Compensation in resting metabolism for experimentally increased activity

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Abstract To study zebra finch allocation of energy to day and night at two different workloads, we assessed the daily energy turnover from: (1) metabolizable energy of the food, and (2) doubly-labeled water. In both experiments we imposed two levels of activity on captive zebra finches (*Taeniopygia guttata*), by applying different computer-controlled workload schedules. A low workload required 20 hops, and a high workload 40 hops to obtain 10 s access to food. In experiment 1, we further measured nocturnal energy expenditure by overnight oxygen consumption. From experiment 2 we derived an estimate of the costs of hopping activity, from inter-individual association of daily amount of hopping and daily energy expenditure. Surprisingly, the daily energy budget was, on average, reduced slightly when birds were subjected to a high workload. Since hopping activity was 50% higher during the high workload than during the low workload, the birds apparently compensated, even over-compensated, for the increased energetic demands of activity. Nocturnal energy expenditure was indeed reduced for the high workload, which was largely due to a reduction in resting metabolic rate. Economizing on energy was more than could have been accomplished by a reduction in mass alone, and we discuss the occurrence and potential mechanisms of physiological compensation. The amount of energy saved during the night did account for part of the total amount of energy saved. We surmise that the strategy of energetic compensation observed during the night was extended into the inactive hours of the day.

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Introduction

To study the trade-off between current and future reproduction, brood sizes of birds have been experimentally manipulated; parent birds are thereby subjected to an increase in energetic demands (Williams 1987; Dijkstra et al. 1990; Deerenberg et al. 1995), and can respond with two strategies to balance the energy budget: (1) adjust the intake of energy, and (2) compensate, i.e., reduce other energy intensive activities or processes. Compensation may be achieved by behavioral adaptation in time-budget, or choice of environment, and/or by physiological mechanisms.

Brood size manipulation experiments with great tits (*Parus major*, Verhulst and Tinbergen 1997) and starlings (*Sturnus vulgaris*, J. Wright personal communication) have demonstrated an increase of parental feeding rates with brood size. They failed to show a positive relation between brood size and parental energy turnover rates, as established by stable isotopes. In both studies, raising an experimentally manipulated brood entailed fitness costs due to reduced incidence of second clutches. In a study with captive zebra finches (*Taeniopygia guttata*) it was demonstrated that parents face a time cost after raising a manipulated brood. This cost of reproduction was associated with energy turnover of females during the nestling phase, which increased with experimental brood size; energy turnover of male parents, however, was not related to experimental brood

size or subsequent costs (Deerenberg 1996). A comparable time cost was observed following episodes of increased activity not related to parental care; at a higher level of activity, daily food intake of experimental birds was reduced, indicating a reduced daily energy budget (Deerenberg 1996). We hypothesize that this reduced level of energy turnover was achieved by energetic compensation. Such compensation of excess energy expenditure during activity may have important consequences. In time-energy-budget approaches in behavioral ecology, it is usually assumed that each behavioral state has a fixed energetic cost and that time devoted to different behaviors can be directly translated into energy (e.g., Schartz and Zimmerman 1971; Masman et al. 1988); this assumption would be rendered invalid by compensation. We therefore explored the occurrence and the nature of compensation in experiments where hopping activity of zebra finches was manipulated. We report on the overall level of energy expenditure in relation to activity, allocation of energy to day and night, and resting metabolism.

Materials and methods

Adult zebra finches ($n = 26$) with breeding experience were kept individually in cages which were placed in a room with a constant temperature of 25 °C, relative humidity of 60%, and in a light-dark cycle of 14:10 (lights on from 0800 hours to 2200 hours). Each cage had two perches 56 cm apart, with no nest or special roosting place. Hopping activity between the two perches was recorded continuously (DataQuest System III, Data Sciences) as interruptions of an infra-red beam placed in front of one of the perches. Spontaneous activity was about 2000 hops per day. The birds had drinking water ad libitum, and a mixture of dry seeds was supplied according to an experimental protocol (see below). Food was provided through an automatic feeder, and access to food was controlled by a central processing unit (Series III System, GE). The birds were weighed once a week, and food was weighed daily to assess gross food intake.

