2018

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Original Publication Citation

Fowler, M., Champagne, C., & Crocker, D. (2018). Adiposity and fat metabolism during combined fasting and lactation in elephant seals. *Journal of Experimental Biology*, 221(Supp1), 1-10. doi:10.1242/jeb.161554

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REVIEW

Adiposity and fat metabolism during combined fasting and lactation in elephant seals

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ABSTRACT

Animals that fast depend on mobilizing lipid stores to power metabolism. Northern elephant seals (*Mirounga angustirostris*) incorporate extended fasting into several life-history stages: development, molting, breeding and lactation. The physiological processes enabling fasting and lactation are important in the context of the ecology and life history of elephant seals. The rare combination of fasting and lactation depends on the efficient mobilization of lipid from adipose stores and its direction into milk production. The mother elephant seal must ration her finite body stores to power maintenance metabolism, as well as to produce large quantities of lipid and protein-rich milk. Lipid from body stores must first be mobilized; the action of lipolytic enzymes and hormones stimulate the release of fatty acids into the bloodstream. Biochemical processes affect the release of specific fatty acids in a predictable manner, and the pattern of release from lipid stores is closely reflected in the fatty acid content of the milk lipid. The content of the milk may have substantial developmental, thermoregulatory and metabolic consequences for the pup. The lactation and developmental patterns found in elephant seals are similar in some respects to those of other mammals; however, even within the limited number of mammals that simultaneously fast and lactate, there are important differences in the mechanisms that regulate lipid mobilization and milk lipid content. Although ungulates and humans do not fast during lactation, there are interesting comparisons to these groups regarding lipid mobilization and milk lipid content patterns.

KEY WORDS: *Mirounga angustirostris*, Milk, Pinniped, Insulin, Lipolysis

Introduction: simultaneous fasting and lactation

Periods of nutrient restriction are common for most free-ranging animals (Secor and Carey, 2016; McCue, 2010). Some animals routinely fast (i.e. abstain from nutrient intake) as part of their life history and often display physiological adaptations that involve a reduction in energy expenditure, such as hibernation, torpor or adaptations in nutrient allocation (Champagne et al., 2012b; Tøien et al., 2011; Florant and Healy, 2012). Lactating females are particularly impacted by nutrient availability. Of all reproductive costs, lactation demands the most energy; with many lactating mammals increasing their energy intake by 60–200% (Gittleman and Thompson, 1988). Thus, females who need to provision their offspring during periods of nutrient deprivation are confronted with conflicting metabolic challenges: to fast, nutrient stores need to be rationed, whereas lactation requires increased nutrient intake and/or fuel mobilization. Owing to these conflicting demands, the combination of fasting and lactation as a life-history strategy is rare. Only three groups of animals are known to simultaneously fast and lactate: phocid seals, mysticete whales and bears.

Investigating the metabolic adaptations that reconcile these conflicting demands can provide insight into physiological adaptations for nutrient allocation. The northern elephant seal (NES; *Mirounga angustirostris*) is a particularly well-studied species that fasts concomitant with energetically expensive activities at several life-history stages. Fuel stores are accumulated over two long foraging trips thousands of kilometers out to sea (Robinson et al., 2012) and are used to power their activities while fasting ashore. During the breeding season, female NES give birth to a single pup and nurse the pup for ∼26 days while abstaining completely from nutrient intake. This intense, short investment period is characterized by rapid nutrient transfer from mother to pup in the form of milk that is ∼25% lipid in early lactation, increasing to >55% lipid shortly before weaning (McDonald and Crocker, 2006; Fowler et al., 2016; Crocker et al., 2001) (Table 1, Fig. 1). Pups are weaned abruptly when the mother departs to the sea to swim to distant foraging grounds. Pups remain ashore after weaning and fast for 2.5–3 months (Champagne et al., 2012b). The mass gained during the suckling period is a crucial fuel source required for subsequent developmental changes needed before foraging independently (Champagne et al., 2012b).

This Review will focus primarily on the mobilization of lipid from maternal body stores and the production of lipid-rich milk. A suite of metabolic adaptations enables simultaneous fasting and lactation, and the ability to efficiently use fat as fuel while sparing protein stores is a crucial component. The substantial lipid stores gained at sea power >90% of the energy requirements of lactation (Crocker et al., 2001). Females typically weigh ∼470 kg after giving birth (Crocker et al., 2014b; Fowler et al., 2016) (Table 1) and continue to grow throughout their lifetime. Shortly after giving birth, females are approximately 36% body fat and catabolize ∼50% of their initial body energy during lactation (Crocker et al., 2014a). The energy expended for maternal metabolism, beyond that required for milk energy expenditure, can be considered ‘metabolic overhead’ (Crocker et al., 2001; Fedak and Anderson, 1982). Older, larger females can reduce their metabolic overhead by reducing their maintenance metabolism, reducing their time ashore and increasing the rate of energy transfer to the pup (Crocker et al., 2001).

