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Rapid changes in cell physiology as a result of acute thermal stress House sparrows, *Passer domesticus*

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ABSTRACT

Given that our climate is rapidly changing, Physiological Ecologists have the critical task of identifying characteristics of species that make them either resilient or susceptible to changes in their natural air temperature regime. Because climate change models suggest that heat events will become more common, and in some places more extreme, it is important to consider how extreme heat events might affect the physiology of a species. The implications of more frequent heat wave events for birds have only recently begun to be addressed, however, the impact of these events on the cellular physiology of a species is difficult to assess. We have developed a novel approach using dermal fibroblasts to explore how short-term thermal stress at the whole animal level might affect cellular rates of metabolism. House sparrows, *Passer domesticus* were separated into a "control group" and a "heat shocked" group, the latter acclimated to 43 °C for 24 h. We determined the plasticity of cellular thermal responses by assigning a "recovery group" that was heat shocked as above, but then returned to room temperature for 24 h. Primary dermal fibroblasts were grown from skin of all treatment groups and the pectoralis muscle was collected. We found that glycolysis (ECAR) and oxygen consumption rates (OCR), measured using a Seahorse XF 96 analyzer, were significantly higher in the fibroblasts from the heat shocked group of House sparrows compared with their control counterparts. Additionally, muscle fiber diameters decreased and, in turn, Na^+–K^+-ATPase maximal activity in the muscle significantly increased in heat shocked sparrows compared with birds in the control group. All of these physiological alterations due to short-term heat exposure were reversible within 24 h of recovery at room temperature. These results show that acute exposure to heat stress significantly alters the cellular physiology of sparrows, but that this species is plastic enough to recover from such a thermal insult within 24 h.

1. Introduction

The warming trends predicted for earth will generate a serious challenge for survival in some populations of animals (Hansen et al., 2006; McKechnie and Wolf, 2010). In the decades to come, if animals are to survive while dealing with increasing ambient temperatures, they will need to alter their distribution patterns, change their behavior, or adjust their physiology, to compensate for increasing thermal demands (Angilletta, 2009; Chown et al., 2010; Williams et al., 2012). If evolutionary or phenotypic adjustments are impossible or slower than the rate required to keep up with environmental change, populations of animals could become locally extinct (Quintero and Wiens, 2013). When making predictions about how a species will respond to increases in temperature in their environment, one important consideration is how animals alter their physiology when exposed to elevated body temperatures (reviewed by Helmuth et al., 2005; Kearney and Porter, 2009).

Because climate change models suggest that heat wave events will become more common, and in some places more extreme, it is important to consider how extreme heat events might affect the physiology of a species (Meehl and Tebaldi, 2004; McKechnie and Wolf, 2010). A critical task is to identify the characteristics of species that make them either resilient or susceptible to episodes of heat. Small birds would seem especially vulnerable to heat wave events, because they are prone to dehydration in extreme heat, because of their diurnal habits, which expose them to the highest air temperature during the day, and because of their limited use of thermally amenable microsites (McKechnie and Wolf, 2010; Williams et al., 2012).

Whole-animal responses to heat stress vary considerably from some small temperate birds that die at ambient temperatures of 42 °C to desert birds that are able to withstand ambient

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temperatures of 50 °C for short periods (Tieleman et al., 2002; Somero 2010; Pörtner et al., 2012; Boyles et al., 2012). However, the precise molecular mechanisms initiated by cells to guard against damage from elevated body temperature remain unclear. Linking cellular mechanisms to the generalized stress response at higher levels of biological organization is crucial in furthering our understanding of how animals will respond to global climate change and episodic heat events. Recently, studies have emphasized mechanistic approaches to predict the effects of climate change on endotherms by examining the interaction of physiological characteristics of the organism and its environment, however, these studies are few (Humphries et al., 2002; Monaghan et al., 2009; Kearney et al., 2010; McKechnie and Wolf 2010; Molnár et al., 2010; Porter et al., 2010). Studies that incorporate whole-animal responses to heat stress with the effects of the animals′ cellular physiology are still rare.

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 the 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 stress resistance (Harper et al., 2007, Salmon et al., 2008), on growth control properties (Seluanov et al., 2008), on relationships between longevity of fibroblasts in culture and life an of mammal donors (Röhme, 1981). 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. Isolated primary dermal fibroblasts seem to have high rates of oxygen consumption (Lemons et al., 2010). To determine the physiological consequences of episodic heat events in birds at the cellular-level, we made use of primary dermal fibroblast tissue culture methods widely used in the medical field, but largely ignored in eco-physiological studies.

