Food restriction decreases thermoregulation in the silky starling
*Sturnus sericeus* (Aves: Passeriformes)

L.-Y. MAO#, J.-Y. Xu#, L. Shi, W.-H. Zheng, & J.-S. Liu*

*College of Life and Environmental Sciences, Wenzhou University, Wenzhou, China*

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**Abstract**

The capacity of small birds to sustain periods of food shortage largely depends on their ability to adaptively regulate expenditure in response to reduced food supply. To investigate changes in thermoregulation during periods of food restriction, we measured changes in the body mass, basal metabolic rate (BMR), organ mass, thyroid hormone levels, and several physiological and biochemical markers, of silky starlings (*Sturnus sericeus*) subject to 16 days food restriction. Birds in the control group were provided with food *ad libitum* whereas those in the food restriction (FR) group were provided with half of the usual quantity of food. Oxygen consumption was measured using an open-circuit respirometry system. Mitochondrial state-4 respiration and cytochrome c oxidase (COX) activity in the liver and pectoral muscle were measured with a Clark electrode. The concentrations of T3 and T4 in plasma were determined by radioimmunoassay. Starlings in the FR group had lower body mass and BMR compared to the control group. Starlings in the FR group also had lower state-4 respiration and COX activity in the liver and muscle, and lower T3/T4 ratio in plasma, relative to the control group. BMR was positively correlated with body mass, state-4 respiration and COX activity, in liver and muscle tissue. These results indicate that silky starlings can decrease their energy metabolism during periods of food shortage by making a suite of adjustments from the organismal to the biochemical level. These morphological, physiological and biochemical responses could be adaptations that allow starlings to adjust to seasonal changes in food abundance, such as the typically lower food abundance over winter.

**Keywords:** Cytochrome c oxidase, food restriction, state-4 respiration, *Sturnus sericeus*, thermogenesis

**Introduction**

Food abundance is an environmental cue for the seasonal acclimatization of thermoregulation in birds (Hiebert 1991; Foster 1997). It has been demonstrated that an animal’s body mass (Kelly & Weathers 2002), energy balance (Liang et al. 2015), and basal metabolic rate (BMR) (Gutiérrez et al. 2011) all are affected by food. In the face of unpredictable food shortages, birds often adopt different strategies, including energy storage, entering torpor or conserving energy, to survive (Hiebert 1991). For example, Liang et al. (2015) found that when food is limited, Chinese bulbuls (*Pycnonotus sinensis*) consume energy reserves, reduce their body mass, body fat and organ mass, in order to maintain essential metabolic functions. In contrast, Rufous hummingbirds (*Selasphorus rufus*) increased their body mass and spent more time in torpor when food was restricted, but became lighter and more active as soon as *ad libitum* feeding was resumed (Hiebert 1991). No matter which strategy is used, maintaining energy balance is one of the most important survival strategies for birds and it is clear that temporary fluctuations in food abundance can result in fluctuations in energy metabolism.