The challenge that fasting lactating NES face is to ration lipid stores to prolong supporting maternal metabolism in the absence of exogenous nutrients while also mobilizing extraordinary amounts of lipid into milk for provisioning the offspring. A key characteristic of the rapid, high-intensity lactation of phocid seals is the production of some of the highest milk-fat content known among mammals (Costa, 1991, 1993). Across mammals, diet and the duration of lactation have driven the evolution of milk composition (Skibiel et al., 2013). In pinnipeds, the constraints of fasting have favored a short lactation duration and rapid nutrient transfer; this combination has driven milk composition that is primarily lipid with

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Journal of Experimental Biology (2018) 221, jeb161554. doi:10.1242/jeb.161554

Table 1. Summary of body composition, milk and plasma hormonal and metabolite changes of adult female northern elephant seals from early (day 5 post parturition) to late lactation (day 21 post parturition)

	Early lactation	Late lactation
[1]Mass (kg)*	471±16	335±15
[2]Adiposity (% fat)*	36.3±1.3	23.4±0.9
[3]Milk lipid (%)*	25.2±5.8	51.5±0.9
[2]NEFA (mmol l^{-1})*	1.28±0.42	3.11±0.99
[2]Insulin (pmol l^{-1})*	14.1±2.9	7.8±2.0
[2]Cortisol (nmol l^{-1})*	146.2±54.1	285.6±82.5

Values are means±s.d.
*The study reported a significant difference.
Data consolidated from: [1]Fowler et al., 2016; Champagne et al., 2006; McDonald and Crocker, 2006; [2]Crocker et al., 2014a; [3]Fowler et al., 2015; Fowler et al., 2016; McDonald and Crocker, 2006.

only trace levels of carbohydrate (Costa, 1991; Oftedal, 1993). The increase in milk lipid content over the fast combined with the exceptional rate of milk production, ∼4 kg day^{-1} for 26 days in the NES (Crocker et al., 2001), indicates high metabolic activity by the mammary gland. In addition to milk lipid deposition, females deliver on average 53 liters of water to their pup during lactation (Costa et al., 1986). Since females do not drink during lactation, this water must be made available by metabolic water production from lipid oxidation. This water production is combined with a highly efficient urine-concentrating ability in females (Crocker et al., 1998) and minimal respiratory water loss to ensure the water balance of the mother while providing water to the pup in milk. Maternal protein catabolism is generally low, but increases in late lactation, representing a possible limit to lactation duration (Crocker et al., 1998). Despite the increased maternal protein catabolism late in lactation, milk protein content remains constant and milk water decreases (Fig. 1). Water conservation is another challenge for fasting animals, and especially those also directing water to milk production. Some fasting animals increase protein catabolism specifically for water production (Gerson and Guglielmo, 2011). However, this scenario is only applicable in animals capable of highly concentrated urinary nitrogen excretion (e.g. uricotelic animals) and is not a viable source of water production in phocid seals. In animals that are fasting, and, hence, lacking a dietary input of lipid, there are two general routes for the deposition of lipid in milk: either the mammary gland synthesizes lipids *de novo* or the lipid is deposited from circulating maternal plasma.

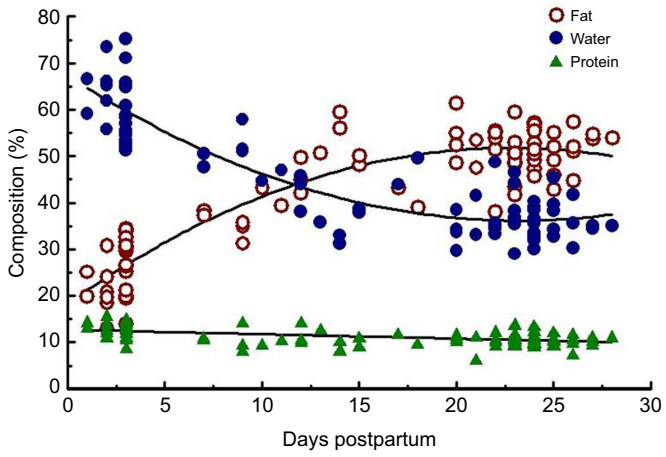

Fig. 1. Changes in the proximate composition of the milk of northern elephant seals over the lactation period. After Crocker et al. (2001).

Many species synthesize milk lipids *de novo* in the mammary gland from non-lipid precursors (Neville and Picciano, 1997). Measurements of lipid synthesis in free-ranging seal mammary tissue have not been attempted; however, we can infer the approximate quantity of *de novo* synthesis by assessing the fatty acid composition of the milk. In most mammals, short and medium chain fatty acids (≤16 carbons) can be synthesized *de novo* in the mammary gland (Neville and Picciano, 1997; Dils, 1983). However, seal milk contains only trace amounts of fatty acids with fewer than 16 carbons, indicating that there is little, if any, *de novo* production from glucose or ketone precursors within the mammary gland (Fowler et al., 2014). Thus, the source of lipid in pinniped milk is likely derived predominantly from maternal lipid stores rather than *de novo* synthesis. The high degree of similarity between fatty acids in blubber and milk suggests minimal modification from maternal blubber to milk production (some differences are discussed below) (Fowler et al., 2014) (Fig. 2).

