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PLASMA METABOLITE LEVELS PREDICT INDIVIDUAL BODY-MASS CHANGES IN A SMALL LONG-DISTANCE MIGRANT, THE GARDEN WARBLER

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ABSTRACT.—Change in body mass is an important measurement in many studies addressing changes in energy stores or condition. Usually, change in body mass is measured in birds caught twice, but this has a number of drawbacks (e.g. low number of retraps, retraps not representative of all first captures, adverse effects of first capture on body-mass development). Therefore, we investigated whether plasma metabolites correlate with body-mass change, and which metabolites could be used to predict body-mass change in birds caught once. In an experiment, 20 Garden Warblers (*Sylvia borin*) were given different amounts of food to induce stable, increasing, and decreasing body masses. Most of the eight plasma metabolites we examined were significantly correlated with the change in body mass between early morning and midday, the time of blood sampling, but not with body mass or various measures of activity. Metabolites that are known to characterize resorption were elevated in birds gaining body mass and metabolites characteristic of fasting were elevated in birds losing body mass. Triglycerides and β-hydroxy-butyrate together explained 61% of the variation in body-mass change (triglycerides alone 44% and β-hydroxy-butyrate alone 51%). These metabolites may be used to predict body-mass change in birds caught once, provided that the reliability and sensitivity of this method are checked in field tests. *Received 27 September 1993, accepted 30 January 1994.*

BIRDS MAY FACE DEFICITS in their energy balance during the nonactive period of the day, during periods of several days, or even over a series of weeks. They usually build up energy stores in anticipation of such periods and as an adaptive compromise to different selective pressures (e.g. Lima 1986, Lehikoinen 1987, Moreno 1989, Ekman and Hake 1990, Rogers and Smith 1993). This results in fluctuating energy stores between foraging and nonforaging periods (usually day and night) superimposed on longer periods of net energy storage and net energy expenditure (King 1972). For instance, cold and short winter days, bad weather, incubation, feeding young, and migration may all be associated with decreasing energy stores and preceded by energy storage.

Body mass is a crude but widely used estimate of energy stores because it is easy and harmless to measure. If corrected for size, body mass may be used as an indication of condition (e.g. Blem 1990). Changes in body mass may give a fairly accurate picture of the fluctuations in energy stores (e.g. Rogers and Rogers 1990). The measurement of changes in body mass of free-living birds is important in a variety of contexts. For instance, it may allow the assessment of the effects of food availability on energy storage in different habitats, under different weather conditions, and under different degrees of competition or predation (e.g. Bibby et al. 1976, Bibby and Green 1983, Buchanan et al. 1985, Lima 1985, Lindström 1990, Moore and Yong 1991). Body-mass changes may indicate periods of constraints and periods of adaptive mass variations during the annual cycle (Moreno 1989). Evaluation of body-mass changes may allow study of the pattern and environmental influences of energy storage during stopovers by migrants (e.g. Bibby et al. 1976, Mehlum 1983a, b, Biebach 1985, Biebach et al. 1986, Alerstam and Lindström 1990).

In free-living birds, changes in body mass generally are measured in birds caught at least twice, usually at the same place. This, however, has a number of drawbacks: (1) changes in body mass are measured only in the subsample of retraps and this may reduce the sample size drastically, especially when trap shyness is a factor; (2) a certain group of birds may not be recaught and, thus, the sample of retraps may not be representative (e.g. Bibby et al. 1976, Biebach et al. 1986, Veiga 1986, Winker et al. 1992); and (3) first capture may have an adverse

effect on the development of body mass (e.g. Rogers and Odum 1966, Nisbet and Medway 1972, Ens et al. 1990, Winker et al. 1992).

Therefore, it would be helpful to have a method that allows the estimation of body-mass changes of birds caught only once. This would allow insights into the dynamics of the condition of the bird (i.e. the potential of an individual to meet its future energy requirements; Evans and Smith 1975). Winker et al. (1992) used the daily pattern of body mass of birds caught once to estimate mass gain. This method, however, provides a mean mass gain for the sample and not estimates for individual birds. Plasma metabolites are likely to reflect body-mass change in individuals, since they show pronounced daily variation dependent on food intake.

To test whether plasma metabolite levels predict individual body-mass changes, we chose to study a long-distance migrant, the Garden Warbler (*Sylvia borin*). This species naturally shows pronounced endogenous changes in body mass during the migratory season that are well studied in experimental situations (Klein et al. 1971, Berthold et al. 1972a, b, 1974, Berthold 1976, Schindler et al. 1981, Gwinner et al. 1988) and in the field (Bairlein 1987, 1991). We examined whether one or more of eight plasma metabolites studied reflect changes in body mass or whether they are dependent on other factors such as body mass and activity.

Material and Methods

Animals.—Twenty first-year Garden Warblers were caught in the Engadin valley, Switzerland, from 4–7 August 1990. They were kept singly in cages in a room with artificial light that simulated the natural photoperiod. The two perches in each cage were mounted on microswitches that were connected to a personal computer and counted the hops for each interval of 15 min. Disturbances of the birds (feeding, cleaning, examinations) were recorded and taken into account in the analyses. Molt and the visible amount of subcutaneous fat in the furcular depression were determined about every fourth day.

The birds were allowed to accustom to the cages during two weeks with food given *ad libitum*. During this time nearly all birds finished their postjuvenile molt and started nocturnal migratory restlessness at the onset of migration in free-living conspecifics (Klein et al. 1973, Jenni and Jenni-Eiermann 1987). After the experiments, birds were released.

