Physiological responses to food deprivation in the house sparrow, a species not adapted to prolonged fasting

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Khalilieh A, McCue MD, Pinshow B. Physiological responses to food deprivation in the house sparrow, a species not adapted to prolonged fasting. Am J Physiol Regul Integr Comp Physiol 303: R551–R561, 2012. First published July 11, 2012; doi:10.1152/ajpregu.00076.2012.—Many wild birds fast during reproduction, molting, migration, or because of limited food availability. Species that are adapted to fasting sequentially oxidize endogenous fuels in three discrete phases. We hypothesized that species not adapted to long fasts have truncated, but otherwise similar, phases of fasting, sequential changes in fuel oxidation, and similar changes in blood metabolites to fasting-adapted species. We tested salient predictions in house sparrows (Passer domesticus biblicus), a subspecies that is unable to tolerate more than ~32 h of fasting. Our main hypothesis was that fasting sparrows sequentially oxidize substrates in the order carbohydrates, lipids, and protein. We dosed 24 house sparrows with [13C]glucose, palmitic acid, or glycine and measured 13CO2 in their breath while they fasted for 24 h. To ascertain whether blood metabolite levels reflect fasting-induced changes in metabolic fuels, we also measured glucose, triacylglycerides, and β-hydroxybutyrate in the birds’ blood. The results of both breath 13CO2 and plasma metabolite analyses did not support our hypothesis; i.e., that sparrows have the same metabolic responses characteristic of fasting-adapted species, but on a shorter time scale. Contrary to our main prediction, we found that recently assimilated 13C-tracers were oxidized continuously in different patterns with no definite peaks corresponding to the three phases of fasting and also that changes in plasma metabolite levels accurately tracked the changes found by breath analysis. Notably, the rate of recently assimilated [13C]glycine oxidation was significantly higher (P < 0.001) than that of the other metabolic tracers at all postdosing intervals. We conclude that the inability of house sparrows to fast for longer than 32 h is likely related to their inability to accrue large lipid stores, separately oxidize different fuels, and/or spare protein during fasting.

house sparrow; fasting; starvation; blood metabolites; 13CO2; stable isotopes; breath testing

MANY WILD ANIMALS endure long fasts during reproduction, molting, hibernation, or migration (53), or simply because food is scarce (7, 21, 41, 43). The physiological and behavioral responses of species that are adapted to long fasts have been the subjects of much attention; in brief, animals adapted to fasting tend to preferentially mobilize carbohydrates and lipids, while sparing precious body proteins (2, 14, 17, 18, 24). By contrast, little attention has been paid to the responses to food deprivation in species that are not considered well adapted to fasting (27).

Fasting is a common occurrence in the lives of many avian species. Most long distance migrants cannot feed in flight and must exploit endogenous fuel deposits to meet energy demands (5, 34, 57, 60, 67). Some of these birds fly nonstop for thousands of kilometers, most notably shorebirds and waders that cross oceans on their migration (26, 56, 57). Migrants are not the only birds that experience long fasts. Many species of marine birds (e.g., blue petrels, Halobaena caerulea, short-tailed shearwaters Puffinus tenuirostris, and sooty shearwaters, P. griseus) forage in areas far from their nests, and some of their trips can last for as long as 14 days (15, 25, 58). During these periods, the chicks must fast until their parents return to feed them (3, 9). King penguin (Aptenodytes patagonicus) chicks fast for as long as 5 mo during the winter when the parents leave them inland on land to forage in the ocean (16). Small birds inhabiting temperate habitats often face severe environmental conditions during winter, such as cold temperatures, snowfalls and storms, long nighttime roosting, reduced foraging time, and unpredictable food resources that force them to fast (7, 20, 43, 63). The adaptations of these birds to fasting are crucial elements in their survival and involve a variety of behavioral, physiological, and biochemical responses (34, 66).