Experiment 1

The experimental protocol

Experiment 1 comprised three 4-week episodes of individual exposure to a constant workload, with ca. 5 weeks between the episodes during which the birds were allowed to breed (Deerenberg 1996). We imposed two workloads: a low workload consisting of 20 hops for each 10 s access to the feeder, and a high workload of 40 hops for 10 s access to food. In this experiment, we estimated the total energy budget from metabolizable energy of the food (see below). Nocturnal energy expenditure (Enight) (see below) was measured twice for each bird during each workload episode. After completion of a workload episode, pairs were reunited and reproductive behavior was recorded (see Deerenberg 1996). A few days after hatching of the eggs, we removed the brood and separated male and female again to expose them to a new episode of work. All birds were maintained on both workloads in the sequence low-high-low ($n = 6$), or high-low-high ($n = 7$), thus allowing intra-individual comparisons.

Metabolizable energy intake

Daily metabolizable energy (DME) was assessed from gross daily food intake, recorded for all birds and translated to metabolizable energy on the basis of food balance trials. We measured the food balance of seven individuals, two on a low workload and five on a high workload, to assess metabolizable energy of the food. We assessed energetic density of complete seeds, hulls (not eaten), and feces using bomb calorimetry. During the trials, the amount of seeds taken was measured by weighing food supply daily, while the remaining seeds, hulls and feces produced were also collected and weighed; there were no seeds left outside the feeder. Hulls accounted for 29% of the weight of seeds. Energy content of seeds was 19.95 kJ·g^{-1}, and of hulls, 18.09 kJ·g^{-1}. One gram of seeds thus provided 14.70 kJ of energy. On average 0.08 g (SD = 0.02) dry weight of feces was produced per gram of seeds taken, with an energy density of 18.2 kJ·g^{-1} (SD = 0.06). Metabolizable energy amounted to 13.19 kJ·g^{-1} (SD = 0.26) per gram seeds taken. Assimilation efficiency (average 0.90) did not differ between the workloads (Kolmogorov-Smirnov test for small samples, Siegel and Castellan 1988: $D_{2,5} = 13.5$, critical value $c_{0.10} = 10$).

Enight and resting metabolic rate

Enight was measured as oxygen consumption in an open air flow system during the nocturnal period of the experiment (from 2200 hours until 0800 hours). Birds were placed in a metabolic chamber of 2 l, in darkness at 25 °C. We conducted the overnight respirometry measurements at this temperature, which is below thermoneutrality (29.5–40 °C, Calder 1964); we kept the birds at this temperature throughout the experiment. Dry air was pumped through the chamber, with flow rates set at ca. 20 l·h^{-1}. Flow rate was measured on the outlet air with a mass-flow controller (type 5850E Brooks) to an accuracy of 0.1%. Outflowing air was dried over a molecular sieve (3 Å, Merck). Gas analysis was done with a zirconium oxide sensor (S3A/II Applied Electrochemistry) to an accuracy of 0.01%, and recorded automatically at 6 min intervals. Oxygen consumption was calculated using equations from Hill (1972), and converted to energy expenditure (kJ) on the basis of an appropriate respiratory quotient (RQ) for granivorous birds, of 0.83 (Gessaman and Nagy 1988), and an energy equivalent of 20.24 kJ·l^{-1} O$_2$. Resting metabolic rate (RMR) was calculated as the lowest value of a 30 min running mean.

Experiment 2

The experimental protocol

In this experiment the same birds were exposed again to a low and a high workload, each for 2–3 weeks, without intermittent breeding. We estimated the total energy budget from energy expenditure using the turnover of stable isotopes (see below). We also assessed the energy costs of activity from the relationship between number of hops and energy expenditure; all birds were exposed again to both workloads.

