Comparative Biochemistry and Physiology, Part A 209 (2017) 10–15

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Stable isotope analysis of CO_2 in breath indicates metabolic fuel shifts in torpid arctic ground squirrels

Trixie N. Lee[a,*], Melanie M. Richter[b,1], Cory T. Williams[a,2], Øivind Tøien[a], Brian M. Barnes[a], Diane M. O'Brien[a], C. Loren Buck[b,2]

[a] Institute of Arctic Biology, 311 Irving I, University of Alaska Fairbanks, Fairbanks, AK 99775, USA
[b] Department of Biological Sciences, University of Alaska Anchorage, 3101 Science Circle, Anchorage, AK 99508, USA

ARTICLE INFO

Keywords:
Arctic ground squirrel
Breath carbon isotopes
Hibernation
Metabolic fuel
Stable isotopes

ABSTRACT

Stable carbon isotope ratios ($\delta^{13}C$) in breath show promise as an indicator of immediate metabolic fuel utilization in animals because tissue lipids have a lower $\delta^{13}C$ value than carbohydrates and proteins. Metabolic fuel consumption is often estimated using the respiratory exchange ratio (RER), which has lipid and carbohydrate boundaries, but does not differentiate between protein and mixed fuel catabolism at intermediate values. Because lipids have relatively low $\delta^{13}C$ values, measurements of stable carbon isotopes in breath may help distinguish between catabolism of protein and mixed fuel that includes lipid. We measured breath $\delta^{13}C$ and RER concurrently in arctic ground squirrels (*Urocitellus parryii*) during steady-state torpor at ambient temperatures from −2 to −26 °C. As predicted, we found a correlation between RER and breath $\delta^{13}C$ values; however, the range of RER in this study did not reach intermediate levels to allow further resolution of metabolic substrate use with the addition of breath $\delta^{13}C$ measurements. These data suggest that breath $\delta^{13}C$ values are 1.1‰ lower than lipid tissue during pure lipid metabolism. From RER, we determined that arctic ground squirrels rely on nonlipid fuel sources for a significant portion of energy during torpor (up to 37%). The shift toward nonlipid fuel sources may be influenced by adiposity of the animals in addition to thermal challenge.

1. Introduction

Animals use three primary metabolic fuels to meet energy demands: protein, carbohydrate, and lipid. Protein is typically reserved for structural and functional roles and does not contribute significantly to energy metabolism during periods of energy balance (Robbins, 2001). Carbohydrates are often directly metabolized for immediate energy and are not stored in large quantities in mammals (Vock et al., 1996). Lipid is the most energy-dense fuel, providing 8 to 10-times more energy on a wet mass basis than carbohydrate or protein (McWilliams et al., 2004). Thus, lipid, which is stored as white adipose tissue, is used over relatively long time frames after carbohydrate stores are depleted. Several factors influence which fuels are selected for metabolism, including exercise intensity, training status, and diet (reviewed in Holloszy et al., 1998). Animals enduring fasting or starvation exhibit a well-defined pattern of metabolic fuel selection, with timing of shifts between fuels based on the amount of lipid available and catabolism of protein as a last resort (Robbins, 2001).

Mammalian hibernation is a strategy used by a variety of species to conserve energy in anticipation of and during periods of insufficient food resources. During hibernation, animals often do not eat for months and rely entirely on endogenous stores of metabolic fuel. Some animals can conserve as much as 90% of the energy they would otherwise use by spending most of the hibernation season in torpor, the low-metabolism, energy-saving phase of hibernation (Karpovich et al., 2009; Wang and Wolowyk, 1988). Most hibernators support their metabolic demand from large lipid stores which are accumulated during the pre-hibernation fattening period (Dark, 2005), but this appears insufficient for animals hibernating in extreme environmental conditions, such as the Arctic (Buck and Barnes, 2000).

