means that for a given stage, individual variation in organ mass should be accompanied by positively correlated variation in total oxidative capacity (i.e. larger organs have higher total oxidative capacity), suggesting that within a physiological stage, an increase in the mass of a high-intensity organ may truly result in an elevation of BMR. However, overall organ metabolic activity can be very different from one stage to another and this may or may not be associated with a change in organ mass. Therefore, a given organ may have a very different metabolic rate at different times and thus its relationship with BMR may be apparent only at certain specific points in the life cycle of the animal. Our data may thus explain some of the inconsistencies regarding which organs are significantly related to BMR in the literature (see Table 1 in Piersma 2002).

Recently, attention has focused on the role that varying ecological conditions play in reversible physiological adjustments, i.e. phenotypic flexibility (Piersma & Lindström 1997; Piersma 2002; Piersma & Drent 2003). These adjustments can occur at many levels of organization and be relatively independent of each other. In terms of an animal's energy budget, it must be remembered that metabolic flexibility at the organ level (metabolic intensity) may confound the interpretation of the variability recorded at the whole animal level. Our study highlights the importance of considering variation in tissue metabolic intensity, as well as variation in organ mass in the interpretation of changes in basal or resting metabolic rates, both for differing ecological conditions and physiological states that the animal experiences. It is already well known that organ mass varies in response to various ecological factors (Geluso & Hayes 1999; Vezina & Williams 2002; Hammond, Szewczak & Krol 2001; Hilton *et al*. 2000). Here we show that these changes in organ mass do not necessarily mean a change in organ energy consumption, and this

is especially important for studies making assumptions about organ mass variations to interpret measured changes in energy expenditure or vice versa (see for example Hilton *et al.* 2000; Nilsson 2002). Findings for one specific ecological or physiological condition cannot simply be extrapolated to other conditions, and this should be taken into account in future evolutionary and ecological studies investigating the fitness consequences of energy management strategies.

Acknowledgements

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