Ontogeny and Nutritional Status Influence Oxidative Kinetics of Nutrients and Whole-Animal Bioenergetics in Zebra Finches, *Taeniopygia guttata*: New Applications for ^{13}C Breath Testing

Marshall D. McCue[1,*]
Scott R. McWilliams[2]
Berry Pinshow[1]
[1]Mitrani Department of Desert Ecology, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, 84990 Midreshet Ben-Gurion, Israel; [2]Department of Natural Resources Science, University of Rhode Island, 1 Greenhouse Road, Kingston, Rhode Island 02881

Accepted 7/30/2010; Electronically Published 11/2/2010

ABSTRACT

Rapidly growing animals or those that are recovering from nutritional stress may use exogenous nutrients differently from well fed adults. To test this possibility, we compared the rates of exogenous nutrient oxidation among fledgling, fasted adult, and refed adult zebra finches using a technique called breath testing, where animals are fed ^{13}C-labeled nutrients and ^{13}C in the exhaled breath is collected and quantified. In order to identify the possible mechanisms responsible for differences in oxidative kinetics of ingested nutrients, we also compared body mass (m_b), organ mass, core body temperature (T_b), and metabolic rate (MR). We found that fasted birds had lower T_b, relative liver and intestine masses, MR, and respiratory exchange ratios (RERs) than fed adults. Adult birds recovering from nutritional stress had much lower rates of exogenous nutrient oxidation than fed birds; this difference was particularly evident for fatty acids. Differences in oxidative kinetics were correlated with reduced RER, m_b, and liver mass, suggesting that previously fasted birds were using recently assimilated nutrients to replenish exhausted fuel stores. Rapidly growing fledglings oxidized exogenous nutrients as quickly as fed adults, despite their significantly lower m_b and T_b. We suggest that fledglings had higher mass-specific rates of exogenous nutrient oxidation because they must compensate for the relatively low conversion efficiency of feather production and other lean tissue growth, which was not taking place in the adults. Although this study demonstrates that ontogeny and nutritional status influence the way that birds oxidize exogenous nutrients, it also underscores the likelihood that environmental and endogenous factors shape how other types of animals spend the nutrients they ingest.

* Corresponding author. Present address: Department of Biological Sciences, St. Mary's University, One Camino Santa Maria, San Antonio, Texas 78228; e-mail: mmccue1@stmarytx.edu.

Physiological and Biochemical Zoology 84(1):000–000. 2011. 1522-2152/2011/8401-10065$15.00. DOI: 10.1086/657285

Introduction

All animals eat to satisfy their daily energy and nutrient requirements; however, these daily requirements can be affected by nutritional history, activity levels, environmental conditions, and developmental state (Klasing 1998; Karasov and Martinez del Rio 2007; Barbosa et al. 2009; Raubenheimer and Simpson 2009). Birds require relatively more energy and nutrients than other vertebrates of comparable body mass (m_b) because of their exceptionally high mass-specific basal metabolism and the power requirements of flapping flight (Tucker 1971; Dawson et al. 1983; Alerstam and Lindstrom 1990; Jenni and Jenni-Eiermann 1998; Gannes 2001; Bairlein 2002; McWilliams et al. 2004). To cope with their high energy demands, many small birds exhibit remarkable phenotypic flexibility in their digestive physiology that may reduce energetic requirements when food is limited and maximize uptake rates when food is not limiting (Karasov and Hume 1997; Piersma 1998; Karasov and McWilliams 2005; Bauchinger et al. 2009). How this phenotypic flexibility is related to whole-animal energetics and the metabolism of exogenous nutrients is poorly understood, especially in relation to changing environmental conditions that affect food availability.

The rates at which animals oxidize exogenous fuels to meet immediate energy demands can be quantified using an approach called breath testing (Schoeller et al. 1980; Amarri and Weaver 1995; Bodamer and Halliday 2001; Geboes et al. 2004). After an animal consumes an isotopically enriched meal, the isotopic signature of the exhaled carbon dioxide ($\delta^{13}CO_2$) can be measured to quantify the oxidation rates of exogenous metabolic substrates (Fig. 1). The extent to which exogenous materials are used to fuel postprandial metabolic requirements have been recently quantified in nectarivores, including bats (Voigt et al. 2003; Voigt and Speakman 2007) and hummingbirds (Welch et al. 2006, 2008; Welch and Suarez 2007), but is less well understood in animals that consume mixed diets (Hatch et al. 2002; Ishihara et al. 2002; Starck et al. 2004; Voigt et al. 2008; Waas et al. 2010).

In this study, we characterized the oxidative kinetics of com-

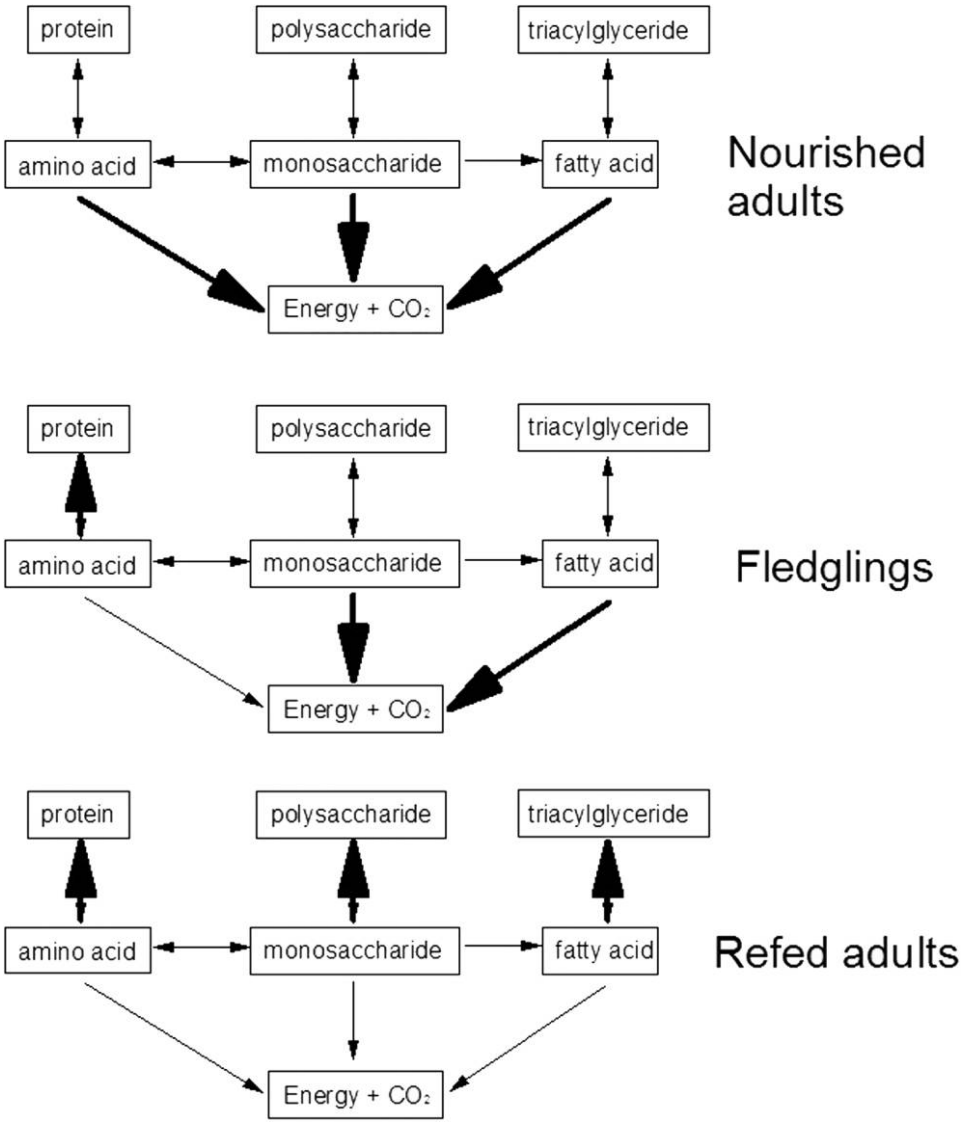

Figure 1. Hypothesized fates of different classes of exogenous nutrients (amino acids, monosaccharides, and fatty acids) in fed adult, juvenile, and refed adult zebra finches. The sizes of the arrowheads correspond with the predicted relative magnitudes of the flux between nutrient pools.

