ELECTRONIC SUPPLEMENTARY MATERIAL

MATERIAL AND METHODS AND STATISTICAL DETAILS

In experiment 1, we measured the fractional absorption of ^{14}C radiolabeled L-glucose (*N*=8) following Karasov and Cork (1994). Tests with ^{14}C labeled L-glucose find that it is absorbed by non-mediated pathways, not catabolized, excreted quantitatively, and nearly all (>90%) label remains on the mother compound (Chang *et al.* 2004). Fractional absorption (F) was calculated as:

$$F = (S \bullet P \bullet k_{el}) \bullet I^{-1} \qquad \text{(eq. 1)}$$

where S is the probe distribution space (µl plasma), P is the steady-state feeding concentration of ^{14}C L-glucose in plasma (dpm μl^{-1}), k_{el} is the elimination rate constant for L-glucose ($time^{-1}$), and I is the label intake rate (dpm $time^{-1}$). To obtain these parameters, we conducted two trials. In the first, we determined k_{el} and S by injecting birds in the pectoralis muscle with 9.25 x 10^4 Bq of [1-^{14}C]-L-glucose (Moravek Biochemicals, Brea, CA, USA) dissolved in 10-15 µl of deionized water. We determined k_{el} as the exponent of exponential decay functions fitted to the relationship between the concentration of label in excreta and time (Hall *et al.* 1977; Karasov & Cork 1994) assuming that the label disappearance rate from plasma is matched by its rate of appearance in excreta (Hartman Bakken *et al*. 2004). Probe distribution space was determined in five birds by taking a single blood sample (~10 µl) by clipping a toenail 2-3 h post-injection, and using the k_{el} values obtained from excreta to calculate the theoretical time-zero plasma label concentration ($A_i(0)$, dpm μl^{-1}). In the second trial, birds were fed a 584 mM sucrose solution containing approximately 1.83 x 10^4 Bq ml^{-1} of [1-^{14}C]-L-glucose to determine P and I. Birds were allowed to feed for 2-3 h before a single blood sample was taken. Steady state feeding was verified by measuring food intake rate and label concentration in excreta for several hours after labeled food was presented.

Hummingbirds increase their food intake when the sugar concentration of their food decreases (Martínez del Rio *et al.* 2001). To vary food intake rate in experiment 2, we fed birds 292 (*N*=5) and 876 mM (*N*=4) sucrose solutions containing approximately 1.35 x 10^4 and 2.37 x 10^4 Bq ml^{-1} of [1-^{14}C]-L-glucose, respectively, and measured P and I as described for experiment 1. Because this experiment simply tested for a diet treatment effect, we assumed that S and k_{el}

did not change with treatment. This was confirmed by finding that L-glucose distribution pool size and k_{el} for the same individual birds under the same treatments (T_a = 24±1 °C, 292 and 876 mM sucrose diets) obtained in previous separate experiments (Hartman Bakken *et al.* 2004) did not differ significantly between diet treatments ($F_{1,7}$ = 1.16, p = 0.32 and $F_{1,7}$ = 1.99, p = 0.2, respectively). Because the birds gained considerable body mass and significantly increased their body fat stores during the six months between the measurements done by Hartman Bakken *et al.* (2004) and those reported in the present study, we could not use these values to calculate L-glucose fractional absorption in experiment 2 directly.

Analysis of variance (ANOVA) or covariance (ANCOVA) were used to test for treatment and body size effects in experiment 2. Proportional data were arcsin(sqrt) transformed before analysis (Sokal & Rohlf 1995). In all other cases, we used linear models on non-transformed data to assess significance. Values are reported as mean ± 1 s.e.m.

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