Energy expenditure

We measured daily energy expenditure (DEE) of each bird on both low and high workloads with the doubly labeled water (DLW) technique (Lifson and McClintock 1966, Nagy 1980) over 48 h. The birds were injected intraperitoneally with approximately 0.15 ml of a mixture of H$_2$^{18}O and ^{2}H$_2$O; initial blood samples were taken 1 h after injection. The zebra finches stayed in their home cages during the interval between injection and initial blood sampling. The isotopic enrichments of the blood samples were determined by means of mass spectrometry, and corrected for natural isotopic abundance (background) in the body water. Total body water volume was assessed from the dilution space of ^{18}O (Schoeller et al. 1986), using the extrapolation method. Carbon dioxide production was calculated using formulas given by Lifson and McClintock (1966). DEE was then calculated using an RQ of 0.83, and an energetic equivalent of CO$_2$ of 24.4 kJ·l^{-1}, based on

nutrient catabolism of a typical granivorous diet with 15% protein (Gessaman and Nagy 1988). The seed mixture fed to the zebra finches contained 14.4% of dry weight protein (Kjeldahl analysis). For a detailed description of application of the DLW-method to zebra finches, see Deerenberg (1996).

Data analysis

Average differences between the two workloads were tested intra-individually using paired *t*-tests. We estimated the relations of energy expenditure (DEE and RMR) with workload, hopping activity and/or body mass with ANCOVA using a repeated measurements design.

Results

Workload and the daily energy budget

The average daily hopping activity, body mass and food intake during both low and high workloads are listed for both experiments in Table 1. In accordance with the aim of the imposed workloads, daily hopping activity was significantly higher during the high workload than during the low workload. While activity was increased on the high workload, both body mass and daily gross food intake (g seeds and hulls removed from feeder) were reduced in both experiments. To examine whether body mass remained stable during each workload episode, changes in body mass were analyzed using a repeated measurements ANOVA test on weekly averages. Multivariate tests (Wilks) indicated that there was no significant variation among the weekly measures ($F = 2.46$, $df = 3,28$, $P = 0.08$), while average body mass differed between the two workloads ($F = 3.47$, $df = 3,28$, $P = 0.03$). This result suggests an active adjustment of mass to the current workload, rather than an effect of an unbalanced diet requiring the mobilization of stored reserves.

In experiment 1 the daily energy budget was estimated from DME intake only; DME was calculated from gross daily food intake using conversion factors obtained in the food balance trials. Surprisingly, the average food intake and thus DME was smaller on the high workload (Table 1), but DME data may have been slightly biased either way due to unobserved changes in assimilation rate.

In experiment 2, DME was lower than in experiment 1 on both low and high workloads. This probably resulted from lower average body masses in combination with lower hopping rates during the second experiment (Table 1), considering that both mass and activity were major contributors to the energy budget. In addition to DME, in experiment 2 we estimated the daily energy budget by assessing DEE using the DLW method. We successfully obtained data on the turnover of isotopes from 11 birds on the two workloads. Both parameters, intake and expenditure of energy, corresponded closely (low workload: $r = 0.73$, $P = 0.01$; high workload: $r = 0.79$, $P < 0.01$), although estimates of DEE were consistently higher than those of DME (Fig. 1).

Energy costs of hopping activity

To assess the impact of the experimentally manipulated activity levels on the daily energy budget, we examined the contribution of hopping activity to DEE during the
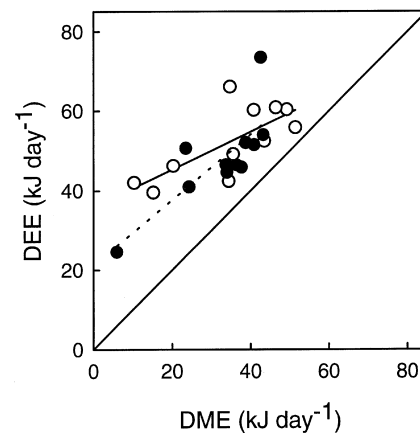

Fig. 1 Association of daily energy expenditure (DEE) and daily metabolic energy (DME) (converted from daily food intake). *Open symbols* represent birds on the low workload schedule, *filled symbols* the same birds on the high workload schedule

Table 1 Descriptives of workloads. Values are given as means ± SD. (*DEE* daily energy expenditure, *DME* daily metabolic energy)

