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Interaction between organ mass and citrate synthase activity as an indicator of tissue maximal oxidative

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 bolic

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 $P > 0{\cdot}1$ 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.

Results

VARIATION IN ORGAN MASS AND CS ACTIVITY WITH BREEDING STAGE

Pectoral muscle

Fresh pectoral muscle varied in mass between breeding stages ($F_{2,107}$ = 44·2, P < 0·0001) 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: $F_{1,107}$ = 8·4, P < 0·005; NRBM: $F_{1,107}$ = 139·5, P < 0·0001; interaction year – NRBM: $F_{1,107}$ = 9·3, P < 0·005). 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 < 0·0001 in both cases; Fig. 1a). Mass-specific CS activity was also affected by breeding stages ($F_{2,98}$ = 10·2, P < 0·0001; 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 < 0·0005) 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 = 0·2, 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 = 0·3 in all cases). Total pectoral muscle CS activity varied with stage ($F_{2·97}$ = 22·7, P < 0·0001; no significant year effect) and was affected by NRBM ($F_{1·97}$ = 18·8, P

group and NB birds (post-hoc comparison $P < 0{\cdot}01$ 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 $P < 0{\cdot}0001$; overall model $F_{2,97} = 10{\cdot}55$, $P < 0{\cdot}0001$, 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 ($F_{2,96} = 10{\cdot}05$, $P < 0{\cdot}0001$, no effect of year, NRBM or interaction) and showed the same pattern (post-hoc comparison $P < 0{\cdot}01$; Fig. 3b). Protein-corrected CS activity was independent of fresh kidney mass in NB and CK birds when controlling for year ($P = 0{\cdot}2$ in both cases). However, there was a positive but weak relationship between the two variables in LY – 1 individuals ($r = 0{\cdot}3$, $n = 41$, $P < 0{\cdot}05$; 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 ($P = 0{\cdot}7$, 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 ($P = 0{\cdot}4$ in both cases).

Liver

Fresh liver mass changed between breeding stages and the difference varied between years (year–breeding stage interaction term; $F_{\text{ecl.0419 62Tm (= 10·05,) Tj /F2 1 Tf 0.5}}$

liver mass was independent of breeding stage ($P = 0{\cdot}09$, 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 $P < 0{\cdot}0001$ in both cases; overall model $F_{2,51} = 15{\cdot}3$, $P < 0{\cdot}0001$, no NRBM effect or interaction). Mass – specific and protein-specific CS activity were dependent on breeding stage (tissue: $F_{2,95} = 4{\cdot}9$, $P < 0{\cdot}01$, protein $F_{2,95} = 4{\cdot}0$, $P < 0{\cdot}05$, no year effect in both cases).We also found a significant breeding stage by NRBM interaction (tissue: $F_{2,95} = 5{\cdot}8$, $P < 0{\cdot}005$, protein $F_{2,95} = 4{\cdot}2$, $P < 0{\cdot}05$) 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 ($P < 0{\cdot}0001$ in both cases, Fig. 4b). The same pattern was found for protein-corrected activity, but in this case differences were significant for all groups ($P < 0{\cdot}01$ 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 ($P = 0{\cdot}1$ in both cases), but the variables were positively correlated in CK ($r = 0{\cdot}6$, $n = 20$, $P < 0{\cdot}005$). Total liver CS activity was also affected by breeding stage ($F_{2,95} = 3{\cdot}8$, $P < 0{\cdot}05$) and an interaction between stage and NRBM ($F_{2,95} = 4{\cdot}1$,

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 locomotor 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 (Véz3.9s

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