Food consisted of mealworms and a mixture described by Berthold et al. (1990). During the experimental period, the birds were given an amount of food that induced the desired change in body mass. In addition, fruits (*Frangula alnus, Sambucus nigra, S. racemosa, Lonicera xylosteum*) found in natural habitats of Garden Warblers always were available.

Experimental protocol.—The experimental design was to take blood samples during four different body-mass conditions: (a) during a period of stable body mass of about 18 g (corresponding to premigratory mass); (b) during a period of mass gain; (c) during a period of stable high body mass of about 21 g; and (d) during a period of mass loss. For keeping body mass stable at a low level (ca. 18 g), 5 to 6 g of mealworms and food mixture were given; for keeping body mass stable at a high level (ca. 21 g), 10 to 11 g of food were provided. For increasing body mass, food was provided *ad libitum* and, for inducing a decrease in body mass, food was reduced to 3 to 4 g. The amount of food given was adjusted individually. One-half of the food was given in the early morning, the other half at midday.

Ten birds followed the original experimental design and went through the body-mass conditions in the order a-b-c-d (group 1, Fig. 1). The other 10 birds did not increase body mass despite food *ad libitum* and went through the body-mass conditions in the order a-a-d-b (group 2, Fig. 1). Hence, although the four body-mass conditions were comparable between the two groups, the absolute body mass and the sequence of body-mass changes differed.

Data collection.—Birds were weighed daily between 0630 and 0730, and at midday. This allowed us to determine the change in body mass between midday of the day before blood sampling and morning of the day of blood sampling (D-MASS-Y), and between morning and midday of the day of blood sampling (D-MASS-M).

Locomotor activity recorded was expressed as: sum of hops (HOPS-M) and mean number of hops per 15 min (ACTIV-M) during morning from light until blood sampling; sum of hops (HOPS-N), mean number of hops per 15 min (ACTIV-N), and percent of 15-min intervals with hopping activity (ACTIV-P) during night before blood sampling. There was a wide variation in these variables (HOPS-M, range 1,221–13,488; HOPS-N, 4–16,302; ACTIV-M, 42–409; ACTIV-N, 0.11–407; ACTIV-P, 2.5–100). Although all individuals were housed in the same room and could hear one another, there was no significant difference in any activity measure among days, but night-activity measures nearly reached a significance level (i.e. $P = 0.05$). This was partly due to a general increase of night activity during the migratory season (cf. Berthold et al. 1972a, 1974).

Blood was taken at midday of the day when the individual bird attained the desired body-mass condition. Thus, individual birds were sampled on different days. Blood samplings were never taken less than three days apart. Blood was sampled by punc-