If the mammary gland is not synthesizing fatty acids *de novo*, then fatty acid transport to, and their uptake by, the mammary gland is likely a determining factor in milk lipid content. There is potential for regulatory mechanisms at either mammary gland uptake or the release of precursors from the fat stores. Lipoprotein lipase (LPL) hydrolyzes circulatory triglycerides, and the resulting fatty acids are available for uptake by the mammary gland and incorporation into milk. In some phocid species (grey and hooded seals), LPL in the mammary gland is hypothesized to be the primary regulatory point for milk composition (Iverson et al., 1995; Mellish et al., 1999b). However, in NES, mammary LPL levels are stable from early to late lactation, and unrelated to increasing milk content (McDonald and Crocker, 2006). Thus, mammary LPL does not appear to be responsible for the regulation of milk composition in NES. A remaining hypothesis for mothers to regulate the composition of milk lipid is at the level of release from lipid reserves in the adipose tissue (Fowler et al., 2016, 2015).

If females do not regulate milk lipid content via mammary LPL, regulation may occur at the level of the precursor supply – including the release of fatty acids from lipid stores and the availability to the mammary gland. Body size and composition are independent of plasma non-esterified fatty acid (NEFA) levels and are inversely related to milk lipid content; as females catabolize adipose tissue they become leaner while milk lipid content increases (Fowler et al., 2016). Although specific fatty acids can be important in the development of offspring (Jump, 2002), lactating NES mobilize individual fatty acids according to their structure – the degree of unsaturation and carbon-chain length – and not in a preferential manner based on the demands of the pup (Fowler et al., 2014). Additional avenues regulating milk lipids are mechanisms that affect lipolysis and the uptake and use of fatty acids by other tissues before they are taken up by the mammary gland. The rate at which NES mobilize and then utilize their lipid stores is an important component of reconciling the conflicting demands of fasting and lactation.

There are presumably strong selective advantages for females to provide adequate milk energy to offspring. The health and developmental consequences for the pup are multi-faceted, including the duration of the post-weaning fast and, thus, adequate time for important developmental changes [e.g. increases in respiratory pigments (Somo et al., 2015)], and the development of cardiovascular control and skeletal muscle features (Castellini et al., 1994) needed for their initial foraging trip at sea as well as potential thermoregulatory considerations (Noren et al., 2003, 2008; Kanatous et al., 2008). The provision of specific milk

Journal of Experimental Biology (2018) 221, jeb161554. doi:10.1242/jeb.161554

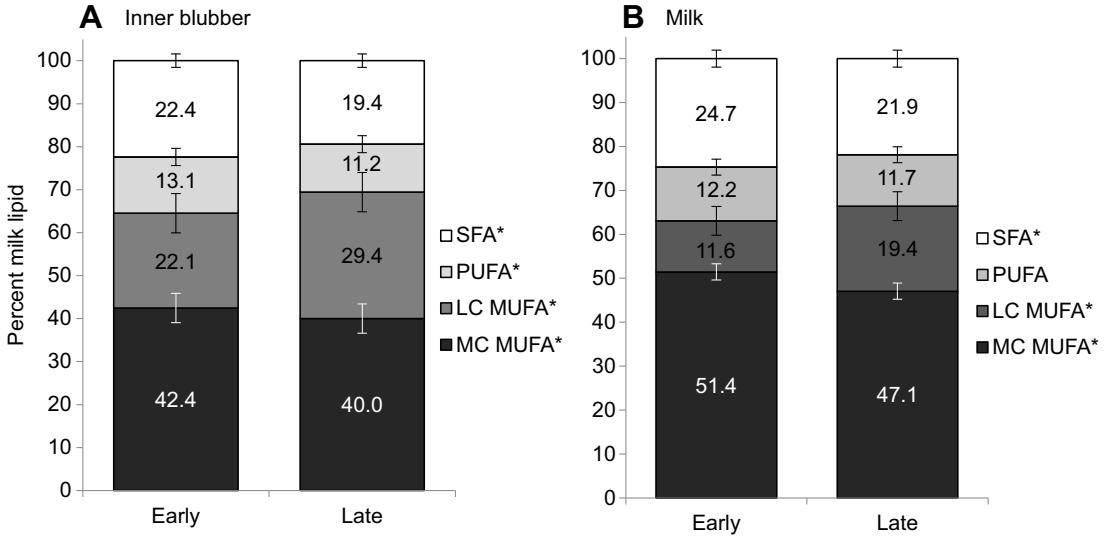

Fig. 2. Change in the proportion of fatty acid classes in the inner blubber and milk of northern elephant seals across the lactation period. (A) Blubber and (B) milk fatty acid compositions are similar. The only class of fatty acids to increase in milk across the lactation period were LC MUFA; the other classes remained stable or decreased. Values are means±s.d. Asterisks indicate that the proportion of the fatty acid class present at the early stage of lactation was significantly different from that at the late stage. SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; LC MUFA, long-chain monounsaturated fatty acid (≥20 C); MC MUFA, medium-chain monounsaturated fatty acid (≤18 C). After Fowler et al. (2014).

fatty acids to pups may affect neural development (Larque et al., 2002) as well as potentially altering muscle development (Trumble et al., 2010; Wheatley et al., 2008b). Thus, not only energy content but also specific fatty acids are important for pup development.

