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Published in:
Royal Society of London. Proceedings B. Biological Sciences

DOI:
10.1098/rspb.2002.2071

2002

Link to publication

Citation for published version (APA):
Nilsson, J.-Å. (2002). Metabolic consequences of hard work. *Royal Society of London. Proceedings B. Biological Sciences*, *269*(1501), 1735-1739. https://doi.org/10.1098/rspb.2002.2071

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Received 17 January 2002
Accepted 1 May 2002
Published online 25 July 2002

Metabolic consequences of hard work

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When an animal has to meet increased demands on its working capacity, for example, for thermoregulation or parental care, two strategies are available. The animal can reallocate energy from costly maintenance processes—such as immunological defence or DNA repair systems (compensation hypothesis)—or it may try to increase the rate of energy intake or efficiency of digestion by increasing the size of the alimentary tract (increased-intake hypothesis). By manipulating brood size, I affected parental effort among marsh tits (*Parus palustris*) as demonstrated by a significant increase in parental feeding rate with experimental brood size. Basal metabolic rate (BMR) increased both with manipulated brood size and individual feeding rate, supporting the predictions from the increased-intake hypothesis. Furthermore, I found a direct positive relation between BMR and energy expenditure, measured with the help of the doubly labelled water technique. The cost of achieving a higher working capacity is substantial since BMR increases more quickly than the surplus energy available for work. Since the cost of a high sustained workload was not primarily dependent on a reallocation of energy away from maintenance, such a cost should be searched for among the detrimental effects of a high metabolic rate *per se*, for example, an increased oxidative damage to DNA, proteins and lipids.

Keywords: basal metabolic rate; doubly labelled water; energy expenditure; oxidative stress; parental effort

1. INTRODUCTION

Life-history theory is founded on the 'Principle of Allocation', i.e. an increase in the energy allocated to one process must result in a decrease to another process (Stearns 1992). This principle is, however, only valid if intake rate is constant. Thus, when an animal needs to increase its working capacity to be able to expend more energy e.g. for thermoregulation, migration or reproduction, two strategies are available: (i) increasing the rate of energy intake (increased-intake hypothesis); or (ii) reallocating energy from costly maintenance processes to work (compensation hypothesis). According to the increased-intake hypothesis, individuals will be able to ingest and process more food due to an increased size of the alimentary tract (Kersten & Piersma 1987; Hume & Biebach 1996; Piersma & Lindström 1997; Chappell *et al.* 1999; Hammond *et al.* 2000). The metabolic activity of organs in the alimentary tract, such as gut, intestines and liver, is high and contributes significantly to the basal metabolic rate (BMR) (Alexander 1999), resulting in an increase in general BMR with increases in the alimentary tract (Daan *et al.* 1990; Lindström & Kvist 1995; Lindström *et al.* 1999). Thus, the increased-intake hypothesis predicts a positive relation between BMR and energy expenditure to support high levels of sustained workload (Bennett & Ruben 1979).

According to the compensation hypothesis, the animal makes trade-offs between different parts of the existent energy budget to meet elevated energetic demands (Deerenberg *et al.* 1998). If the animal compromises physiological maintenance processes, e.g. immunological defence systems or DNA repair systems (Sheldon & Verhulst 1996; Deerenberg *et al.* 1997), a negative relation between BMR and work rate is predicted.

The two hypotheses outlined above are not mutually exclusive: both may well be in effect at the same time. The aim of this study was to test the predictions generated by the hypotheses and endeavour to establish which of the two strategies is most important to meet increased demands of work during reproduction. More specifically, my aim was to investigate how females respond to an increase in the need for parental effort. (i) Are females willing to increase their workload? (ii) Will the BMR be affected by workload? (iii) Will an increase in parental effort result in an increased overall energy expenditure?