During episodic heat events, the cells of birds, including their muscle cells, experience high body temperature (T_b) because heat flows from the environment to the animal, and because bouts of activity, such as flying, produces additional endogenous heat that can elevate T_b (Hudson and Bernstein, 1981). How an elevation in T_b influences muscle structure and function in endotherms remains poorly understood. When exposed to heat, muscle cells often respond with an up-regulation of heat shock proteins (HSPs) that protect proteins within the cell from denaturing (Liu and Steinbacker, 2001). Muscle atrophy has been commonly linked to the expression of HSPs, thus, an up-regulation of heat shocked proteins during thermal stress may decrease muscle fiber size (Sakuma et al., 1998; Baracos et al., 1995; Medina et al., 1995; Liu et al., 2006). There seems to be a strong link between fiber size and cost of ion pumping, where muscle fiber SA:V and rate of cell metabolism are proportional to rates of Na^+–K^+-ATPase activity, making larger muscle fibers less metabolically costly to maintain (Jimenez et al., 2011; 2013; Kielhorn et al., 2013; Johnston et al., 2003). Because Na^+–K^+ ATPase activity in muscle sarcolemma makes up 19–40% of resting muscle metabolic rate (Gregg and Milligan, 1982; Rolfe and Brown, 1997; Milligan and McBride, 1985), and muscle makes up to 30% of the total body mass of animals (Zurlo et al., 1990), any alteration in SA:V of muscle fibers as a result of thermal stress would have implications for the cost of maintaining the muscle mass and in turn, basal metabolic rate of the animal.

In this study, we pursued the linkages between acute whole organism heat stress, and any compensatory changes in cellular metabolic parameters in primary dermal fibroblasts and muscle cells from House sparrows, *Passer domesticus*. Our temperature treatments were meant to promote acute physiological changes that would occur during a heat wave event for birds. We found that fibroblast cells from sparrows that were heat shocked for 24 h had significantly higher rates of glycolysis, oxygen consumption, and an increased density of mitochondria. We also found that pectoralis muscle fibers from heat shocked sparrows had a smaller diameter and, in turn, a higher Na^+–K^+-ATPase activity. We initially hypothesized that the decreases we observed in muscle fiber diameter of sparrows that were heat shocked would be related to an up-regulation of HSP70 in muscle tissue. However, we found no differences in HSP70 expression levels in muscle between heat shocked and control treatment groups. After 24 h of recovery from the heat shock event by adults, all of these increases in cellular metabolic rate had returned to control values. These acute cellular changes due to thermal stress at the whole-animal level, though plastic, seem to be metabolically costly to House sparrows and may be significant during the recovery period for adult energy balance.

2. Materials and methods

2.1. Collection of birds

We collected House Sparrows by mist net under an Ohio Division of Wildlife permit (number 15–29) during January and February 2013 in Columbus, OH. The Institutional Animal Care and Use Committee of Ohio State University (protocol IACUC2004A0093) approved all procedures. We realize that winter birds from Ohio would not be exposed to similar thermal conditions as those used in these experiments. The reason for using sparrows during the winter was that we wanted to induce a maximal cellular response due to a large thermal variation. Additionally, the rapidity of the response to heat exposure and the fact that even winter birds were able to completely reverse the cellular effects from the heat stress within 24 h is remarkable.

2.2. Temperature treatment and heat stress

We separated sparrows into four groups, a "control" group of 10 birds that were sacrificed after collection, a "heat shocked" group of 11 birds that were acclimated to 43 °C for 24 h in a PERCIVAL environmental chamber and then sacrificed by cervical dislocation, a "recovery group" of 8 birds that was heat shocked as above, but then returned to room temperature for 24 h, and a control recovery group of 6 birds that remained at room temperature. All treatments were repeated with two sets of House sparrows to confirm our results. Animals were fed and watered *ad libidum* and were kept in captivity for a maximum of 48 h. After we sacrificed the bird, we measured body temperature (T_b) using a themorcouple thermometer and a 40 gauge copper–constantan thermocouple placed 2 cm into the rectum. Skin and pectoralis muscle were collected from each individual and processed as described below.

2.3. Establishment of fibroblast cell lines

Immediately after birds were sacrificed, we plucked their feathers and the exposed skin was washed with anti-microbial soap. We excised a 5×5 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, 2% heat-inactivated chicken serum, and antibiotics [100 U/mL pen/strep], containing 10 mM HEPES).

We established primary dermal fibroblast cell cultures after the skin was exposed to 0.5% Collagenase B solution overnight (Harper