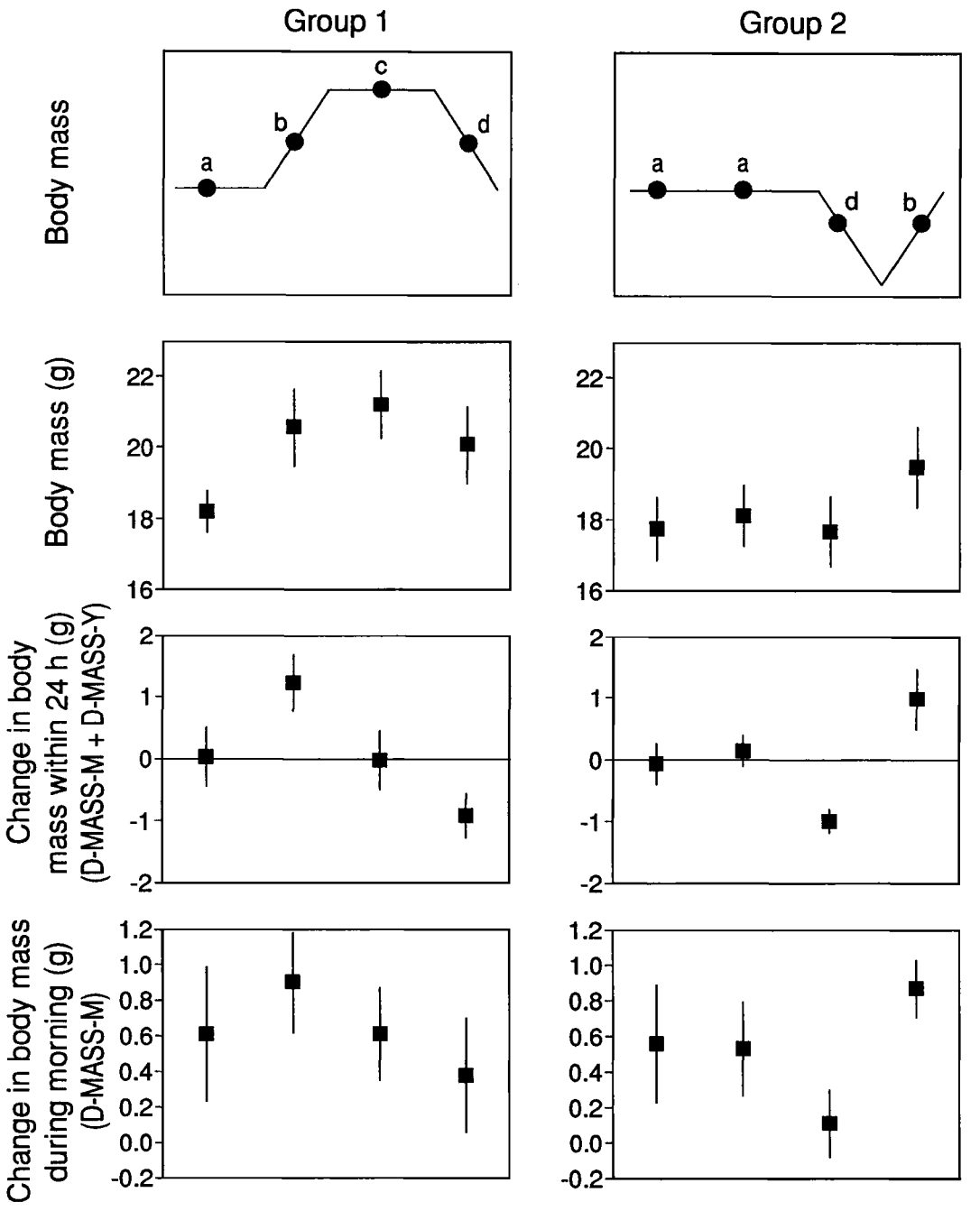


Fig. 1. Schematic representation of experimental design of body-mass conditions (top), and of body mass and body-mass changes of two experimental groups. Sample size 10 for each mean value, except for body mass (condition a) of group 1, where it was 9. $\bar{x} \pm$ SD indicated by square and vertical lines.

turing the vena ulnaris and the blood drops were collected with a capillary system (Microvette CB300 Fluore, Sarstedt). The blood was centrifuged and the plasma stored at −20°C until analysis. An aliquot of plasma was stored at 4°C for protein and lipoprotein electrophoreses, which were completed within two days. In a few cases, the amount of blood sampled was insufficient for the determination of all metab-

olites. Therefore, sample sizes differ slightly for the different metabolites.

Metabolites were determined in the plasma (in hemolyzed blood for glucose) using standard test combinations as modified for handling small amounts of plasma (3–10 μl per determination): enzymatic UV-test for glycerol (Boehringer Mannheim GmbH), glucose (Merck) and β-hydroxy-butyrate (Sigma Diagnostics), enzymatic colorimetric tests for uric acid, free fatty acids (Boehringer Mannheim GmbH), and triglycerides including free glycerol (Roche Diagnostica, Basel). Plasma triglycerides were calculated as:

$$(\text{triglycerides} + \text{glycerol}) - \text{glycerol}.$$

Lipoproteins and plasma proteins were evaluated using the standard agarose gel electrophoresis system Paragon (Beckman) and following precisely the instructions given by the producer. The proportions of the peaks were quantified by densitometric scanning (Appraise Junior Densitometer, Beckman).

Lipoprotein electrophoreses showed a maximum of five peaks. Peak 1 (LIPO1), situated at the origin of the electrophoresis, is found only in resorptive birds (unpubl. data) and represents the chylomicrons. Peak 2 (LIPO2) was shown to represent the very-low-density lipoproteins VLDL (fraction 1 in Jenni-Eiermann and Jenni 1992). For the purpose of this study, the sum of LIPO1 + 2 was considered, since chylomicrons and VLDL are directly related to fat transportation to the adipose tissues.

Plasma protein electrophoresis showed a maximum of six peaks. Peak 1 (PROT1) is considered in our study, because it represents albumin (unpubl. data), which is the main carrier of free fatty acids in the blood.

Data analysis.—By varying the amount of food, it was possible to induce the planned body-mass conditions in general (Fig. 1). However, the body-mass changes could not be controlled very precisely, probably because the birds could always choose among food types of different energy contents, and because they differed in activity levels. This resulted in a range of body-mass changes. Therefore, plasma metabolite levels were analyzed both by evaluating differences between experimental body-mass conditions and by looking at correlations with body-mass changes. One bird of group 1 decreased in body mass when sampled for condition a, and was excluded for comparisons between body-mass conditions.

Because of their large departure from a normal distribution, β-hydroxy-butyrate concentrations were transformed into ln(β-hydroxy-butyrate + 0.5), HOPS-N into ln(HOPS-N) and ACTIV-N into ln(ACTIV-N). The proportions of LIPO1 + 2 and PROT1 were transformed using the arcsine-square-root transformation (Linder and Berchtold 1976).

Differences in plasma metabolite levels between treatments (conditions of body mass) were tested by two-way ANOVAs without replication (Sokal and Rohlf 1981). Linear multivariate regression was used to analyze the dependence of the metabolite levels on body mass, body-mass changes (D-MASS-Y, D-MASS-M), activity (HOPS-M, HOPS-N, ACTIV-M, ACTIV-N, ACTIV-P), experimental group (dummy variable, 1 = group 1, 2 = group 2), and individuals. The 20 individuals were entered into the multivariate-regression analyses as a set of 19 dummy variables to test for individual differences. Independent variables were added to the model stepwise and the significance of the variable added was tested after each step using the *F*-test (Sokal and Rohlf 1981).

Results

Effects of body-mass conditions.—The plasma metabolite levels differed significantly between the experimentally induced body-mass conditions with the exception of glucose, protein, LIPO1 + 2 (group 1) and PROT1 (Fig. 2). Glycerol, free fatty acids, and β-hydroxy-butyrate are high during fasting (Jenni-Eiermann and Jenni 1991), and they also showed low levels with increasing body mass and high levels with decreasing or stable body mass here.

