Glucose utilization rate and pancreatic hormone response to oral glucose loads are influenced by the migratory condition and fasting in the garden warbler (*Sylvia borin*)

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Abstract

Substrate utilization and regulatory mechanisms of metabolism were studied in migratory garden warblers by measuring plasma levels of glucose, free fatty acids (FFAs), β -hydroxybutyrate, insulin and glucagon in response to oral glucose loads. Three different physiological states were examined: (a) the autumnal migratory period on a high and (b) on a fasted low body mass level, and (c) the postmigratory period with low body mass.

Glucose tolerance was better in the postmigratory lean than fat condition. However, total food deprivation of 5–7 days with fat birds reaching their lean body mass further reduced the glucose utilization rate. Initial levels of FFAs were highest in the starved, intermediate in the fat and lowest in the lean condition. Changes in plasma FFAs during glucose tolerance tests were opposite to those of the

glucose levels. Ten minutes after the glucose load plasma glucagon levels decreased and insulin increased. These effects were larger in the fat than in the postmigratory lean condition. There were no differences between sexes.

It appears that during premigratory and migratory periods glucose utilization may be inhibited by a more favorable oxidation of fatty acids as would be predicted by the glucose fatty acid cycle. However, the inhibition of glucose utilization seems to be counterregulated by stronger responses of insulin and glucagon. These results may be important also in the consideration of food selection during premigratory periods and refueling abilities of birds crossing ecological barriers.

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Introduction

Prior to their long-distance flights garden warblers (*Sylvia borin*) like many other migratory birds accumulate large amounts of subcutaneous fat as fuel for migration (Blem 1980, Bairlein 1987). During this premigratory fat deposition, food intake and body mass increase up to 100% (Berthold 1996) and food preference (Bairlein & Simons 1995) and utilization efficiency (Bairlein 1985, Hume & Biebach 1996) can change. These effects are controlled by an endogenous time program (Berthold *et al.* 1972, Bairlein & Gwinner 1994).

In the garden warbler the fat deposition coincides with shifts of plasma metabolite levels (Bairlein 1983, Totzke 1996). Correspondingly, migrating garden warblers show significantly higher plasma levels of glucose and fatty substrates at stop-over sites in the Sahara than in the breeding grounds (Bairlein & Totzke 1992). Such changes are unusual for vertebrates adapted to extreme endurance performances; hence a new pathway for fuel supply in small passerines during long-distance migration has been postulated (Jenni-Eiermann & Jenni 1992). Nonetheless,

there are obvious similarities to processes involved in human obesity. From this we proposed that increased glucose levels in fat birds may be the result of an inhibition of carbohydrate metabolism by the higher plasma levels and oxidation of fatty acids (Totzke 1996). In humans the main cause of carbohydrate metabolism disorders are malfunctions of pancreatic hormones. A defect in insulin production leads to type I diabetes mellitus (Cudworth 1976) and type II diabetes mellitus is characterized by insulin resistance causing decreased peripheral glucose uptake in muscle and adipose tissue as well as increased hepatic glucose production (Gerich 1991, DeFronzo 1992). Because of competitive oxidation, fatty acids can influence glucose oxidation (Randle *et al.* 1963). Recent studies indicate that the insulin-induced suppression of hepatic glucose production is mediated by fatty acids (Mittelman *et al.* 1997). A possible mechanism, involving both the regulation of glucose oxidation by the glucose fatty acid cycle (Randle *et al.* 1963) as well as the reliance of lactate production on glucose oxidation, resulting in substrate-induced changes in the rate of gluconeogenesis

in the Cori-cycle, has been discussed by Hübinger *et al.* (1997).

The role of pancreatic hormones in domestic birds seems to deviate in some respects from that in mammals (Hazelwood 1986), but in spite of the large implications this may have for migratory birds little comparative work has been done (Wingfield *et al.* 1990).

In this study, the relationship of fat and carbohydrate metabolism and its control by glucagon and insulin were investigated in the migratory garden warbler comparing three different physiological states: (a) during a (pre) migratory phase with high body mass, or (b) after a period of continuous fasting which simulated the crossing of large ecological barriers, and (c) during postmigration with low body mass. The aim was to examine whether seasonal fattening, fasting and preprogrammed fat loss in migratory birds parallel changes of glucose utilization and insulin sensitivity known from mammals during obesity and starvation.

