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U. Totzke · A. Hübinger · J. Dittami · F. Bairlein

The autumnal fattening of the long-distance migratory garden warbler (*Sylvia borin*) is stimulated by intermittent fasting

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Abstract To investigate the proximate influence of a changing food availability on the seasonal fattening of migratory birds, garden warblers (*Sylvia borin*) following postnuptial moult were food restricted once a week. Body mass, food intake, plasma hormone and metabolite levels were measured and compared to birds which always had ad libitum food access. The food-restricted birds increased their body mass significantly earlier than the controls. The accelerated fattening was initially not accompanied by hyperphagia and may be due to either an increased food utilisation efficiency or a reduced metabolic rate. An increase of basal glucagon and corticosterone and a decrease of insulin levels prior to fattening were not significant, however, they resulted in a significant decrease of the insulin:glucagon ratio. This ratio was also lower in food-restricted birds than in control birds and may account for the difference in the fattening progress. We conclude that seasonal fattening may be stimulated by a catabolic impulse which could be imposed in free-living birds by a decrease of food availability and/or by an increase of energy expenditure. A negative energy balance is hypothesised to be a common proximate factor affecting migratory as well as winter fattening.

Key words Fattening · Food availability · Insulin · Glucagon · Corticosterone

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U. Totzke (✉) · F. Bairlein
Institut für Vogelforschung 'Vogelwarte Helgoland',
An der Vogelwarte 21,
26386 Wilhelmshaven, Germany
e-mail: ifv@ifv.terramare.de
Tel.: +49-4421-96890; Fax: +49-4421-968955

A. Hübinger
Diabetes-Forschungsinstitut, Aufm Hennekamp 65,
40225 Düsseldorf, Germany

U. Totzke · J. Dittami
Institut für Zoologie, Abteilung Ethologie,
Althanstrasse 14, 1090 Vienna, Austria

Introduction

Many bird species accumulate body fat reserves prior to seasonal events with high energy demands or low food availability, such as incubation (Boismenu et al. 1992; Meijer et al. 1996), migration (Berthold 1993), or harsh winter conditions (Baldwin and Kendeigh 1938). In passerines, fat is mainly accumulated prior to spring and autumnal migration and in resident species during winter. While winter fattening shows a high plasticity and may be under the control of environmental factors such as temperature and/or food predictability (Biebach 1996), the migratory fattening is part of a more or less rigid innate circannual programme which is regarded as being predominantly synchronised by changes of photoperiod (Gwinner 1986; Berthold 1996). However, the migratory fattening in spring and autumn seems to be controlled by different endocrine factors (Wingfield et al. 1990) and with respect to fattening the concept of photoperiodic control has shown several deficiencies (Bairlein and Gwinner 1994).

A proximate influence of decreasing food availability has been shown for winter fattening (Witter et al. 1995) and has also been suggested to influence migratory fattening (Hiebert 1991; Berthold 1993). Despite evidence for an increase of catabolic enzyme activities in preparation for migration (Lundgren and Kiessling 1985, 1986) and for an acceleration of lipogenesis after starvation (Ramenofsky 1990), this has not yet been investigated. Therefore, this study aimed to investigate: (a) the influence of food shortage on the initial phase of autumnal fattening and (b) the direct endocrine and metabolic adjustments involved.

This study was carried out on the garden warbler (*Sylvia borin*), a European-African migrant with a marked fattening even under laboratory conditions (Bairlein 1987). Garden warblers fatten not only due to hyperphagia, but also with an increased food utilisation efficiency (Bairlein 1985; Hume and Biebach 1996) and a shift in diet selection (Bairlein and Simons 1995). Along with fattening they also display metabolic changes such as a lower glucose utilisation rate and several other symptoms of mammalian obesity, both in the field (Bairlein and Totzke 1992) and captivity (Totzke and Bairlein 1998; Totzke et al. 1998). These data suggest a close relationship between metabolic adjustments and migratory disposition and, further, that the garden warbler is a suitable species for an investigation of the effect of food availability on fattening and its related metabolic changes in long-distance migratory passerines.

