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Cellular metabolic rates in cultured primary dermal fibroblasts and myoblast cells from fast-growing and control *Coturnix* quail

Ana Gabriela Jimenez [a,*], Clara Cooper-Mullin [a], Nicholas B. Anthony [b], Joseph B. Williams [a]

[a] Department of Evolution, Ecology and Organismal Biology, Ohio State University, 318 W. 12th Ave., Columbus, OH 43210, USA
[b] Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA

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ABSTRACT

Fibroblast cells have been extensively used in research, including in medicine, physiology, physiological-ecology, and conservation biology. However, whether the physiology of fibroblasts reflects the physiology of other cell types in the same animal is unknown. Dermal fibroblasts are responsible for generating connective tissue and involved in wound healing, but generally, this cell type is thought to be metabolically inactive until it is required at the site of tissue damage. Thus, one might question whether fibroblasts are a representative model system to portray the metabolic profile of the whole organism, as compared with cells isolated from other tissues, like muscle, brain or kidneys. To explore whether fibroblasts have the same metabolic profile as do myoblast cells, we cultured cells from day-old chicks of quail (*Coturnix coturnix japonica*) selected for fast-growth or normal growth (our control group). Our results suggest that isolated primary fibroblasts and myoblast cells had higher rates of glycolysis, oxygen consumption and more mitochondria in the fast-growing line than in the control line. Our findings lend support for the idea that fibroblasts are a representative cell system to characterize the whole organism metabolic signature at the cellular-level. These data are striking, however, because fibroblasts had higher rates of metabolism for every parameter measured than myoblast cells isolated from the same individuals.

1. Introduction

Whole-animal metabolism encompasses the summation of each tissue's metabolic rate (Krebs, 1950). Some tissues, those of the brain, liver and kidneys, have high mass-specific metabolic rates, whereas others like bone, intestine, and skeletal muscle have relatively low mass-specific rates of metabolism (Martin and Fuhrman, 1955; Zurlo et al., 1990). However, muscle makes up the largest fraction of total body mass, so its contribution to whole-organism resting metabolic rate is higher than that of other tissues (Zurlo et al., 1990). Mature skeletal muscle is post-mitotic and well-known for its capacity to adjust to changing physiological demands such as training and injury (Hawke and Garry, 2001). The repair or plasticity of muscle tissue is largely dictated by the migration of mononucleated satellite cells, which reside between the sarcolemma and basal lamina of muscle cells, into the existing muscle fiber (Mauro, 1961; Campion, 1984; Cheek, 1985; Hawke and Garry, 2001; Bruusgaard et al., 2003; Zammit et al., 2006). In response to stimuli such as trauma or training, satellite cells become activated, proliferate, and integrate into the existing muscle cell (Hawke and Garry, 2001). Once isolated from the animal and placed in culture, satellite cells differentiate into myoblasts; these can be distinguished in culture because satellite cells express the protein Pax7, whereas myoblasts co-express Pax7 and MyoD (Zammit et al., 2006). Myoblasts retain the fiber-type properties of the muscle fiber from which they have been isolated, and consequently, a population of myoblasts isolated from muscle tissue will be representative of the fiber-types in the whole animal (Feldman and Stockdale, 1991).

In contradistinction to muscle tissue, fibroblasts, the cells responsible, in part, for production of connective tissue, are thought to make a small contribution towards whole animal metabolic rate (Krebs, 1950). Fibroblasts orchestrate the synthesis of extracellular matrix constituents in the connective tissue, and in the presence of tissue injury, these cells promote wound closure and tissue repair (Sorrell and Caplan, 2004). However, fibroblasts are still considered a heterogeneous population of cells (Fries et al., 1994), where phenotypic differences are manifested in a variety of ways including, extracellular matrix production and organization, production of growth factors/cytokines and participation in inflammatory responses (Limeback et al., 1982; Doane and Birk, 1991; Fries et al., 1994; Smith et al., 1997). Nevertheless, connective tissue is uniform and ubiquitous throughout the body, thus, fibroblasts are one of the most common cell types that are found in organs and tissues (Langevin et al., 2004; Sorrell and Caplan, 2004). Several lines of evidence suggest that fibroblasts in the body are not separate cells, but rather linked together in a reticular network extending through an animals' body (Langevin et al., 2004). Similarly, in culture, these cells also form a reticular matrix and are still able to respond to mechanical forces