The basal metabolic rate (BMR) is the minimum maintenance metabolic rate of a normothermic resting endotherm and one of the fundamental physiological standards for assessing the energetic cost of thermoregulation (Williams & Tieleman 2000; McKechnie et al. 2006; Swanson et al. 2017).
Birds subject to food restriction or fasting often, though not always, have a lower BMR than birds fed ad libitum (Brzez & Konarzewski 2001; Pierce & McWilliams 2004). This has been demonstrated in a variety of species, such as rufous hummingbirds (Selaphorus rufus) (Hiebert 1991), blackcaps (Sylvia atricapilla) (Karavov et al. 2004), Eurasian tree sparrows (Passer montanus) (Yang et al. 2010), the little ringed plover (Charadrius dubius) (Gutiérrez et al. 2011), and Chinese bulbuls (Liang et al. 2015; Zhang et al. 2018). The association between reduced BMR during food restriction/fasting reveals a possible functional link between minimum sustained energy expenditure and BMR (Prinzinger & Siedle 1988; Pierce & McWilliams 2004; Liang et al. 2015). In addition to adjusting body mass and BMR, reducing organ function and cellular activity can also be effective ways of adjusting to unpredictable, or reduced, food resources (Vézina & Williams 2003; Liknes & Swanson 2011). Skeletal muscle is the main source of metabolic heat in birds (Bicudo et al. 2001). Meanwhile, organs such as the liver, kidneys and digestive tract, which have a high mass-specific energy metabolism, may contribute significantly to a bird’s BMR (Piersma et al. 1996; Williams & Tieleman 2000). At the cellular level, mitochondrial respiration produces heat from its imperfect coupling to ADP phosphorylation (Else et al. 2004). Furthermore, adjusting catabolic enzyme activity in oxidative pathways may improve heat production by enhancing cellular aerobic capacity (Liknes & Swanson 2011; Zheng et al. 2013). Levels of state-4-respiration and cytochrome c oxidase (COX) activity have commonly been used as BMR indicators at the cellular level (Venditti et al. 2004; Zheng et al. 2008a, 2014a; Swanson et al. 2014). Finally, the thyroid hormones thyroxine T4 and triiodothyronine T3 play pivotal roles in the energy metabolism of endothermic animals (Decuyper et al. 2005; Liu et al. 2006). These hormones can affect adaptive thermogenesis by influencing several aspects of energy metabolism, such as substrate cycling, ion cycling, and mitochondrial proton leakage (Yen 2001; Decuyper et al. 2005; Zheng et al. 2008a, 2014b). The silky starling (Sturnus sericeus) is resident in vast areas of southern and southeastern China, South Korea, North Vietnam and the Philippines (MacKinnon & Phillipps 2000). This species primarily feeds on insects, fruits and seeds, and nests in caves. Studies have found that male silky starlings have higher body mass, body temperature and thermal conductance, than females, and a relatively low BMR with a relatively wide thermal neutral zone (Zhang et al. 2006; Zhao et al. 2013). The silky starling also has high metabolic water production (MWP) and evaporative water loss (EWL) (Bao et al. 2014), and is able to adjust thermogenesis in response to seasonal changes in temperature, food quality and energy requirements (Li et al. 2017). This suggests that the ability of this species to adjust thermogenesis may be important to its ability to maintain energy balance in a changing natural environment. However, the effect of food restriction on thermogenesis in the silky starling is currently unknown. In a previous study we found that thyroid hormones play an essential role in thermoregulation in Chinese bulbuls during seasonal changes in temperature (Zheng et al. 2010, 2014b). However, we don’t know if thyroid hormones are involved in the thermoregulation of small birds during food shortages. To investigate these questions, we measured the metabolic rate, organ mass, cellular respiratory activity and T3, T4 levels of silky starlings that were subject to food restriction and compared these parameters with those of a control group that was fed ad libitum. We predicted that silky starlings would reduce thermogenesis and decrease thyroid hormone levels in plasma in order to decrease energy expenditure when food was limited.

Materials and methods

Animals

This study was carried out in Wenzhou City, Zhejiang Province (27°29’N, 120°51’E, 14 m in elevation), China. Wenzhou has a warm–temperate climate with an average annual rainfall of 1,500 mm, spread across all months, with slightly more precipitation during spring and summer. Daily minimum and maximum ambient temperatures vary greatly, ranging from 25.4 to 38.6°C in summer, and from 0.9 to 14.8°C in winter (Wenzhou Bureau of Meteorology).

Adult male silky starlings were captured in mist nets in Wenzhou City in October 2014, transported to a laboratory where they were maintained in individual enclosures (60 × 60 × 30 cm³) at 25 ± 1°C on a 12:12-h light-dark photoperiod with lights on at 06:00 a.m. Food (commercial starling pellets: 20% crude protein, 6% crude fat, 4% crude fiber, 1% calcium; Xietong Bioengineering, Jiangsu, China) and water were supplied ad libitum. Birds were kept under these conditions for two weeks to acclimatize, then randomly assigned to one of two groups; a control group (n = 11), which had access to food ad libitum, and a food restriction group (FR) (n = 11), which was provided with 50% of the usual
quantity of food for 16 days (Karavos et al. 2004; Liang et al. 2015). One bird that would have been assigned to the FR group died during the acclimatization period and was therefore excluded from data analyses. All experimental procedures were approved by the Animal Care and Use Committee of the Wenzhou University (Protocol 20130012).