Materials and methods

Experimental design

Twenty garden warblers were housed in outdoor aviaries in Wilhelmshaven (53°3N, 8°1E) until the end of August. They were then brought indoors (week 1) and kept in single cages at the same long-day photoperiod (L:D 15:9) experienced outdoors, at a constant temperature of 20 ± 2 °C and with constant food (Bairlein 1986). After a 4-week accommodation phase during which the birds completed postnuptial moult, an experimental group was restricted to approximately 50% of the average spontaneous daily food intake (2.5 g dry mass/day) on a fixed day once a week (from week 5 onwards). A control group received the same food always ad libitum. From mid-September (week 6) onwards the length of the light-phase was shortened by 15 min/day until photoperiod reached L:D12:12 similar to the photoperiodic changes experienced in nature. The food regimen was continued until week 11 when birds began fattening. From then onwards all birds received food ad libitum.

Every 2–3 weeks, blood was taken in the morning immediately following lights-on, for the determination of standardised preprandial plasma hormone and metabolite levels. From weeks 5–11, these measurements were conducted in the morning following the day of food restriction in the experimental group. Body mass and food intake (g dry matter/day) were recorded daily in the morning (Totzke and Bairlein 1998). Birds of the experimental group always consumed restricted food amounts completely.

Blood sampling and analysis

Birds were bled by puncture of the wing vein. The blood was collected with heparinised capillary tubes and centrifuged for 10 min at 8900*g* and 4 °C. The plasma was measured for insulin, glucagon (Totzke et al. 1997) and corticosterone (Palme and Möstl 1997) with validated radioimmunoassays or enzyme-immunoassays; for glucose, triglycerides, cholesterol, urea, and uric acid with the EKTACHEM DT II analysing system (Kodak, Stuttgart, Germany), and for free fatty acids and β-hydroxybutyrate photometrically with standard kits from Boehringer (Mannheim, Germany) and Sigma (Deisenhofen, Germany), respectively (Totzke and Bairlein 1998). The blood sampling procedure was completed within 4 min from entering the room and within 2 min from catching the individual bird, respectively. Therefore, a distortion of measurements due to the influence of stress was negligible even for corticosterone (Wingfield et al. 1982; Schwabl et al. 1991).

Data analysis

For the statistical analysis, individual body mass changes were standardised in relation to the body mass at week 5 (end of accommodation phase) to compensate for size-related inter-individual variability. Means of body mass changes and food intake were tested using: (a) the Student's *t*-test for differences between experimental groups and (b) the paired *t*-test to compare within-group differences prior to and after an experimental change, such as the change of photoperiod or the cessation of food restriction. To avoid pitfalls with adjustments for multiple testing (Cabin and Mitchell 2000) integrals of the curves along the time axis were calculated for each individual and mean values of groups were compared by *t*-test.

Since blood parameters for individual birds could be collected only in time intervals of several weeks, mean values were calculated for each bird monthly. Blood parameters were tested by a mixed model two-way analysis of variance (ANOVA) with groups of birds and months as factors. However, since resulting sample sizes were often very small the effect of replication could finally be neglected. Hormone levels were additionally tested for differences between groups by comparing the mean integrals of curves along the time axis and for temporal changes by a Spearman rank correlation analysis.

