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Fatty acid analyses may provide insight into the progression of starvation among squamate reptiles

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ABSTRACT

Fasting-induced changes in fatty acid composition have been reported to occur within the body lipids of several types of animals; however, little is known about the changes in fatty acid profiles exhibited by reptiles subjected to prolonged fasting. This study characterizes the fatty acid profiles of six reptile species subjected to sublethal periods of fasting lasting 0, 56, 112, and 168 days. Analyses of fatty acid methyl esters (FAMEs) conducted on the total body lipids of rattlesnakes (*Crotalus atrox*), ratsnakes (*Elaphe obsoleta*), pythons (*Python regius*), boas (*Boa constrictor*), true vipers (*Bitis gabonica*), and monitor lizards (*Varanus exanthematicus*) revealed that all of the species exhibited similar characteristic changes in their fatty acid profiles during starvation stress. According to ANOVAs, the four most effective indicators of the onset of starvation were significant increases in the [1] fatty acid unsaturation index as well as ratios of [2] linoleic to palmitoleic acid, [3] oleic to palmitic, and [4] arachidonic to total fatty acid concentrations. The results of this study suggest that FAME analyses might be useful for identifying nutritional stress and/or starvation among squamate reptiles; however, forthcoming studies will be required to validate the generality of these responses. I also review the potential limitations of this approach, and suggest experiments that will be important for future applications of FAME analyses. Ultimately, it is hoped that FAME analyses can be used in conjunction with current practices as an additional tool to characterize the prevalence of starvation experienced by free-living reptiles.

1. Introduction

Lipids, particularly their constituent fatty acids, are a primary source of cellular energy during periods of food limitation among animals (Young and Scrimshaw, 1971; Owen et al., 1979; Castellini and Rea, 1992; Navarro and Gutierrez, 1995; Drackley, 2000; Wang et al., 2006). Studies examining a wide range of animals subjected to starvation report significant changes in the amounts of body lipid and levels of circulating fatty acids (Zammit and Newsholme, 1979; Groscolas, 1986; Da Silva and Migliorini, 1990; Nordoy et al., 1993; Hervant et al., 2001; Freitas et al., 2003). Unfortunately, measurements of circulating lipid metabolites (*e.g.* triacylglycerides, free fatty acids, glycerol, *etc.*) provide little information about the rates at which specific circulating substrates are turned over, and are therefore unable to differentiate between a situation where a given metabolite is being mobilized into circulation at an increased rate, or one where a given metabolite is being catabolized at a reduced rate (Arab, 2003; McCue, 2007a). Moreover, inferring the onset of starvation from body lipid levels is difficult because information about the lipid content of most reptile species remains unavailable (but see Zain and Zain-ul-Abedin, 1967; Afroz et al., 1971; Blem, 1997; Brian et al., 1972; Shine and Madsen, 1997), and even less is known about the degree to which different reptiles mobilize lipid stores during starvation (Herman and Oliw, 1998, but see McCue, 2007b).

Several studies of starving invertebrates and/or vertebrates report changes in the fatty acid profiles of stored lipids (Bryden and Stokes, 1969; Estevez et al., 1998; Stuart et al., 1998; Smith et al., 2002; Perez-Velasquez et al., 2003; Zak et al., 2005; Schlechtriem et al., 2006; Wen et al., 2006, 2007). While these reported changes are too varied to permit reliable generalizations that apply to all animals, the best evidence that fasting-induced alterations in fatty acid profiles might be used as bioindicators of starvation come from studies involving aquacultured fishes. Several studies have determined that fishes demonstrate characteristic, repeatable modifications in their fatty acid composition during fasting (Iverson, 1972; Jezierska et al., 1982; Tidwell et al., 1992; Zamal and Ollevier, 1995; Abi-Ayad et al., 2000; Aidos et al., 2002; Ji et al., 2003).

The direction and magnitude of shifts in fatty acid concentrations during fasting are typically reported using an index that involves comparisons of the relative abundance of one type of fatty acid to another fatty acid. Sometimes, directional changes in these indices are used to characterize the progression of fasting among animals, but some researchers have proposed that 'cut-off points' or 'critical limits' be developed to increase the ability for such metrics to

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indicate the onset of nutritional stress (Holman, 1960; Morhauer and Holman, 1963).

Because different animals apparently exhibit unique changes in specific fatty acid concentrations during fasting, the literature is replete with various indices of fatty acid concentrations (Table 1). Some of these indices are based on comparisons of fatty acids that only occur in trace amounts, and whose values could be highly sensitive to sampling and/or analytical errors. Other indices tend to be species-specific, involving fatty acids that are rarely found among vertebrates. It remains unknown whether existing fatty acid indices are useful in identifying starvation stress among reptiles.

The present study is part of a larger comparative project designed to characterize the physiological, compositional, and morphological responses to fasting and starvation exhibited by reptiles. The primary goal of this study was to determine if squamate reptiles subjected to starvation demonstrated characteristic changes in their fatty acid profiles. The secondary goal was to develop a series of indices that might be used to identify the physiological progression of fasting, particularly the onset of starvation. It is hoped that these indices can ultimately be combined with current practices to aid in the assessment of the nutritional status of wild reptile populations.