2. MATERIAL AND METHODS

(a) *Animals*

I studied the BMR of female marsh tits (*Parus palustris*) during the breeding seasons of 1999 and 2000 in a population in the Revinge area, 20 km southeast of Lund, southern Sweden. During the breeding season of 1999, I also measured the field metabolic rate (FMR) using the doubly labelled water technique. The study area (64 km^2) consists of small deciduous forests and groves, interspersed in a landscape of permanent pastures and agricultural fields. All marsh tits used in this study bred in nest-boxes.

(b) *Brood-size manipulation*

When nestlings were nine days old, I experimentally enlarged and reduced broods by moving nestlings that had hatched at the same date, between boxes. Non-manipulated broods were used as controls. In neither year did clutch size differ between the experimental categories (ANOVA: $p > 0.5$). The aim was to increase brood size in enlarged broods by 50%. Following manipulation, the mean realized increase in number of nestlings in enlarged broods was 46% (s.e. = 1.7; $n = 16$) and the mean decrease in reduced broods was 48% (s.e. = 2.2; $n = 14$). This resulted in a significant difference in the number of fledged young between experimental categories (mean ± s.e.; 1999: reduced, 4.2 ± 0.66, $n = 5$; control, 7.8 ± 0.49, $n = 5$; enlarged,

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10.7 ± 0.61; ANOVA, $F_{2,13} = 29.7$; $p < 0.001$. 2000: reduced, 4.4 ± 0.29, $n = 9$; control, 8.6 ± 0.48, $n = 7$; enlarged, 12.7 ± 0.26, $n = 10$; ANOVA, $F_{2,2} = 168.6$; $p < 0.001$). Due to lack of time and space in the respirometer (see § 2d), two reduced broods, one in each year, were removed from the experimental protocol.

(c) *Feeding rate*

Female feeding rate was used as a measure of female effort during the nestling phase of breeding. Both males and females were equipped with a uniquely identifiable microchip (transponder) glued to their colour rings. Feeding rate was recorded on a datalogger (Trovan, AEG ID, Ulm, Germany) connected to a circular antenna placed around the entrance hole of the nest-box. During both years, feeding recordings were conducted when nestlings were 11 days old and continued for two consecutive days (mean (± s.e.) duration of a recording was 54 ± 1.1 h). Feeding rate was calculated as the mean feeding rate between 06.00 and 19.00, a time-period with a constant feeding rate (Råberg *et al.* 2000). During 2000, the mean feeding rate of a female was based, on average, on 29 h (s.e. = 0.4; $n = 23$; range, 33–25) of feeding recordings. This included one female tending a reduced brood, which did not feed at all during this time-period. However, she was observed feeding a few times before 06.00 as well as occasionally both before and after the recording period. In 1999, the last of the two recording days was used to measure feeding rate after the administration of doubly labelled water (see below). Thus, undisturbed feeding rate was based on a shorter time-period during this year (mean, 14 h; s.e. = 1.4; $n = 12$; range, 8–24). Due to the malfunctioning of some of the recording devices, feeding rate was not measured in four and three broods, respectively, in 1999 and 2000.

(d) *Basal metabolic rate measurements*

BMR was measured in terms of oxygen consumption during the night in an open-circuit respirometer. During the evening when nestlings were 14 ($n = 33$) or 15 ($n = 6$) days old, females were captured and placed individually in sealed respirometer chambers (1.6 l) that were placed in the darkness of a climate cabinet (Hereaus) at 25 °C (for a description of the measurement procedure see Nilsson & Råberg (2001)). Three females during 2000 evaded capture and were not measured in the respirometer.

(e) *Field metabolic rate measurements*

In 1999, 12 females caring for 12- or 13-day-old nestlings were captured and their FMRs were measured using the doubly labelled water (DLW) technique (Speakman 1997). Females were weighed, to the nearest 0.1 g, and then injected intraperitoneally with 0.1 ml of a mixture containing 4.4086 g of 94.0 AP $H_2{}^{18}O$ and 2.4001 g of 99.9 AP D_2O. Females were then kept in a cloth bag for *ca.* 1 h to allow the isotopes to mix with the body fluids. Before release, an initial blood sample was taken from the brachial vein. After *ca.* 24 h (mean: 24 h 10 min ± 5 min), all females except one were recaptured and another blood sample was taken from the brachial vein in the opposite wing. The female that evaded capture 24 h after the initial blood sample was instead captured 48 h 10 min after the initial blood sample. All females were weighed before release. All blood samples were kept in flame-sealed, heparinized capillary tubes until analysed for ^{18}O and ^{2}H concentrations at the Centre for Isotope Research, University of Groningen, The Netherlands.

