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The demands of lactation promote differential regulation of lipid stores in fasting elephant seals

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ABSTRACT

Fasting animals must ration stored reserves appropriately for metabolic demands. Animals that experience fasting concomitant with other metabolically demanding activities are presented with conflicting demands of energy conservation and expenditure. Our objective was to understand how fasting northern elephant seals regulate the mobilization of lipid reserves and subsequently milk lipid content during lactation. We sampled 36 females early and 39 at the end of lactation. To determine the separate influences of lactation from fasting, we also sampled fasting but non-lactating females early and late (8 and 6 seals, respectively) in their molting fasting period. Mass and adiposity were measured, as well as circulating non-esterified fatty acid (NEFA), triacylglycerol (TAG), cortisol, insulin and growth hormone levels. Milk was collected from lactating females. Milk lipid content increased from 31% in early to 51% in late lactation. In lactating females plasma NEFA was positively related to cortisol and negatively related to insulin, but in molting seals, only variation in cortisol was related to NEFA. Milk lipid content varied with mass, adiposity, NEFA, TAG, cortisol and insulin. Surprisingly, growth hormone concentration was not related to lipid metabolites or milk lipid. Suppression of insulin release appears to be the differential regulator of lipolysis in lactating versus molting seals, facilitating mobilization of stored lipids and maintenance of high NEFA concentrations for milk synthesis. Milk lipid was strongly impacted by the supply of substrate to the mammary gland, indicating regulation at the level of mobilization of lipid reserves.

1. Introduction

Periods of nutrient restriction are common for most free ranging animals (McCue, 2010). For some animals this reduction in food availability occurs seasonally (e.g. winter or migration) (Battley et al., 2001; Florant and Healy, 2012). Animals that routinely fast as part of their life-history often display physiological adaptations that involve a reduction in energy expenditure such as hibernation, torpor or adaptations in nutrient allocation (Castellini and Rea, 1992; Florant and Healy, 2012; Tøien et al., 2011). Some animals, however, combine natural periods of nutrient restriction with activities that impose large nutrient demands.

Northern elephant seals (*Mirounga angustirostris*) experience fasting concomitant with energetically expensive activities in several life history stages. Fuel stores are accumulated over two long foraging trips thousands of kilometers out to sea in each age class (Le Boeuf et al., 2000; Robinson et al., 2012). Adult males forage for ~4 months for both the post breeding and post molting trips to sea (Le Boeuf et al., 2000). The post breeding foraging trip for adult females lasts 2.5–3 months, while the post molting trip lasts approximately 7 months (Robinson et al., 2012). When elephant seals are ashore, they fast for several consecutive weeks or months. Adult males and females employ a capital breeding strategy, subsisting on stored reserves during breeding (Costa et al., 1986; Deutsch et al., 1990). Pregnant female elephant seals begin to arrive on shore in late December/early January (Le Boeuf and Laws, 1994). A single pup is born several days after arrival and the mother fasts while nursing the pup for approximately 26 days. Lactation ends when the mother returns to the ocean to forage, leaving the newly weaned pup onshore. Breeding occurs in synchrony, with the majority of the pups born before the end of January and most pups weaned by early March (Le Boeuf and Laws, 1994). Elephant seals also fast during the catastrophic molt,

Abbreviations: NEFA, non esterified fatty acids; TAG, triacylglycerol; GH, growth hormone; LPL, lipoprotein lipase.

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a process which involves a 3–4 weeks fast onshore while all skin and hair is sloughed and regrown from onboard reserves, with adult females beginning their molt in late April (Worthy et al., 1992). Additionally, pups fast during postweaning development, after they are weaned by their mothers, but before going to sea for their first foraging trip (Ortiz et al., 1978).

Due to conflicting metabolic demands, the combination of fasting and lactation is especially rare, occurring in only three groups, phocid seals, mysticete whales and bears. Of all reproductive costs, lactation demands the most energy; with many lactating mammals increasing their energy intake by 60–200% (Gittleman and Thompson, 1988). Animals such as the elephant seal that lactate while fasting must synthesize milk exclusively from nutrients mobilized from body reserves. This is extremely unusual, as in contrast, most lactating mammals obtain the majority of precursors for milk synthesis from dietary input (Neville and Picciano, 1997). The pairing of fasting and lactation necessitates metabolic alterations of milk production as compared to mammals that feed during lactation.

Both fasting and lactation prioritize use of stored lipids for metabolism. In contrast to other large mammals that lactate for months or years, phocid seals lactate for 4 weeks or less, produce very energy rich milk and transfer a large amount of energy to the offspring in the short nursing period (Costa et al., 1986). The rapid, high intensity lactation of phocid seals is enabled by having one of the highest milk fat contents among taxa (Costa, 1991). On average elephant seal milk approaches 55% fat content by the end of lactation (Crocker et al., 2001). Phocid milk is very high fat, very low carbohydrate and has a relatively high protein content (Oftedal, 2000). Females use lipid oxidation to fuel about 90% of their energy needs during fasting and lactation (Crocker et al., 2001), while balancing the preservation of vital protein stores with the necessity to provide amino acid precursors to the mammary gland (Crocker et al., 1998, and see Champagne et al., 2012b; Crocker et al., 2014a for a more comprehensive review of the metabolic alterations during fasting). Thus, the combination of the demands of fasting with the composition of milk require that females must deliver large amounts of lipid and protein substrates to the mammary gland for milk synthesis, while supporting their own maintenance metabolism with fatty acid oxidation, and minimizing oxidation of carbohydrates and proteins.