Fasting females are constrained to using the onboard lipid stores for milk lipid synthesis; thus, the milk fatty acid content is highly impacted by blubber fatty acid availability. The fatty acid composition of maternal lipid stores is heavily dependent on the food source (Iverson et al., 2004); and the fatty acid composition deposited in milk closely mirrors those that have been mobilized from the blubber, indicating that much of what is mobilized from maternal blubber is directed unmodified to milk (Fowler et al., 2014) (Fig. 2). However, the mother must also use the mobilized fatty acids for her own metabolism, in addition to deposition in milk. The few discrepancies between blubber and milk fatty acid content indicate that mothers may preferentially use medium-chain monounsaturated fatty acids and saturated fatty acids to meet their energy requirements, resulting in greater proportions of long-chain monounsaturated fatty acids deposited into milk (Fowler et al., 2014) (Fig. 2). The interplay between maternal diet, metabolism and milk production indicates that the different food sources available to the mother may have consequences for the fatty acids available to the pup for development.

Patterns among fatty acids used by different tissues (mammary, blubber, skeletal tissue) may depend on physiological mechanisms. Partitioning among tissues could be a result of tissue-specific changes in fatty acid binding and transport proteins (Clegg et al., 2001; Glatz et al., 2010). Fasting NES pups show decreases in adipose fatty acid transporters across the fast (Viscarra et al., 2012) and several fatty acid transport and binding proteins have been quantified in skeletal muscle (Robbins, 2014). The transport dynamics of fatty acids in multiple tissues in adult, lactating NES are unknown.

Lipid mobilization

The magnitude of lipid mobilized by lactating NES has prompted the inspection of lipolytic mechanisms. Two enzymes are principally responsible for lipolysis within adipose tissue – adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL). ATGL catalyzes the removal of the first fatty acid from triacylglycerol and HSL is responsible for the hydrolysis of the second. These enzyme activities were assessed in blubber samples collected from NES at the beginning and end of lactation. HSL appears to be of minimal importance during fasting and lactation given that its activity was essentially non-detectable (Fowler et al., 2015). Conversely, ATGL expression increased during lactation (Fowler et al., 2015). Despite the increase in expression, the activity of the enzyme in hydrolyzing triacylglycerol substrate *in vitro* was similar at the beginning and end of lactation. Although the expression of ATGL correlated with plasma NEFA, the lipolytic activity of ATGL was independent of plasma NEFA or milk lipid composition. Furthermore, although ATGL is the primary lipase during fasting and lactation in NES, neither the expression nor the lipase activity of ATGL appears to be directly regulating milk lipid composition (Fowler et al., 2015).

Regulatory mechanisms may explain the stable ATGL activity concomitant with increased expression. The stable lipolytic activity of ATGL in blubber across lactation is consistent with stable whole-body lipolysis rates (Houser et al., 2007). Several molecular regulators that modulate ATGL lipolytic activity are potential points of regulation. For example, the chaperone protein CGI-58 is required for ATGL activity (Lass et al., 2006) and its expression may affect *in vivo* activity. Furthermore, the expression and phosphorylation of perilipin, a molecule that blocks access to the surface of the lipid droplet (Subramanian et al., 2004; Watt and Steinberg, 2008) could be an avenue of regulation. An additional potential regulatory point in fasting seals may be the protein GS02, an inhibitor of ATGL (Yang et al., 2010). Brown bears are similar to NES in that they also undergo periods of rapid weight gain, followed by periods of fasting, lactation and lipid dependence. Levels of GS02 were highly upregulated during lipogenesis and decreased to undetectable during hibernation to facilitate increased lipolysis during hibernation (Jessen et al., 2016). Thus, GS02 may be a regulatory point in fasting elephant seals.

Other factors must be driving changes in the rates of lipolysis during lactation. For example, 5′-adenosine monophosphate-activated protein kinase (AMPK) functions as a cellular energy sensor and influences several metabolic pathways, acting to modulate lipid metabolism (Gauthier et al., 2008). Long-term elevation of activated AMPK increases lipolysis and fatty acid oxidation; in addition, ATGL synthesis is increased and re-esterification is decreased (Gaidhu et al., 2009). These changes work in concert to control the catabolism of lipid stores and increase the oxidation of these fuel substrates, while decreasing lipogenesis. Weaned NES exhibit an increase in adipocyte AMPK late in the post-weaning fast, concomitant with increased ATGL expression (Viscarra et al., 2011a, 2013). These increases parallel findings in fasting laboratory rats (Gaidhu et al., 2010). Although adipocyte AMPK has not been quantified in adult female elephant seals, it may be a putative mechanism contributing to increased ATGL expression and decreased re-esterification. Interestingly, in humans on a high-fat diet, the above described 'typical' modulation of the lipid metabolism by AMPK is dysregulated (Gaidhu et al., 2010). Seals, although obese by human standards, do not appear to follow the pattern of dysregulated lipid metabolism.