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

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even in the absence of a wound (Banes et al., 1995; Stoltz et al., 2000; Langevin et al., 2004). Consequently, fibroblasts have been established as a useful model system because of their ease of use in tissue culture (Morell and Froesch, 1973). This cell type has been used in many scientific disciplines, including medicine (cancer research: i.e. Turner and Grose, 2010; Erez et al., 2010; Gonda et al., 2010; Marsh et al., 2012, aging research: i.e. Ungvari et al., 2011; Lima et al., 2011; Csiszar et al., 2012, immunology research: i.e. Wynes et al., 2011; Barnas et al., 2010; Liang et al., 2011, stem cell research: i.e. Doi et al., 2009; Han et al., 2012; Feng et al., 2009), research in cell physiology (i.e. Finkel et al., 2009; Vierbuchen et al., 2010; Semenza, 2012), physiological ecology (i.e. Salmon et al., 2008; Harper et al., 2011; Jimenez et al., 2013), and conservation biology (i.e. Li Chen et al., 2009; Wise et al., 2010; Selvaraj et al., 2011). Even though research using these cells is pervasive, it remains relatively unknown how the metabolic profile of fibroblasts compare with that of other cells isolated from the same individual. Researchers use fibroblasts with the implicit assumption that the metabolic patterns of these cells are the same as other cells in the body, but this assumption has never been tested. Here, for the first time, we looked at metabolic profiles of myoblasts and fibroblasts isolated from the same individual. When making inferences about metabolic rate comparisons between whole animals and cells, one might question whether cells with seemingly low metabolic demand, such as fibroblasts, represent a model system that characterizes the metabolic profile of other more metabolically active tissues like the muscle.

Evidence suggests that growth rate is correlated to whole-animal metabolism and by inference cell metabolism. For example, slow-growing precocial birds have a lower mass-specific metabolic rate compared with fast-growing altricial birds which suggests a low cell metabolism in slow growing birds (Ricklefs, 1974; Klaassen and Drent, 1991). In this study we purposed to compare the metabolic profile of fibroblasts and myoblasts from cells taken from intrinsically fast growing and control quail, which we thought might have different rates of cellular metabolism. We isolated myoblasts from fast-growing quail chicks (*Coturnix coturnix japonica*) and compared them with primary dermal fibroblasts from the same animal, and we isolated myoblasts from a control line of slower growing quail chicks and compared them with primary dermal fibroblasts of the same individual. This comparison allowed us to make comparisons between two different cell types, myoblast and fibroblasts, that would theoretically show differences in glycolysis and oxygen consumption rates, and allowed us to compare cellular metabolic parameters to different growth rates. We have found that the pattern of the metabolic profile between myoblast cells and fibroblasts was the same, showing lower cellular metabolic rates for the control line compared with the fast-growth line. Our results suggest that isolated fibroblasts represent a model system that provides patterns of metabolism similar to other tissues of the organism.