mon metabolic substrates in order to understand how birds alter whole-animal bioenergetics when exogenous nutrients may be limited. Specifically, we compared the biochemical, physiological, and morphological responses of rapidly growing juvenile birds with those observed in adult birds in various nutritional states (i.e., fed, fasted, and refed). We hypothesized that the energy expenditure of birds and the extent to which they oxidize exogenous metabolic fuels change with developmental state and nutritional status (Fig. 1). We tested the following specific predictions stemming from this hypothesis by measuring $^{13}CO_2$ emission before and after birds were administered doses of isotopically labeled nutrients (i.e., glucose, leucine, and palmitic acid). To understand how variables related to whole-animal energetics are affected by these factors, we also measured rates of oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), body temperature (T_b), m_b, and masses of digestive organs in a small granivorous songbird, the zebra finch (*Taeniopygia guttata*).

Fasted Adults

As fasting birds exhaust glycogen stores (Castellini and Rea 1992; Milinkovic-Tur et al. 1996), they rapidly shift from carbohydrate-dominated to lipid-dominated catabolism (McCue 2010). We thus expected respiratory exchange ratios (RERs) to decrease in these birds, as has been reported for other species (Chwalibog and Thorbek 1989; Walsberg and Wolf 1995; Laurila et al. 2005). Moreover, fasting-induced reductions

in body temperature (T_b) set point have been documented for many animals presumably to minimize metabolic expenditure during lean times (Bicego et al. 2007). We therefore predicted that the birds would reduce T_b while fasting. Finally, because the organs of fasting passerine birds are well known to undergo disproportionate changes in mass (Hume and Biebach 1996; Starck 2003; Karasov et al. 2004; Bauchinger et al. 2005; McCue 2010), we predicted decreases in the relative masses of digestive organs with high metabolic rates (MRs), such as liver and intestine. Such fasting-induced changes in T_b and organ masses may permit fasted finches to adaptively reduce overall metabolic expenditure, as has been reported for several species of nonpasserine birds (Shapiro and Weathers 1981; Cherel et al. 1988; Chwalibog and Thorbek 1989; Boismenu et al. 1992; Klaassen and Biebach 1994; Mata et al. 2001).

Refed Adults

Birds are exceptional in being able to rely on lipids to fuel metabolic demands while fasting; however, some level of protein (Hannaford et al. 1982; Cherel et al. 1991, 1992; Thouzeau et al. 1999; Lamsova et al. 2004) and carbohydrate (Cherel and Le Maho 1985; Knowles et al. 1995; Milinkovic-Tur et al. 1996; Alsonso-Alverez and Ferrer 2001; McWhorter et al. 2004) loss is inevitable (McCue 2010). We therefore predicted that birds recovering from a fast have lower rates of exogenous fuel oxidation than fed individuals because these refed individuals route more exogenous substrates to replenish exhausted protein, lipid, and carbohydrate stores rather than to directly fuel metabolism (Fig. 1). Moreover, given the high metabolic costs of protein synthesis during refeeding (Grisolia and Kennedy 1965; Brown and Cameron 1991; McCue 2006), we predicted that individuals recovering from nutritional stress have higher mass-specific MR than do fed individuals.

Fledglings

Given the high costs of protein synthesis (Coulson et al. 1978; Reeds et al. 1998; Hochachka and Somero 2002) and the fact that fledglings are rapidly growing feathers, building flight muscles, and increasing lean m_b, we predicted that fledglings have higher total metabolic demands than fed adults (Fig. 1). Moreover, because this tissue growth represents a sink for exogenous nutrients, we predicted that fledglings have lower rates of exogenous substrate oxidation than fed adults. Given the high protein content of keratin and flight muscle (Murphy and King 1986; Grubb 1991; Cherel et al. 1994), this pattern is expected to be particularly evident for exogenous amino acids as opposed to fatty acids and carbohydrates.

Methods

Animals

We used adult male ($n = 89$) and fledgling ($n = 46$) zebra finches, *Taeniopygia guttata*, from our captive breeding population on the Sede Boqer Campus of Ben-Gurion University, Midreshet Ben-Gurion, Israel. The birds were banded with uniquely numbered plastic leg bands and maintained in a large, permanent outdoor aviary (4 m × 3 m × 2.5 m [length × width × height]), where they were fed a diet of mixed millet seeds (~12% protein and 5% lipid as dry mass; Williams and Ternan 1999; McCue et al. 2009) and provided with tap water ad lib. Crushed chicken egg shells, vitamin-supplemented water, and fresh lettuce were also provided once a week.

Metabolic Rates

Fed adult and fledglings with full crops were collected from aviaries, and their MRs were measured within 30 min. MRs of fasted birds were measured after holding them in cages without food for 19–22 h, starting at 1700–1800 hours on the previous day. Note that this period included the scotophase, when these birds do not normally eat. MRs were measured in fasted birds and in birds that fasted and were then permitted to feed ad lib. for 5 min (hereafter referred to as refed birds), sufficient time for them to refill their crops.

After weighing birds to ±0.1 g, resting rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured over five 6-min periods between 1000 and 1600 hours at 24° ± 1°C by indirect calorimetry in an open-flow system consisting of a Sable Systems eight-channel multiplexer connected to an Applied Electrochemistry S3-A oxygen analyzer and an Ametek CD-3A carbon dioxide analyzer. $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated as described by McCue et al. (2010). Allometric relationships between m_b and MR of birds were compared among treatment groups using ANCOVAs (Packard and Boardman 1988; Jackson and Somers 1991; Beaupre and Dunham 1995; Raubenheimer 1995). RER was calculated as the ratio of $\dot{V}CO_2$ to $\dot{V}O_2$.

Body Temperature and Organ Masses

After measurements of MR, T_b of fed and fasted adults were measured to the nearest 0.1°C using a small, polyvinyl chloride–coated type T thermocouple inserted approximately 5 mm into the rectum. Birds were then decapitated, and whole intestine (from gizzard to cloaca) and whole liver were extracted. The intestines were removed and emptied of their contents by gently squeezing them out and flushing them with water. Tissues were placed into preweighed containers and dried in a convection oven at 90°C for 48 h.

$^{13}CO_2$ Analyses and Calculations

All tracer experiments were done at the same time of day that MR was measured, although not on the same days. Background $^{13}CO_2$ levels were determined by placing four birds in 800-mL metabolic chambers at 24° ± 1°C with dry CO_2-free air pumped through each chamber at a rate of 150–200 mL min^{-1}, a flow rate that allowed the fractional concentration of CO_2 in the air excurrent (F_e CO_2) from the metabolic chambers to reach slightly less than 1% (Buyse et al. 2004; Hughes et al. 2008).

Table 1: Results of ANCOVA (*F* values, *P* values comparing slope and intercepts) of dry organ mass versus body mass in fed adults, fledglings, and fasted adults in zebra finches

		Slope		Intercept	
Treatment	Organ	*F*	*P*	*F*	*P*
Adult vs. fledglings	Intestine	1.406	.2512	3.525	.0759
Adult vs. fasted	Intestine	.17	.6842	12.73	**.0016**
Fledglings vs. fasted	Intestine	1.218	.2828	39.187	**<.0001**
Adult vs. fledglings	Liver	.257	.6183	.269	.6099
Adult vs. fasted	Liver	.72	.4052	19.237	**.0002**
Fledglings vs. fasted	Liver	3.172	.0901	15.557	**.0007**

Note. Boldface indicates significant results.