Fatty acids, released from blubber stores via enzymes and hormones, are transported to target tissues in the bloodstream as NEFA. Intriguingly, the plasma NEFA levels at the beginning of lactation have doubled by late lactation, whereas lipolysis rates remain high and stable (Houser et al., 2007). Fatty acids released by lipolysis that are not used immediately for β-oxidation may be re-incorporated into triglycerides through re-esterification. Re-esterification rates have not been measured in NES adipose tissue; however, stable levels of NEFA released from lipid stores, in the face of decreasing re-esterification rates, may contribute to the decoupling of lipolysis rates and plasma NEFA (Crocker et al., 2014a). An intermediate mechanism involved in decreased re-esterification may involve transporting plasma NEFA into adipocytes, which in turn affects their availability for re-esterification. If lactating NES exhibit decreased fatty acid transporter expression similar to weanlings (Viscarra et al., 2012), plasma NEFA levels may be impacted. In such a scenario, NEFA transport from the plasma into adipocytes for re-esterification would decrease over the fasting duration, contributing to the decoupling of lipolysis and plasma NEFA (Crocker et al., 2014a). This decoupling of plasma NEFA availability from the magnitude of fat reserves may facilitate the dramatic increase in milk lipid content late in lactation despite significant depletion of adipose reserves.

Hormonal regulation of metabolism

Hormones play a crucial role in regulating fuel metabolism. Although adipose tissue is unequivocally the primary energy source in fasting seals, it also functions as an endocrine organ – secreting a variety of chemical signals that the body uses to integrate metabolic status (Rasouli and Kern, 2008). Although little to no data have been collected regarding adipocytokines in adult female NES, there are limited data for other age classes.

Several candidate adipocytokines have been measured in other age classes of NES, including leptin, apelin, adiponectin and tumor necrosis factor alpha (TNFα). In most mammals, leptin is strongly correlated with adipose stores and functions in a complex signaling cascade to indicate energy balance (Ahima and Flier, 2000; Harvey and Ashford, 2003). In fasting adult male and weaned pup NES (Viscarra et al., 2011a; Crocker et al., 2012), leptin concentration is independent of fat mass. These patterns are surprising – we would expect reduced leptin levels with declining fat mass with the progression of fasting, given the relationship between leptin and adipose stores in other mammals. In humans, adiponectin is inversely related to fat mass, contrary to most of the other adipocytokines (Hotta et al., 2001), whereas apelin increases with fat mass (Boucher et al., 2005). Both adiponectin and apelin are typically associated with improved insulin sensitivity (Okamoto et al., 2008; Yue et al., 2010) and both decrease over the fasting duration in NES weanlings (Viscarra et al., 2011a,b, 2012; Suzuki et al., 2013). NES weanlings display insulin resistance (Viscarra et al., 2011a), thus the decrease in these adipocytokines is consistent with decreased insulin sensitivity. TNFα is associated with increased lipolysis (Yang et al., 2011; Souza et al., 1998) and insulin resistance (Chen et al., 2009). Fasting NES require continued lipolysis and indeed display insulin resistance (Viscarra et al., 2013; Fowler et al., 2008). Adipocyte TNFα expression levels are stable throughout the fast and increase in the muscle of NES weanlings (Suzuki et al., 2013; Viscarra et al., 2013). These expression patterns from early to late fasting in NES weanlings align with observed metabolic patterns such as elevated lipolysis and insulin resistance. Much remains to be elucidated regarding the involvement of adipocytokines in lipid metabolism in all age classes of NES.

Specific endocrine glands other than adipose tissue also play crucial roles in maintaining homeostasis. Growth hormone has an established lipolytic effect (Djurhuus et al., 2004) and is used extensively in the dairy industry (Bauman, 1999); thus, it might be an ideal candidate to regulate lipolysis and deposition into milk. However, growth hormone shows only a modest, increasing trend (that is not statistically significant) in fasting, lactating NES. Circulating growth hormone is not associated with milk lipid content or circulating NEFA, indicating that growth hormone is not a main driver of lipolysis or milk lipid deposition (Fowler et al., 2016).

Cortisol, among other metabolic actions, has lipolytic effects (Djurhuus et al., 2004). Despite the additional metabolic consequence of stimulating protein catabolism (Brillon et al., 1995), the increasing levels of cortisol and decreasing secretion of insulin together drive plasma NEFA and milk lipid content in lactating NES (Fowler et al., 2016) (Fig. 3). Insulin is an important regulator of lipid metabolism in NES and is a potent inhibitor of lipolysis (Frayn et al., 1994; Choi et al., 2010), which has clear implications for an animal highly dependent on lipid mobilization. Alterations in insulin concentration also have important implications for carbohydrate metabolism (Aronoff et al., 2004) (discussed below). Lactating NES exhibit low circulating insulin, which decreases even further with fasting (Fowler et al., 2008, 2016; Champagne et al., 2006; Houser et al., 2007). The decreased insulin in fasting elephant seals likely removes its anti-lipolytic effect, facilitating lipid mobilization. Early in lactation, NES exhibit an insulin response to exogenous administrations of glucose as well as glucagon. The rate of subsequent glucose clearance is very low, indicating insulin resistance. As adiposity decreases in late lactation, the insulin response to exogenous glucose and glucagon is abolished, suggesting a decreased level of pancreatic β-cell responsiveness (Crocker et al., 2014a; Fowler et al., 2008). The lack of insulin secretion in combination with peripheral insulin resistance may promote lipolysis to facilitate both maternal metabolism and milk lipid deposition. The negative relationship between insulin and both plasma NEFA and milk lipid content in lactating females suggests that the removal of the inhibition of insulin on lipolysis is crucial to the supply of NEFA to the mammary gland (Fowler et al., 2016) (Figs 3 and 4). These patterns