To support the fat based metabolism exhibited by fasting mammals, lipid must be mobilized from storage molecules (triacylglycerol, TAG) in adipose/blubber and released into the bloodstream as non-esterified fatty acids (NEFAs) for uptake and use by other tissues; notably, the mammary gland during lactation. Circulating NEFA may also be taken up by the liver, incorporated into TAG/lipoprotein complexes and secreted back into the bloodstream. Circulating NEFA can diffuse passively across membrane borders or be facilitated by transport proteins (Glatz et al., 2010), while TAG/lipoprotein complexes must be hydrolyzed before entering tissue. Lipoprotein lipase (LPL) is the primary enzyme enabling tissues to hydrolyze circulating TAG/lipoproteins (Frayn et al., 1995). In some species, mammary LPL facilitates milk lipid production by hydrolyzing TAG/lipoprotein complexes so that the lipids are available for uptake by the mammary gland. McDonald and Crocker (2006), however, found low and stable mammary LPL activity in northern elephant seals across lactation, and found no relationship to milk lipid, which suggests that circulating NEFA may contribute more to milk lipid than TAG. Maternal lipid reserves directly influence the milk energy delivered to pups (Crocker et al., 2001) and the ability of the female to spare body protein during lactation (Crocker et al., 1998). Catabolizing maternal stores during fasting to levels that are difficult to recoup when foraging could affect future survival and reproduction (Arnbom et al., 1997; McMahon et al., 2000).

Elephant seals undergoing fasts in different life history stages experience different challenges regarding the partitioning of their lipid and protein reserves. Due to the large role hormones play in managing fuel catabolism, there is the possibility of differential hormonal regulation of lipid metabolism between life history stages. Growth hormone (GH), cortisol and insulin can affect the mobilization and use of fuel stores. While both GH and cortisol can increase lipolysis (Djurhuus et al., 2004), they have opposing effects on protein metabolism; GH promotes lean tissue accretion (Norrelund et al., 2001), while cortisol promotes protein catabolism (Brillon et al., 1995).

Given the varied fasting metabolic demands of different life history stages in elephant seals (e.g. lactation/development/molting), it is not surprising there are different patterns of GH levels in different age classes (Crocker et al., 2012a; Kelso et al., 2012; McDonald, 2003; Ortiz et al., 2003). These differences in baseline GH with changing metabolic demands suggest either potentially differential action of GH between life history stages, or the importance of other hormones to lipolysis. The impact of GH on lipolysis and milk production in fasting, lactating elephant seals has not been clearly elucidated. GH has been shown to affect milk lipid content in domestic cattle (Bitman et al., 1984; Eppard et al., 1985), which makes it a primary candidate for enacting large effects in fasting and lactating seals. Similarly, elephant seals exhibit differences in fasting baseline cortisol levels in different life history stages (Champagne et al., 2005, 2006; Crocker et al., 2012b; Engelhard et al., 2002; Kelso et al., 2012), again raising the question of differential action in different age classes. The combined effect of lipolytic hormones on milk lipid content is also unclear.

Insulin, an anti-lipolytic hormone (Frayn et al., 1994), also likely affects various age classes of fasting seals differently. The importance of reducing circulating insulin levels in lactating elephant seals was highlighted by the abolishment of the insulin response to a glucose challenge by late lactation (Fowler et al., 2008), suggesting that facilitating lipid mobilization with low insulin levels supersedes the need to regulate an increase in circulating glucose late in lactation. Similarly, low insulin levels may facilitate high levels of circulating NEFA by reducing re-esterification of fatty acids in adipose tissue (Crocker et al., 2014a). Low levels of insulin may be important to maintaining high rates of lipid mobilization for the demands of milk synthesis, but this has not been directly assessed.

Given the diverse effects of these regulatory hormones and their wide variation among northern elephant seal life-history stages, our understanding of metabolic regulation during lactation remains superficial. The mechanisms underlying nutrient mobilization from reserves and delivery to the mammary gland are crucial in linking foraging success at sea to parental investment on land in capital breeding phocids. Variation in nutrient mobilization potentially affects both the magnitude and composition of milk production and the resulting level of parental investment in offspring. Similarly, the factors influencing the mobilization and use of body reserves may impact the physiological state of the female, influencing the fitness costs of reproduction and future survival.

Our objective was to investigate how stored reserves are partitioned during simultaneous fasting and lactation in the northern elephant seal. We evaluated the effects of hormones on lipid mobilization and subsequent allocation of lipid to milk in fasting and lactating seals. For comparison, we examined the effect of hormones on lipid mobilization in seals that are fasting and molting, but not lactating. We hypothesized that GH and cortisol, both lipolytic hormones, would be positively related to NEFA, as well as to milk lipid content. Insulin was expected to be negatively related to NEFA and milk lipid. Circulating NEFA was also hypothesized to be positively related to milk lipid content, while TAG levels were not expected to be related to milk lipid content.