**Measures of metabolic rate**

Oxygen consumption was measured using an open-circuit respirometry system (S-3A/1, AEI technologies, Pittsburgh, PA, USA) (Zheng et al. 2014b; Wu et al. 2015). The metabolic chamber was 1.5 l in volume and contained a perch for birds to stand on (Smit & McKechnie 2010). Individual birds were allowed to rest in the metabolic chamber, which was housed in a temperature–controlled cabinet capable of regulating temperature to ±0.5°C (Artificial Climatic Engine BIC–300, Shanghai, China). Air was scrubbed of H₂O and CO₂ by passing it through a silica gel/soda lime/silica column, before, and after, passing through the metabolic chamber. The fractional concentrations of oxygen in excurrent gas were measured from the metabolic chamber with an oxygen sensor (AEI Technologies N-22M, USA). The pump was located downstream of the metabolic chamber and air was pulled through the chamber at 300 ml min⁻¹ by the pump during metabolic rate measurements. This maintained a fractional concentration of O₂ in the respirometry chamber of about 20%, calibrated to ±1% accuracy with a general purpose thermal mass flow-meter (TSI 4100 Series, USA) (McNab 2006). Oxygen consumption rates were measured at 25 ± 0.5°C, which is within the thermal neutral zone of the silky starling (Bao et al. 2014). Baseline O₂ concentrations were obtained before and after each test (Wu et al. 2015). All gas exchange measurements were obtained during the resting phase of the birds’ circadian cycle (between 20:00 and 04:00) in dark chambers. Food was removed 4 h before each test to create post-absorptive conditions (to minimize the heat increment associated with feeding). Birds were in the metabolic chamber for an average of 4 hours. We allowed an equilibration period of at least 1 h within the metabolic chamber before we initiated metabolic measurements. We measured the metabolic rate of all birds for a period of at least 2 h following the equilibration period, and calculated 5 min running mean values for oxygen consumption over the test period using equation 2 of Hill (1972), with the lowest 5 min running mean designated as the basal metabolic rate (Zheng et al. 2014b; Wu et al. 2015). Metabolic rates were expressed as ml O₂ h⁻¹ and corrected to STPD conditions (Schmidt-Nielsen 1997). Body mass was measured to the nearest 0.1 g before and after experiments; mean body mass was used in calculations.

**Measurements of organ mass, tissue state-4 respiration, and cytochrome c oxidase (COX) activity**

Birds were euthanized by cervical dislocation at the end of the experimental period, and their pectoral muscle, brain, heart, lungs, liver, kidneys, gizzard and small intestine, extracted and weighed (Zheng et al. 2008a; Liknes & Swanson 2011). Sub–samples of liver and muscle were used to measure state-4 respiration and COX activity in these tissues (Zhou et al. 2008b, 2014a). State-4 respiration in liver and pectoral muscle tissue was measured at 30°C in 1.96 ml of respiration medium (225 mM sucrose, 50 mM Tris-HCl, 5 mM MgCl₂, 1 mM EDTA and 5 mM KH₂PO₄, pH 7.2) with a Clark electrode (Hansatech Instruments, UK; DW-1). State–4 respiration was measured over a 1-hour period under substrate-dependent conditions, with succinate as the substrate (Zhou et al. 2016). COX activity in liver and pectoral muscle tissue were measured polarographically at 30°C using a Clark electrode. The respiration medium contained 100 mM KCl, 20 mM TES, 1 mM EGTA, 2 mM MgCl₂, 4 mM KH₂PO₄, 60 mM BSA, at pH 7.2. A 10 ml aliquot was taken from the supernatant, and 30 ml of cytochrome c (37.9 mg/ml) were added to the electrode, after which COX activity was measured in a final volume of 2 ml (Zheng et al. 2013; Zhou et al. 2016). Both measurements were expressed as whole organ activity, μmol O₂·min⁻¹·organ⁻¹ (Zhou et al. 2016; Hu et al. 2017).

**Thyroid hormone concentration**

The concentrations of T₃ and T₄ in plasma were determined by radioimmunoassay, using RIA kits designed for measuring the levels of these hormones in humans provided by the China Institute of Atomic Energy. Antigens for T₃ and T₄ were labeled with ¹²⁵I (Liu et al. 2006). The kits were validated for silky starlings by cross-activity. Intra- and inter-assay coefficients of variation were 2.4% and 8.8% for the T₃ and 4.3% and 7.6% for T₄, respectively.

