Comparative Biochemistry and Physiology, Part A 176 (2014) 41–48

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Cellular metabolic rates from primary dermal fibroblast cells isolated from birds of different body masses

Ana Gabriela Jimenez *, Joseph B. Williams

Department of Evolution, Ecology and Organismal Biology, Ohio State University, 318 W. 12th Ave., Columbus, OH 43210, USA

ARTICLE INFO

Article history:
Received 1 May 2014
Received in revised form 14 June 2014
Accepted 9 July 2014
Available online 16 July 2014

Keywords:
Cellular metabolism
Birds
Body mass
Metabolic rate

ABSTRACT

The rate of metabolism is the speed at which organisms use energy, an integration of energy transformations within the body; it governs biological processes that influence rates of growth and reproduction. Progress at understanding functional linkages between whole organism metabolic rate and underlying mechanisms that influence its magnitude has been slow despite the central role this issue plays in evolutionary and physiological ecology. Previous studies that have attempted to relate how cellular processes translate into whole-organism physiology have done so over a range of body masses of subjects. However, the data still remains controversial when observing metabolic rates at the cellular level. To bridge the gap between these ideas, we examined cellular metabolic rate of primary dermal fibroblasts isolated from 49 species of birds representing a 32,000-fold range in body masses to test the hypothesis that metabolic rate of cultured cells scales with body size. We used a Seahorse XF-96 Extracellular flux analyzer to measure cellular respiration in fibroblasts. Additionally, we measured fibroblast size and mitochondrial content. We found no significant correlation between cellular metabolic rate, cell size, or mitochondrial content and body mass. Additionally, there was a significant relationship between cellular basal metabolic rate and proton leak in these cells. We conclude that metabolic rate of cells isolated in culture does not scale with body mass, but cellular metabolic rate is correlated to growth rate in birds.

1. Introduction

The rate of metabolism is the speed at which organisms use energy, an integration of all energy transformations within the body; it governs biological processes that influence rates of growth and reproduction, and may have a direct or indirect influence on life span (Kleiber, 1975; McNab, 2003). Early work found that as species increased in body size, mass-specific metabolism decreased in a non-linear fashion, best described as a power function (Kleiber, 1975). These comparisons also generated awareness that animals of the same body size, such as a mouse, shrew, and bat, could have markedly different rates of mass-specific metabolism and associated life-history parameters, and therefore, by inference, natural selection must be able to adjust factors that influence metabolism in dramatic ways (Calder, 1984; Mueller and Diamond, 2001). Renewed interest in the underlying causes of variation in metabolic rates of organisms of different body masses emerged with the putative idea that oxygen delivery systems follow universal scaling laws (West et al., 2003), but while these attempts to identify such laws have provided new ideas for exploration, they have also failed to explain many key elements of the variation of metabolic rates among organisms (Kozlowski and Konarzewski, 2005; Chown et al., 2007). Progress in understanding functional linkages between whole organism metabolic rate and underlying mechanisms that influence its magnitude has been slow despite the central role this issue plays in evolutionary and physiological ecology (Bennett, 1988; Speakman, 2005).

The connection between whole-animal metabolic rate and the metabolic rate of cells isolated from animals and cultured remains controversial (West et al., 2003; Brown et al., 2007; Wheatley, 2007). Coulson et al. (1977) first proposed that the metabolic rate of cells in animals was dictated by the rate of delivery of nutrients from their circulatory system. From a review of the literature, Agutter and Wheatley (2004) concluded that differences in mass-adjusted metabolism of mammals of different body sizes did not reside in cellular function but at higher levels of physiological organization. West et al. (2003) argued that metabolic scaling followed a fractal-like design, and predicted that the metabolic rate of cells isolated from an animal would be uniform and independent of animal body mass. Additionally, some have argued that metabolically active cells will lose their in situ scaling characteristics when they are grown in a common nutrient environment, and thus, cells placed in culture from animals with different rates of mass-specific metabolism will assume a uniform rate of cellular O_2 consumption (West et al., 2003). When they measured metabolic rate of dermal fibroblasts for 10 species of different-sized mammals including lab mice, humans, domestic cows and horses, Brown et al. (2007)

* Corresponding author.
E-mail address: jimenez.102@osu.edu (A.G. Jimenez).

http://dx.doi.org/10.1016/j.cbpa.2014.07.009
1095-6433/

did not find a significant relationship between metabolic rate of dermal fibroblasts from long-term culture and body size, in support of West et al. (2003). However, their results have been criticized because they did not culture primary fibroblast cells, but rather acquired them from other facilities (Wheatley, 2007). Thus, the length of time that cells had been dividing in culture conditions varied among species, and cells experienced differences in culture conditions. Slight differences in culture conditions among sources of the fibroblasts could have impacted results.

On the other hand, the quantum metabolism theory, which attempts to infer whole organism metabolic rates from the metabolic activities of component cells, posits that cells isolated from larger animals with low rates of whole-organism metabolism will maintain a low rate of cellular metabolism (Demetrius, 2006; Wheatley, 2007). The mass-specific decline in metabolic rate of endotherms of large body size can be partially accounted for by a reduction in the O_2 consumption of cells (Rolfe and Brown, 1997; Porter, 2001). Krebs (1950) demonstrated that O_2 consumption of liver slices from mice was 7.4 times greater than that of liver slices from horses, a result supported by work of Porter and Brand (1995) on hepatocytes. These results together support the notion that intrinsic differences among cells from different species can, in part, be responsible for differences in organismal metabolic rate.