The sequential physiological responses to fasting have been categorized into discrete phases, delimited by the types of metabolic fuel being oxidized, levels of blood metabolites, or changes in rates of mass loss (2, 14, 16, 18, 39, 40, 46). After the absorption of the last meal, Phase I begins wherein glycogen is apparently the predominant source of energy (2, 24, 66). In some cases, stored glycogen may be completely exhausted to maintain blood glucose levels (reviewed in Ref. 46). Phase II is typically the longest phase in fasting-adapted species. In this phase, lipid oxidation is ostensibly the major source of energy and protein is spared from catabolism (13, 16). As a consequence of increasing lipid catabolism, the levels of free fatty acids, glycerol, and β-hydroxybutyrate in blood plasma generally increase, and triacylglycerides decrease (14, 32). In Phase III, the terminal phase of fasting, blood plasma concentrations of nitrogenous waste increase and lipid metabolites decrease (13, 14, 18). From what is known about the metabolic changes that take place during fasting in birds and mammals adapted to long fasts, we hypothesized that bird species that do not typically fast for long periods in nature also have three distinct fasting phases; however, these are truncated in time. In other words, such birds should undergo the same sequential changes in fuel oxidation and blood metabolites during fasting as those documented in fasting-adapted species, but each phase is proportionally shorter. We examined this possibility using both 13CO2 breath testing and the more traditional approach of blood metabolite chemical analyses, in fasting house spar-
rows (Passer domesticus biblicus), a subspecies that is generally unable to survive >32 h of fasting (Khalilieh and McCue, unpublished observations).

Before the onset of fasting, carbon atoms in an animal’s diet can be stored in its tissues, released in excreta, or oxidized and exhaled in CO₂. Assuming that the physiological fates of 13C-labeled tracers are essentially the same as nonlabeled ingested nutrients, by measuring 13C in exhaled breath, we recently used organic compounds (glucose, fatty acids, and amino acids) artificially enriched with 13C I) to track recently assimilated fuel oxidation in well-fed adult birds during the postprandial phase (50) and 2) to examine how postprandial fuel oxidation changed with the developmental and nutritional status of birds (49). The theoretical framework for using enriched tracers to quantify directly nutrient oxidation during prolonged fasting was recently summarized by McCue (47), who also found that the three tracers we used were routed preferentially into tissue within 2 h of administration in house sparrows (51). The present study is the first to employ this technique experimentally.

With the above assumption in mind, we tested three predictions regarding fuel oxidation in fasting house sparrows following meals labeled with artificially enriched 13C molecules. First, fasted birds, dosed with [13C]glucose have exhaled breath 13CO₂ enrichments during Phase I of fasting (Fig. 1A). Second, fasted birds dosed with artificially enriched [13C]palmitic acid have exhaled breath 13CO₂ enrichments during fasting Phase II reflecting fatty acid oxidation (Fig. 1A). Third, fasted birds dosed with artificially enriched [13C]glycine, a glucogenic and nonessential amino acid, have exhaled breath 13CO₂ enrichments during fasting Phase III reflecting amino acid oxidation (Fig. 1A). With respect to blood metabolites, we predicted that 1) fasting birds maintain constant blood glucose levels during Phases I and II; however, it decreases in Phase III; 2) the concentration of β-hydroxybutyrate increases throughout Phase II but decreases during Phase III, while 3) the concentration of triacylglycerides decreases slowly and steadily during the three phases of fasting (Fig. 1B). Since changes in blood metabolites are traditionally used as an indirect measure for changes in metabolic fuels (30–33, 35), we also predicted that blood plasma metabolite concentrations correspond to the sequential changes in recently assimilated substrates, as determined by breath testing.

MATERIALS AND METHODS

Permits. This research was done under permit no. 9017/2008 of the Israel Nature and National Parks Protection Authority and under permit BGU-R-06-2009 (to B. Pinshow) of the BGU Animal Care and Ethics Committee.

Study organism. Sixty adult house sparrows were captured using mist nets at Midreshet Ben-Gurion (30°52′ N, 34°47′ E), Israel, in July 2010. Each bird was banded with a uniquely numbered plastic leg band (Red Bird, Mt. Aukum, CA). The birds were randomly divided into groups of 10 and maintained in six outdoor aviaries (2.5 m × 1.5 m × 1.5 m), where they were provided with a diet of unhusked millet seeds (~12% protein and 5% lipids by dry mass; (65)) and tap water ad libitum. Once a week, the birds were provided also with crushed chicken egg shells, vitamin-supplemented water, and fresh lettuce. Thus the birds were acclimatized to seminatural conditions. One month before experiments, that began in October 2010 and ended in February 2011, the birds were gavaged twice with Baytril (Enrofloxacine, 0.1 ml), one week apart, to reduce the risk of infections that might influence the overall health of birds and the oxidative dynamics of tracers. All birds used were first fasted for 24 h to identify any weak or sick individuals, and they were given a minimum of 12 days to recover before subsequent fasting experiments. Perceptibly weak individuals were released and replaced. The number of individuals used differed according to the sample size requirements for each type of measurement. None of the birds were used more than once in any 13C tracer experiment; however, in cases where birds used for both 13C tracer measurements and metabolite measurements, they were first given a minimum of 19 days to recover. None of the birds were reproductive or molting during experiments.