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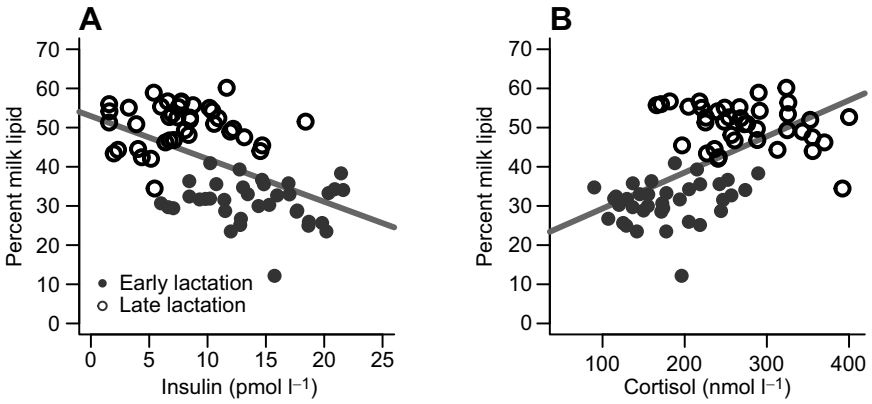

Fig. 3. Effects of insulin and cortisol on the milk lipid content of northern elephant seals. Regression parameters were extracted from a mixed model with 'seal ID' and 'Year' as random effects. (A) Insulin was negatively related to milk lipid content across the lactation period ($F_{167.1}$=16.3, P<0.001). (B) Cortisol was positively related to milk lipid content across the lactation period ($F_{168.4}$=34.0, P<0.001). Filled circles represent early lactation; open circles represent late lactation. See Fowler et al. (2016) for details of statistical analysis.

combined with the lack of regulation of milk content at the level of mammary LPL (McDonald and Crocker, 2006) supports the hypothesis that the regulation of fatty acid mobilization from blubber for delivery to the mammary gland is a crucial feature regulating milk lipid composition.

The characteristics displayed by lactating NES are reminiscent of human metabolic syndrome in some respects (Houser et al., 2013). Metabolic syndrome is a pathological condition in humans, characterized by dyslipidemia, altered glucose metabolism, insulin resistance, hypertension, an increased risk of type 2 diabetes and often obesity (Povel et al., 2013; Silva et al., 2014). In humans, as type 2 diabetes progresses, insulin secretion declines. Hyperinsulinemia accompanying early metabolic syndrome eventually exhausts the ability of the pancreatic cells to maintain insulin secretion to compensate for insulin resistance (Ehses et al., 2009). NES share some characteristics with these metabolic syndrome conditions, including decreased insulin sensitivity, fasting hyperglycemia and obesity. In contrast to the hyperinsulinemia in human pathological conditions, lactating NES exhibit low insulin levels, which decrease even further with fasting (Fowler et al., 2008; Champagne et al., 2006).

Understanding the mechanisms behind insulin secretion may be instructive to forming hypotheses about patterns of plasma insulin in NES. Circulating fatty acids stimulate β-cell insulin secretion on an acute time frame (Cen et al., 2016). Although some studies support the idea that chronically elevated NEFA impairs β cell insulin secretion, effects can vary by species, current metabolic state and genetic background (Rebelos et al., 2015; Carpentier et al., 2000; Yaney and Corkey, 2003). Insulin stimulates LPL to facilitate the uptake of circulating lipids (Kraemer et al., 1998) and stimulates transcription factors for lipogenesis (Ito et al., 2013). We have previously suggested that the relationship between adiposity and insulin secretion may facilitate continued lipolysis as fuel stores are depleted (Crocker et al., 2014a,b; Fowler et al., 2008). The attenuation of the glucose-stimulated insulin response in late lactation may well be a result of continued exposure to high levels of circulating NEFA (Houser et al., 2013) as adipose stores are mobilized. In contrast to lactating females, fasting NES weanlings exhibit an insulin response to glucose both early and (albeit blunted) late in the fast (Viscarra et al., 2011b), indicating that some pancreatic β-cell function is maintained throughout fasting. As discussed above, many other factors such as adipose-derived signaling molecules can modulate lipid metabolism and insulin secretion. Thus, one part of the relationship between adiposity, insulin and lipid metabolism may be facilitated by NEFA suppression of insulin secretion, further enabling lipolysis in adipose tissue.