After 15 min, 10 mL of excurrent air was subsampled by drawing it into a gas-tight syringe at a rate of ~60 mL min^{-1}. The syringes were emptied into glass Exetainer vials (Labco, High Wycombe, Buckinghamshire) previously flushed with helium.

After we collected baseline CO_2 samples, each bird was gavaged with 5 mg of one of three isotopically labeled molecules—1-^{13}C D-glucose, 98%–99%; 1-^{13}C L-leucine, 99%; and 1-^{13}C palmitic acid, 99% (Cambridge Isotope Laboratories, Andover, MA)—mixed with 100 μL of carrier. Leucine and palmitic acid were suspended in sunflower seed oil, and glucose was dissolved in distilled water. We recently found that oxidative kinetics of a glucose tracer does not differ when administered either as a lipid suspension or as a solution (McCue et al. 2010). The tracer plus carrier mixtures were quantitatively administered using a 15-g silicon-tipped polyethylene feeding tube (FTP-15-78; Instech Solomon, Plymouth Meeting, PA) attached to a 1.0-mL tuberculin syringe. After administration, the birds were returned to the metabolic chambers, and exhaled CO_2 samples were taken at 15-min intervals over the next 1.5 h.

All isotope analyses were done within 4 wk of collection (McCue et al. 2010). The carbon isotope composition of the CO_2 in the vials was measured using gas source isotope ratio mass spectrometry (GS-IRMS) through a GasBench II interface (Thermo-Fisher Scientific, Waltham, MA). The $\delta^{13}CO_2$ in each gas sample was calculated according to the Craig equation:

$$\delta^{13}C_{PDB} = \left[\frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{std}}{(^{13}C/^{12}C)_{std}}\right] \times 10^3, \quad (1)$$

where $^{13}C/^{12}C$ is the ratio (R) of the heavy and the light carbon isotopes in the sample, compared with the ratio in a standard (Craig 1957). Our standard was Pee Dee Belemnite (PDB) with a value of $R_{PDB} = 0.1112329$. We determined the precision of measurements to be 0.14‰.

Since $\delta^{13}C_{PDB}$ is neither an SI unit nor an appropriate metric for isotope tracer studies (Slater et al. 2001; Wetzel 2005), we corrected $\delta^{13}C$ to atom percent ($AP^{13}C$) using equation (5) modified from Slater et al. (2001):

$$AP^{13}C = 10^2 \times \left[\frac{1}{(\delta^{13}C_{PDB}/10^3 + 1) \times R_{PDB}} + 1\right]^{-1}. \quad (2)$$

To correct for background isotopic signatures and to avoid reporting small percentages, the atom fraction excess of the tracer ($y \times 10^6$ AFE ^{13}C) was calculated using equation (6) modified from Slater et al. (2001):

$$y \times 10^6 \text{ AFE } ^{13}C = [(AP^{13}C)_E - (AP^{13}C)_B] \times 10^3, \quad (3)$$

where $(AP^{13}C)_B$ and $(AP^{13}C)_E$ refer to background and enriched values, respectively. The instantaneous rates of tracer oxidation

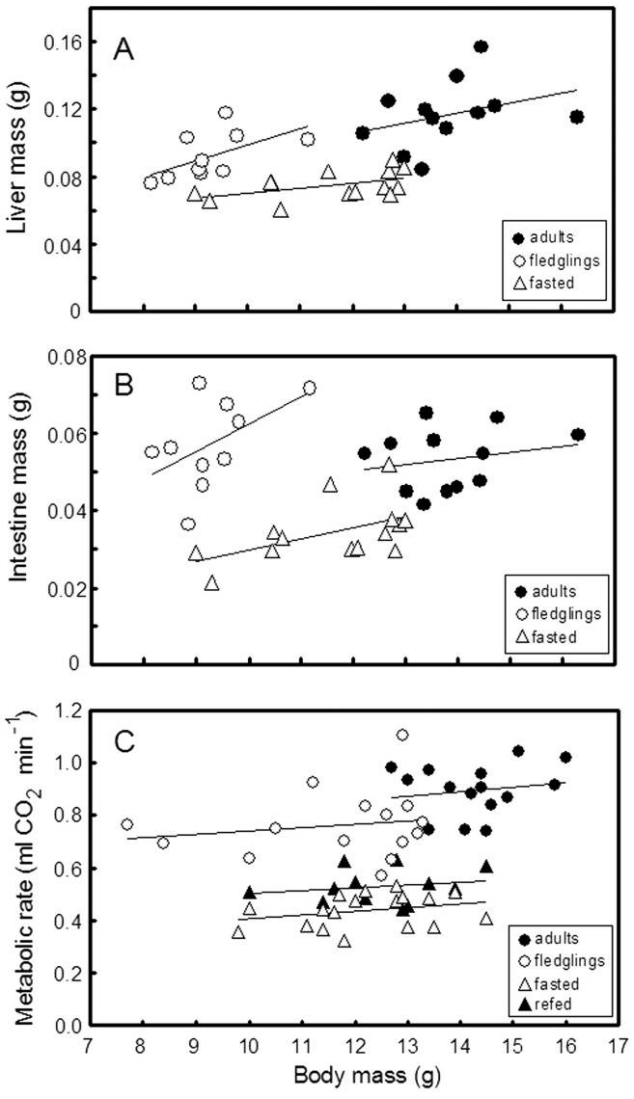

Figure 2. Dry liver mass (*A*) and intestine mass (*B*) from fed adult, refed adult (fasted), and fledgling zebra finches. Results of ANCOVA are presented in Table 1. *C*, Rates of carbon dioxide production ($\dot{V}CO_2$) for fledgling, fed adult, fasted adult, and refed adult zebra finches. Results of ANCOVA are presented in Table 2.

Table 2: Results of ANCOVA (F values, P values comparing slope and intercepts) on bilogarithmic functions of carbon dioxide production versus body mass in fed adults, fledglings, fasted adults, and refed adult zebra finches

	Slope		Intercept	
Treatment	F	P	F	P
Adult vs. fledglings	.026	.8743	4.048	.0546
Adult vs. fasted	.08	.7792	114.557	**<.0001**
Adult vs. refed	.001	.9972	70.399	**<.0001**
Fasted vs. refed	.115	.7372	13.483	**.001**
Fledglings vs. refed	.037	.85	39.88	**<.0001**
Fledglings vs. fasted	.037	.5475	100.576	**<.0001**

Note. Boldface indicates significant results.

(T; nmol min^{-1}) were calculated using the following equation from McCue et al. (2010):

$$T = \frac{(\{\dot{V}_{CO_2}[(y \times 10^6 \text{ AFE } {}^{13}\text{C})/10^2]/k\} \times 10^3) \times (\theta \times \text{BRF})^{-1}}{m \times 10^3}. \quad (4)$$

Here BRF is the bicarbonate retention factor (i.e., a correction factor that accounts for the loss of ^{13}C tracer into the circulating bicarbonate pool) for birds (0.86; Tabiri et al. 2002), θ is the number of isotopically enriched atoms per tracer molecule, m is molar mass, and k is the volume of CO_2 (mL) produced per milligram of tracer oxidized. The value of k was calculated for each tracer molecule with the following equation, which combines the stochiometry of uric acid with the ideal gas law (McCue et al. 2010):

$$k = \frac{[C - (1.2 \times N)] \times 22.4}{m}, \quad (5)$$

where 22.4 is the volume of 1 mol of gas (L) at standard temperature and pressure and C and N are the number of carbon and nitrogen atoms in each tracer molecule, assuming nitrogen is converted to uric acid (Griminger and Scanes 1986).