**Data analysis**

Data were analyzed using SPSS (version 19.0). The normality of all variables was assessed using the
Kolmogorov-Smirnov test, and non-normally distributed data were log10-transformed prior to analysis. The statistical significance of differences in body mass at 5 different times points during the experiment (e.g. days 0, 4, 8, 12 and 16) were assessed using a repeated-measures ANOVA. To eliminate confounding effects of individual differences in body mass, changes in metabolic rate on these 5 different time days were assessed with a repeated-measures ANCOVA with tarsus length as a covariate (Suárez et al. 2005; Zhou et al. 2016). The statistical significance of differences in metabolic rate and organ mass between treatment groups was analyzed by ANCOVA with tarsus length as a covariate. Because the T3/T4 ratio is a percentage, which is typically not normally distributed, we applied an arcsin-square root transformation to these data before analyzing them. We used least-squares linear regression to test for allometric correlations between log body mass, log organ mass and log RMR. For organ mass allometric regression we used body mass minus the wet organ mass of the organ in question to avoid statistical problems with part-whole correlations (Christians 1999). If allometric correlations for organ mass were significant, we calculated residuals from the allometric equations and regressed log organ mass residuals against log RMR residuals to determine if the mass of various organs was significantly correlated with RMR. If allometric correlations were not significant, we regressed raw values for log organ mass against log RMR to test for RMR–organ mass correlations (Zheng et al. 2013, 2014b). Least-squares linear regression was used to evaluate the relationship between log BMR and log body mass, and between log BMR, log state-4 respiration, and COX, between treatment groups was evaluated with the Student-Newman-Keul’s post hoc test, whereas the statistical significance of differences in metabolic rate and organ mass between treatment groups was analyzed by ANCOVA with tarsus length as a covariate. Because the T3/T4 ratio is a percentage, which is typically not normally distributed, we applied an arcsin-square root transformation to these data before analyzing them. We used least–squares linear regression to test for allometric correlations between log body mass, log organ mass and log RMR. For organ mass allometric regression we used body mass minus the wet organ mass of the organ in question to avoid statistical problems with part-whole correlations (Christians 1999). If allometric correlations for organ mass were significant, we calculated residuals from the allometric equations and regressed log organ mass residuals against log RMR residuals to determine if the mass of various organs was significantly correlated with RMR. If allometric correlations were not significant, we regressed raw values for log organ mass against log RMR to test for RMR–organ mass correlations (Zheng et al. 2013, 2014b). Least-squares linear regression was used to evaluate the relationship between log BMR and log body mass, and between log BMR, log state-4 respiration, and COX. Values are expressed as mean ± SE; P-values < 0.05 were considered statistically significant.

Results

Body mass and basal metabolic rate (BMR)

No between-group differences in body mass (F1,19 = 0.003, P = 0.957; Figure 1(a)) or BMR (F1,18 = 0.351, P = 0.561; Figure 1(c)) were found prior to food restriction. However, birds in the FR group underwent a significant decrease in body mass (F1,103 = 52.449, P < 0.001; Figure 1(b)) and BMR (F1,103 = 49.172, P < 0.001; Figure 1(d)) compared to the control. Significant group-by-time interactions were also found for body mass (F4,76 = 17.397, P < 0.001; Figure 1(a)) and BMR (F4,72 = 7.140, P < 0.001; Figure 1(c)). A significant decrease in both body mass (F1,19 = 7.809, P = 0.012) and BMR (F1,18 = 6.548, P = 0.020) was apparent in the FR group after 4 days, and these decreases were sustained for the duration of the experiment (Figure 1(a) and (c)). A positive correlation was found between log body mass and log BMR (r = 0.674, P < 0.001; Figure 1(e)).