13C isotope measurements and calculations. Breath tests were done on a total of 24 adult house sparrows (12 males and 12 females, mean mass (m₀) = 27.4 ± 1.84 g). In each sequence of measurements, four birds were used that were allowed to feed from dawn (~0600 h) until 0800 h. They were then transferred to the laboratory and background 13CO₂ levels were measured. The birds were placed in 850-ml metabolic chambers made from transparent, hermetically sealable plastic containers (model HPL808, Lock&Lock, Hana Cobi, Korea) maintained in a room where the air temperature was 25 ± 0.5°C. We chose this temperature because it is at the low end of the bird’s thermal-neutral zone [22.5–35°C; (29), explained in Ref. 4]; the temperature within the chambers never measurably exceeded 25.5°C. Dry, CO₂-free air was pumped through the chambers at 90–140 ml/min, a rate high enough to prevent hypercapnic conditions (12, 50), yet sufficient to ensure detectable levels of CO₂ for isotope analysis. The inlet and outlet ports were on opposite sides of the chamber, at sparrow head height. After 30 min, a 50-ml sample of excurrent air was collected from each chamber using a gas-tight, glass syringe (Perpektum, Popper, New Hyde Park, NY) and was injected in to the analyzer immediately (see below).

After collection of background 13CO₂ samples at 0900 h (experiment hour 0), the birds were gavaged with 20 mg of one of three 13C-labeled tracers, namely Δ-[1-13C]glucose, 98–99%; [1-13C]palmitic acid, 99%; or [1-13C]glycine, 99% (Cambridge Isotope Laboratories, Andover, MA), suspended in 240 μl of sunflower seed oil, using a 1.0-ml syringe.
attached to a 15-g silicon-tipped polyethylene feeding tube (FTP-15-78; Instech Solomon, Plymouth Meeting, PA). Immediately after administration, the birds were returned to the metabolic chambers, and excurrent gas samples were collected 30 min later and then at hourly intervals over the next 24 h. Since birds were gavaged about 2 min apart, the timing for each bird was standardized to the same 0 hour for the purposes of calculations and analyses. This sequence was repeated so that the sample size for each tracer was eight.

The $^{13}$CO$_2$ content of collected breath samples ($^{13}$CO$_2$) was determined using an infrared $^{13}$CO$_2$ analyzer (HeliFiANplus, Fischer Analyseinstrumente, Leipzig, Germany). For quality control, some randomly chosen breath samples were collected into 9-ml glass Vacutainers (Greiner Bio-One, Kremsmunster, Austria) and analyzed by conventional gas source isotopic ratio mass spectrometry (GS-IRMS) through a Gas Bench II interface (Thermo-Fisher Scientific, Waltham, MA). Delta ($\delta$) through Craig’s equation (19):

$$\delta^{13}C_{\text{PDB}} = \left( \frac{^{13}C}{^{12}C}_{\text{sample}} - \frac{^{13}C}{^{12}C}_{\text{std}} \right) \times 10^3$$

where $\left( ^{13}C/^{12}C \right)_{\text{sample}}$ is the ratio of $^{13}$C to $^{12}$C atoms in the sample and $\left( ^{13}C/^{12}C \right)_{\text{std}}$ is the ratio of the $^{13}$C to $^{12}$C atoms in the standard. We used the international standard for $\left( ^{13}C/^{12}C \right)_{\text{std}}$, namely that of Pee Dee Belemnite with a value of 0.01112329, in the calculations.