We converted units to μmol and modeled the cumulative oxidation, $(\int_0^t f(x)dx) \times 10^{-3}$, using a single-compartment, two-parameter exponential equation for amino acid and carbohydrate tracers:

$$f(t) = a \times (1 - e^{-bt}), \quad (6)$$

where t is time (min) and a and b are empirically determined coefficients and exponents, respectively. The cumulative oxidation of fatty acids could not be modeled using an exponential model and was therefore described using a three-parameter, logistic model of the following form:

$$f(t) = a \times (1 - e^{-bt})^c \quad (7)$$

where a, b, and c are empirically determined values. Raw data, not mean values, at each time point were used for modeling.

We used StatView (SAS Institute, Cary, NC) for ANCOVA and SigmaPlot 11 (Systat, Chicago) for ANOVAs, post hoc tests, t-tests, and curve fitting. In situations where data were not normally distributed, we used Kruskal-Wallis one-way ANOVA on ranks. For ANOVA that yielded significant interactions, pairwise comparisons were made between treatments using Dunn's test. Critical α was set at 0.05, but Bonferroni-corrected P values were used for multiple comparisons. Values refer to means ± SD unless otherwise noted.

Results

Fed and Fasted Adults

The m_b of fasted adults was on average 10.2% less than that of fed adults (14.1 ± 1.1 and 12.1 ± 1.2 g, respectively; df = 52, $P < 0.0001$). The T_b of fasted adults was on average 2.3°C lower than that of fed adults (41.0° ± 0.6° and 38.7° ± 0.7°C, respectively; df = 29, $P < 0.0001$). Liver mass of fasted adults, corrected for differences in m_b, was on average 35% less than that of fed adults (115 ± 19 and 75 ± 8 mg, respectively; Table 1; Fig. 1*A*). Intestine mass of fasted adults, corrected for dif-

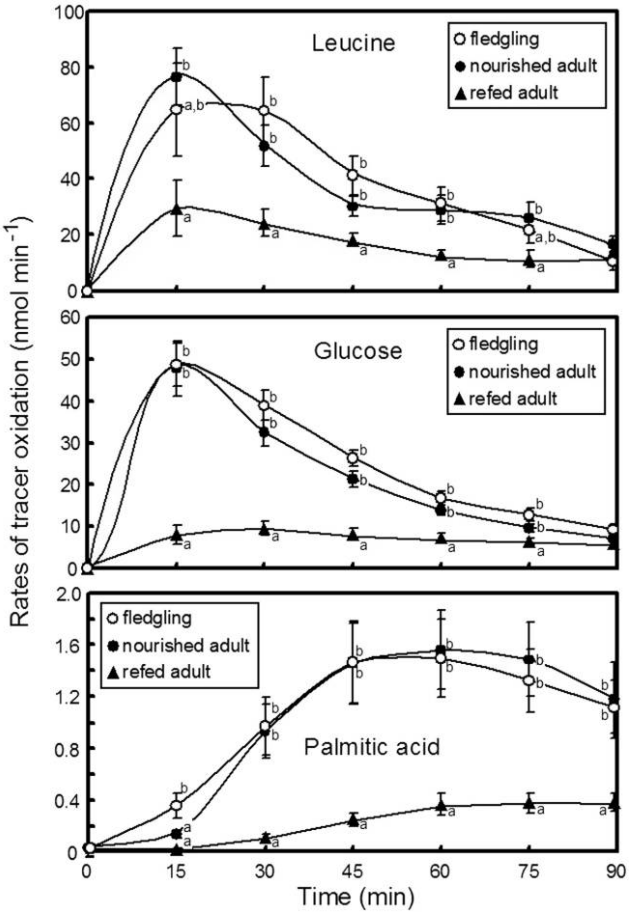

Figure 3. Instantaneous rates of exogenous tracer oxidation in fledgling, fed adult, and refed adult zebra finches. Different letters indicate significant differences between treatment groups at a given time according to ANOVA and Dunn's post hoc pairwise comparisons.

Table 3: Net volume of CO_2 produced per milligram of tracer oxidation (k)

	k (mL CO_2 mg^{-1})	a	b	c	R^2
Leucine:					
Adult	.82	3.5064 (.3955)	.0249 (.0064)	...	.6215
Refed	.82	1.8872 (.3983)	.0204 (.0085)	...	.4367
Fledgling	.82	3.5094 (.5281)	.0269 (.0096)	...	.4550
Glucose:					
Adult	.746	1.8740 (.1491)	.0337 (.0075)	...	.6883
Refed	.746	1.0238 (.4293)	.0115 (.0073)	...	.5843
Fledgling	.746	2.2586 (.1910)	.0297 (.0064)	...	.7308
Palmitic acid:					
Adult	1.398	.1331 (.0642)	.0262 (.0258)	2.6280 (2.3537)	.5938
Refed	1.398	.0317 (.0177)	.0277 (.0264)	3.8858 (3.9650)	.6287
Fledgling	1.398	.1331 (.0734)	.0237 (.0270)	2.1884 (1.9621)	.5436

Note. Values (SD) a, b, c, and R^2 are parameters of the cumulative tracer oxidation models, following equations (6) and (7).

ferences in m_b, also averaged 35% less than that of fed adults (52 ± 10 and 34 ± 7 mg, respectively; Table 1; Fig. 1*B*).

MRs of adults were positively related to m_b but were also influenced by nutritional status (Fig. 2*C*). The $\dot{V}CO_2$ of fasted adults (0.437 ± 0.062 mL CO_2 min^{-1}) was 51.3% lower than that of fed adults (0.897 ± 0.096 mL CO_2 min^{-1}; Fig. 2*C*), and the $\dot{V}CO_2$ of fasted adults (0.721 ± 0.102 mL O_2 min^{-1}) was 46.4% lower than that of fed adults (1.345 ± 0.159 mL O_2 min^{-1}; df = 31; $P < 0.0001$). ANCOVA of the bilogarithmic relationship between $\dot{V}CO_2$ and m_b confirmed that $\dot{V}CO_2$ was significantly lower in fasted adults than in fed adults (Table 2). The RER of fasted adults was also significantly lower than that of fed adults, decreasing from 0.67 ± 0.3 to 0.61 ± 0.2 (df = 31, $P < 0.0001$).

Before tracer administration, fasting adults had significantly lower $\delta^{13}CO_2$ values than those of fed adults (−15.2‰ ± 0.2‰ and −12.8‰ ± 0.03‰, respectively; df = 67, $P < 0.0001$). After administration, fed adults oxidized exogenous leucine (76 ± 37 nmol min^{-1}) more rapidly than glucose (48 ± 18 nmol min^{-1}; df = 19, $P = 0.0282$) or palmitic acid (1.6 ± 0.8 nmol min^{-1}; df = 19, $P < 0.0001$). The peak exogenous nutrient oxidation occurred at approximately 15 min for glucose and leucine and at approximately 60 min for palmitic acid (Fig. 3).

Refed Adults

The $\dot{V}CO_2$ of refed adults (0.529 ± 0.063 mL CO_2 min^{-1}) was significantly lower than that of fed adults (0.897 ± 0.096 mL CO_2 min^{-1}; Fig. 2*C*). ANCOVA of the bilogarithmic relationship between $\dot{V}CO_2$ and m_b, using nutritional status as a covariate, showed that $\dot{V}CO_2$ was significantly lower in refed adults than in fed adults but that the $\dot{V}CO_2$ of refed adults did not differ from that of fasted adults (Table 2). The RER of refed adults (0.69 ± 0.3) was not significantly different from that of fed adults (0.67 ± 0.3; df = 25, $P = 0.128$).