Blood samples from three females not included in the experiment were used for determining background levels of the two isotopes. As an estimate of body water volume, the average for passerine birds, i.e. 66.2% of body mass (Speakman 1997), was used. Daily CO_2 production was derived from fractional turnovers of the two isotopes using procedure (i) of Speakman (1997). This is a revised version of the Lifson & McClintock (1966) single pool equation.

The reaction to handling and being held in captivity for 1 h differed greatly between females. Continuous registration of their provisioning behaviour made it possible to evaluate how accurately the method measured normal FMR. Six females resumed feeding within 1 h after release from the initial blood sampling. The other six females did not start to feed for 4–10 h (mean: 5.7 h) after release. Since this reduction in female effort occurred during the time between 06.00 and 19.00 (the period of high and constant feeding rate), this period of high energy expenditure was reduced by, on average, 46%. Furthermore, feeding rate during the period of FMR measures was significantly lower, by, on average, 9.5 feedings h^{-1} (paired *t*-test: $t = 4.85$; $p = 0.005$), than during the day before administration of the DLW in the group with delayed resumption of feeding. In the six females that started to feed within 1 h of release, feeding rate before and after handling did not differ significantly (*t*-test: $t = 1.19$; $p = 0.3$). Thus, only the sample of six females (two tending reduced broods and four tending enlarged broods) that continued with their normal activities within 1 h of DLW administration was used in analyses concerning FMR.

3. RESULTS

(a) *Female effort*

Number of feedings per hour increased significantly over the three experimental broods, a relation that did not differ between years (ANOVA: year, $F = 2.6$, $p > 0.1$; experimental category, $F = 7.5$, $p = 0.002$). Taking the two years together, females on average (± s.e.), fed their young 9.1 ± 1.2 times h^{-1} in reduced broods ($n = 13$), 14.6 ± 2.5 times h^{-1} in control broods ($n = 8$) and 16.7 ± 1.0 times h^{-1} in enlarged broods ($n = 14$).

(b) *Basal metabolic rate*

To investigate the relation between BMR and mass, the logarithm of these two variables was calculated, corresponding to the general allometric equation of the form $y = aM^x$, where M is mass, a is the intercept (the metabolic rate when mass = 1) and x is the allometric exponent. As commonly observed, BMR was shown to be significantly influenced by mass (figure 1); however, this influence differed between years (multiple regression, effect of year: $t = 3.15$, $n = 39$, $p = 0.003$). In 2000, the BMR was higher than in 1999, especially at low masses (figure 1). In the following analyses involving BMR, I have used the residuals from the allometric equation for each specific year.

Body mass-adjusted BMR increased with manipulated brood size (ANOVA with ordered heterogeneity test; Rice & Gaines (1994): $F_{2,36} = 1.14$; $r_s P_c = 0.7$; $p = 0.05$), as well as with female feeding rate (figure 2). This was not an effect of systematic differences in mass between females assigned to different brood sizes. Female mass, as measured after the night in the respirometer (see § 2d), did not differ between the experimental categories (ANOVA:

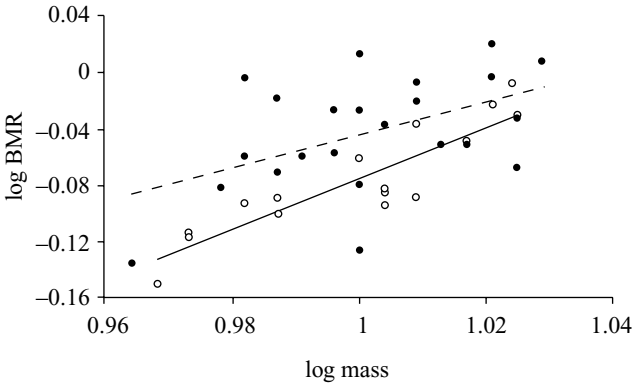

Figure 1. The relation between log BMR and log mass of female marsh tits for 1999 (open circles, solid line) and 2000 (filled circles, broken line). Each relation was tested with regression analysis: 1999: $t = 6.87$; $n = 16$; $p < 0.001$. 2000: $t = 2.56$; $n = 23$; $p = 0.018$. Equations for the lines:
1999: log BMR = 1.76 log mass − 1.83;
2000: log BMR = 1.14 log mass − 1.18.

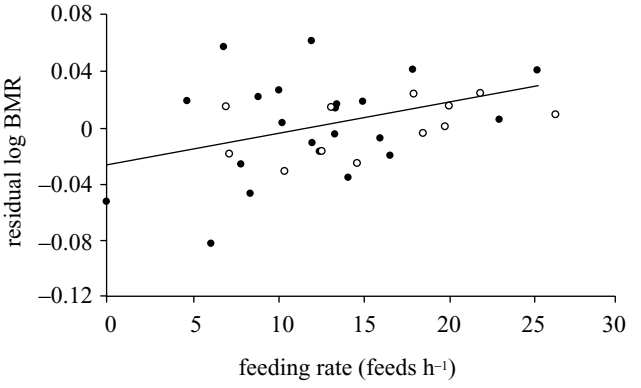

Figure 2. The relation between residuals from the regression between log BMR and log mass of female marsh tits and their feeding rate during 1999 (open circles) and 2000 (filled circles). Year did not affect the relation ($p = 0.6$) and was excluded from the model. Tested with regression analysis: $t = 2.09$, $n = 34$, $p = 0.045$.

$F_{2,36} = 0.83$; $p = 0.45$). Average mass (± s.e.) for females tending reduced broods was 10.0 ± 0.12 g ($n = 14$), control broods: 9.9 ± 0.12 g ($n = 10$) and enlarged broods: 10.1 ± 0.09 g ($n = 15$).

(c) *Field metabolic rate*

Energy expenditure during 24 h of the six female marsh tits that resumed normal feeding rates within 1 h of handling (see § 2) ranged between 2.48 and 2.89 kJ h^{-1}, with a mean of 2.69 kJ h^{-1} (s.e. = 0.05). If the energy expenditure of individual females is presented as a multiple of their BMR (factorial metabolic scope; FMR/BMR), the average is 3.17 × BMR (s.e. = 0.07), varying between 3.02 and 3.51 × BMR.

The FMR did not vary in any systematic way with mass (regression analysis: $t = 0.003$; $n = 6$; $p = 1.00$). Female marsh tits expended more energy tending enlarged broods (2.75 ± 0.05 kJ h^{-1}) compared with reduced broods (2.58 ± 0.10 kJ h^{-1}), although the difference was not significant, presumably due to low sample size (*t*-test: $t = 1.61$; d.f. = 4; $p = 0.18$). The number of feeding trips made by the females during the FMR sampling period may be a better measure of female reproductive effort than the experimental category. In line with this, I found a strong positive relation between the FMR and the number of feeding trips (figure 3). Furthermore, I found a strong positive relation between the FMR and BMR (figure 4). This relation was also significant when mass-specific FMR and BMR were used (regression analysis: $t = 3.07$; $n = 6$; $p = 0.037$).

