Comparative Biochemistry and Physiology, Part A 212 (2017) 1–8

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Mitochondrial oxidative phosphorylation efficiency is upregulated during fasting in two major oxidative tissues of ducklings

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ARTICLE INFO

Keywords:
Mitochondria
Oxidative phosphorylation
Bioenergetics
Starvation
Birds
Skeletal muscle
Liver

ABSTRACT

Fasted endothermic vertebrates must develop physiological responses to maximize energy conservation and survival. The aim of this study was to determine the effect of 1-wk. fasting in 5-wk. old ducklings (*Cairina moschata*) from whole-body resting metabolic rate and body temperature to metabolic phenotype of tissues and mitochondrial coupling efficiency. At the level of whole organism, the mass-specific metabolic rate of ducklings was decreased by 40% after 1-wk. of fasting, which was associated with nocturnal *T*b declines and shallow diurnal hypothermia during fasting. At the cellular level, fasting induced a large reduction in liver, gastrocnemius (oxidative) and pectoralis (glycolytic) muscle masses together with a fuel selection towards lipid oxidation and ketone body production in liver and a lower glycolytic phenotype in skeletal muscles. At the level of mitochondria, fasting induced a reduction of oxidative phosphorylation activities and an up-regulation of coupling efficiency (+ 30% on average) in liver and skeletal muscles. The present integrative study shows that energy conservation in fasted ducklings is mainly achieved by an overall reduction in mitochondrial activity and an increase in mitochondrial coupling efficiency, which would, in association with shallow hypothermia, increase the conservation of endogenous fuel stores during fasting.

1. Introduction

Fasted endothermic vertebrates can develop several physiological responses to maximize energy conservation and survival, which involves *supply-* and/or *demand-side* regulation of the energy balance (McCue, 2007). This is best illustrated by king penguin chicks that are evolved to survive several months of starvation (Cherel and Le Maho, 1985). Their ability to withstand prolonged periods of starvation is driven both by *supply-side* regulations (*e.g.* high capacity to store, mobilize and oxidize fat as fuel) and by *demand-side* mechanisms, which minimize heat dissipation and energy expenditure (growth arrest, low basal metabolic rate, reduced lipid-induced thermogenesis), and induce shallow hypothermia (Barré, 1984; Duchamp et al., 1989; Cherel et al., 1993, 2004; Eichhorn et al., 2011; Teulier et al., 2013). Short-term fast also triggered the development of an economical management of endogenous energy substrates in king penguin chicks by decreasing the cost of ATP production at the level of skeletal muscle mitochondria (Monternier et al., 2014). This finding is of interest because mitochondrial activity remains the main physiological link between *supply-side* (endogenous fuel store and oxidation) and *demand-side* mechanisms (energy allocated to animal activities and survival).

Energy saving may be achieved by down-regulating the activities (reduced "demand") and/or up-regulating the efficiency of mitochondrial oxidative phosphorylation for the "supply" side. For instance, an early active suppression of mitochondrial metabolism and subsequent metabolic suppression has been suggested to trigger body temperature falls in hibernating mammals (Staples, 2014). An up-regulation of mitochondrial efficiency has been recently reported in birds fasting in the cold (Monternier et al., 2014, 2015). As mentioned above, an improvement of mitochondrial energy coupling efficiency would reduce the demand for substrate oxidation, triggering an economical management of endogenous fuel store. Finally, it is worth noting that starvation-induced decrease in body temperature is the most common energy saving mechanism documented in mammals and birds, including species that do not hibernate (McKechnie and Lovegrove, 2002; McCue, 2010; Eichhorn et al., 2011). In this context, large benefits would further arise from the passive effects of low temperature on mitochondrial bioenergetics, such as a lower energy cost of ATP synthesis (Monternier et al., 2014; Jarmuszkiewicz et al., 2015). At the crossroads of environmental resources and animal performances, the

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http://dx.doi.org/10.1016/j.cbpa.2017.06.018
Received 20 February 2017; Received in revised form 23 May 2017; Accepted 19 June 2017
Available online 21 June 2017

regulation of mitochondrial activity and/or efficiency may contribute to save energy in organisms living in environments with seasonal or unpredictable shortages in resource supplies. The aim of the present study was to investigate the effect of 1-wk. fasting in 5-wk. old ducklings (*Cairina moschata*) from whole-body resting metabolic rate and body temperature to metabolic phenotype of tissues and mitochondrial coupling efficiency. Ducklings (*Cairina moschata*, Linnaeus 1758) were reared at thermoneutrality and results from ducklings fed *ad libitum* were compared with those from birds that were fasted for 1 week. Resting metabolic rate and body temperature were measured *in vivo*. Mitochondrial oxidative phosphorylation activity and coupling efficiency (ATP/O ratios) and the activities of enzymes involved in energy metabolism were assayed in liver and in both pectoralis (glycolytic) and gastrocnemius (oxidative) muscles. In addition, plasma (glucose, triglycerides, glycerol, non-esterified fatty acids, β-hydroxybutyrate and uric acid) and tissue metabolites (triglycerides and glycogen) were quantified.