Triglycerides, uric acid and LIPO1 + 2, which have high values during resorption (Jenni-Eiermann and Jenni 1991, unpubl. obs.), exhibited the reverse pattern. They were high when body mass increased and low when body mass decreased.

Glucose and protein levels did not differ significantly between body-mass conditions. PROT1 showed no significant differences between body-mass conditions, although its pattern was similar to the metabolites associated with fasting.

Effects of body mass, body-mass changes, and activity.—In a first set of multiple-regression analyses, the individual birds always were included in the model as a set of dummy variables to test for individual differences, but the experimental group was excluded. In all regression models tested, the set of dummy variables representing the individual birds had no significant influence on the metabolite concentrations. In a second set of multiple-regression analyses, the individuals were excluded and the experimental group was tested for significance.

Table 1 shows the final models that best explained the variation in plasma metabolite levels. The mass change between morning and midday of the day of blood sampling (D-MASS-

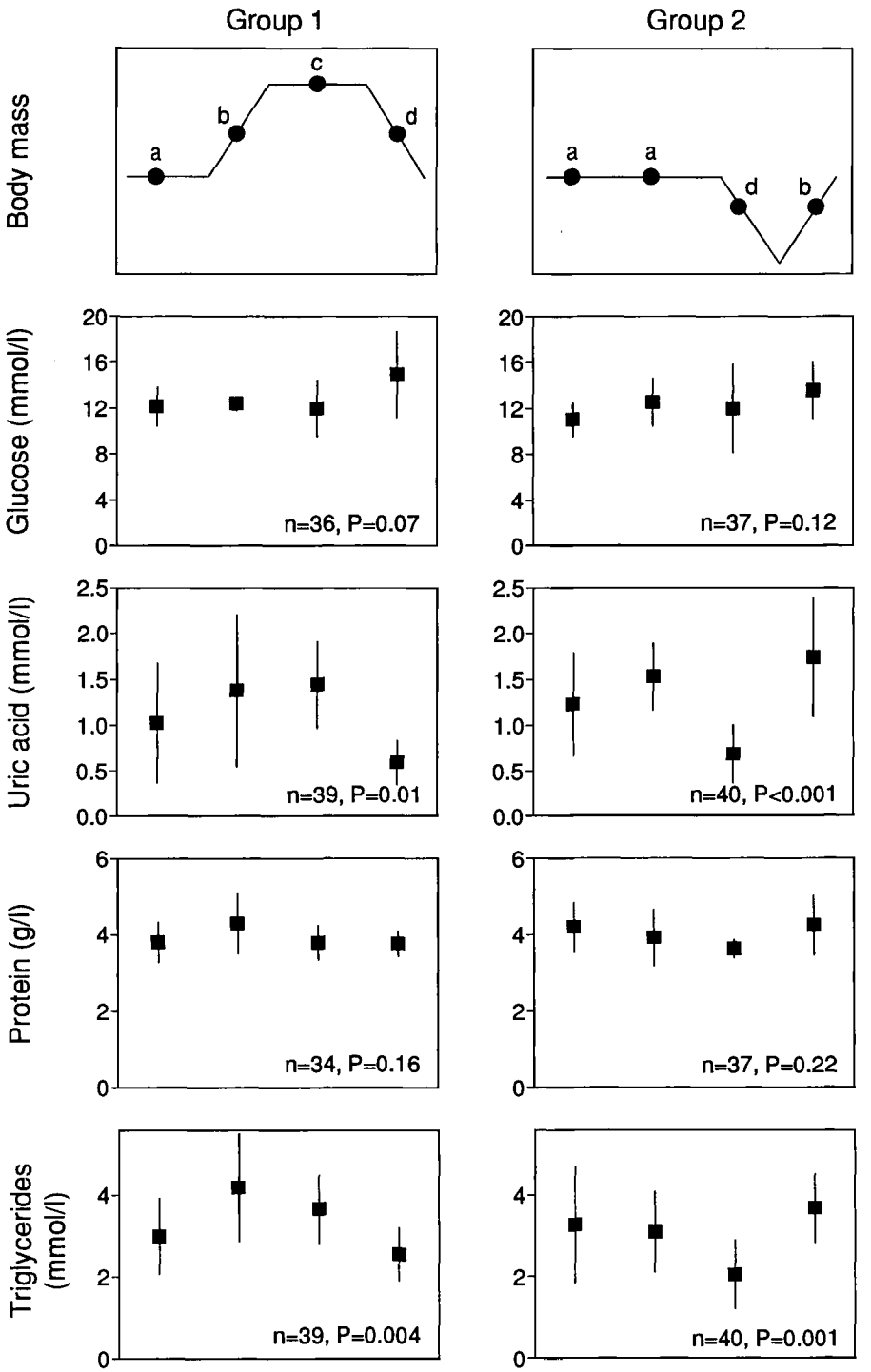
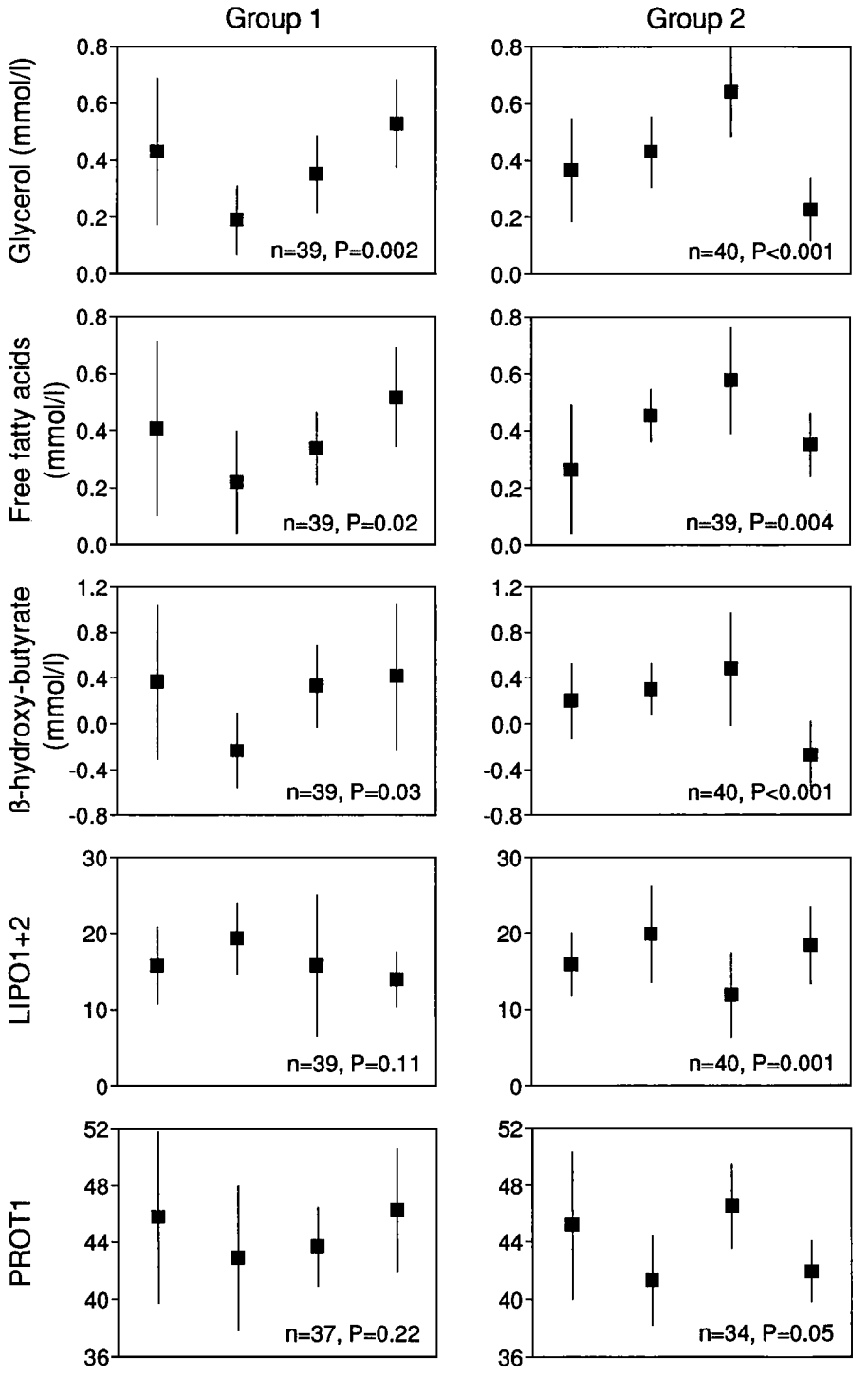