Organ mass

ANCOVA (with tarsus length as the covariate) detected significant differences between the pectoral muscle and the gizzard mass of the FR and control group (Table I). The average mass of the pectoral muscle of birds in the FR group was 16% lower than that of birds in the control group. However, the average wet mass of the gizzard of birds in the FR group was 15% higher than that of birds in the control group. Log BMR was significantly and positively associated with log body mass (log BMR = −0.214 + 1.27 × log body mass; Figure 1(e)). Allometric relationships between log organ mass and log body mass (minus organ wet mass) were positive for all organs, but only the mass of the pectoral muscle, liver and heart were significantly correlated with body mass (Table II). BMR residuals were significantly and positively associated with pectoral muscle mass residuals, and BMR was significantly and positively correlated with lung mass, but no other organ mass or organ mass residuals were significantly correlated with BMR residuals or BMR (Table II).

Tissue state-4 respiration and cytochrome c oxidase (COX) activity

By the end of the experiment, the FR group had significantly lower state-4 respiration in the liver (t19 = 3.274, P = 0.004) and pectoral muscle (t19 = 2.614, P = 0.017) (Figure 2(a)), and reduced COX activity in the liver (t19 = 4.098, P = 0.001) and pectoral muscle (t19 = 7.527, P < 0.001) (Figure 2(b)), compared to the control group. There were positive correlations between log BMR and log state-4 respiration in the liver (r = 0.497, P = 0.022; Figure 3(a)) and pectoral muscle (r = 0.631, P = 0.002; Figure 3(b)), and between log BMR and log COX activity in the liver (r = 0.474, P = 0.030; Figure 3(c)) and pectoral muscle (r = 0.621, P = 0.003; Figure 3(d)).

Thyroid hormone concentration

There were no significant between-group differences in plasma T3, but the FR group had a significantly
Figure 1. Temporal trends in body mass and the basal metabolic rate (a and c), overall differences in body mass and basal metabolic rate (b and d), and relationship between body mass and basal metabolic rate (e), in food-restricted and control (ad libitum) silky starlings (*Sturnus sericeus*) over 16 days. Data are group means ± SE, *: \( p < 0.05 \), **: \( p < 0.01 \), ***: \( p < 0.001 \). BMR = basal metabolic rate.

Table I. Parameter estimates derived from an ANCOVA model comparing mean differences in silky starling organ masses after 16 days of food restriction. In the models, tarsus length was included as a covariate.

| Variable            | Control group | FR group | Significance |
|---------------------|---------------|----------|-------------|
| Sample size (n)     | 11            | 10       |             |
| Pectoral muscle (g) | 12.780 ± 0.315| 10.987 ± 0.331 | \( F_{(1, 19)} = 7.662; P = 0.004 \) |
| Liver (g)           | 2.392 ± 0.145 | 2.279 ± 0.152 | \( F_{(1, 19)} = 1.239; P = 0.313 \) |
| Heart (g)           | 0.804 ± 0.029 | 0.696 ± 0.031 | \( F_{(1, 19)} = 3.049; P = 0.072 \) |
| Lung (g)            | 0.650 ± 0.019 | 0.581 ± 0.020 | \( F_{(1, 19)} = 3.246; P = 0.063 \) |
| Kidney (g)          | 0.670 ± 0.020 | 0.692 ± 0.021 | \( F_{(1, 19)} = 0.264; P = 0.771 \) |
| Brain (g)           | 1.662 ± 0.028 | 1.643 ± 0.029 | \( F_{(1, 19)} = 0.104; P = 0.901 \) |
| Gizzard (g)         | 1.483 ± 0.058 | 1.670 ± 0.061 | \( F_{(1, 19)} = 4.575; P = 0.025 \) |
| Small intestine (g) | 2.005 ± 0.109 | 2.093 ± 0.115 | \( F_{(1, 19)} = 0.335; P = 0.719 \) |
| Rectum (g)          | 0.093 ± 0.009 | 0.088 ± 0.101 | \( F_{(1, 16)} = 0.564; P = 0.580 \) |
| Entire digestive tract (g) | 3.519 ± 0.142 | 3.792 ± 0.158 | \( F_{(1, 16)} = 1.277; P = 0.307 \) |

Data are mean ± SE; bold type indicates statistical differences.
higher T₄ and lower T₃/T₄ ratio, than the control group (Table III). A positive correlation was found between log BMR and log T₃/T₄ ratio (r = 0.522, P = 0.009; Figure 4).