Materials and Methods

Twelve garden warblers, seven males and five females, were caged under constant indoor conditions (20 °C, with a ratio of 12 h light to 12 h darkness, with no light during the night to suppress migratory restlessness (Czeschlik 1977) and standard insect food (Bairlein 1986)) from September to June. Body mass and food intake were recorded daily in the morning, fat level (Kaiser 1993) and molt intensity (0=none, 1=less than one-third, 2=up to two-thirds, 3=more than two-thirds of the feather tracts) were estimated weekly (Berthold *et al.* 1970).

Glucose utilization and the pancreatic hormone response were examined in freely feeding birds after reaching a relatively constant high body mass level during autumnal fat deposition. To simulate the fasting situation of long-distance flights, birds which were still fat after both tests were food-deprived for 5–7 days until they reached a body mass around 20 g and tested for glucose utilization again. Finally, birds were re-examined at a constant low body mass level when the endogenous time program for the fall migration had been completed.

Oral glucose loads (100 µl of a solution containing approximately 80 mg glucose) were administered to the birds preprandially during the morning immediately after light onset.

For the hormone response test (HRT) blood samples were taken at light onset once prior to and once 10 min after glucose application. Because of the plasma amounts required for the hormone measurements (≥ 75 µl), samples at 0 min and 10 min from each bird were taken on different days, but in each condition within 2 weeks.

For the glucose tolerance test (OGTT) plasma glucose, free fatty acids (FFAs) and occasionally β -hydroxybutyrate were determined. Since smaller sample sizes were suf-

ficient, samples were taken from each bird immediately prior to and 1 and 2 h after glucose application. Time schedules of both tests were adjusted to standards in human medicine. However, due to the bird's size and the amount of blood, measurements of stimulated hormone levels were carried out only once at 10 min after glucose administration. Other studies on larger bird species have shown that maximum responses could be expected at this time (Simon & Rosselin 1978, Minick *et al.* 1996).

Since annual changes in the nutrient utilization efficiency of the garden warbler are known (Bairlein 1985, Hume & Biebach 1996), glucose absorption was determined by simultaneously collecting feces in which glucose was quantified enzymatically (Boehringer, Mannheim, Germany).

Blood was collected in capillaries (containing heparin or EDTA/trasyol in a 10%-amount of the blood volume to be taken) by puncturing the wing vein. Blood samples were centrifuged (15 min, 10 000 r.p.m., 4 °C) and the supernatant plasma was assayed for glucagon (according to Unger modified by Weir *et al.* 1973) and insulin (Pharmacia, Uppsala, Sweden) with recently validated heterologous RIAs (Totzke *et al.* 1997) and for glucose (Kodak Ektachem, Stuttgart, Germany), FFAs (Boehringer) and β -hydroxybutyrate (HBA; Sigma Chemical Co., St Louis, MO, USA).

For the statistical analysis of glucose tolerance tests the areas enclosed by the glucose and FFA curves and the line through the respective initial level were calculated by integration. Mean values of dependent samplings were tested according to Friedman and Wilcoxon tests, and correlations were described by Spearman's rank correlation coefficients (r_s). Non-parametric tests were used since the kind of data distribution in the small sample size was not clear with certainty. For blood analysis results from both sexes were pooled.

Results

Circannual fat deposition

There were no differences in the occurrence and the extent of fat deposition between sexes. The mean levels of body mass of the birds on the experimental days and the average food intake and body mass change during the preceding day as well as fat and molt scores are shown in Table 1.

Glucose and FFA levels

Basal levels of FFAs were highest in fasted (1.34 ± 0.24 mmol/l, $n=9$), intermediate in fat (1.08 ± 0.10 mmol/l, $n=12$) and lowest in lean birds (0.72 ± 0.06 mmol/l, $n=12$, average mean $\pm$ s.e.m. of all birds in HRT or OGTT, Friedman: $P<0.05$, $n=5$;

Table 1 Mean levels of body mass of the birds on the experimental days and the average food intake and body mass change during the preceding day, and fat and molt scores. Values are means $\pm$ S.E.M. Significant differences are given by the superscripted letters, which refer to the physiological conditions given in brackets in the first column