The instantaneous rates of the substrate oxidation ($T$; nmol/min) was calculated using the following equation that combine equations 4 and 5:

$$T = \left[ \frac{\text{APE} \times V_{\text{CO2}}}{k \times m \times \theta \times \text{BRF}} \right] \times 10^4$$

where APE is the atom percent excess of $^{13}$CO$_2$ above background levels, $V_{\text{CO2}}$ is the carbon dioxide production (ml CO$_2$/min), $k$ is the volume of CO$_2$ (ml) produced per milligram of tracer oxidized, $m$ is the molar mass of each tracer, $\theta$ is the number of isotopically enriched atoms per tracer molecule, and BRF is the bicarbonate retention factor [0.86; (62)]. The cumulative oxidation, $f(T)dT$ (in mmol), was calculated using equation 5:

$$f(T)dT = \left[ \int_0^{1440} f(T)dT \right] \times 10^{-3}$$

Blood metabolites. Fifty-six house sparrows (26 males and 30 females) were weighed to ±0.1 g, after being fed from dawn to ~0800 h. Fourteen birds were used in each of four sequences of blood metabolite measurements. In each sequence, the birds were placed individually in the 850-ml metabolic chambers, exposed to room air at 25 ± 0.5°C, and allowed 15 min to habituate. Then blood samples (~50 μl) were collected into heparinized capillary tubes from two birds, by puncturing the brachial vein with a sterile, 26-g needle; the process taking about a minute. The blood samples were analyzed for plasma levels of triacylglycerides, glucose, and β-hydroxybutyrate. The 12 remaining birds were gavaged with 20 mg of unlabeled glucose suspended in 240 μl of sunflower seed oil using the method describe above. After dosing, beginning at 0900 h, blood samples were collected from pairs of birds at intervals during the 24-h fast: at 30 min, 1, 2, 3, 4, 5, 6, 7, 8, and 9 h. To help maintain plasma volume, the birds were gavaged with 2 ml water 5 h after the first blood sampling. Blood was sampled again from each pair of birds in the same order at 10.5, 13.5, 15.5, 20, 22, and 24 h and was not sampled more than twice from any individual. Plasma concentration of triacylglycerides, glucose, and β-hydroxybutyrate were measured using a blood chemistry analyzer (model ST, CardioCheck, Maria Stein, OH) periodically calibrated with standard samples on strips supplied by the manufacturer. The analyzer’s lower detection limit of triacylglyceride concentration is 50 mg/dl.

Carbon dioxide production. Since $V_{\text{CO2}}$ is a key input variable to equation 2, we measured it in 21 sparrows (11 males and 10 females) sparrows randomly chosen from our captive birds at 25 ± 0.5°C, the same ambient temperature at which all our measurements were made. Individuals were weighed to ± 0.1 g, after being fed for about 2.5 h between dawn and 0830 h. $V_{\text{CO2}}$ was measured starting at 0900 h (~10 min) and continued for 24 h while fasting. $V_{\text{CO2}}$ data were collected from seven birds at a time and background, using an open-flow, 8-channel, multiplexed, respirometry system (Qubit Systems, Kingston, Ontario, Canada). The measurement sequence was 5 min per bird and 5 min background air between birds, thus $V_{\text{CO2}}$ was measured for 6 birds each hour. To calculate $V_{\text{CO2}}$ (as ml gas/min) we used equation 4, appropriate for this system (42):

$$V_{\text{CO2}} = V_i \times (F_i\text{CO2} - F_e\text{CO2})$$

where $V_i$ is the flow rate of CO$_2$-free dry air flowing into the metabolic chamber (in ml/min), and $F_i\text{CO2}$ and $F_e\text{CO2}$ are, respectively, the fractional concentrations of CO$_2$ in incident and excurrent (dried) air of the metabolic chambers. Flow rates were verified with a bubble flow calibrator (Sensydne, Gilian Gilibrator 2, Clearwater, FL), and FC02 was calibrated against dry nitrogen and O$_2$, CO$_2$, and N$_2$ mixtures supplied by a three-way gas mixing pump (model M301/2-F, H. Wösthoff, Bochum, Germany).

Data organization and statistical analysis. After careful visual inspection of the data, we divided the measurements for each tracer into 5 phases: 1) the postprandial phase (experiment hours 0 to 3, i.e., 0900–1200 h); 2) the postabsorptive phase, until the end of the first photophase (hours 4 to 7); 3) fasting: the transition between the first photophase and the scotophase (hours 8 to 11); 4) fasting: scotophase (hours 12 to 20); and 5) fasting: the transition between the scotophase into the second photophase (hours 21–24). Breath samples were taken from eight birds, four times in stages 1, 2 and 3, and 5, and nine in stage 4.