Fig. 2. Mean values (± SD) of seven metabolite concentrations, and proportion of two electrophoresis peaks for the four body-mass conditions of each experimental group. Probability values concern differences among body-mass conditions (two-way ANOVA without replication). There were no significant added variance components among birds, except for triglycerides of group 2 ($P = 0.004$) and for LIPO1 + 2 (group 1, $P =$



0.012; group 2, $P = 0.004$). Sample size 10 for each mean value, except for glucose, protein and PROT1 for which about one-half of the means have sample size between 6 and 9. β-hydroxy-butyrate, LIPO1 + 2, and PROT1 are transformed (see Methods).

TABLE 1. Dependence of metabolites (dependent variables) on body mass, body-mass change (D-MASS-M, D-MASS-Y), activity (HOPS-M, ACTIV-M, HOPS-N, ACTIV-N, ACTIV-P), group, and individuals. Result of final step of multiple-regression analyses is given. Only those independent variables with significant contributions are listed. β-hydroxy-butyrate was entered in its transformed form ln(β-hydroxy-butyrate + 0.5), and LIPO1 + 2 and PROT1 in their arcsine-square-root transformations. Regression coefficient (r) with T-value and level of significance (asterisks) given. Adjusted correlation coefficient (r^2_{adj}), F-value, and significance (asterisks) of the final model, and sample size (n) provided in parentheses following dependent variable.

Dependent variable Independent variable	b	T
Glucose (0.060, 5.60*, 73)		
Body mass	0.441	2.37*
Constant	4.095	1.14
Uric acid (0.247, 13.96***, 80)		
D-MASS-M	0.573	3.02**
D-MASS-Y	0.311	2.79**
Constant	1.033	6.73***
Protein (0.152, 7.38***, 72)		
D-MASS-M	0.793	3.80***
Body mass	−0.101	−2.16*
Constant	5.436	6.42***
Triglycerides (0.429, 60.30***, 80)		
D-MASS-M	2.138	7.77***
Constant	1.960	10.66***
Glycerol (0.574, 54.33***, 80)		
D-MASS-M	−0.315	−6.91***
D-MASS-Y	−0.119	−4.43***
Constant	0.515	13.96***
Free fatty acids (0.373, 16.07***, 77)		
D-MASS-M	−0.226	−4.05***
HOPS-M	2.4×10^{-5}	3.24**
ACTIV-P	1.7×10^{-3}	2.10*
Constant	0.305	3.89***
β-hydroxy-butyrate (0.528, 45.23***, 80)		
D-MASS-M	−1.067	−9.47***
Group	−0.177	−2.19*
Constant	1.08	7.22***
LIPO1 + 2 (0.125, 6.52**, 78)		
D-MASS-Y	2.161	2.02*
ACTIV-M	−0.016	−2.34*
Constant	20.264	15.09***
PROT1 (0.111, 9.83**, 72)		
D-MASS-M	−4.312	−3.14**
Constant	46.656	50.11***