Arctic ground squirrels (*Urocitellus parryii*) are the most northern small hibernator in North America and experience hibernacula temperatures averaging −8.9 °C and as low as −23 °C over the 6–8 month hibernation season (Buck and Barnes, 1999b). Arctic ground squirrels defend a minimum body temperature as low as −2.9 °C for weeks at a time during torpor (Barnes, 1989), which requires them to be con-

Abbreviations: $\delta^{13}C$, carbon isotope ratio; RQ, respiratory quotient; RER, respiratory exchange ratio; T_a, ambient temperature

* Corresponding author at: Biology Department, Box 12251, Harding University, Searcy, AR 72149, USA.
E-mail address: tnlee@harding.edu (T.N. Lee).
[1] Present Address: Biology Department, 1906 College Heights Blvd., #11080, Western Kentucky University, Bowling Green, KY 42101, USA.
[2] Present Address: Department of Biological Sciences and Center for Bioengineering Innovation; 617 S. Beaver St., Northern Arizona University, Flagstaff, AZ 86011-5640, USA.

http://dx.doi.org/10.1016/j.cbpa.2017.04.004
Received 16 January 2017; Received in revised form 4 April 2017; Accepted 5 April 2017
Available online 07 April 2017
1095-6433/

tinuously thermogenic due to the still lower burrow temperatures (Buck et al., 2008). During the short active season, arctic ground squirrels increase lipid (Buck and Barnes, 1999a) and lean mass (Boonstra et al., 2011; Sheriff et al., 2013), and significant proportions of both tissues are used over winter, as indicated by body composition estimates before and after hibernation (Buck and Barnes, 1999b). Use of lean mass during hibernation is also indicated by a shift toward increased reliance on mixed fuel metabolism during torpor at decreasing ambient temperatures (T_a), as indicated by respirometry (Buck and Barnes, 2000). Carbohydrate (glucose) is needed to facilitate lipid oxidation associated with brown adipose tissue thermogenesis (Cannon and Nedergaard, 1979; Vallerand et al., 1990), to support anaplerosis (Owen et al., 2002), and to fuel certain organs such as the kidneys (Berg et al., 2002). The primary precursor to glucose in most hibernators is glycerol, a byproduct of triglyceride catabolism (Galster and Morrison, 1975; Staples and Hochachka, 1998). However, it has been suggested that the increasing demand for carbohydrates by thermogenesis surpasses the supply of glucose formed from glycerol and requires the additional breakdown of protein for gluconeogenesis (Buck and Barnes, 2000; Galster and Morrison, 1975; Krilowicz, 1985). This hypothesis is supported by dramatic upregulation during hibernation of the gene *PCK1*, which codes for a crucial enzyme in gluconeogenesis from pyruvate, lactate, or amino acid precursors (Williams et al., 2011). Whether amino acids are catabolized directly for fuel use during torpor has not yet been determined.

Respirometry is the traditional method used to differentiate metabolic fuel use, but it cannot differentiate between protein and mixed fuel catabolism. The respiratory quotient (RQ), the ratio of carbon dioxide (CO_2) produced to oxygen (O_2) consumed (Kleiber, 1961), is approximately 0.7 during lipid oxidation while carbohydrate oxidation results in an RQ of 1.0. Oxidation of proteins yields an intermediate RQ of 0.83 (Kleiber, 1961), similar to that expected from mixed lipid and carbohydrate metabolism. Respiratory exchange ratios (RER), collected from whole-animal respirometry, are used in this study to approximate RQ values, and we will discuss RQ and RER as a singular concept for the purposes of this study. Alternative measures of fuel use in addition to RQ may help to better distinguish the proportions of metabolic contribution among carbohydrate, protein, and lipid.

Stable carbon isotope measurements are becoming more common in studies of substrate use, as respired CO_2 is a direct product of the animal's metabolism (Hatch et al., 2002; Voigt et al., 2008a; reviewed in: McCue and Welch, 2016; Welch et al., 2016). Lipid has a lower carbon isotope ratio ($\delta^{13}C$) than other metabolites (DeNiro and Epstein, 1977; reviewed in McCue and Welch, 2016). Animals that are fasting shift toward lower $\delta^{13}C$ values in respired CO_2 (Perkins and Speakman, 2001; Schoeller et al., 1984; Voigt et al., 2008a, 2008b), which is consistent with an increase in the proportion of lipid utilization during food deprivation (McCue and Pollock, 2013; Robbins, 2001). Investigations of metabolism in plants have shown a strong correlation between RQ and $\delta^{13}C$ values in respired CO_2 (Pataki, 2005; Tcherkez et al., 2003), but few studies in animals have combined measurements of RER and breath $\delta^{13}C$ values from naturally distinct, endogenous substrates (Gautier et al., 1996; Schoeller et al., 1984).