Discussion

Food abundance is considered one of the most important environmental factors influencing thermoregulation and is thought to drive the evolution of a suite of morphological, physiological, and behavioral adaptations (Gutiérrez et al. 2011). We found that 16 days of food restriction caused silky starlings to decrease their body mass and BMR. Actually, decreased body mass and BMR can be explained by the food restriction, which, in turn caused the reduced energy expenditure. We also found a decrease in state-4 respiration and COX activity in the liver and muscle after 16 days of food restriction, which suggests decreased substrate oxidation system activity. Furthermore, we observed an increase in T₄, and a decrease in the plasma T₃/T₄ ratio, after 16 days of food restriction.

Effects of food restriction on body mass, BMR and organ mass

Body mass is an important index that reflects an animal’s nutritional status (Kelly & Weathers 2002) and its stability depends on the balance between energy intake and expenditure (Hegemann et al. 2012). Our results show that 16 days of food restriction was sufficient to cause silky starlings to undergo a significant decrease in body mass. Similar results have been found in white
throated sparrows (*Zonotrichia albicollis*) (Pierce & McWilliams 2004), Eurasian tree sparrows (Yang et al. 2010) and the Chinese bulbul (Liang et al. 2015). We also found that the FR group underwent a significant decrease in body fat compared to the control group (FR group, 5.05 ± 0.48 g; control group, 8.39 ± 0.55 g). Fat reserves can supply emergency energy reserves during periods of food shortage (Zhang et al. 2018). Increased body fat levels are common in many temperate passerines during winter, enabling these birds to meet their thermoregulatory demands and provide a nutritional buffer against temporary restrictions on foraging activity caused by inclement weather (Dawson & Marsh 1986; Li et al. 2017). The FR birds may have had to consume their fat reserves to compensate for their decreased energy intake (Liang et al. 2015; Zhang et al. 2018). Furthermore, our data show that FR birds had a lower BMR than control birds, indicating that they had lower heat output than the latter. BMR is a widely-accepted benchmark of metabolic expenditure for endotherms and is commonly used as a measure of the energetic cost of thermoregulation (McKechnie 2008; Swanson 2010; Swanson et al. 2017). Decreased BMR in response to food shortage, under either experimental or natural conditions, has been reported previously in other avian species (Prinzinger & Siedle 1988; Liang et al. 2015; Zhang et al. 2018). As BMR is directly related to the baseline for comparisons of the metabolic costs of activities among species or species groups, Schew and Ricklefs (1998) argue that there is an adaptive, and active, decrease in energy expenditure in response to food restriction. These results suggest that the ability to decrease BMR is an important adaptation used by silky starlings to survive periods of food shortage. In addition, decreased body mass may have contributed to the observed decrease in BMR, as indicated by the positive correlation between these two variables. Such a reduction in body mass generally reduces maintenance requirements, thereby resulting in a decrease in whole-body energy expenditure. Food restriction has also been associated with changes in organ size and mass. One idea is that energetically challenged birds decrease their food intake, and at the same time, reorganize their internal organs to improve thermal efficiency (Brzek & Konarzewski 2001). Several authors have suggested that much of the energy used in basal metabolism is consumed by visceral organs (Daan et al. 1990; Piersma et al. 1996), including the small intestine (storage, digestion and absorption), the heart and lungs (oxygen transport), the liver (catabolism), and the kidneys (waste elimination) (Daan et al. 1990; Li et al. 2017). The liver, heart, kidney, and digestive tract comprise less than 10% of total body mass, but consume 50–70% of total energy expenditure (Daan et al. 1990; Rolfe & Brown 1997).
Clapham 2012). For example, food restriction caused the songbird (Zonotrichia albicollis) to have a lighter liver, gizzard and digestive tract than control birds (Pierce & McWilliams 2004). This suggests that food deprivation has an effect on the body composition of birds. However, we did not find that the mass of the heart, kidneys, and digestive tract decreased significantly in the FR group relative to the control. Similar results were found in Chinese bulbuls (Liang et al. 2015) and small mammals including the striped hamster (Cricetulus barabensis) (Zhao et al. 2018). The studies cited above may be evidence of species- and organ-specific changes in organ morphology in response to food restriction. In the case of silky starlings, the only tissue significantly correlated with BMR was pectoral muscle (residual correlation). Skeletal muscle mass is less often associated with BMR, likely because of its low metabolic output at rest. However, a positive correlations between BMR and skeletal muscle mass has been documented for pectoral muscle in house sparrows (Chappell et al. 1999; Zheng et al. 2014a). Thus, the positive correlation we found between pectoral muscle mass and BMR in silky starlings is not without precedent. The proportionately large size of locomotory muscle mass in birds may facilitate a significant contribution to BMR variation, despite the typically low metabolic output of these muscles at rest (Chappell et al. 1999; Zheng et al. 2014a).