Within each stage, we tested for normality of distribution using the Shapiro-Wilk test and for homogeneity of variance using Bartlett’s test. Where necessary, we transformed the data to comply with the assumptions of the parametric tests used. To test for differences in oxidation rates of tracers (13C breath test) we used repeated measures (RM)-ANOVA. To test for differences in blood metabolite concentrations we used one-way ANOVA. Where ANOVA and RM-ANOVA showed significant differences, we used the Tukey HSD post hoc test to ascertain which means at which times were different. Data are reported as means ± SD, and null hypotheses were rejected at P < 0.05. All statistical tests were done using Statistica 10 software (StatSoft, Tulsa, OK), and all graphs were plotted using SigmaPlot 11.0 (Systat Software, San Jose, CA).

RESULTS

All the results that follow, based on equation 2 required $V_{\text{CO2}}$ as an input variable. The average values for $V_{\text{CO2}}$ during 24 h of fasting, used in our calculations are shown in Fig. 2 that also clearly illustrates the difference between awake (photophase) and resting (scotophase) birds.

$^{13}$CO$_2$ breath tests. The rates at which the different tracers were oxidized was affected by fasting time. The instantaneous rate of glucose tracer oxidation reached a peak of 132.3 ± 35.7 nmol/min during the postprandial phase, 30 min after tracer administration (Fig. 3A). It then declined over the next 6.5 h that included the last 2.5 h of the postprandial phase and 4 h more, until hour 7 of the photophase (RM-ANOVA from hour 0.5 until hour 3 of fasting, F$_{3,21}$ = 147.90, P < 0.0001; Tukey HSD, P < 0.0001; RM-ANOVA from hour 4 until hour 7 of fasting, F$_{3,21}$ = 44.996, P < 0.0001, Tukey HSD, P < 0.0001) (Fig. 3, A and B). However, glucose tracer oxidation briefly stabilized between hours 8 and 9 (from 4.39 ± 1.10 to
rate of palmitic acid tracer oxidation occurred between hours 10 and 11 (RM-ANOVA from hour 8 until hour 11 of fasting, F3,21 = 36.04, P < 0.0001, Tukey HSD, P < 0.0002). During the scotophase (hours 12–20) the rate of palmitic acid tracer oxidation decreased between hours 12 and 14 (RM-ANOVA from hour 12 until hour 20 of fasting, F3,21 = 8.083, P < 0.0001, Tukey HSD, P < 0.0367), after which it stabilized for the remainder of the scotophase (Tukey HSD, P = 0.9065) and by the subsequent morning (RM-ANOVA from hour 21 until hour 24, F3,21 = 0.3685, P = 0.7765), again unlike the glucose tracer. During the experiment, sparrows oxidized the palmitic acid tracer less extensively than glucose, i.e., only 1.53 ± 0.43% of the tracer was recovered in the bird’s breath (Fig. 4C).

Glycine tracer oxidation peaked at 2705.3 ± 687.4 nmol/min during the postprandial phase, 30 min after its administration (Fig. 5A). A rapid decrease in oxidation rate occurred during the first 3 h (RM-ANOVA from hour 0.5 until hour 3 of fasting, F3,21 = 63.56, P < 0.0001, Tukey HSD, P < 0.0001), yet this decrease significantly declined over the next 4 h of fasting (RM-ANOVA from hour 4 until hour 7 of fasting, F3,21 = 378.7, P < 0.0001, Tukey HSD, P < 0.0001) (Fig. 5, A and B). Nevertheless, the rate of glycine tracer oxidation stabilized as the birds entered the transition phase between photophase and scotophase at approximately hour 8 (30.5 ± 3.1 nmol/min) and remained relatively constant for the next hour (RM-ANOVA from hour 8 until hour 11 of fasting, F3,21 = 58.23, P < 0.0001, Tukey HSD, P = 0.8950). However, the rate of oxidation decreased significantly between hours 9 and 11 (Tukey HSD, P < 0.0002) during the same phase. During the scotophase, the rate of glycine tracer oxidation stabilized between hours 12 and 15 (RM-ANOVA from hour 12 until hour 20 of fasting, F3,21 = 5.078, P < 0.0001, Tukey HSD, P = 0.5242), yet decreased thereafter until hour 19 (Tukey HSD, P < 0.002) and stabilized for the next hour (Tukey HSD, P = 0.9214). During the photophase of the next morning, the rate of glycine tracer oxidation increased significantly until the end of the experiment (RM-ANOVA from hour 21 until hour 24 of fasting, F3,21 = 5.078, P < 0.0003, Tukey HSD, P < 0.002). Over the 24-h fast, the sparrows oxidized the glycine tracer more extensively than either glucose or palmitic acid tracers, and 61.8 ± 7.6% of the tracer was recovered in their breath (Fig. 5C).