2. Materials and methods

2.1. Terminology

The term *starvation* is difficult to define in a manner that can be usefully applied to all animals; however, in this paper I use the term *starvation* to refer to a biologically realistic period of inanition (*sensu* McCue, 2007b) that could pose a significant risk to the overall fitness of an animal. In the case of the squamate reptile species employed in this study, this period was defined as six months of complete fasting under thermal conditions that approximate their active/feeding seasons.

2.2. Experimental animal

In 2005 and 2006, I obtained 100 subadult reptiles ($n=16$ western diamondback rattlesnakes, *Crotalus atrox*; $n=17$ gray ratsnakes, *Elaphe obsoleta*; $n=16$ ball pythons, *Python regius*; $n=16$ boa constrictors, *Boa constrictor*; $n=19$ gaboon vipers, *Bitis gabonica*; $n=16$ savannah monitors, *Varanus exanthematicus*) from commercial breeders and/or suppliers (Diamond Reptile, FL; LLL Reptile, CA;

Table 1

Fatty acid indices used to track the progression of nutritional stress (*sensu* Karasov and Martinez del Rio, 2007) among different animals

Index	Fatty acid name	Organism	Reference
n3 : n6		Clam, perch	Caers et al. (1999), Abi-Ayad et al. (2000)
		Abalone, seabream	Durazo-Beltran et al. (2004), Pinto et al. (2007)
		Artemia, lobster	Smith et al. (2002, 2003), Ritar et al. (2003)
		Seabream, snail	Tandler et al. (1989), Stuart et al. (1998)
n3 : n9		Seabream	Tandler et al. (1989)
n6 : n3		Tuatara, lizard	Cartland-Shaw et al. (1998), Simandle et al. (2001)
n6 : n9		Seabream	Tandler et al. (1989)
n9 : n3		Rat	Pan and Storlien (1993)
SFA : UFA		Trout, grouse	Jezierska et al. (1982), Hissa et al. (1990)
		Lizard	Geiser and Learmonth (1994), Simandle et al. (2001)
MUFA : PUFA		Flatfish, snail	Hayashi and Yamada (1975), Stuart et al. (1998)
Sum of 20 to 22 FA		Rat	Pan and Storlien (1993)
Average chain length		Rat, snail	Pan and Storlien (1993), Stuart et al. (1998)
Unsaturation index		Trout, lizard	Jezierska et al. (1982), Geiser et al. (1992)
		Rat, snail	Pan and Storlien (1993), Stuart et al. (1998)
		Lizard	Simandle et al. (2001)
Triene : Pentaene		Rat, lizard	Morhauer and Holman (1963)
PL : P	16:1 n7 / 16:0	Human	Zak et al. (2005)
O : S	18:1 n9 / 18:0	Human	Zak et al. (2005)
O : P	18:1 n9 / 16:0	Squamate reptiles	this study
LA : PL	18:2 n6 / 16:1 n7	Squamate reptiles	this study
GLA : LA	18:3 n6 / 18:2 n6	Human	Zak et al. (2005)
DHLA : LA	20:3 n6 / 18:2 n6	Human	Zak et al. (2005)
DHLA : GLA	20:3 n6 / 18:3 n6	Human	Zak et al. (2005)
MA : AA	20:3 n9 / 20:4 n6	Rat	Holman (1960), Morhauer and Holman (1963)
MA : EPA	20:3 n9 / 20:5 n3	Mammals, carp	Alfin-Slater and Aftergood (1968), Csengeri (1996)
MA : DHA	20:3 n9 / 22:6 n3	Trout	Castell et al. (1972)
AA	20:4 n6	Squamate reptiles	this study
AA : LA	20:4 n6 / 18:2 n6	Rat	Pan and Storlien (1993)
AA : DHLA	20:4 n6 / 20:3 n6	Human	Zak et al. (2005)
EPA : AA	20:5 n3 / 20:4 n6	Artemia, lobster	Estevez et al. (1998), Smith et al. (2002, 2003)
EPA : DHA	20:5 n3 / 22:6 n3	Seabream	Pinto et al. (2007)
DHA : EPA	22:6 n3 / 20:5 n3	Artemia, perch	Estevez et al. (1998), Abi-Ayad et al. (2000)
		Artemia, lobster	Smith et al. (2002, 2003), Ritar et al. (2003)

AA, Arachidonic acid.
DHA, Docosahexaenoic acid.
DHLA, dihomo-gamma Linolenic acid.
EPA, Eicosapentaenoic acid.
GLA, gamma-Linolenic acid.
LA, Linoleic acid.
MA, Mead acid.
MUFA, Monounsaturated fatty acids.
O, Oleic acid.
P, Palmitic acid.
PL, Palmitoleic acid.
PUFA, Polyunsaturated fatty acids.
S, Stearic acid.
V, Vaccenic acid.