In addition to impaired insulin secretion, NES also display insulin resistance (Viscarra et al., 2011b; Fowler et al., 2008). This resistance revolves around the ability of the cell to respond to insulin and one method of assessment involves measuring the intermediate steps of the signaling cascade. Chronically elevated circulating NEFA is associated with impaired insulin secretion, as well as peripheral insulin resistance in rats (Yu et al., 2002). Administration of insulin increased glucose clearance in fasting NES weanlings, demonstrating the maintenance of some level of insulin sensitivity. Tissue level insulin signaling has not been quantified in adult females; however, weanling adipose tissue exhibits decreased

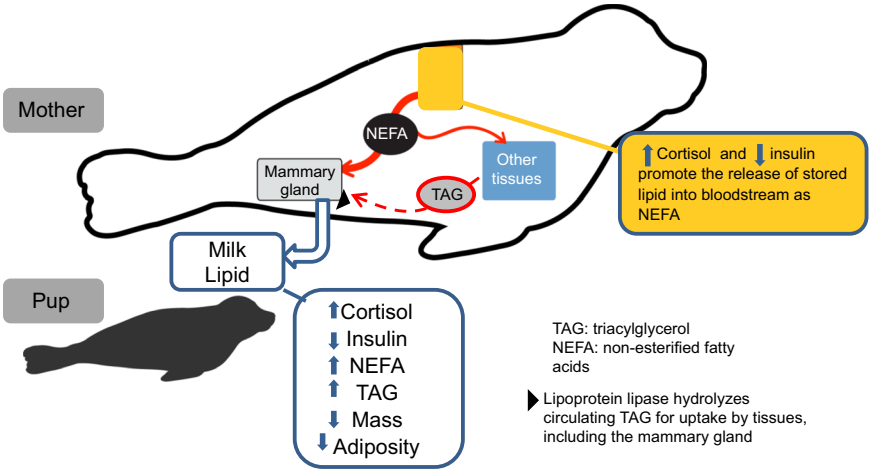

Fig. 4. Summary of processes involved during the simultaneous fasting and lactation of northern elephant seals. Arrows indicate the pattern of change from early to late lactation.

Journal of Experimental Biology (2018) 221, jeb161554. doi:10.1242/jeb.161554

insulin signaling with time fasting, whereas skeletal muscle maintains some insulin signaling, despite decreased glucose clearance rates (Viscarra et al., 2013). Fasting weanling muscle cells respond to insulin, whereas adipose cells do not, which in turn affects how quickly glucose is removed from the bloodstream. Lactating NES exhibit a much more dramatic change in insulin secretion (abolished) and insulin sensitivity. While NES weanlings appear to maintain β-cell functionality, as well as peripheral insulin sensitivity in some tissues, their mothers display decreased β-cell responsiveness (lack of insulin secretion) and increased insulin resistance, potentially as a result of the increased demands of lactation in addition to fasting. Tissue-specific insulin signaling in lactating NES could shed light on whether they are further depressing insulin signaling and action via similar but more dramatic measures than fasting weanlings.

Coordinated substrate metabolism

Contrary to humans, NES do not display the pathological conditions associated with modifications in glucose or lipid metabolism. Modification to the hormonal regulation of glucose metabolism is likely an adaptive mechanism in NES (Viscarra and Ortiz, 2013; Houser et al., 2013) and linked with facilitating lipid metabolism. A low level of insulin secretion facilitates lipolysis, and insulin resistance promotes lipid oxidation and restricts glucose uptake to glucose-dependent tissues, such as the central nervous system. One pathological issue confronted by humans who display increased dependence on lipolysis (both in diabetes and while fasting) is ketoacidosis, which can lead to serious metabolic disorders (Fery and Balasse, 1985). Despite their increased dependence on lipid catabolism and extended fasting durations, NES maintain surprisingly low ketone levels compared with other fasting species and avoid ketoacidosis by unknown mechanisms (Champagne et al., 2006; Crocker et al., 2014b; Houser et al., 2007).