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

M) correlated best with every metabolite, except for glucose and LIPO1 + 2. However, LIPO1 + 2 also showed a correlation with D-MASS-M alone ($P = 0.03$). The metabolites characteristic of fasting (i.e. glycerol, free fatty acids, β-hydroxy-butyrate, and PROT1) showed a negative relationship with D-MASS-M, while the metabolites characteristic of resorption (protein, uric acid, triglycerides, and LIPO1 + 2) showed a positive relationship with D-MASS-M.

Glucose was the only metabolite that was only significantly correlated with body mass. Body mass had also a weak positive correlation with protein. In addition to D-MASS-M, uric acid showed a positive and glycerol a negative relationship with D-MASS-Y. LIPO1 + 2 correlated negatively with the amount of activity during the morning and free fatty acids positively (and to a lesser extent with night activity). Differences between the two experimental groups were only found in β-hydroxy-butyrate.

From the analyses reported above, it appears that D-MASS-M is strongly related to all metabolites examined, except glucose. However, these relationships may not be causal, since other factors correlated with D-MASS-M could determine the plasma metabolite levels. Experimentally, we controlled for age, molt, time of day, and season. However, body mass and activity were correlated with D-MASS-M (body mass, $r = 0.403$, $P = 0.0002$; HOPS-M, $r = -0.332$, $P = 0.003$; ACTIV-M, $r = -0.358$, $P = 0.001$; no significant correlation with HOPS-N, ACTIV-N, ACTIV-P) and, thus, could be factors causally related to metabolite levels. These were analyzed in more detail.

In a first analysis, we selected body-mass condition a and c of the experimental group 1, because they showed similar D-MASS-M values ($\bar{x} = 0.61 \pm$ SD of 0.38 g and 0.61 ± 0.26 g; one-way ANOVA, $F = 0.0007$, $P = 0.98$), but a marked difference in body mass (18.2 ± 0.58 g and 21.2 ± 0.95 g; see Fig. 1). If mass and not the change in body mass influences the plasma metabolite concentrations, the metabolite levels should differ between body-mass condition a and c. However, no significant difference was found (Table 2).

In a second analysis, we selected the central third of the cases with respect to the distribution of D-MASS-M. These cases had D-MASS-M values between 0.43 and 0.69 g, thus covering only 18% of the total range in D-MASS-M (−0.19 to 1.29 g), but 64% of the total range in body

TABLE 2. *F*-value from one-way ANOVA of differences in metabolite levels comparing body-mass condition a and c of experimental group 1 ($P > 0.12$ for all ANOVAs).

Metabolite	*n*	*F*
Glucose	19	0.03
Uric acid	19	2.58
Protein	18	0.00
Triglycerides	19	2.74
Glycerol	19	0.73
Free fatty acids	19	0.43
β-hydroxy-butyrate	19	0.02
LIPO1 + 2	19	0.00
PROT1	18	0.89

mass (16.2 to 21.1 g of total range of 15.9 to 23.5 g). Again, no correlation between body mass and the plasma metabolites could be detected (Table 3).

A third analysis examined the influence of the activity during the morning on the plasma metabolites in more detail, and we selected again those cases with D-MASS-M between 0.43 and 0.69 g. They covered 100% of the total range in HOPS-M and ACTIV-M. Only free fatty acids correlated strongly with HOPS-M and ACTIV-M (Table 3). Hence, only free fatty acids showed a strong correlation with the amount of activity during the few hours before blood sampling. This confirms the results of the multiple-regression models (Table 1). The correlation with HOPS-M is apparently strong enough to resist the intercorrelation with D-MASS-M.

In summary, these analyses confirm that D-MASS-M is the best predictor for most plasma metabolite levels. Regarding the multiple-regression models (Table 1), no intercorrelations between D-MASS-M and body mass or morning activity obscured a relationship between body-mass change and the metabolites.

Predicting body-mass change from metabolite levels.—Since most plasma metabolites studied showed relatively high correlations with the change in body mass during the hours before blood sampling, the change in body mass of a bird caught once may be predicted by one or a set of plasma metabolites measured at the time of capture. The data from our study provide a calibration for this prediction.

In a stepwise multiple-regression analysis, D-MASS-M was tested for dependence on all the plasma metabolites studied and on the five measures of activity. The plasma concentrations of triglycerides and β-hydroxy-butyrate contributed significantly to explain variation in D-MASS-M (Table 4). Together they explained 61% of the variation in D-MASS-M, triglycerides alone 44% and β-hydroxy-butyrate alone 51% (Fig. 3). The association of D-MASS-M with triglycerides is not significantly different from the association with β-hydroxy-butyrate (test of homogeneity between two correlation coefficients, $t = 0.65$, $P = 0.48$; Sokal and Rohlf 1981).