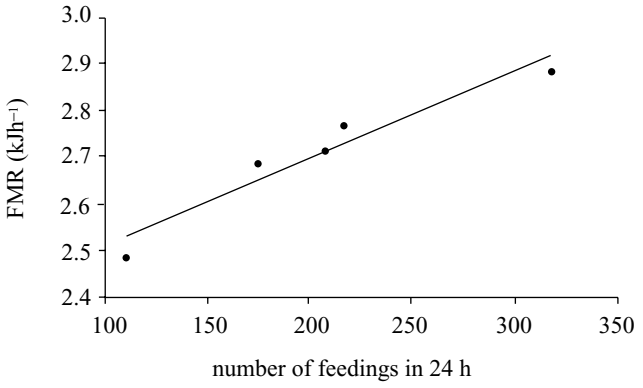

Figure 3. Relation between female FMR and number of feeding trips to the nest during the period of DLW measurements. Tested with regression analysis: $t = 6.26$, $n = 5$, $p = 0.008$.

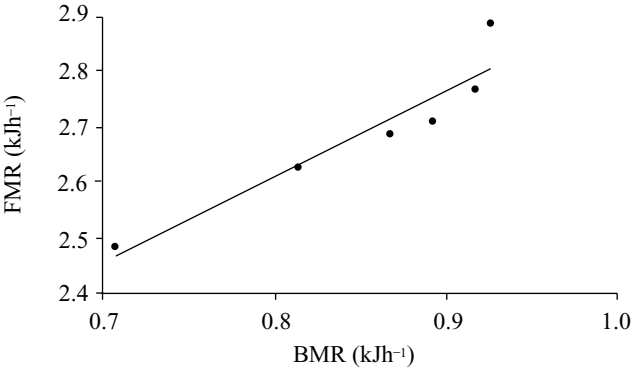

Figure 4. Relation between FMR and BMR of nestling-feeding marsh tit females in 1999. Tested with regression analysis: $t = 6.08$, $n = 6$, $p = 0.004$.

The simultaneous measures of BMR and FMR on the same individual made it possible to estimate the cost of increasing the FMR in terms of the BMR. As the BMR increases so does the scope for energy expenditure (figures 2 and 4). However, this increase is accomplished at a diminishing rate since the factorial metabolic scope decreases significantly with an increase in BMR (figure 5). If the BMR is increased to be able to sustain a high work rate, I predict that the surplus energy available for work, i.e. absolute metabolic scope (FMR–BMR), will increase with BMR. This was also the case (one-tailed regression analysis: $t = 2.17$; $n = 6$; $p = 0.047$). However, the slope of this relation was only 0.56.

4. DISCUSSION

(a) *Basal metabolic rate*

The results of this study strongly support the predictions from the increased-intake hypothesis. The BMR was

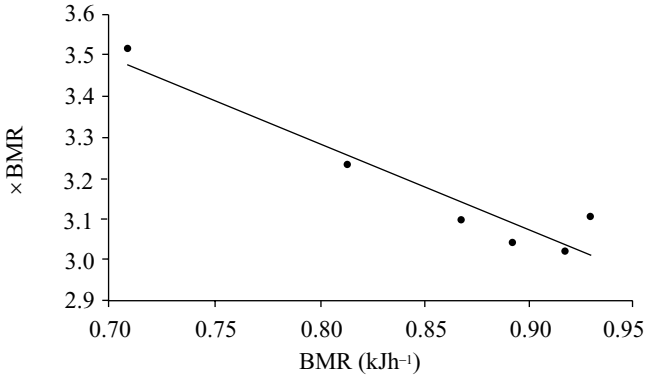

Figure 5. Relation between FMR, expressed as a multiple of BMR(× BMR), and BMR in nestling-feeding marsh tit females in 1999. Tested with regression analysis: $t = -6.35$, $n = 6$, $p = 0.003$.

positively related to both workload (figure 2) and actual energy expenditure (figure 4). Thus, a breeding female cannot support a high workload by reallocating energy from maintenance processes, but rather has to accept a high level of energy expenditure. Even if the females were allocating energy away from maintenance processes, this would not have been enough to cover an increased demand on working capacity. The physiological adjustments of important digestive organs, enabling females to improve food processing, are very rapid since measurements of FMR and BMR were taken only 4–5 days after the brood-size manipulation. Equally fast, or even faster adjustments of the digestive machinery with an increased BMR as a consequence, have been observed in migrating birds during fuel deposition (Hume & Biebach 1996; Lindström *et al.* 1999; Kvist & Lindström 2000). The BMR of hard-working birds may also increase as a result of the increased costs of DNA repair systems (Perez-Campo *et al.* 1998), incurred by the increased production of free radicals (see § 4c).