Experiment	Workload	Low	High	T	*P*
1	Sample size	13	13		
	Activity (hops · day^{-1})	5904 ± 2154	8497 ± 2618	5.60	< 0.001
	Body mass (g)	13.84 ± 1.40	13.36 ± 1.34	3.36	< 0.01
	Food intake (g · day^{-1})	3.75 ± 0.44	3.39 ± 0.37	4.20	= 0.001
	DME (kJ · day^{-1})	49.43 ± 5.86	44.73 ± 4.89		
2	Sample size	11	11		
	Activity (hops · day^{-1})	5050 ± 1969	6506 ± 2329	2.22	= 0.05
	Body mass (g)	13.54 ± 1.25	13.18 ± 0.85	1.38	ns
	Food intake (g · day^{-1})	2.63 ± 1.05	2.49 ± 0.84	0.46	ns
	DME (kJ · day^{-1})	34.70 ± 13.89	32.80 ± 11.06		
	DEE (kJ · day^{-1})	52.27 ± 9.00	48.23 ± 11.56	1.20	ns

48 h of the DLW measurements in experiment 2. Most birds lost weight during the DLW measurements, on average −0.52 g (SD = 0.46) and −0.55 g (SD = 0.70) over 48 h for low and high workloads respectively; thus they derived energy from body mass in addition to energy derived from food. However, the DLW-method assessed the turnover of energy, regardless of its source. The marginal cost of hopping was therefore estimated from the slope of the regression of DEE on the number of hops per day.

Among individuals, average DEE over the two workloads increased with average hopping activity ($F = 4.76$, $df = 1, 9$, $P = 0.06$) with a coefficient of 2.85 J per hop. Within individuals, an increase in workload effected an increase in DEE of 6.85 kJ$\cdot$d^{-1} ($F = 9.62$, $df = 1,9$, $P = 0.01$), which was associated with concomitant differences in hopping activity between the workloads ($F = 9.29$, $df = 1,9$, $P = 0.01$); the coefficient of the intra-individual regression yielded an estimation of 3.76 J per hop.

Enight and RMR

We estimated energy expenditure from oxygen consumption over complete dark phases (2200–0800 hours) of experiment 1. Average Enight was 11.70 kJ (SD = 0.87, $n = 13$) and 10.33 kJ (SD = 1.25, $n = 13$) for low and high workloads respectively. The within-individual reduction on the high workload was significant ($t = 3.74$, $P < 0.01$). This reduction may have come about behaviorally by a reduction in nocturnal activity. In order to judge whether the metabolic rate was also physiologically reduced in episodes of complete inactivity, we determined the RMR, while taking the contribution of reduced body mass into account. Average RMR was 0.282 Watt (SD = 0.02), and 0.231 Watt (SD = 0.03) during low and high workload, respectively; RMR was significantly reduced on the high workload ($t = 5.01$, $P < 0.001$). Within-individual differences in RMR between the workloads were apparently achieved not only by the observed reduction in body mass of, on average, 0.56 g ($F = 14.69$, $df = 1,11$, $P < 0.01$), but in combination with an additional reduction due to workload ($F = 11.18$, $df = 1,11$, $P < 0.01$; Fig. 2).

Discussion

Nocturnal and diurnal compensation?

We experimentally manipulated workload by changing the amount hopping activity of zebra finches. Hopping activity on the high workload was indeed higher than on the low workload, but, in spite of an increase in diurnal energy expenditure due to hopping activity, the daily energy budget was not increased on the high workload. On the contrary, the DME based on daily food intake in
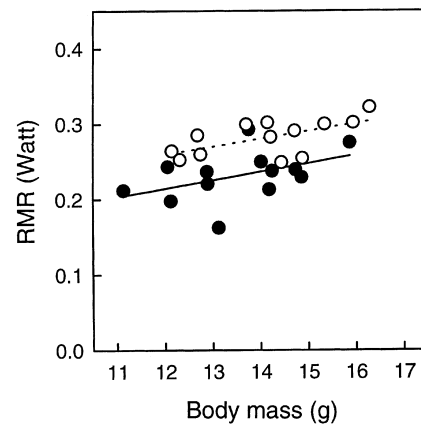

Fig. 2 Variation in resting metabolic rate (RMR) as a function of body mass and workload. *Open symbols* indicate birds on a low workload, *filled symbols* the same birds while exposed to a high workload

Experiment 1 showed a significantly reduced energy budget on the high workload, while the DEE in experiment 2 was indistinguishable between low and high workloads. The birds apparently (over-)compensated for the experimentally manipulated increase in energy expended on activity.