Results

Fattening and food intake

Food-restricted garden warblers started fattening significantly earlier than control birds (Fig. 1). The control

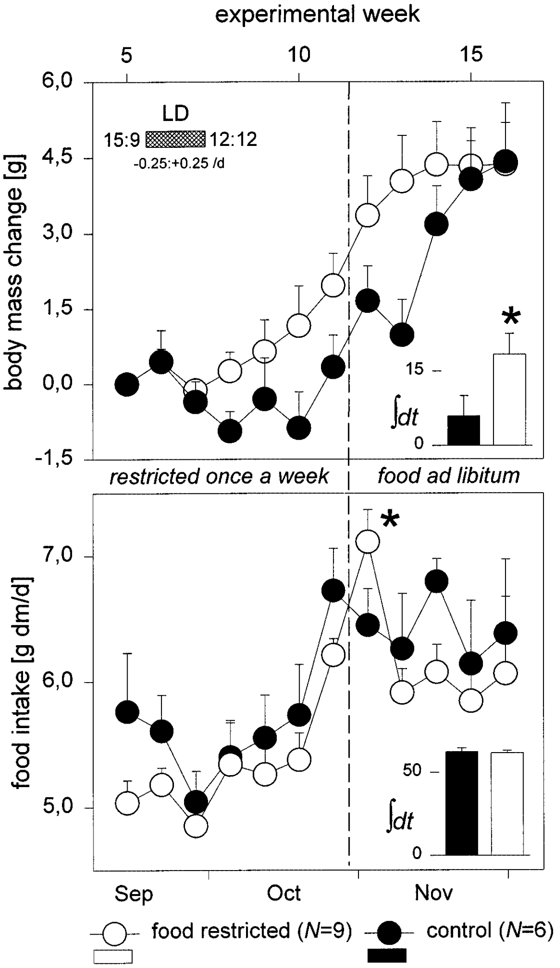

Fig. 1 Body mass change and food intake (means ± SE) of the food-restricted and ad libitum-fed garden warblers during migratory fattening in autumn. The *hatched bar* indicates the time period during which the light phase was reduced by daily shifts of 0.25 h from 15 h/day to 12 h/day. The *dashed vertical line* indicates the end of food restriction in the experimental group. The *bar chart* on the lower right of each panel shows the mean integrals (±SE) of the corresponding curves between week 5 and week 15. *Asterisks* mark significant differences between the control ($n = 6$) and the food-restricted group ($n = 9$). Non-fattening birds were excluded (three of the control, and one of the food restricted group)

birds even showed an initial decrease in body mass in response to the reduction of the light phase (mean body mass ± SE, week 6: 19.8 ± 0.8 g vs. week 8: 18.9 ± 0.6 g, paired t-test: $P < 0.014$, $n = 9$; Fig. 1). Irrespective of food restriction, average daily food intake per week was almost identical (Fig. 1). During the first week after food restriction had ceased, the experimental birds showed a significantly higher food intake than controls (Fig. 1). Both groups maintained a significantly higher food intake during the high body mass phase as compared to the low body mass phase (food intake, weeks 5–10: 5.2 ± 0.2 g dry matter/day vs. weeks 11–16: 6.0 ± 0.2 g dry matter/day, paired t-test: $P < 0.001$, $n = 18$, Fig. 1).

Endocrine and metabolic responses

From August to November in both groups, glucagon and corticosterone levels increased and insulin levels decreased (Figs. 2, 3). The Spearman rank correlations of time with corticosterone ($r_s = 0.30$; $n = 51$) and insulin ($r_s = -0.33$; $n = 45$) were significant (both $P < 0.05$), but monthly variations tested by ANOVA were not (both $P > 0.05$). The insulin:glucagon ratio decreased in both groups significantly (ANOVA: $P < 0.01$) and appeared lower in food-restricted birds than in control birds (Fig. 2).