(b) *The scope of sustained work rate*

Factorial metabolic scope (FMR/BMR) decreased with increasing BMR (figure 5; see also Chappell *et al.* 1999; Hammond *et al.* 2000). Thus, as the FMR increases in response to increasing demands on the work rate, BMR will constitute an increasing proportion of the FMR. Furthermore, the absolute metabolic scope (FMR–BMR), i.e. the surplus energy that can be used for work, increases with BMR but only at a slope of approximately 0.5. Thus, to increase the scope for sustained work is expensive. For each increase of one unit of energy to be directed to work, the female marsh tit has to pay two units of energy in increased BMR.

(c) *Implications for life-history theory*

This study represents, to my knowledge, the first experimental test of the relationship between workload or FMR and BMR in a wild population of vertebrates performing natural behaviours at a highly sustained workload. Previous observational and laboratory studies have produced varying results (Hayes & Garland 1995; Fyhn *et al.* 2001). Experiments relating BMR to maximal short-term power production (Chappell *et al.* 1999; Hammond *et al.* 2000) may not be comparable with the results in this study since the increased-intake hypothesis predicts a positive relation between BMR and sustained workload, emphasizing the physiological adjustments necessary to sustain a high-power production over longer time-periods than of the order of minutes.

Deerenberg *et al.* (1998) found evidence for the compensation hypothesis in zebra finches (*Taeniopygia guttata*), working at different rates to get food. This is the only laboratory experiment where the animals had unrestricted access to food. Since increasing the scope for work by physiological adaptations is energetically expensive (this study), it might only be feasible in situations when the foraging cost is low, e.g. at high food availabilities, as shown interspecificly among *Peromyscus* mice (Mueller & Diamond 2001). This is probably normally the case during the chick-rearing phase in temperate birds (Nilsson & Råberg 2001).

So, what are the energetic implications for a reproductive cost? Breeding birds are commonly able to raise more young than they actually do, as demonstrated by brood-size manipulations, with a concomitant increase in FMR (Sanz & Tinbergen 1999, but see Tinbergen & Verhulst 2000). The fitness consequences of a high FMR have been reported in the form of reduced future reproductive success (Daan *et al.* 1996; Sanz & Tinbergen 1999). Thus, although food is abundant enough for increased parental effort, females still produce a clutch size that is decided by an optimization process rather than by physiological limits. One set of hypotheses, i.e. reduced energetic investment in maintenance in favour of work rate (Sheldon & Verhulst 1996; Deerenberg *et al.* 1997), appear inapplicable to female marsh tits. However, several detrimental effects of a high metabolic rate *per se* have been suggested, mainly through the action of oxidative metabolites and free radicals (von Schantz *et al.* 1999). These are highly reactive by-products of metabolism that cause oxidative stress, i.e. oxidative damage to DNA, proteins and lipids. In this way, a high level of metabolism may increase the rate of degenerative cell destruction and tissue damage underlying senescence and death (Sacher 1978; Ricklefs & Finch 1995; Perez-Campo *et al.* 1998). Thus, the functional base for a cost of reproduction seems to be the detrimental effects of high metabolism rather than energy allocation between maintenance and work rate. Variation in clutch size between females may thus depend on differences in overall prey abundance between territories, affecting general levels of FMR or individual differences in antioxidant defence systems, which, in turn, affect the ability to take care of produced free radicals.

I thank Henk Visser, Centre for Isotope Research, Nijenborgh, Groningen, The Netherlands for analysing the doubly labelled water samples. I also thank Lars Råberg and Martin Stjernman for valuable comments on the manuscript. The study was supported by grants from the Swedish Natural Science Research Council.

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