Cumulative oxidation over time for leucine and glucose was accurately modeled with both an exponential model (eq. [6]) and a logistic model (eq. [7]) for palmitic acid (Table 3). During the 90-min measurement period, refed birds oxidized only half of the leucine (1.54 ± 0.69 μmol) than that of fed adults (3.17 ± 0.87 μmol; df = 23, $P < 0.0001$; Fig. 3), and they oxidized less exogenous glucose (0.65 ± 0.26 μmol) than did fed adults (1.79 ± 0.47 μmol; df = 14, $P < 0.0001$; Fig. 3). Fed adult birds oxidized (0.101 ± 0.052 μmol) over four times more palmitic acid than did refed adults (0.022 ± 0.012 μmol; df = 14, $P = 0.0012$; Fig. 3).

Fledglings

The m_b of fledglings (11.7 ± 1.8 g) was on average 17% less than that of fed adults (df = 38, $P < 0.0001$); however, their m_b did not differ from that of fasted adults (df = 41, $P = 0.3655$). ANCOVA of the relationship between organ mass and m_b, using age class as a covariate, confirmed that liver mass (92 ± 14 mg) and intestine mass (57 ± 11 mg) of fledglings were not significantly different from those of fed adults (Table 1; Fig. 2*A*, 2*B*). In contrast, the liver and intestine masses of fledglings were significantly greater than those of fasted adults (Table 1; Fig. 2*A*, 2*B*). The T_b of fledglings was 39.2° ± 0.9°C and was significantly lower than that of fed adults (41.0° ± 0.6°C; df = 23, $P < 0.0001$); however, the T_b of fledglings did not differ from that of fasted adults (0.61 ± 0.2; df = 24, $P = 0.1163$).

The total $\dot{V}CO_2$ of fledglings (0.763 ± 0.132 mL CO_2 min^{-1}) was lower than those of fed and fasted adults (0.897 ± 0.096 and 0.721 ± 0.102 mL O_2 min^{-1}, respectively; Fig. 2*C*); however, ANCOVAs of the bilogarithmic relationships between $\dot{V}CO_2$ and m_b indicated that $\dot{V}CO_2$ did not differ between fledgling and fed adults (Table 2). The mean RER of fledglings was 0.74 ± 0.1 and was significantly higher than those of fed (0.67 ± 0.3; df = 28, $P = 0.0002$), fasted (0.61 ± 0.2; df = 31, $P < 0.0001$), and refed (0.69 ± 0.3; df = 25, $P = 0.0074$) adults.

Cumulative tracer oxidation during the 90-min measurement period did not differ between fledglings and fed adults (Fig. 4; Table 3). Nevertheless, fledglings oxidized significantly

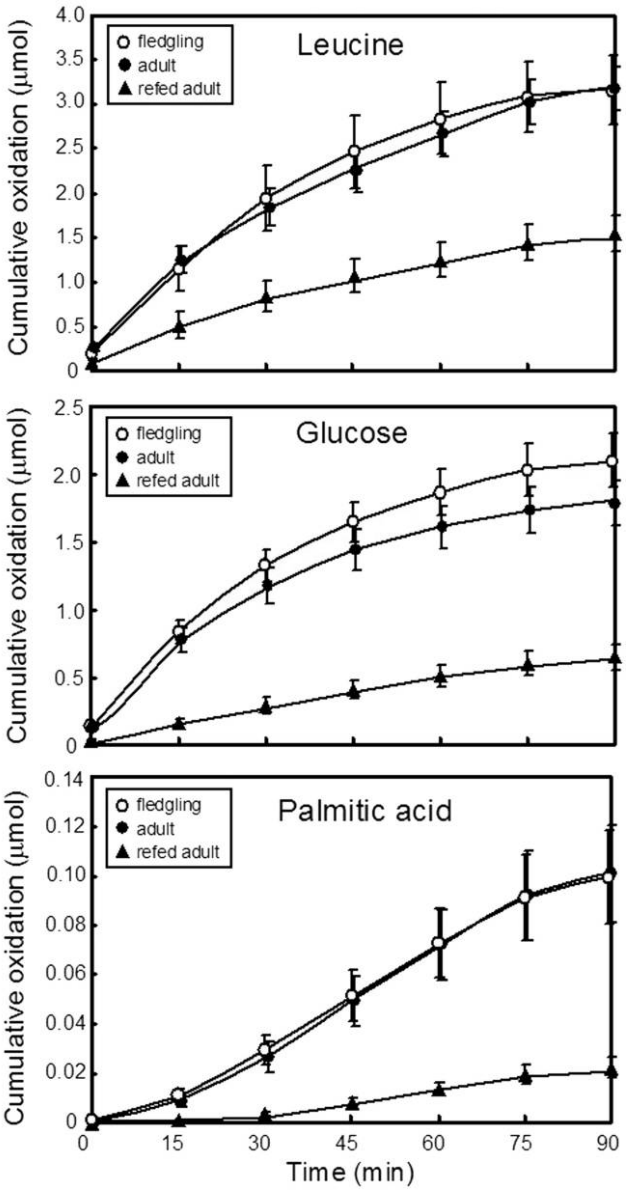

Figure 4. Cumulative rates of exogenous tracer oxidation in fledgling, fed adult, and refed adult zebra finches. Data points are slightly offset for clarity. See Table 3 for model parameters.

more leucine (3.16 ± 1.29 μmol) than did refed adults (1.54 ± 0.69 μmol; df = 23, $P < 0.0001$). They also oxidized more glucose (2.10 ± 0.57 μmol) and palmitic acid (0.099 ± 0.053 μmol) than did refed adults (0.65 ± 0.26 μmol, df = 14, $P < 0.0001$; 0.022 ± 0.012 μmol, df = 14, $P < 0.0001$).

Discussion

We found that nutritional state affects the metabolism of exogenous nutrients. As predicted (Fig. 2), fasted birds oxidized exogenous carbohydrates, fatty acids, and amino acids less extensively than did regularly fed birds. The differences in nutrient oxidation between fed and fasted adults were correlated with differences in m_b, T_b, and relative organ mass. Fasted birds lost approximately 10% of their m_b; however, during the same period, liver and intestine mass were reduced by 35%. This disproportionately large decrease in digestive organs that have relatively high tissue-specific MR has been documented in other species (Parker and Holm 1990; Hume and Biebach 1996; Thouzeau et al. 1999; Karasov et al. 2004; Lamsova et al. 2004) and probably accounts for a significant proportion of the hypometabolic response observed in the MR in fasted, compared with fed, adults. Nevertheless, additional mechanisms—such as hypothermia, enzyme downregulation, hormonal adjustment, and cellular and tissue restructuring—may also contribute to the apparent fasting-induced hypometabolism exhibited by the fasted adults (Cherel et al. 1988; Hervant et al. 2001; Mata et al. 2001; McCue 2007*a*, 2007*b*)

Fasting birds are able to tolerate large decreases in the relative masses of digestive organs (Brady et al. 1978; Hume and Biebach 1996; Thouzeau et al. 1999; Karasov et al. 2004; Lamsova et al. 2004). Small passerine birds require 1–2 d to rebuild these digestive organs after fasting (Starck 1999; Bauchinger et al. 2009), and this rebuilding is often associated with comparatively slow m_b gain (Karasov and McWilliams 2005; McWilliams and Karasov 2005). A key result of our study of finches is that the reduction in digestive tract (i.e., liver, intestine) was associated with a significantly slower rate and extent of oxidation of certain exogenous substrates. Given that during migration birds regularly alternate feeding at migratory stopovers with in-flight fasting, any reduction in rate of oxidation of exogenous substrates would exacerbate the gut limitation imposed by reduction in digestive tract tissue (McWilliams and Karasov 2005). Examination of the nonoxidative fates of exogenous nutrients in birds recovering from a fast will be useful to elucidate how such mass gain is partitioned between rebuilding of digestive organs and rebuilding of storage tissues (reviewed in McCue 2011).