2. Materials and methods

2.1. Animals

The quail (*C. coturnix japonica*) lines were derived from random bred control (RBC) populations maintained at the University of Arkansas, Department of Poultry Science. The control line was established in 1992 from a composite of the Eastern Shore RBC and a random bred control line from The Ohio State University (Anthony et al., 1996). The fast-growing line was established in 2011 as a composite of a heavy line (HW) (Nestor et al., 1982) and a P-line (Marks, 1978). At the time of creation of the fast growing line, the parental lines had experienced 65 (HW) and 110 generations (P-line) of selection for 4 week body weight. Selection environments differed between HW and RBC lines. The newly formed heavy RBC had completed 6 generations of random mating prior to the initiation of this study. In general, there is a greater than two fold difference in body weight at maturity with the control line having an adult body weight of 120 g and the fast line 265 g. All procedures were approved by the Institutional Animal Care and Use Committee of the Ohio State University (protocol IACUC2004A0093). Eggs from a fast-growing and a control line of quail (*C. coturnix japonica*) were shipped from the Department of Poultry Science at the University of Arkansas to our laboratory at the Ohio State University, where they were incubated for 16 days until hatching. There was no difference in time of hatch between the fast growing line and the control line. Additionally, every bird was sacrificed and processed immediately after hatching. At hatch, body mass averaged 9.4 ± 0.4 g (N = 14) for the fast-growing chicks and 8.2 ± 0.2 g (N = 13) for the control group chicks, values significantly different (P = 0.006). We collected one sample of muscle and one sample of skin per individual.

2.2. Establishment of cell lines

Immediately after hatching, chicks were sacrificed, their feathers plucked, and their skin washed with anti-microbial soap. We excised a $5 \times 5\ mm^2$ piece of skin and placed it into cold complete bird cell culture media (Dulbecco's modified Eagle medium [DMEM], high-glucose variant [4.5 mg/mL], with sodium pyruvate [110 mg/L], supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2% heat-inactivated chicken serum (CS), and antibiotics [100 U/mL pen/strep], containing 10 mM HEPES). After the skin was exposed to 0.5% collagenase B solution overnight, we plated fibroblast cells onto culture dishes (Harper et al., 2007). Cells were grown in culture flasks at 37 °C in an atmosphere of 5% CO_2 and 5% O_2 (Harper et al., 2011). When cells reached 90% confluence, they were trypsinized (0.25%), counted, pelleted and cryopreserved at 10^6 cells/mL in DMEM mixed with 40% fetal bovine serum and dimethylsulfoxide (DMSO) at a final concentration of 10%. We stored vials of cells in liquid N_2 for up to 12 months prior to assessment of their metabolic profiles. Cell lines were thawed, re-suspended and allowed to grow 5 days before oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) experiments. We measured O_2 consumption of cells at passage 2 (P2).

Myoblasts were cultured using a technique modified from Velleman et al. (2000). We excised pectoralis and gastrocnemius muscle from quail chicks and then exposed them to 0.8 mg/mL Pronase solution for 40 min to liberate satellite cells from the basal lamina. Following enzymatic digestion, cells were separated from muscle debris by differential centrifugation at 2700 rpm for 6 min, then 1350 rpm for 10 min, three times each and then filtered through 28 μm Nitex cloth. Because fibroblasts adhere to plastic culture dishes more rapidly than do muscle satellite cells, we pre-plated our cell mixture for 2 h to allow fibroblasts to adhere, before we poured the media from the culture dishes onto a second plate. This method was successful in obtaining a pure preparation of muscle satellite cells. Cells were optimized for maximal proliferation by growing on gelatin-coated plates in the presence of plating media [DMEM, 10% CS, 5% horse serum (HS), and antibiotics (100 U/mL pen/strep and 0.1% gentamicin)]. Cells were incubated overnight at 37 °C, in an atmosphere of 5% CO_2, 5% O_2, the remainder N_2.

After 24 h, the plating media was replaced with feeding media [McCoy's 5A, 10% CS, 5% HS] antibiotics (100 U/mL penicillin/streptomycin and 0.1% gentamicin) and 20 ng/mL fibroblast growth factor (FGF), which stimulates skeletal muscle cell proliferation, and prevents the differentiation of these cells (Velleman et al., 2000). Cells were passaged at 60% confluence and cryopreserved at 10^6 cells/mL in DMEM and dimethylsulfoxide (DMSO) at a final concentration of 10%. Once isolated from the animal and placed in culture, we have shown that these satellite cells express Pax7 and MyoD, and are thus differentiating myoblasts (Zammit et al., 2006; data not shown).

2.3. Metabolic profiles

Most cells possess the ability to shift dynamically from glycolysis and oxidative phosphorylation for ATP production, adjusting to changes in their environment. We used a Seahorse XF-96 Extracellular flux