**Blood metabolites.** Fasting time significantly affected plasma concentrations of glucose, β-hydroxybutyrate, and triacylglycerides during the 24-h experiment (Fig. 6, A–C). Plasma glucose concentration was relatively stable (Fig. 6A) during the first 5.5 h; however, it decreased significantly between hours 7 and 8 (ANOVA, from hour 0 until the end of the experiment, F12,91 = 7.9, P < 0.0001, Tukey HSD, P < 0.003). After this point, the concentration of plasma glucose seemingly increased until hour 13.5, although the trend was not significant (Tukey HSD, P = 0.520). A second gradual decrease in plasma glucose concentration occurred between hours 13.5 and 24, where minimum mean concentration reached 229.8 ± 43.4 mg/dl at hour 24 (Tukey HSD, P < 0.006).

The concentration of plasma β-hydroxybutyrate increased continuously from the background level of 11.55 ± 1.80 mg/dl (n = 8) at 0 h, to 17.4 ± 2.92 30 min after glucose administration, to 23.7 ± 3.29 after 1 h, reaching 64.30 ± 5.30 mg/dl at hour 8 (Fig. 6B). Although the increase is unambiguously
clear due to the use of ANOVA on all the data at once, statistical significance is apparent only at hour 3.5, just as the birds became postabsorptive (ANOVA, from hour 0 until the end of the experiment, $F_{12,91} = 47.7, P < 0.0001$, Tukey HSD, $P < 0.0005$). Thereafter, the concentration of plasma ketone bodies stabilized, with slight variations, from hour 8 until the end of the 24 h of fasting (Tukey HSD, $P < 0.4206$).

Plasma triacylglyceride concentration declined rapidly from 30 min after glucose administration until hour 5.5 of fasting (Fig. 6C); however, the decline only became statistically significant at hour 2 during the postprandial phase (ANOVA, from hour 0 until the end of the experiment, $F_{12,91} = 16.227, P < 0.0001$, Tukey HSD, $P = 0.0102$). Nevertheless, the concentration of triacylglycerides remained relatively constant after that, with slight variations, up to hour 20 of the experiment (Tukey HSD, $P = 0.9997$). By hour 22, some of the triacylglyceride values fell below the range of detection (50 mg/dl), precluding statistical comparisons.

**DISCUSSION**

Since there are at least 12 subspecies of the house sparrow (4), and some of them are resident in cold climates, we hesitate to generalize about the responses we see in *Passer domesticus biblicus*, although Blem (8) reports mean body fat between 5 and 12% in *P. d. domesticus*, increasing from low to high latitudes in North America. With our initial assumptions and the results of McCue et al. (51) in mind, the results of both breath $^{13}$CO$_2$ and plasma metabolite analysis in the present study did not support our hypothesis that *P. d. biblicus* have the same metabolic responses characteristic of fasting-adapted species of similar body mass, but on a shorter time scale.
Recently assimilated tracers were oxidized continuously in different patterns and did not exhibit the sequential changes corresponding to the three phases of fasting that we predicted. In addition, changes in plasma metabolite levels did not follow the typical pattern of fasting-adapted species.

The rates of tracer oxidization during the postprandial phase (hours 0–3) were highest during the 24 h of fasting. The magnitude of oxidization rates differed greatly among the three tracers, but the timing of peak glucose and glycine oxidization were similar (~30 min after administration), occurring before the peak oxidization of palmitic acid (~1 h after administration). Similar results for the oxidization of exogenous fuels in house sparrows were found in an additional study, where overall fuel catabolism during the postprandial phase in house sparrows was examined (50).