Growth rate, a fundamental parameter of an organism's life history, varies some 30-fold across all bird species (Ricklefs, 1984; Williams et al., 2007; Dmitriew, 2011). Growth rate is a key life history trait that influences fitness, and shapes the basic physiology of organisms (Lack, 1968; Russell et al., 2004; Dmitriew, 2011). Predation risk, length of the breeding season, and food limitation are factors that are thought to ultimately shape growth rates in birds (Arendt, 1997; Fontaine et al., 2007; Martin et al., 2011). Metabolic rate, and growth rate have been studied in organisms, but the link between an organism's growth rate and metabolic rate of its cells remains unknown. Cell lines held in a common environment, but established from bird species that grow at different rates allow a test of the idea that intrinsic differences in the cellular metabolic profile as a result of differences in growth rates can be observed in vitro. The idea that growth rate and metabolic rate of cultured cells should be related is in direct opposition to the concept presented by Brown et al. (2007) of cells losing their in situ metabolic scaling when isolated from the animal.

Primary dermal fibroblasts are often used as a “model cell,” not because they provide an infallible surrogate for other cell types of interest (muscle, liver, brain, etc.), but because of convincing data showing that these cells, in vitro, retain properties that distinguish species that differ in life history patterns and physiology. The evidence includes published reports on cellular stress resistance of fibroblasts from different species (Kapahi et al., 1999; Harper et al., 2007; Salmon et al., 2008; Jimenez et al., 2013), on growth control properties (Seluanov et al., 2008), on relationships between longevity of fibroblasts in culture and life span of mammal donors (Röhme, 1981), and on cellular metabolic rates as they related to life-history trade-offs (Jimenez et al., 2014a). Primary dermal fibroblasts are responsible for generating connective tissue and are involved in wound healing (Sorrell and Caplan, 2004), but generally this cell type is thought to be metabolically inactive until it is required at the site of tissue damage.

Here, we explore whether cells isolated from birds with different body masses might have different rates of cellular metabolism. We examined primary dermal fibroblasts from 49 species of birds of varying body masses for their cellular metabolic rate, fibroblast cell size and mitochondrial content. We failed to find any significant differences between cellular metabolic rate and body mass of birds, supporting the findings of Brown et al. (2007), and consistent with West et al. (2003). However, we found a significant correlation between basal O_2 consumption of these cells and their proton leak. We also found a significant correlation between growth rates and cellular basal metabolic rates.

2. Materials and methods

2.1. Collection of birds

Birds were collected in and around Ohio, USA (latitude: 41.28° and longitude: 83.1°). All birds were adults of unknown age and were collected under an Ohio Division of Wildlife permit number 15-29. All procedures were approved by the Institutional Animal Care and Use Committee of The Ohio State University (protocol IACUC2004A0093). Additionally, The Wilds and the Columbus zoo provided feather samples from Red-crowned Cranes, Ostriches, Rainbow Lorikeets, Guinea Fowl and Flamingos.

2.2. Establishment of cell lines

Immediately after birds were sacrificed, we plucked their feathers, and washed their skin with anti-microbial soap. We excised a 5 × 5 mm^2 piece of skin from the chest and abdomen, and placed it into cold complete bird cell culture media (Dulbecco's modified Eagle medium [DMEM], high-glucose variant [25 mM], with sodium pyruvate [1 mM], supplemented with 10% heat-inactivated fetal bovine serum, 2% heat-inactivated chicken serum, and antibiotics [100 U/mL penicillin/streptomycin], containing 10 mM HEPES). Feather shafts from zoo animals were placed in cold complete bird cell culture media.

We established primary dermal fibroblast cell cultures after the skin or pieces of feather pulp were exposed to 0.5% collagenase B solution overnight (Harper et al., 2007). Cells were grown in culture flasks at 37 °C in an atmosphere of 5% O_2 (Harper et al., 2011). When cells reached 90% confluence, they were trypsinized (0.25%) and cryopreserved at 10^6 cells/mL in DMEM supplemented with 40% fetal bovine serum and dimethylsulfoxide (DMSO) at a final concentration of 10%. We stored cells in liquid N_2 for up to 12 months prior to assessment of their metabolic profile. All O_2 consumption measurements were conducted using cells at passage 2 (P_2). All cell lines were thawed, resuspended and allowed 5 days to recover from freezing before oxygen consumption rate (OCR) experiments.

2.3. Metabolic profiles

A Seahorse XF-96 Extracellular flux analyzer was used to measure the rate of O_2 uptake in isolated primary dermal fibroblast cells from all birds. Assays were performed prior to experiments to determine the optimal cell seeding density, and optimal concentrations of each compound used. We seeded 20,000 cells per well per individual into XF-96 cell culture plates 24 h before experiments. All individuals were run in duplicate. OCR was determined following Nicholls et al. (2010), using XF-96 FluxPaks (37 °C) from Seahorse Bioscience.

2.4. Oxygen consumption rates (OCRs)

We measured OCRs after cells were equilibrated to running media for 1 h, which contained 25 mM glucose and 1 mM sodium pyruvate in DMEM. Because the blood of birds has 2–4 times higher glucose concentration than that of mammals, we elevated the glucose concentration of our media 3× than is commonly used for mammals (Holmes et al., 2001). Baseline measurements of OCRs were made three times prior to injection of 30 μM oligomycin A, which inhibits ATP synthesis by blocking the proton channel of the F_o portion of the ATP synthase. This can be used to distinguish the percentage of O_2 consumption devoted to ATP synthesis and the O_2 consumption required to overcome the natural proton leak across the inner mitochondrial membrane plus any non-mitochondrial O_2 consumption. Proton leak is driven by the magnitude of the proton motive force across the inner mitochondrial membrane (Δp) and is determined by the inner membrane conductivity to protons at a given Δp (Brown and Brand, 1986). Notably, however, oligomycin induces a respiratory condition similar to that of state-4