	<i>n</i>	Body mass (g)	Δ Body mass (g/day)	Food intake (g dwt/day)	Fat level	Molt
Fat (a)	12	28.0 $\pm$ 0.5 ^{b,c}	0.05 $\pm$ 0.07 ^b	4.8 $\pm$ 0.4 ^b	7.2 $\pm$ 0.1 ^{b,c}	0.1 $\pm$ 0.03 ^c
Fasted (b)	9	19.2 $\pm$ 0.2 ^{a,c}	-1.30 $\pm$ 0.04 ^{a,c}	0.0 $\pm$ 0.0 ^{c,a}	6.0 $\pm$ 0.3 ^{a,c}	0.0 $\pm$ 0.0
Lean (c)	12	17.7 $\pm$ 0.4 ^{a,b}	0.04 $\pm$ 0.09 ^b	4.7 $\pm$ 0.2 ^b	2.1 $\pm$ 0.3 ^{a,b}	0.6 $\pm$ 0.2 ^a
Friedman	<i>P</i>	<0.001	<0.001	=0.001	<0.001	>0.1

Figure 1 Plasma glucose (GLUC) and FFA levels of garden warblers during OGTT in lean, fat and fasted birds. On the left, plasma levels (mean $\pm$ S.E.M.) 0, 1, and 2 h after an oral glucose (GLUC) load are shown. On the right integrals of the areas enclosed by the curves and the horizontal line of the respective initial level are shown. Since GLUC levels were increasing and FFA levels decreasing during OGTT, integrals were positive for GLUC and negative for FFA. At the bottom right, the relationship between the integrated GLUC and FFA changes is shown.

Wilcoxon, lean-fat: $P < 0.05$, lean-fasted: $P < 0.05$; Figs 1 and 2). Basal glucose levels changed similarly but not significantly (Friedman: $P = 0.06$, $n = 9$; Figs 1 and 2).

After glucose administration, lean birds had lower plasma levels and higher utilization rates than fat birds (Figs 1 and 2). However, the latter were further reduced after fat birds had been food deprived until they had almost

Figure 2 Plasma glucagon (GLN), insulin (INS), glucose (GLUC) and FFA levels (mean $\pm$ S.E.M.) in lean and fat birds during HRT. Significant differences between 0 and 10 min after oral glucose loads are given above bars, while those between the lean and the fat condition are marked by asterisks: * $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$ (Wilcoxon).

reached their corresponding lean body mass (Fig. 1, Table 1). Integrals of plasma glucose increases were positively correlated with initial plasma FFA levels ($r_s = 0.65$, $P < 0.001$, $n = 29$). One hour after an oral glucose load mean plasma FFA levels decreased in all groups. After 10 min decreases were only found in the lean condition (Fig. 2). After 2 h, FFA levels tended to recover faster than plasma glucose (Fig. 1). Integrals of plasma FFA changes were opposite to those of glucose ($r_s = -0.73$, $P < 0.001$, $n = 29$; Fig. 1). In non-fasting birds none of them correlated significantly with food intake or body mass change on the preceding day ($P > 0.1$).

Glucose absorption varied without any relationship to the bird's physiological condition between 70 and 100%.

Hormone response

There were no significant differences in the basal levels of glucagon, insulin and insulin:glucagon ratios among lean and fat birds (Fig. 2). Ten minutes after glucose administration, birds showed higher insulin and lower glucagon mean levels (Fig. 2), but for both hormones changes were smaller in the lean than in the fat condition (Fig. 2). They were only significant for the latter (Wilcoxon, lean: each $P > 0.1$, fat: each $P \leq 0.01$). Ten minutes after the oral glucose loads, glucagon levels tended to be lower in the fat than in the lean condition (Wilcoxon: $P = 0.059$). The average changes of hormone levels relative to corresponding glucose increases were nearly twice as high in fat birds than in lean birds, but differences were not significant ($\Delta\text{insulin}/\Delta\text{glucose}$, lean: 0.21 ± 0.14 , fat: 0.38 ± 0.17 ; $\Delta(\text{insulin:glucagon})/\Delta\text{glucose}$, lean: 0.44 ± 0.20 , fat: 0.86 ± 0.26 (mean $\pm$ s.e.m.); Wilcoxon, sign-test, paired t -test: $P > 0.1$, $n = 8$).