2. Materials and methods

2.1. Animals and tissues sampling

All experiments were conducted in accordance with animal care guidelines and were approved by the Ethics Committee of Lyon University and the Ministère de la Recherche et de l'Enseignement Supérieur. Male Muscovy ducklings (*Cairina moschata*) were obtained from a commercial stockbreeder (Eclosion Grimaud Frères, Roussay, France). Ducklings were reared at 25 °C for 4 weeks in a constant photoperiod (light/dark: 8/16). Thereafter, three protocols were carried out on ducklings that were either fed *ad libitum* or fasted for 1 week. The first protocol was dedicated to measuring body temperature over the course of fasting. The second protocol was set up to measure resting metabolic rate with blood sampling. The third protocol was dedicated to biochemical measurements (plasma and tissue metabolites, enzymes activities) and mitochondrial bioenergetics. For this purpose, animals were stunned by cranial percussion and killed by decapitation. Blood was collected and centrifuged (5 min, 800 × *g*) at 4 °C and the resulting plasma was stored at − 80 °C until assays were conducted. Liver and skeletal muscles (pectoralis and the red part of gastrocnemius muscles) were rapidly withdrawn and weighed. Noted that growing ducklings used in the present study display rather an immature and poorly developed flight muscles (*e.g.* pectoralis muscle) compared with the well-developed and functional leg muscles (*e.g.* gastrocnemius muscle). Small sample of each tissue was freeze-dried (Christ® ALPHA 1-4LD lyophilisator) and stored at − 80 °C until assayed for metabolites contents or enzymes activities. The remainder of the skeletal muscle and liver tissues were freshly used for mitochondrial isolation.

2.2. Plasma and muscle metabolites

Plasma metabolites were assayed by the biochemical department of Lyon Sud Hospital using standard enzymatic methods and routine analysis systems. Extraction and assays of glycogen, triglycerides and proteins from freeze-dried tissue samples were processed as previously done (Monternier et al., 2015). Briefly, total lipids were extracted by homogenizing muscle samples in 20 volumes of a chloroform-methanol mixture (2:1 vol/vol), and triglycerides were measured using the enzymatic triglycerides (GPO Trinder) test from Sigma-Aldrich (France). To measure glycogen content, tissues samples were homogenized in 10 volumes of 1 N NaOH. Glycogen was subsequently measured using the enzymatic Glucose HK (Sigma-Aldrich, France) kit. All assays were performed using a spectrophotometer (Thermo Electron Corporation, AQUAMATE) at room temperature.

2.3. Body temperature

Five days prior to the experimental period, thirteen ducklings ($N = 5$ in the fed group; $N = 8$ in the fasted group) were force fed a temperature data logging device (ANIPILL system®; Body-cap, Caen, France) as previously used in ducklings (Tattersall et al., 2016). The device has been designed for use in birds to lodge within the gizzard, providing access to an internal body temperature (T_b) measurement (data logged at 15 min intervals). For the fasted group, we subsequently divided the experiment into two time periods: 4-day of *ad libitum* feeding and subsequent fasting for 1 week.

2.4. Metabolic rate

Twelve ducklings were used to measure resting metabolic rate (RMR) at 25 °C within the thermoneutral zone for these birds (Teulier et al., 2014). This protocol was performed after an overnight fast for ducklings fed *ad libitum* (Fed group, $N = 6$) or after 1-wk. of fast (Fasted group, $N = 6$). RMR was measured between 9:00 and 11:00 AM by indirect calorimetry using an open-circuit as already used in ducklings (Barré et al., 1986; Teulier et al., 2010). The fractional concentrations of oxygen were measured using a Servomex 1100 paramagnetic gas analyzer (Taylor Instrument Analytics Ltd., Sussex, UK). Carbon dioxide concentrations were measured using a Servomex 1400 infrared gas analyzer. The oxygen analyzer was calibrated with pure nitrogen gas and atmospheric air assuming oxygen content of 20.93%. The carbon dioxide analyzer was calibrated with pure nitrogen gas and a known mixture of 0.502% carbon dioxide. The rates of oxygen consumption and carbon dioxide production were calculated as previously described (Teulier et al., 2010). Blood samples were collected at the end of metabolic measurements, centrifuged at 4 °C and the resulting plasma was stored at − 80 °C until assays were conducted.

2.5. Mitochondrial isolation

Twelve ducklings were used to assess mitochondrial bioenergetics (Fed group, $N = 6$; Fasted group, $N = 6$). Mixed skeletal muscle mitochondrial populations were isolated from either pectoralis and the red part of gastrocnemius in an ice-cold isolation buffer (100 mM sucrose, 50 mM KCl, 5 mM EDTA, 50 mM Tris-base, and pH 7.4 at 4 °C) as previously described (Monternier et al., 2015). Briefly, the mitochondrial isolation procedure involved Potter homogenization, protease digestion and differential centrifugations, with all steps at 4 °C. Liver mitochondrial population was isolated by differential centrifugations in an ice-cold isolation buffer (250 mM sucrose, 1 mM EGTA, 20 mM Tris-HCl, and pH 7.3 at 4 °C) as previously described (Salin et al., 2010). Finally, both skeletal muscles and liver mitochondria were pelleted at 8700 × *g* (10 min) and the protein concentration of mitochondrial suspension was determined by a Biuret method with bovine serum albumin as standard.

2.6. Mitochondrial oxidative phosphorylation activity and efficiency

Mitochondrial oxidative phosphorylation efficiency was assessed at 40 °C by measuring the rates of oxygen consumption and ATP synthesis in respiratory buffer (120 mM KCl, 5 mM KH_2PO_4, 1 mM EGTA, 2 mM $MgCl_2$, 3 mM Hepes, 0.3% fatty acid-free bovine serum albumin, pH 7,4) supplemented with hexokinase (1.6 U/ml) and glucose (20 mM). Respiration was initiated by adding a mixture of respiratory substrates consisting of pyruvate (5 mM), malate (2.5 mM) and succinate (5 mM). This combination of substrates was chosen to generate a convergent electron flow at the coenzyme-Q junction of the respiratory chain, which would reconstitute the physiological citric acid cycle function in isolated mitochondria, by generating simultaneously NADH and succinate in the mitochondrial matrix (Gnaiger, 2009). Thereafter, mitochondrial ATP synthesis was initiated by the addition of 100 μM,