The zebra finches partly compensated behaviorally for the increased workloads imposed on them. It has been shown elsewhere (Deerenberg 1996) that the birds increased their efficiency – by reducing the surplus amount of hopping over the required amount (40 hops) – while subjected to the high workload. We evaluated the effect of the imposed workloads by daily hopping rates. While imposed rates had a ratio 1:2, based on 20 or 40 hops required to gain access to the feeder, the actual number of hops showed an average ratio of 1:1.5. Birds were observed to be very efficient indeed, because they hardly spent any time on the floor, thereby escaping registration of activity, and because they had quickly learned during the initial entrainment to the workloads to react to the noise of the motor opening the feeder before descending to feed (personal observations).

To estimate the full extent of physiological compensation, we reconstructed allocation of energy to daytime activity (Eda) and of Enight for the low and the high workload in both experiments. In experiment 1, DME and Enight were measured directly; the difference (37.7 kJ$\cdot$d^{-1} for low workload; 34.4 kJ$\cdot$d^{-1} for high workload) represents the energy expended in daytime (Ed). When we apply the lowest (inter-individual) estimate of the marginal cost of activity (2.85 J hop^{-1}), the energy allocated to Eda becomes 16.8 kJ$\cdot$d^{-1} for low workload and 24.2 kJ$\cdot$d^{-1} for high workload. The difference (Ed-Eda) is the estimate for the energy allocated to inactivity during daytime (Edr): 20.9 kJ$\cdot$d^{-1} for low workload 10.2 kJ$\cdot$d^{-1} for high workload, representing a reduction of 51% (Fig. 3, left). In experiment 2, DEE was measured directly and we applied the data from experiment 1 for Enight, which yielded estimates of
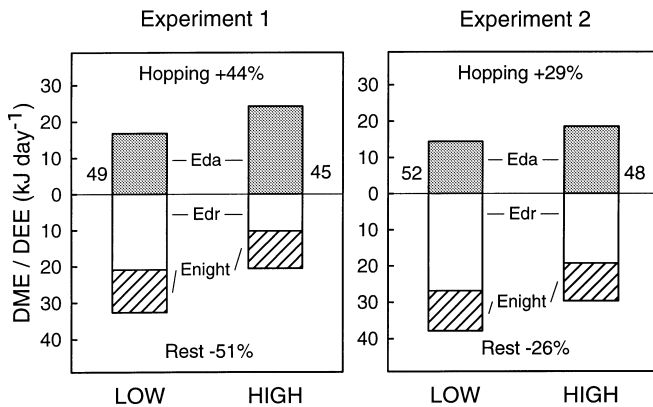
Fig. 3 Allocation of daily energy to diurnal hopping activity (*gray area* above horizontal axis), and diurnal (*open area* below axis) and nocturnal inactive behavior (*hatched area* below axis). The numbers left and right of the bar graphs in each panel give the total energy budget in $kJ \cdot d^{-1}$, as assessed from DME (Experiment 1) and DEE (Experiment 2)

$11.0 \ kJ \cdot d^{-1}$ and $10.3 \ kJ \cdot d^{-1}$ for the low and high workloads, respectively, after correcting for body mass differences. Applying the same marginal activity cost, we arrived at Eda estimates of $14.4 \ kJ \cdot d^{-1}$ and $18.5 \ kJ \cdot d^{-1}$ for the low and high workloads, respectively. The estimates of Edr, $26.2 \ kJ \cdot d^{-1}$ and $19.4 \ kJ \cdot d^{-1}$, now represent a reduction of 26% in the high compared to the low workload (Fig. 3, right).

The zebra finches thus appear to have down-regulated the energy expended on non-hopping behavior. Although Enight was significantly reduced for the high workload, this was not sufficient to balance the costs of increased activity during the day. Additional compensation may have been achieved by extension of a reduced RMR during the inactive hours of the day. Furthermore, the birds may have compensated by changing the relative frequencies of non-hopping behavior, such as preening or sleeping, towards higher relative frequencies of energetically cheaper behaviors. The estimated minimum duration of hopping activity (based on the maximum number of 200 hops per 2-min interval) was ca. 1 h (low workload) or 1.5 h (high workload), thus leaving 13 h, and 12.5 h, respectively, for diurnal non-hopping behavior.