Interestingly, we found no support for the prediction that fledglings, which are rapidly growing feathers and increasing m_b in general, would oxidize exogenous substrates more than nourished adults in steady state. We suggest that the lack of difference in oxidative kinetics between fed adults and growing fledglings reflects the combined costs of higher mass-specific MRs in smaller individuals and the high energetic costs of new tissue growth (Pullar and Webster 1977; Krikwoon 1991; Lindstrom et al. 1993; Reeds et al. 1998; Montes et al. 2007), both of which are paid for with exogenous nutrients (McCue 2006). It is worth noting that a shift in the proportion of nutrients immediately oxidized and those allocated to tissue growth is not the only mechanism to support rapid growth in animals. For example, reduced T_b in fledglings could reduce maintenance costs, thereby permitting a greater proportion of the oxidized nutrients to pay for costs of growth. Moreover, hyperphagia during refeeding (Russell and Wootton 1992; Wang et al. 2000; Tian and Qin 2003, 2004) or seasonal fattening (Totzke et al. 2000; Butler and Woakes 2001; Bairlein 2002; Cooper 2007) may increase available nutrients and facilitate rapid growth without a change in nutrient allocation between

oxidative or nonoxidative fates. We did not quantify daily food intake in this study and so are unable to evaluate the role of hyperphagia in supporting more rapid growth of fledglings. Nevertheless, our results provide some support for the reduced T_b mechanism in that fledglings had lower T_b than fed adults; this response was similar to the strategy used by the fasting birds to conserve energy (Laurila et al. 2005; Wang et al. 2006; Bicego et al. 2007; Ben-Hamo et al. 2010; McCue 2010).

In conclusion, a central goal of physiological ecology is to understand the mechanisms by which organisms marshal and allocate environmental resources that are often unpredictably available while maintaining an acceptable level of homeostasis (Tracy and Turner 1982; Boggs 1992; Gurney and Middleton 1996; Goldstein and Pinshow 2006; Lillywhite and Navas 2006). Our results demonstrate that birds adjust their metabolism of ingested nutrients in response to extreme changes in food availability (i.e., fasted, refed, ad lib.) and during growth. The differences in nutrient oxidation demonstrated by these birds underscore the fact that m_b, whole-animal MR, organ masses, T_b, nutritional status, developmental stage, and so on, work in an integrated fashion to regulate how much and which foodstuffs are catabolized for fuel. Because of the large number of confounding variables, accurate predictions of these responses based on first principles may be precluded, and it is thus useful to quantify these patterns empirically. The analytical approach for quantifying rates of nutrient oxidation described here can be applied to virtually any terrestrial animal on which MRs can be measured. Although these experiments focused on the specific effects of ontogeny and nutritional stress, future studies using this approach might examine how countless other endogenous and exogenous factors— such as season, photoperiod, activity level, reproductive status, nutritional habituation, thermal acclimation, and water stress—shape the ways in which animals "spend" the metabolic fuel on which they all depend.

Acknowledgments

We are grateful to Anastasia Mihailov, Efrat Eliani, and Orit Sivan for help with analyzing breath samples and members of the Pinshow lab for helping with animal care. We also thank A. Munoz-Garcia and three anonymous reviewers for their comments on earlier drafts of this manuscript. This work was funded by a U.S.–Israel Binational Science Foundation Grant 2005119 awarded to B.P. and S.R.M. and a Blaustein Postdoctoral Fellowship awarded to M.D.M. All experiments were done under permit 30993 of the Israel Nature and National Parks Protection Authority. This is publication 690 of the Mitrani Department of Desert Ecology.

Literature Cited

Alerstam T. and A. Lindstrom. 1990. Optimal bird migration: the relative importance of time, energy, and safety. Pp. 331–351 in E. Gwinner, ed. Bird Migration: The Physiology and Ecophysiology. Springer, Berlin.

Alsonso-Alverez C. and M. Ferrer. 2001. A biochemical study of fasting, subfeeding, and recovery processes in yellow-legged gulls. Physiol Biochem Zool 74:703–713.

Amarri S. and L.T. Weaver. 1995. ^{13}C-breath tests to measure fat and carbohydrate digestion in clinical practice. Clin Nutr 14:149–154.

Bairlein F. 2002. How to get fat: nutritional mechanisms of seasonal fat accumulation in migratory songbirds. Naturwissenschaften 89:1–10.

Barbosa P.S., K.L. Parker, and I.D. Hume. 2009. Integrative Wildlife Nutrition. Springer, Berlin.

Bauchinger U., H. Kolb, D. Afik, B. Pinshow, and H. Biebach. 2009. Blackcap warblers maintain digestive efficiency by increasing digesta retention time on the first day of migratory stopover. Physiol Biochem Zool 82:541–548.

Bauchinger U., A. Wohlmann, and H. Biebach. 2005. Flexible remodeling of organ size during spring migration of the garden warbler (*Sylvia borin*). Zoology 108:97–106.

Beaupre S.J. and A.E. Dunham. 1995. A comparison of ratio-based and covariance analyses of a nutritional diet. Funct Ecol 9:876–880.

Ben-Hamo M., B. Pinshow, M.D. McCue, S.R. McWilliams, and U. Bauchinger. 2010. Fasting triggers hypothermia and ambient temperature modulates its depth in Japanese quail *Coturnix japonica*. Comp Biochem Physiol A 156:84–91.

Bicego K.C., R.C.H. Barros, and L.G.S. Branco. 2007. Physiology of temperature regulation: comparative aspects. Comp Biochem Physiol A 147:616–639.

Bodamer O.A.F. and D. Halliday. 2001. Uses of stable isotopes in clinical diagnosis and research in the pediatric population. Arch Dis Child 84:444–448.

Boggs C.L. 1992. Resource allocation: exploring connections between foraging and life history. Funct Ecol 6:508–518.

Boismenu C., G. Gauthier, and J. Larochelle. 1992. Physiology of prolonged fasting in greater snow geese (*Chen caerulescens atlantica*). Auk 109:511–521.

Brady L.J., D.R. Romsos, P.S. Brady, W.G. Bergen, and G.A. Levieille. 1978. The effects of fasting on body composition, glucose turnover, enzymes and metabolites in the chicken. J Nutr 108:648–657.

Brown C.R. and J.N. Cameron. 1991. The relationship between specific dynamic action (SDA) and protein synthesis rates in channel catfish. Physiol Zool 64:298–309.

Butler P.J. and A.J. Woakes. 2001. Seasonal hypothermia in a large migrating bird: saving energy for fat deposition. J Exp Biol 204:1361–1367.

Buyse J., B. Geypens, R.D. Malheiros, V.M. Moraes, Q. Swennen, and E. Decuypere. 2004. Assessment of age-related glucose oxidation rates of broiler chickens by using stable isotopes. Life Sci 75:2245–2255.

Castellini M.A. and L.D. Rea. 1992. The biochemistry of natural fasting at its limits. Experientia 48:575–582.

Cherel Y., D. Attaix, R. Rosolowska-Huszcz, R. Belkhou, J. Robin, M. Arnal, and Y. Le Maho. 1991. Whole-body and tissue protein synthesis during brief and prolonged fasting in the rat. Clin Sci 81:611–619.

Cherel Y., J.-B. Charrassin, and E. Challet. 1994. Energy and protein requirements for molt in the king penguin *Aptenodytes patagonicus*. Am J Physiol 35:R1182–R1188.