Glucose correlated negatively with glucagon ($r_s = -0.52$, $n = 40$, $P < 0.001$) and positively with insulin ($r_s = 0.52$, $n = 40$, $P < 0.001$) during the hormone response tests and negatively with both FFA and β -hydroxybutyrate (FFA: $r_s = -0.26$, $n = 87$, $P < 0.05$; HBA: $r_s = -0.61$, $n = 46$, $P < 0.001$) during glucose tolerance tests. FFA levels showed no significant relationships with any of the hormones (each $P > 0.1$), but were related directly to β -hydroxybutyrate ($r_s = 0.55$, $n = 46$, $P < 0.001$).

Discussion

During the premigratory fat deposition, birds store fuel for migration and undergo preadaptive metabolic changes (Marsh 1981, Lundgren & Kiessling 1985, 1986, Ramenofsky 1990), which at least for some species include plasma hyperlipidemia (DeGraw *et al.* 1979, Singh *et al.* 1993, Totzke 1996) and hyperglycemia (Bairlein 1983, Totzke 1996). These processes are similar to human obesity. A special role of triglycerides for the fuel supply during migration has been reported by Jenni-Eiermann & Jenni (1992). Marsh (1981) and Lundgren & Kiessling (1985, 1986) showed a preadaptive change to a more effective oxidation of lipids. Therefore, an inhibition of glucose utilization can be assumed (Totzke 1996), which has been shown for mammalian cells under *in vitro* conditions by the glucose fatty acid cycle (Randle *et al.* 1963). The relevance of this mechanism *in vivo* is still a matter of discussion (Sugden & Holness 1990). However, the close inverse relationship between plasma glucose and FFA levels after oral glucose intake (Fig. 1) during this study provides some evidence for this relevance. Lean and fat birds did not show significant differences in food intake

on the preceding days (Table 1), so that nutritional effects cannot account for the observed differences between both conditions in glucose tolerance and hormone response tests. However, basal levels of glucose, FFAs and plasma glucose increase after an oral application were largest in fasted birds. This indicates the lowest glucose utilization rate and an even stronger reliance on fat as substrate for energy supply than in the fat condition.

An increased basal glucose level as well as a decreased glucose tolerance after food deprivation similar to mammals has been described in the chicken (Simon & Rosselin 1978), although it was assumed to result more from an impaired insulin release than from insulin resistance in contrast to mammals (Penicaud *et al.* 1985).

Insulin resistance could result from the action of corticosterone (Taouis *et al.* 1993, Bisbis *et al.* 1994), which increases in plasma during fasting in several bird species (Harvey *et al.* 1981, Jeffrey *et al.* 1985, Lea *et al.* 1992), including the chicken (Freeman *et al.* 1983). However, this does not seem to apply for seasonal fattening species like the garden warbler, which show no corticosterone increase while drawing on lipid reserves (Cherel *et al.* 1988, Schwabl *et al.* 1991).

Insulin resistance and concurrent hyperinsulinism are also typical features of mammalian obesity (Karam *et al.* 1963). However, garden warblers showed no significant variations in overnight-fasted insulin levels, either during the seasonally or the experimentally induced body mass cycle (Totzke *et al.* 1997). During this study fat birds also showed no deviations in basal hormone levels (Fig. 2), but, actually similar to obese humans, a stronger response in the secretion of pancreatic hormones to oral glucose loads, perhaps in order to compensate for a lack in glucose utilization efficiency. Moreover, the $\Delta\text{insulin}/\Delta\text{glucose}$ ratio indicates a lesser degree of insulin sensitivity in the fat state. However, compared with obese humans the increases of insulin in birds seem smaller and concurred with larger decreases of glucagon (Fig. 2).

In general, the increases in plasma glucose seem to be rather acceptable for birds and less strictly counterregulated than in mammals. However, in contrast to overnight-fasted birds correlations between glucose and pancreatic hormones were highly significant within the first 10 min after application whereas those with FFA were not. This agrees with results in raptors (Minick 1986, Minick *et al.* 1996), and it is indicative of a plasma level control by pancreatic hormones for glucose, but not for FFAs.

In conclusion, the premigratory fat deposition seems to be associated with a switch in peripheral catabolism to the oxidation of fats resulting in a lower glucose utilization. This may influence food selection favoring a preference for lipids during premigratory periods (Bairlein 1987, 1990). Furthermore, this process may be important for birds refueling at stop-over sites, especially in view of fasting during long migratory flights. Still, exercise itself may compensate by improving glucose utilization (Segal *et al.*

1991). Together with the obviously different consequences of human and avian adiposity on metabolism this remains a matter of further investigation.

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