The observed reduction in body mass of birds on the high workload may have been one source of energy savings; body mass was stable during each workload; therefore no additional energy was derived from stored resources. A reduction in body mass is likely to reduce maintenance costs and may also reduce activity costs (Freed 1981, Norberg 1981); the question is whether the reduced mass suffices to account for the reduced energy budget, as we found no evidence for a reduction in the costs of hopping activity. However, maintenance costs estimated by RMR showed an intra-individual reduction of 0.037 Watt per g body mass. With a change of on average 0.59 g (SD = 0.74) between the workloads, this amounted to $1.89 \ kJ \cdot d^{-1}$ saved over a 24-h period. Due

to circadian variation in metabolic rate, with higher rates during the active part of the cycle (α) than during the rest phase (β) (Aschoff and Pohl 1970), there is, however, more potential for energy saving during α. We derive indications for economizing beyond the effects of body mass from the additional unidentified "workload" component contributing to differences in RMR. The exponent of intra-individual change in log (RMR) depending on log (Mass) in this study was 2.30 (SEM = 0.60). It deviates considerably from the interspecific mass exponent of $\frac{2}{3} - \frac{3}{4}$, and is even larger than the intra-individual exponents of 1.8 and 1.5 for male and female Kestrels *Falco tinnunculus* kept in varying nutritional conditions (Daan et al. 1989). This leaves us with the intriguing question of how birds achieve further reduced metabolic rate during inactivity.

The zebra finches may also have compensated for increased energy expenditure due to activity by economizing on the rate of maintenance processes. Hypothermia, as reported for food rationed birds (e.g., Biebach 1977; Shapiro and Weathers 1981; Daan et al. 1989), may be related to down-regulation of enzymatic processes. Birds may further shut down processes of less acute importance; for example, Deerenberg et al. (1997) showed that some zebra finches in energetically demanding situations refrain from an immunological response to a novel challenge. Finally, it cannot be excluded that a larger part of the thermostatic costs of body temperature regulation were covered by activity on the high workload. As the experiments were run at 25 °C, i.e., just below the thermoneutral zone, the scope for such substitution must have been limited.

Potential costs of a compensation strategy

Why would birds apply a strategy of compensation? Why do they not just increase their intake of energy to balance the energy budget? Compensation may be profitable if the environment does not allow an increase in energy intake beyond a certain level due to, e.g., insufficient food availability. Alternatively, there may be risks associated with an increase in intake of energy, e.g., exposure to predators, pathogens, or parasites during foraging. Fitness-related costs of high overall rates of energy turnover, e.g., causing cellular or tissue damage, and metabolic dysfunction (Sjödin et al. 1990) may also exceed those of reallocation of energy.

A strategy of energetic compensation may be accompanied by symptoms of physiological and behavioral curtailments. The observation that the components of the energy budget are variable within an individual suggests that they are subject to a cost-benefit trade-off; otherwise, why would these components not be at a minimum level at all times? Various potential cost factors are anticipated; a reduction in body mass may involve a change in body composition, thus affecting body reserves that usually serve as an insurance against starvation (Lima 1986; Houston and McNamara 1993). Reduction

in (resting or basal) metabolic rates are often associated with a reduction of body temperature (Daan et al. 1989; Rashotte et al. 1995). Hypothermia on the one hand may increase the risk of predation, and on the other hand it may reduce body temperature below the optimal temperature for enzymatic activity, thereby decelerating protein turnover or cellular turnover in general, causing arrears in repair of cellular damage, or in immunological defense mechanisms (Deerenberg et al. 1997). Life history costs, (e.g., costs of reproduction) associated with altered patterns of activity, may well be due to compensation, (e.g., economizing on maintenance processes), instead of increased rates of overall energy expenditure.

We have shown that the energetic consequences of behavior are not constant under changing energetic demands. This finding has important implications for time-energy budgets (TEB). Validation studies sometimes reveal deviations of the daily energy budget when assessed by TEB, in comparison with estimations from turnover of stable isotopes (Nagy 1989; Masman et al. 1988). TEB assumes additivity of energety costs (Schartz and Zimmerman 1971), and more attention should be paid to the possibility of physiological compensation beyond the effects of a reduction in body mass.

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