Cherel Y. and Y. Le Maho. 1985. Five months of fasting in king penguin chicks: body mass loss and fuel metabolism. Am J Physiol 249:R387–R392.

Cherel Y., J. Robin, A. Heitz, C. Calgari, and Y. Le Maho. 1992. Relationships between lipid availability and protein utilization during prolonged fasting. J Comp Physiol B 162:305–313.

Cherel Y., J. Robin, and Y. Le Maho. 1988. Physiology and biochemistry of long-term fasting in birds. Can J Zool 66: 159–166.

Chwalibog A. and G. Thorbek. 1989. Fasting heat production in chickens. Arch Geflugelk 53:54–57.

Cooper S.J. 2007. Daily and seasonal variation in body mass and visible fat in mountain chickadees and juniper titmice. Wilson J Ornithol 119:720–724.

Coulson R.A., J.D. Herbert, and T. Hernandez. 1978. Energy for amino acid absorption: transport and protein synthesis in vivo. Comp Biochem Physiol A 60:13–20.

Craig H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analyses of carbon dioxide. Geochim Cosmochim Acta 12:133–149.

Dawson W.R., R.L. Marsh, and M.E. Yacoe. 1983. Metabolic adjustments of small passerine birds for migration and cold. Am J Physiol 245:R755–R767.

Gannes L.Z. 2001. Comparative fuel use of migrating passerines: effects of fat stores, migration distance, and diet. Auk 118:665–677.

Geboes K.P., B. Bammens, A. Luypaerts, R.D. Malheiros, J. Buyse, P. Evenpoel, P. Rutgeerts, and K. Verbeke. 2004. Validation of a new test meal for protein digestion breath test in humans. J Nutr 134:806–810.

Goldstein D.L. and B. Pinshow. 2006. Taking physiology to the field: using physiological approaches to answer questions about animals in their environments. Physiol Biochem Zool 79:237–241.

Griminger P. and C.G. Scanes. 1986. Protein metabolism. Pp. 326–344 in P.D. Sturkie, ed. Avian Physiology. Springer, New York.

Grisolia S. and J. Kennedy. 1965. On specific dynamic action, turnover, and protein synthesis. Perspect Biol Med 9:578–585.

Grubb T.C. 1991. A deficient diet narrows growth bars on induced feathers. Auk 108:725–727.

Gurney W.S.C. and D.A.J. Middleton. 1996. Optimal resource allocation in a randomly varying environment. Funct Ecol 10:602–612.

Hannaford M.C., L.A. Leiter, R.G. Josse, M.B. Goldstein, E.B. Marliss, and M.L. Halperin. 1982. Protein wasting due to acidosis of prolonged fasting. Am J Physiol 243:E251–E256.

Hatch K.A., B. Pinshow, and J.R. Speakman. 2002. The analysis of $^{13}C/^{12}C$ ratios in exhaled CO_2: its advantages and potential application to field research to infer diet, changes in diet over time, and substrate metabolism in birds. Integr Comp Biol 42:21–33.

Hervant F., J. Meathieu, and J. Durand. 2001. Behavioural, physiological and metabolic responses to long-term starvation and refeeding in a blind cave-dwelling (*Proteus anguinus*) and a surface-dwelling (*Euproctus asper*) salamander. J Exp Biol 204:269–281.

Hochachka P.W. and G.N. Somero. 2002. Biochemical Adaptation: Mechanism and Process in Physiological Evolution. Oxford University Press, New York.

Hughes R.J., D.R. Tivey, and R.N. Butler. 2008. A method for collecting breath samples from individual chickens for analysis of $^{13}CO_2$, H_2, and CH_4. Poult Sci 87:1804–1809.

Hume I.D. and H. Biebach. 1996. Digestive tract function in the long-distance migratory garden warbler, *Sylvia borin*. J Comp Physiol B 166:388–395.

Ishihara K., S. Oyaizu, W. Mizunoya, Y. Fukuchi, K. Yasumoto, and T. Fushiki. 2002. Use of ^{13}C-labeled glucose for measuring exogenous glucose oxidation in mice. Biosci Biotechnol Biochem 66:426–629.

Jackson D.A. and K.M. Somers. 1991. The spectre of "spurious" correlations. Oecologia 86:147–151.

Jenni L. and S. Jenni-Eiermann. 1998. Fuel supply and metabolic constraints in migrating birds. J Avian Biol 29:521–528.

Karasov W.G. and I.D. Hume. 1997. Vertebrate gastrointestinal system. Pp. 409–480 in W. Dantzler, ed. Handbook of Comparative Physiology. American Physiological Society, Bethesda, MD.

Karasov W.H. and C. Martinez del Rio. 2007. Physiological Ecology: How Animals Process Energy, Nutrients, and Toxins. Princeton University Press, Princeton, NJ.

Karasov W.H. and S.R. McWilliams. 2005. Digestive constraints in mammalian and avian ecology. Pp. 87–112 in J.M. Starck and T. Wang, eds. Physiological and Ecological Adaptations to Feeding in Vertebrates. Science, Enfield, NH.

Karasov W.H., B. Pinshow, J.M. Starck, and D. Afik. 2004. Anatomical and histological changes in the alimentary tract of migrating blackcaps (*Sylvia atricapilla*): a comparison among fed, fasted, food-restricted, and refed birds. Physiol Biochem Zool 77:149–160.

Klaassen M. and H. Biebach. 1994. Energetics of fattening and starvation in the long-distance migratory garden warbler, *Sylvia borin*, during the migratory phase. J Comp Physiol B 164:362–371.

Klasing K.C. 1998. Comparative Avian Nutrition. CBA International, New York.

Knowles T.G., P.D. Warriss, S.N. Brown, J.E. Edwards, and M.A. Mitchell. 1995. Response of broilers to deprivation of food and water for 24 hours. Br Vet J 151:197–202.

Krikwoon J.K. 1991. Energy requirements for maintenance and growth of wild mammals, birds and reptiles in captivity. J Nutr 121:S29–S34.

Lamsova D., M. Macajova, and M. Zeman. 2004. Effects of short-term fasting on selected physiological functions in adult male and female Japanese quail. Acta Vet 73:9–16.

Laurila M., T. Pilto, and E. Hohtola. 2005. Testing the flexibility

of fasting-induced hypometabolism in birds: effect of photoperiod and repeated food deprivations. J Therm Biol 30: 131–138.

Lillywhite H.B. and C.A. Navas. 2006. Animals, energy, and water in extreme environments: perspectives from Ithala 2004. Physiol Biochem Zool 79:265–273.

Lindstrom A., G.H. Visser, and S. Daan. 1993. Correction: the energetic cost of feather synthesis is proportional to basal metabolic rate. Physiol Biochem Zool 1993:245.

Mata A.J., M. Caloin, D. Michard-Picamelot, A. Ancel, and Y. Le Maho. 2001. Are non-migrant white storks (*Ciconia ciconia*) able to survive a cold-induced fast? Comp Biochem Physiol A 130:93–104.

McCue M.D. 2006. Specific dynamic action: a century of investigation. Comp Biochem Physiol A 144:381–394.

———. 2007*a*. Snakes survive prolonged fasting by employing supply-side and demand-side economic strategies. Zoology 110:318–327.

———. 2007*b*. Western diamondback rattlesnakes demonstrate physiological and biochemical strategies for tolerating prolonged starvation. Physiol Biochem Zool 80:25–34.

———. 2010. Starvation physiology: reviewing the different strategies animals use to survive a common challenge. Comp Biochem Physiol A 156:1–18.

———. 2011. How animals use the nutrients they ingest: modeling the oxidative and nonoxidative fates of isotopically labeled metabolic tracers. BioScience (forthcoming).