To be certain that levels of triglycerides and β-hydroxy-butyrate were not related to body mass or activity, analogous multiple-regression analyses were performed with body mass or one of the five measures of activity as the dependent variable. Only the plasma concentrations of glycerol and glucose were significantly related to body mass, while free fatty acids were correlated with HOPS-M and ACTIV-M (Table 4).

DISCUSSION

Metabolic responses to body-mass changes.—Our study showed that body-mass changes, induced

TABLE 3. Correlation (*r*) between body mass, HOPS-M, or ACTIV-M and metabolites for those cases with D-MASS-M between 0.43 and 0.69 g.

Metabolite	n^a	Body mass	n^b	HOPS-M	ACTIV-M
Glucose	24	0.32	22	0.02	0.04
Uric acid	26	0.07	24	−0.17	−0.17
Protein	24	−0.10	22	−0.27	−0.28
Triglycerides	26	0.12	24	−0.27	−0.28
Glycerol	26	−0.09	24	0.32	0.32
Free fatty acids	26	−0.06	24	0.62***	0.63***
β-hydroxy-butyrate	26	−0.11	24	0.41	0.38
LIPO1 + 2	26	0.15	26	−0.21	−0.22
PROT1	25	−0.31	25	0.28	0.26

***, $P < 0.001$; others not significant ($P > 0.05$).

[a] Sample size for body-mass comparison.

[b] Sample size for comparisons involving HOPS-M and ACTIV-M.

TABLE 4. Dependence of D-MASS-M, body mass, and activity measures on plasma metabolites. Result of final step of multiple-regression analyses is given. Only those metabolites with significant contributions are listed. β-hydroxy-butyrate was entered in its transformed form ln(β-hydroxy-butyrate + 0.5), PROT2 in its arcsine-square-root transformation. Adjusted correlation coefficient (r^2_{adj}), significance (asterisks), standard error of estimate (SE) and sample size (n) provided in parentheses following dependent variable. For further explanations, see Table 1.

Dependent variable Independent variable	b	T
D-MASS-M (0.608***, 0.226, 80)		
β-hydroxy-butyrate	−0.350	−6.06***
Triglycerides	0.120	4.64***
Constant	0.259	2.81**
Body mass (0.174***, 1.430, 73)		
Glycerol	−2.681	−3.29**
Glucose	0.186	2.82**
Constant	17.893	20.43***
HOPS-M (0.181***, 2,481, 77)		
Free fatty acids	5,742	4.22***
Constant	2,736	4.58***
ACTIV-M (0.187***, 87.77, 77)		
Free fatty acids	206.68	4.29***
Constant	95.12	4.51***

**, $P < 0.01$; ***, $P < 0.001$.

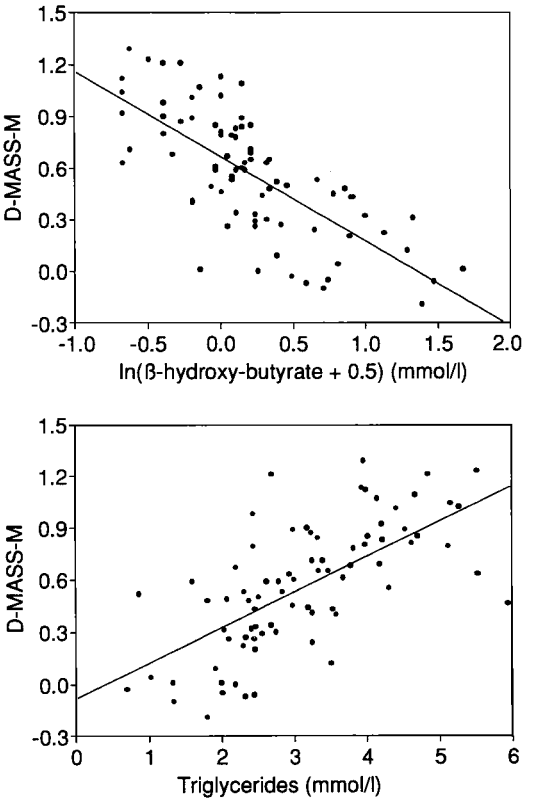


Fig. 3. Dependence of D-MASS-M on β-hydroxy-butyrate and on triglycerides ($n = 80$). Regression on ln(β-hydroxy-butyrate + 0.5) is $Y = 0.669 - 0.495X$ ($r = 0.715$, SE = 0.254, $P < 0.0001$). Regression on triglycerides is $Y = -0.082 + 0.204X$ ($r = 0.660$, SE = 0.273, $P < 0.0001$).

by experimental variation in food intake, are reflected in the metabolic pattern measured amid the foraging period. Most plasma metabolites examined showed a relatively high correlation with the change in body mass between morning and midday (D-MASS-M); the correlations with D-MASS-Y were less strong. This confirms that these metabolites reflect primarily the effects of food intake during a few hours prior to testing.

Chylomicrons, VLDL (summarized in LIPO1 + 2) and triglycerides increased with D-MASS-M (Table 1). This was to be expected since these metabolites increase in the plasma during resorption and transport fat in the blood to the adipose tissues and the energy consuming organs; chylomicrons transport triglycerides that originate directly from the diet, and VLDL triglycerides that were synthesized in the liver. Uric acid, the end product of protein catabolism, also increased with D-MASS-M, which reflects the breakdown of proteins from the diet (Jenni-Eiermann and Jenni 1991).