After the postprandial phase, the glucose tracer was oxidized with no clearly defined peaks during the first 6 h of the birds’ becoming postabsorptive (Fig. 3). The rate of glucose tracer oxidization stabilized between hours 8 and 9 during the transition between photophase and scotophase, but because a similar plateau in rate of oxidation of the other tracers, this is likely related to the animal’s diel metabolic rate adjustment for scotophase. Furthermore, contrary to our prediction, glucose tracer oxidization decreased in rate and remained constant between hours 9 and 21. In other words, we found no peak in \(^{13}\)CO\(_2\) production in [\(^{13}\)C\]glucose-dosed birds during the period that would traditionally be considered Phase II of fasting based on blood metabolites, suggesting the possibilities that (1) only a small amount of glucose was converted to lipids, or (2) carbohydrates were being oxidized along with fatty acids. Furthermore, compound-specific analyses would be necessary to quantify the amount of \(^{13}\)C from the glucose tracer was incorporated into glycogen or recently synthesized lipid pools (10, 45, 55).

Fig. 4. Instantaneous rates of oxidation of palmitic acid tracer by house sparrows. A: during 24 h of fasting; B: data are identical to A, but the postprandial phase was excluded for rescaling; C: cumulative palmitic acid tracer oxidation for the 24 h of fasting. Each point represents the mean for 8 birds ± SD. The experimental period is divided into five phases: postprandial, photophase, photophase-scotophase transition, scotophase, and scotophase-photophase transition (see Materials and Methods for justification). Different lower case letters within each phase indicate significant differences according to post hoc Tukey HSD comparisons. Values are means ± SD.
Unexpectedly, an increase in the rate of $^{13}$CO$_2$ production, following glucose tracer dosing, occurred between hours 21 and 24. This response was not observed for the fatty acid tracer, suggesting that glucose-derived carbon stores were not completely depleted during the first hours of fasting and were conserved as a source of energy for the scotophase of the next day. Glycogen sparing has been documented in ectothermic animals (22, 28, 54), but we are not aware of any studies of birds that indicate that glucose oxidation increases toward the end of a bout of prolonged starvation. Nevertheless, transient increases in glucose oxidation have been reported in flying mammals arousing from torpor (11).

The palmitic acid tracer was continually oxidized after the postprandial phase until hour 8 (Fig. 4). This result does not support our prediction that lipid stores, in particular fatty acids, provide the bulk of the energy needs during what would be considered Phase II of fasting. Nevertheless, the rate of oxidation stabilized between hours 8 and 10 during the transition from photo- to scotophase and into the first hour of the scotophase, unlike the level of glucose tracer oxidation that decreased in this period. The low cumulative oxidation of the palmitic acid tracer may reflect extensive integration of the tracer into a pool of lipids, e.g., structural membrane phospholipids, which is not readily available as an energy source (1, 48, 64). However, since tissue growth is minimal during starvation, this is an improbable explanation for our observations, although tissue turnover might account for some of it. By contrast, unlike the glucose tracer, the oxidation rates of the...
Palmitic acid tracer closely tracked the plasma triacylglyceride values. During fasting, the glycine tracer was continually oxidized at rates far exceeding the other two tracers (Fig. 5). Moreover, the data do not support the prediction that the oxidation of amino acids increases sharply toward the end of the fasting period. Nevertheless, unlike the oxidation of the glucose and palmitic acid tracers, the rate of glycine tracer oxidation did not plateau during the transition from photophase to scotophase. The oxidation rate of glycine tracer was highest during early scotophase, suggesting that house sparrows are limited in their capacity to spare protein during fasting.

Our results indicate that fasting house sparrows do not sequentially oxidize substrates in the order carbohydrates, then lipids, and then protein as typically occur in fasting-adapted species (14, 18, 40). Rather, these birds apparently oxidized simultaneously a combination of recently assimilated substrates, which we assume reflect the three endogenous fuel types (51). Furthermore, the fact that the amino acid tracers were oxidized more extensively than any of the other metabolic tracers indicates that house sparrows do not spare body protein during fasting which, in part, explains the inability of even well-nourished house sparrows to tolerate more than 32 h of fasting at rest. The phenomenon does not seem to be body mass dependent, because many migratory species of similar body mass to house sparrows accumulate large amounts of lipid and can fast for far longer than can house sparrows (6, 30, 36–38, 60).