McCue M.D., O. Amitai, I. Khozin-Goldberg, S.R. McWilliams, and B. Pinshow. 2009. Effect of dietary fatty acid composition on fatty acid profiles of polar and neutral lipid tissue fractions in zebra finches, *Taeniopygia guttata*. Comp Biochem Physiol A 154:165–172.

McCue M.D., O. Sivan, S.R. McWiliams, and B. Pinshow. 2010. Tracking the oxidative kinetics of carbohydrates, amino acids, and fatty acids in the house sparrow using exhaled $^{13}CO_2$. J Exp Biol 213:782–789.

McWhorter T.J., C. Martinez del Rio, B. Pinshow, and L. Roxburgh. 2004. Renal function in Palestine sunbirds: elimination of excess water does not constrain energy intake. J Exp Biol 207:3391–3398.

McWilliams S.R., C.G. Guglielmo, B.J. Pierce, and M. Klaasen. 2004. Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective. J Avian Biol 35:377–393.

McWilliams S.R. and W.H. Karasov. 2005. Migration takes guts: digestive physiology of migratory birds and its ecological significance. Pp. 67–78 in P. Marra and R. Greenberg, eds. Birds of Two Worlds. Smithsonian Institution, Washington, DC.

Milinkovic-Tur S., D. Emanovic, Z. Stoevic, and N.P. Milas. 1996. Effect of fasting on changes in blood glucose concentration and tissues glycogen contents in ducklings. Vet Arh 66:241–249.

Montes L., N. Le Roy, M. Perret, V. De Buffrenil, J. Castanet, and J. Cubo. 2007. Relationships between bone growth rate, body mass, and resting metabolic rate in growing amniotes: a phylogenetic approach. Biol J Linn Soc 92:63–76.

Murphy M.E. and J.R. King. 1986. Composition and quantity of feather sheaths produced by white-crowned sparrows during the postnuptial molt. Auk 103:822–825.

Packard G.C. and T.J. Boardman. 1988. The misuse of ratios, indices, and percentages in ecophysiological research. Physiol Zool 61:1–9.

Parker H. and H. Holm. 1990. Patterns of nutrient and energy expenditure in female common eiders nesting in the high arctic. Auk 107:660–668.

Piersma T. 1998. Phenotypic flexibility during migration: optimization of organ size contingent on the risks and rewards of fueling and flight. J Avian Biol 29:511–520.

Pullar J.D. and A.J.F. Webster. 1977. The energy cost of fat and protein deposition in the rat. Br J Nutr 37:355–363.

Raubenheimer D. 1995. Problems with ratio analysis in nutritional studies. Funct Ecol 9:21–29.

Raubenheimer D. and S.J. Simpson. 2009. Nutritional pharmecology: doses, nutrients, toxins, and medicines. Integr Comp Biol 49:329–337.

Reeds P.J., D.G. Burrin, T.A. Davis, and B. Stoll. 1998. Amino acid metabolism and the energetics of growth. Arch Anim Nutr 51:187–197.

Russell N.R. and R.J. Wootton. 1992. Appetite and growth compensation in the European minnow, *Phoxinus phoxinus* (Cyprinidae), following short periods of food restriction. Environ Biol Fishes 34:277–285.

Schoeller D.A., P.D. Klein, J.B. Watkins, T. Heim, and W.C. MacLean. 1980. ^{13}C abundances of nutrients and the effect of variations in ^{13}C isotopic abundances of test meals formulated for $^{13}CO_2$ breath tests. Am J Clin Nutr 33:2375–2385.

Shapiro C.J. and W.W. Weathers. 1981. Metabolic and behavioral responses of American kestrels to food deprivation. Comp Biochem Physiol A 68:111–114.

Slater C., T. Preston, and L.T. Weaver. 2001. Stable isotopes and the international system of units. Rapid Commun Mass Spectrom 15:1270–1273.

Starck J.M. 1999. Structural flexibility of the gastro-intestinal tract of vertebrates: implications for evolutionary morphology. Zool Anz 238:87–101.

———. 2003. Shaping up: how vertebrates adjust their digestive system to changing environmental conditions. Anim Biol 53:245–257.

Starck J.M., P. Moser, R.A. Werner, and P. Linke. 2004. Pythons metabolize prey to fuel the response to feeding. Proc R Soc B 271:903–908.

Tabiri H.Y., R.F.P. Bertolo, R.O. Ball, and D.R. Korver. 2002. Development of the indicator amino acid oxidation technique in chickens: calibration of oxidation system and determination of bicarbonate retention factor. Poult Sci 81: 1020–1025.

Tian X. and J.G. Qin. 2003. A single phase of food deprivation provoked compensatory growth in barramundi. Aquaculture 224:169–179.

———. 2004. Effects of previous ration restriction on compensatory growth in barramundi *Lates calcarifer*. Aquaculture 235:273–283.

Thouzeau C., J.-P. Robin, Y. Le Maho, and Y. Handrich. 1999. Body reserve dynamics and energetics of barn owls during fasting in the cold. J Comp Physiol B 169:612–620.

Totzke U., A. Hubinger, J. Dittami, and F. Bairlein. 2000. The autumnal fattening of the long-distance migratory garden warbler (*Sylvia borin*) is stimulated by intermittent fasting. J Comp Physiol B 170:627–631.

Tracy C.R. and J.S. Turner. 1982. What is physiological ecology? Bull Ecol Soc Am 63:340–347.

Tucker V.A. 1971. Flight energetics in birds. Am Zool 11:115–124.

Voigt C.C., F. Matt, F. Michener, and T.H. Kunz. 2003. Low turnover rates of carbon isotopes in tissues of two nectar-feeding bat species. J Exp Biol 206:1419–1427.

Voigt C.C., K. Rex, R.H. Michener, and J.R. Speakman. 2008. Nutrient routing in omnivorous animals tracked by stable carbon isotopes in tissue and exhaled breath. Oecologia 157: 31–40.

Voigt C.C. and J.R. Speakman. 2007. Nectar-feeding bats fuel their high metabolism directly with exogenous carbohydrates. Funct Ecol 21:913–921.

Waas S., R.A. Werner, and J.M. Starck. 2010. Fuel switching and energy partitioning during the postprandial metabolic response in the ball python. J Exp Biol 213:1266–1271.

Walsberg G.E. and B.O. Wolf. 1995. Variation in the respiratory quotient of birds and implications for indirect calorimetry using measurements of carbon dioxide production. J Exp Biol 198:213–219.

Wang T., C.C.Y. Hung, and D.J. Randall. 2006. The comparative physiology of food deprivation: from feast to famine. Annu Rev Physiol 68:223–251.

Wang Y., Y. Cui, Y. Yang, and F. Cai. 2000. Compensatory growth in hybrid tilapia, *Oreochromis maxxambicus* × *O. niloticus*, reared in seawater. Aquaculture 189:101–108.

Welch K.C., B.H. Bakken, C. Martinez del Rio, and R.K. Suarez. 2006. Hummingbirds fuel hovering flight with newly ingested sugar. Physiol Biochem Zool 79:1082–1087.

Welch K.C., L. Gerardo-Herrera, and R.K. Suarez. 2008. Dietary sugar as a direct fuel for flight in the nectarivorous bat *Glossophaga sorincia*. J Exp Biol 211:310–316.

Welch K.C. and R.K. Suarez. 2007. Oxidation rate and turnover of ingested sugar in hovering Anna's (*Calypte anna*) and rufous (*Selasphorus rufus*) hummingbirds. J Exp Biol 210: 2154–2162.

Wetzel K. 2005. ^{13}C-Breath Tests in Medical Research and Clinical Diagnosis. Fischer Analysen Instrumente, Leipzig.

Williams T.D. and S.P. Ternan. 1999. Food intake, locomotor activity, and egg laying in zebra finches: contributions to reproductive energy demand. Physiol Biochem Zool 72:19–27.