**Effects of food restriction on biochemical responses in the liver and muscle, and on thyroid hormone levels**

The liver is one of the largest and most metabolically active organs and is thought to make an important contribution to BMR in endotherms (Villarin et al. 2003; Zheng et al. 2008a, 2014b). Indeed, under basal metabolic conditions, the liver has been shown to contribute 25% of total heat production (Li et al. 2001; Zheng et al. 2008a). Skeletal muscle makes up nearly 40% of body mass and is a primary thermogenic tissue in birds (Block 1994; Weber & Piersma 1996; Zheng et al. 2014b; Hu et al. 2017). Levels of state-4 respiration and cytochrome c oxidase (COX; a key regulatory enzyme of oxidative phosphorylation) activity are commonly used as enzymatic markers of change in BMR at the cellular level (Venditti et al. 2004; Zheng et al. 2008a, 2014b; Swanson et al. 2014). In our study, FR birds had lower levels of state-4 respiration and COX activity in the liver and pectoral muscle than the control group. These results are consistent with those of previous studies. For example, FR rats (Rattus norvegicus) showed decreased levels of state-4 respiration and COX in the liver, suggesting that low aerobic capacity in the liver may play an important role in thermogenesis when animals are in a state of semistarvation (Gold & Costello 1975). Likewise, FR bulbuls decreased the oxidative capacity of the pectoral muscles by 28%, and that of the liver by 26%, which could collectively make a significant contribution to thermogenesis (Zhang et al. 2018). Low levels of state-4 respiration and COX activity are related to reduced BMR, a finding that is supported by the significant correlations between state-4 respiration, COX and BMR we found in silky starlings.

Thyroid hormones are a prominent endocrine candidate for thermoregulation because of their role in modulating cellular thermogenesis.
(Decuypere et al. 2005). T₄ is the main product of thyroid secretion. However, T₃ is only a prohormone and must be activated by deiodination to triiodothyronine T₃ in order to initiate thyroid hormone action. Our results show that the FR group had more constant T₃ levels, higher T₄ levels, and a lower T₃/T₄ ratio, than the control group. The constant, or falling, plasma T₃ levels of FR birds are likely to be the result of a shift in the balance between deiodination of T₄ by hepatic type I deiodinase and T₃ degradation by hepatic type III deiodinase (Darras et al. 1992). The results suggest that the increase in T₄ in food-restricted/fasted animals may be due to reduced uptake of T₄ by the organs and decreased utilization of T₄ to T₃ (Decuypere et al. 2005). Furthermore, a lower utilization of T₄ may result in the stabilizing of plasma T₃ levels in food restricted animals.

In conclusion, to survive periods of food shortage, silky starlings appear to undergo a significant decrease in body mass and BMR, a significant decrease in cellular respiratory enzyme activity in liver and muscle and significant variation in plasma T₄ levels, rather than a reduction in organ mass. These changes show that reduced food availability can cause changes at different levels within an organism, from the individual to the cellular and biochemical levels. In addition, indicators of cellular metabolic intensity in liver and muscle were positively correlated with BMR variation in individual starlings, which suggests that respiratory enzyme activity probably contributes to reducing BMR during periods of food shortage. Our data also suggest that T₃ and T₄ may be involved in thermoregulation during periods of food shortage. Collectively, these results indicate that the ability to decrease thermogenic capacity could play a key role in reducing energy consumption, and that this could be an important strategy through which silky starlings maintain energy balance when food resources are limited. Interestingly, silky starlings appear to decrease thermogenesis when food is limited by adjusting cellular metabolic activity and T₄ levels rather than by reducing their organ mass. Further research is needed to explain how thermogenesis is regulated at the molecular level by food restriction.

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Disclosure statement

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