In addition, our results suggest that house sparrows do not increase their stores of lipids much to survive an overnight fast but depend more on protein reserves. This conclusion is supported by the fact that sparrows are not particularly effective at accumulating lipid stores as seen among fasting-adapted species (4, 8). Our data also indicate that our measures of the

Fig. 6. Plasma concentrations of glucose (A), β-hydroxybutyrate (B), and triacylglycerides (C) during 24 h of fasting in house sparrows. Each point represents the mean for 8 birds ± SD. Different lower case letters indicate significant differences according to post hoc Tukey HSD comparisons. Values are means ± SD.
changes in blood metabolites and in tracer oxidation, during the relatively short period of fasting that house sparrows can tolerate, were confounded by regular circadian adjustments in metabolic rate, metabolites, and physiological fuel choice. Consequently, it was difficult to identify clear fasting-phase transitions in these animals.

In the present study of a species that is ostensibly not adapted to prolonged fasting, blood glucose concentration decreased significantly after the postprandial phase up to hour 8 of the experiment. Although blood glucose concentration increased slightly after this point until hour 13.5, it decreased significantly thereafter until the end of the 24-h fast. These results also failed to support our prediction that glucose oxidation is similar to that found in fasting-adapted species but on a shorter time scale.

Surprisingly, β-hydroxybutyrate increased from 30 min after food removal (while the birds were still postprandial!), until hour 8, failing to support our prediction that the concentration of β-hydroxybutyrate does not increase at the onset of fasting. Given the large differences in the extent to which the glycine and the palmitic acid tracers were oxidized, at this time we attribute the source of the ketone bodies to the oxidation of ketogenic amino acids. The level then plateaued with slight fluctuations toward the end of the fasting period. The increase was significant only after 3.5 h. The increase in β-hydroxybutyrate concentration was inversely correlated with the decrease in glucose concentration ($P < 0.001$; Fig. 7). In humans and other mammals the increase in plasma β-hydroxybutyrate initiates a drop in plasma glucose concentration as the rate of glucose production decreases (52, 59), which might explain our results. The high correlation between glucose and β-hydroxybutyrate concentration suggests that β-hydroxybutyrate plays a key role as an energy substitute for glucose, as has been documented in mammals (23, 44, 61).

The concentration of plasma triacylglycerides decreased significantly only after hour 2 of the experiment, during the postprandial phase, and remained low with slight variations, until the end of the 24-h fast. This suggests that, in sparrows, the capacity for lipid storage is meager and alone is not sufficient to meet energy requirements during even a relatively short fast. The breath-test results also failed to support the prediction that house sparrows oxidize mainly lipids during what would be expected to be Phase II of fasting. Moreover, the results of the metabolite analysis suggest that the continued elevation in blood β-hydroxybutyrate concentration is more likely to result from protein catabolism than lipid oxidation since the rate of glycine tracer oxidation was much higher than that of glucose or palmitic acid during the period when ketone levels were highest.

**Perspectives and Significance**

None of the results ($^{13}$CO$_2$ breath-testing or plasma metabolite analyses) supported our prediction that the subspecies of house sparrow that we examined has a truncated, but otherwise similar, blood metabolite pattern to that found in fasting-adapted species. Rather, these results show that these birds, unlike fasting-adapted species, were simultaneously oxidizing recently assimilated carbohydrates, lipids, and amino acids together, albeit at different rates, to meet their energy demands during the first 8 h of fasting. To the best of our knowledge, these are the first direct measurements of oxidation of multiple metabolic fuels during fasting. In addition, we found that the oxidation of the glucose tracer did not follow the same pattern as plasma glucose concentration, rather it was inversely proportional to it, yet the rate of palmitic acid tracer oxidation was reflective of triacylglyceride concentration during fasting. Furthermore, the results of the present study reveal that direct measurements of substrate oxidation can complement more traditional measurements of blood metabolites that, alone, offer limited insight into the physiological transitions among different metabolic fuels.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

Author contributions: A.K. and M.D.M. conception and design of research; A.K. performed experiments; A.K. and M.D.M. analyzed data; A.K., M.D.M., and B.P. interpreted results of experiments; A.K. and B.P. prepared figures; A.K. drafted manuscript; M.D.M. and B.P. edited and revised manuscript; M.D.M. and B.P. approved final version of manuscript.
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