Metabolic processes have the potential to preferentially use one form of isotope over others. This discrimination can lead to differences in the $\delta^{13}C$ values between fuel source and exhaled CO_2. Several experimental studies have found differences between $\delta^{13}C$ values in breath and diet (discrimination factors; surveyed in Table 2 in Voigt et al., 2008a), but the number of metabolic steps that exogenous and endogenous substrates go through before utilization are different and thus differ in their potential for discrimination. In addition, discrimination in the breakdown of endogenous protein and lipid stores likely varies due to the differences in metabolic pathways, but most metabolic processes have not been thoroughly investigated for evidence of discrimination.

Our first objective was to determine whether RER and $\delta^{13}C$ values covary in an animal system utilizing endogenous substrates with naturally distinct $\delta^{13}C$ signatures. To address this objective, we concurrently measured RER and breath $\delta^{13}C$ values in fasting and hibernating arctic ground squirrels, using changes in T_a to induce shifts in fuel use. Previous work on torpid arctic ground squirrels showed a robust, linear increase in RER as T_a decreased below 0 °C (Buck and Barnes, 2000) and a clear difference in $\delta^{13}C$ values between tissue lipids and lean mass (Lee et al., 2012). Our second objective was to determine whether using two 2-endpoint measurements, RER and breath $\delta^{13}C$ values, could help resolve fuel use in torpid arctic ground squirrels. Specifically, if RER values are intermediate and $\delta^{13}C$ values are intermediate, we would conclude that squirrels are using a mix of lipid and other fuels. However, if RER values are intermediate and $\delta^{13}C$ values are high, we would conclude that the animals are using a fuel based on proteins and/or carbohydrates but utilizing little, if any, lipid. Our final objective was to determine whether there was evidence of discrimination between endogenous fuels and breath $\delta^{13}C$ values.

2. Materials and methods

2.1. Animals

Arctic ground squirrels (*Urocitellus parryii*) were captured near Toolik Field Station (68° 38′ N, 149° 36′ W) in the Alaskan Arctic in fall 2008 and summer 2009 and maintained on Mazuri Rodent Chow in captivity at the University of Alaska Anchorage. Each animal had a temperature-sensitive radiotransmitter surgically implanted in its abdomen (~7 g; Data Sciences International, St. Paul, MN, USA, see Richter et al., 2015 for methods). Ground squirrels were initially held at room temperature on an 18L:6D light cycle in 48 × 32 × 32 cm hanging metal cages and were provided ample cotton material from which they constructed nests. They were then moved into environmental chambers at + 2 °C on a 9L:15D light cycle. When an animal began hibernating (body temperature ≤ 30 °C; Buck et al., 2008), it was transferred to a plastic metabolic chamber with a wire lid and placed on a receiver linked to an automated data collection system (Data Sciences International, St Paul, MN, USA) that recorded core abdominal temperature every 10 min.

2.2. Treatment groups

Once a sufficient number of animals began hibernating, they were divided into two treatment groups that followed different schedules of decreasing T_a. The temperature within Chamber 1 (n = 8 animals, 5 males and 3 females) was decreased from + 2 to − 20 °C (this chamber's minimum temperature) in 2 °C increments. The temperature within Chamber 2 (*n* = 9 animals, 6 males and 3 females) was decreased from + 2 °C to 0 °C, then to − 10 °C, and then to − 20 °C, at which point the temperature was lowered in 2 °C increments until − 26 °C, the minimum T_a at which arctic ground squirrels can sustain steady-state torpor (see Richter et al., 2015). This regime allowed us to measure squirrels that still had adequate lipid reserves at T_a < − 20 °C. At each T_a, RER for each animal in the chamber was recorded for 6 h during steady-state torpor (four animals simultaneously; defined in Respirometry section), and excurrent air containing breath from the respirometry chamber was sampled simultaneously. Once all animals in the environmental chamber had been sampled (typically over a period of several days), all were handled and weighed. This handling induced the squirrels to arouse and begin increasing body temperature, ultimately leading to euthermic body temperature. Once squirrels had been handled and movement and/or rapid breathing was observed, they were returned to the environmental chamber set at the next T_a. Here they completed warming and the period of euthermy of an arousal bout (during which they adjusted to the new T_a), then returned to torpor. All animal use procedures were approved by the University of Alaska Anchorage Institutional Animal Care and Use Committee (pro-