Glycerol, free fatty acids and β-hydroxy-butyrate increased with decreasing D-MASS-M (Table 1) and are known to increase during fasting (Jenni-Eiermann and Jenni 1991). Triglycerides from the adipose tissues have to be hydrolyzed to glycerol and free fatty acids before being delivered into the blood. β- hydroxy-butyrate is a keton body synthesized from free fatty acids and replaces glucose, especially in the brain. Since free fatty acids can only be transported in the blood bound to albumin, the observed increase in albumin (PROT1) was also an expected result. The increases in glycerol, free fatty acids and β-hydroxy-butyrate indicate that food intake was not meeting the energy requirements of the birds and that stored fat was catabolized. During fasting, free fatty acids are the main fuel for moderately exercising birds (Brackenbury 1984). Hence, the correlation between the activity during the morning and the level of free fatty acids is an expected result.

In summary, our findings confirmed for a

small passerine that plasma metabolites characteristic of fasting increase during periods of body-mass loss, and metabolites characteristic of resorption increase during periods of body-mass gain. The experiments also showed that this pattern is virtually independent of the actual body mass, at least within the body-mass range examined. Glucose was the only metabolite showing a strong relation with body mass and no relation with body-mass change.

Predicting body-mass changes from metabolite levels.—In our experiments, β-hydroxy-butyrate and triglycerides together explained 61% of the variation in body-mass change, while β-hydroxy-butyrate alone explained 51% and triglycerides alone 44% (Table 4; Fig. 3). In a study of free-living birds, the same two metabolites strongly differentiated between feeding and fasting birds (Jenni and Jenni-Eiermann 1992). The functional relationships between body-mass change and the two metabolites may serve to predict body-mass change of birds caught once in field studies. When applying this method, however, several points listed below have to be observed.

The experiments of our study concerned birds consuming some food during the natural foraging period. Hence, the method to estimate mass change from plasma metabolites should, at this stage, be applied only to birds during the foraging period. In fasting birds that perform high-level activity (e.g. migration without feeding en route), triglycerides are elevated and indicate a special pathway that enhances the delivery of free fatty acids to the muscles (Jenni-Eiermann and Jenni 1992).

Time of year, stage of molt, and age were held constant in our experiments. Further studies should explore whether there is variation in the relationship between the two metabolites and mass change depending on the time of the year (e.g. during reproduction, molt, migratory fattening), and whether there are differences based on sex and/or age.

Time of day also was held constant in the experiments. It is well known that β-hydroxy-butyrate and triglycerides are dependent on the daily pattern of food intake. Triglycerides increase and β-hydroxy-butyrate decreases markedly from the early morning until the afternoon in small day-active passerines (unpubl. data). Therefore, an analysis of plasma metabolite levels of birds sampled over the daylight hours should take into account the daily pattern of the metabolites. Birds in the early morning that have fasted inactively during the night are expected to be of little use for evaluating body-mass change through measurement of plasma metabolites, because differences in food intake are not yet reflected in the metabolite levels. However, it would be of interest to determine whether plasma metabolites measured in the early morning reflect body-mass loss experienced during the overnight fasting period.

Since the levels of plasma metabolites depend strongly on food intake, differences in the daily pattern of food intake (e.g. caused by weather or differences in diet) may cause differences in the daily pattern of the metabolite levels. The metabolite levels might differ depending on the composition of the diet, as suggested for plasma cholesterol, albumin, urea, and uric acid (e.g. Sturkie 1976, Gavett and Wakeley 1986). In our experiments, some birds favored fruits, but others largely neglected them. Although we found no indication of concomitant differences in metabolite levels, this problem needs further study.

For the estimation of body-mass change from plasma metabolites, one may measure both β-hydroxy-butyrate and triglycerides (and use multiple-regression function shown in Table 4), or either of these alone (Fig. 3). β-hydroxy-butyrate does not show a significantly better correlation with D-MASS-M than triglycerides. If only one plasma metabolite is measured, we suggest that investigators evaluate triglycerides because these metabolites are directly related to fat deposition, which is the point of interest for many who determine body-mass change. β-hydroxy-butyrate may be measured in addition, but is more likely to be subject to additional influences than triglycerides. β-hydroxy-butyrate is generally associated with fat catabolism, but is especially high during transitions from one metabolic state to the other (Jenni-Eiermann and Jenni 1991, Jenni and Jenni-Eiermann 1992), since it is involved in regulating glucose metabolism and free fatty acid release (Robinson and Williamson 1980, Féry and Balasse 1983). Furthermore, there may be differences between species of birds fasted for 1 or 2 h (Jenni-Eiermann and Jenni 1991). In our experiments, a weak dependence based on the experimental group was found (Table 1).

The above method for estimating body-mass change from plasma metabolite levels may be employed: (a) to reveal relative differences between samples of birds; and (b) to estimate the

absolute change in body mass from the regression equations provided here. At this stage, we caution against the latter application before field tests have been carried out on several species of birds. Such tests will also reveal the sensitivity of the method together with data on the daily pattern and variability of the metabolites.

Our analyses have established that there is a correlation between the concentrations of some plasma metabolites and body-mass change. In many studies, the point of interest, however, is the change in energy stores or condition. Energy stores may be composed of lipids, protein and carbohydrates, and the composition may vary among species and individuals (Blem 1990, Lindström and Piersma 1993). At present, we do not know whether the plasma metabolites have a higher correlation with one or the other of the energy-store components. It is likely that triglyceride levels are more closely related to changes in fat stores than in overall body mass.

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