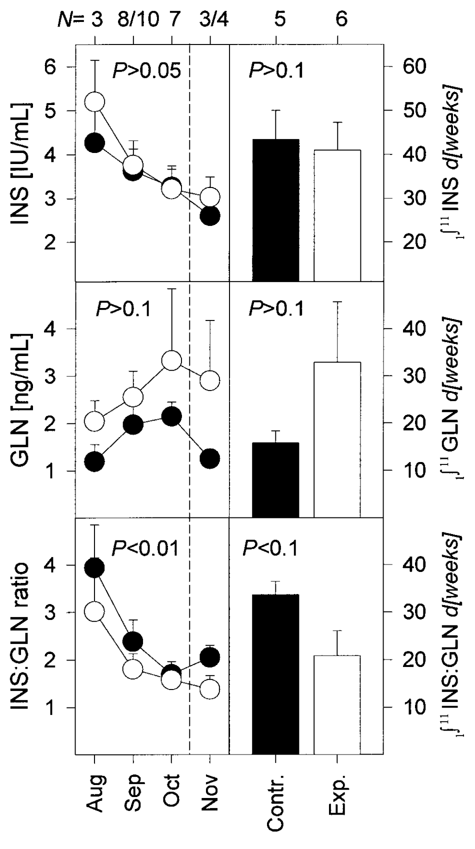

Fig. 2 Plasma pancreatic hormone levels in experimental (*open circles/bars*) and control birds (*solid circles/bars*). On the *left*, plasma levels (means ± SE) from August (immediately after bringing birds indoors) to November with P-values from a two-way ANOVA, referring to differences between months; on the *right*, integrals of the areas beneath curves (means ± SE) with P-values resulting from the independent t-test between groups. The *dashed vertical line* indicates the end of food restriction in the experimental group. Number of measurements is given above the x-axis (if it differs between groups the one of the control group is listed first)

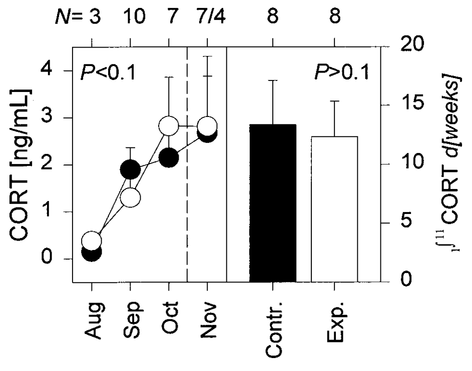

Fig. 3 Plasma corticosterone levels. For explanation see legend of Fig. 2

Glucose levels were higher in the experimental group; cholesterol levels increased in both groups. None of the other plasma metabolite levels varied significantly between or within groups (Table 1).

Discussion

Since the landmark work of E. Gwinner and P. Berthold, behaviour of long-distance migratory passerine bird species has been considered to be under the control of an endogenous circannual programme, triggered ultimately by changes of photoperiod (Gwinner 1986; Berthold 1996). Fattening has always been dealt with as an inherent part of this programme; however, some laboratory findings could not be solely explained by photoperiodic control (Bairlein and Gwinner 1994).

This study provides the first experimental evidence for a proximate influence of a decreasing food availability on migratory fattening: garden warblers which were regularly restricted of food started fattening significantly earlier during autumn, as compared with controls which in turn initially displayed a body mass decrease in response to the reduction of photoperiod.

Since the body mass increase of food-restricted birds started at the same average food intake as the control group, it may either be due to an increase of food utilisation efficiency (Bairlein 1985; Hume and Biebach 1996) or a decrease in metabolic rate. A reduction of the BMR is a widespread fasting adaptation of vertebrates including the garden warbler (Klaassen and Biebach 1994), and it has also been suggested as a mechanism of migratory fattening (Blem 1980). In hummingbirds, torpor, i.e. a profound decrease in metabolic rate, is most likely to facilitate migratory fattening (Hiebert 1991, 1993).

Table 1 Morning plasma metabolite levels (monthly means ± SE) of garden warblers immediately after lights-on. Birds were either food restricted 1 day/week or always had ad libitum food access. Number of measurements are given in brackets. Levels of individual birds were used in a mixed model two-way ANOVA with groups of birds and months as factors. The respective *P*-values are listed in the last two columns

		Food restricted (mmol/l)	Food ad libitum (mmol/l)	ANOVA	
				P_{groups}	P_{months}
Glucose	Sep	15.4 ± 0.4 (6)	14.2 ± 0.3 (6)	0.008	>0.1
	Oct	15.2 ± 0.5 (8)	14.0 ± 0.7 (6)		
	Nov	15.6 ± 0.2 (9)	15.0 ± 0.4 (7)		
Urea	Sep	0.9 ± 0.5 (2)	0.7 ± 0.2 (3)	>0.1	0.073
	Oct	1.7 ± 0.2 (4)			
	Nov	1.5 ± 0.5 (4)	2.1 ± 0.4 (5)		
Uric acid	Sep	0.3 ± 0.1 (6)	0.3 ± 0.03 (7)	>0.1	>0.1
	Oct	0.3 ± 0.1 (8)	0.2 ± 0.03 (6)		
	Nov	0.3 ± 0.1 (9)	0.3 ± 0.04 (7)		
Triglycerides	Sep	3.0 ± 0.4 (6)	2.7 ± 0.2 (7)	>0.1	>0.1
	Oct	2.8 ± 0.2 (8)	2.6 ± 0.5 (6)		
	Nov	3.2 ± 0.3 (9)	3.2 ± 0.4 (7)		
Cholesterol	Sep	5.6 ± 0.5 (6)	5.2 ± 0.5 (7)	>0.1	0.039
	Oct	6.5 ± 0.4 (8)	5.4 ± 0.5 (6)		
	Nov	6.1 ± 0.3 (9)	6.8 ± 0.2 (7)		
Free fatty acids	Sep	1.3 ± 0.2 (6)	1.0 ± 0.2 (7)	>0.1	>0.1
	Oct	1.3 ± 0.2 (8)	1.2 ± 0.2 (6)		
	Nov	1.4 ± 0.1 (8)	1.3 ± 0.2 (7)		
β-hydroxybutyrate	Sep	4.0 ± 0.6 (6)	3.4 ± 0.3 (7)	>0.1	>0.1
	Oct	4.7 ± 0.5 (8)	3.6 ± 0.6 (6)		
	Nov	3.4 ± 0.5 (9)	3.2 ± 0.5 (7)		

Fattening may be stimulated by a catabolic impulse indicated by the non-significant changes of the hormone levels and by the decrease of the insulin:glucagon ratio. Due to food restriction, experimental birds were expected to rely even more on catabolism than the controls, which fasted only overnight. This is reflected by the lower insulin:glucagon ratio of food-restricted birds which appears to result mostly from higher average glucagon levels as compared with controls (Fig. 2). Glucagon has previously been shown to react more sensitively than insulin to environmental changes in the garden warbler (Totzke et al. 1998, 1999). Its hyperglycaemic and lipolytic action (Mialhe et al. 1997; U. Totzke unpublished observations) is in line with higher circulating glucose, free fatty acid and β-hydroxybutyrate levels in food-restricted birds (Table 1). The functional significance of the seasonal increase of plasma cholesterol along with fattening, as shown by both groups, remains unclear but may be related to an increasing need for bile acids during fat assimilation.

Under natural conditions, a catabolic impulse causing a (super-compensating) fattening response may be induced by external food reduction or by an increased energy expenditure during migration, both of which could result in a negative energy balance. Garden warblers, along with many other migratory species, show the largest fattening rates not prior to the start of migration but in front of ecological barriers, such as the Mediterrenean and the Sahara (Bairlein 1991, 1998). Since the onset of migratory activity and fattening evolve independently (Berthold 1996), a short-term temporary negative energy balance may be an additional proximate cue for fattening. Such a mechanism would assure higher flexibility in the unpredictable circumstances faced during migration, and would restrict the duration of carrying high fat loads to times when these are actually required (Alerstam and Lindström 1990; Schaub and Jenni 2000). Whether a negative energy balance may even be a common proximate factor for both winter and migratory fattening deserves further investigation.

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