Fasting animals that are heavily dependent on lipid-based metabolism are faced with the challenge of providing fuel for glucose-dependent tissues and minimizing catabolism of protein stores while avoiding excess ketone accumulation. When carbohydrate stores are depleted, protein is catabolized to produce glucose for glucose-dependent tissues (Goodman et al., 1980). Amino acids from protein stores can feed into the tricarboxylic acid (TCA) cycle and provide gluconeogenic substrates; however, this depletes protein stores and results in compromised tissue function (Owen et al., 1998). Elephant seals exhibit high rates of glucose production despite efficient conservation of protein stores (Champagne et al., 2012a, 2005, 2006; Houser et al., 2012). The high rate of glucose production is counterintuitive because it is expected to be associated with high levels of protein catabolism, which is not typically observed in NES. NES weanlings also exhibit high rates of TCA cycling, which may facilitate lipolysis indirectly through modifications in glucose metabolism (Champagne et al., 2012a; Houser et al., 2012). Rather than catabolizing protein stores, glucose is recycled, in turn facilitating rapid TCA cycling (Fig. 5). Ketones are formed when acetyl-CoA from β-oxidation of lipid accumulates, exceeding the capacity to enter the TCA cycle. Increased TCA cycling may contribute to the avoidance of ketone accumulation (Houser et al., 2012) by allowing excess acetyl CoA to enter the TCA cycle instead of being converted to ketones. Furthermore, rapid TCA cycling is facilitated by high glucose recycling rates and replenishment via pyruvate carboxylase (Champagne et al., 2012a; Tavoni et al., 2013). Thus, high rates of glucose recycling may be coupled to rapid TCA cycling and associated anaplerotic reactions to permit continued entrance of acetyl-CoA into the TCA cycle. Rapid TCA cycling may prevent the accumulation of acetyl-CoA and, thus, ketoacidosis. Rapid glucose cycling may also contribute to protein store preservation by maintaining blood glucose availability for glucose-dependent tissues without degrading protein stores (Fig. 5). Glucagon administration has been shown to increase ketogenesis and protein catabolism in lactating NES and the magnitude of the response varies with adiposity (Crocker et al., 2014b), suggesting the importance of maintaining low glucagon levels while fasting. Insulin and glucagon do not display the usual dynamics in the face of glucose administration in lactating NES (Fowler et al., 2008); thus, low glucagon and the decoupling of the normal insulin–glucagon push–pull interaction may also contribute to decreased ketogenesis.

Inter-specific comparisons of fasting and lactation

Resolving the conflicting demands of fasting and lactation is necessary to enable phocid seals to exploit far-ranging food sources but support terrestrial lactation (Champagne et al., 2012b). Although NES exemplify this strategy, other phocid seals also display this reproductive model. Grey seals (*Halichoerus grypus*) have been extensively studied during their fasting and lactation period. Although there are many similarities between NES and grey seal fasting and lactation (Mellish et al., 2000, 1999a; Mellish and Iverson, 2001; Champagne et al., 2012b; Fowler et al., 2014), some key differences arise. Unlike NES, grey seals appear to regulate milk lipid composition through changes in LPL activity in the mammary gland (Mellish et al., 1999b; Iverson et al., 1995). The only study to quantify insulin in lactating grey seals found stable plasma insulin, NEFA and milk lipid, and no relationship was detected among the three variables. This result is surprising but may be due to low statistical power (Bennett et al., 2015). Despite the differences

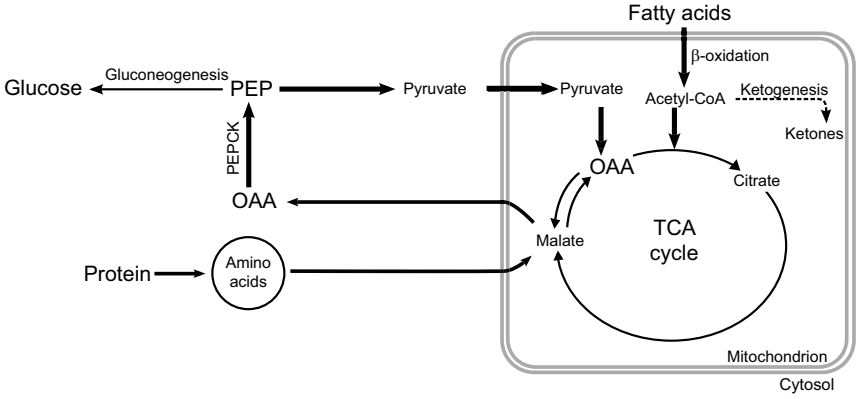

Fig. 5. Seals meet most of their energy needs through fat catabolism. This requires sufficient TCA cycle intermediates to accept acetyl-CoA from β-oxidation. The high rates of carbohydrate metabolism measured in fasting seals may serve to regenerate the intermediates (e.g. oxaloacetate, OAA) that are needed to support continued fatty acid use. This further reduces the need for amino acid influx, which would necessitate protein catabolism. After Houser et al. (2012). PEP, phosphoenolpyruvate; PEPCK, PEP carboxykinase.

Journal of Experimental Biology (2018) 221, jeb161554. doi:10.1242/jeb.161554

Table 2. Interspecific comparison of milk lipid composition

Species	% Milk fat	Reference
Phocids		
Weddell seal (*Leptonychotes weddellii*)	50.0	Wheatley et al., 2008a
Grey seal (*Halichoerus grypus*)	56.0	Iverson et al., 1995
Hooded seal (*Cystophora cristata*)	57.0	Mellish et al., 1999b
Harbor seal (*Phoca vitulina*)	50.0	Lang et al., 2005
Cetaceans		
Fin whale (*Balaenoptera physalus*)	60.0	Borrell et al., 2016
Common dolphin (*Delphinus delphis*)	37.0	Peddemors et al., 1989
Bottlenose dolphin (*Tursiops truncatus*)	25.0	West et al., 2007
Ursids		
Polar bear (*Ursus maritimus*)	36.0	Derocher et al., 1993
Grizzly bear (*Ursus arctos*)	18.0	Farley and Robbins, 1995
Black bear (*Ursus americanus*)	22.0	Iverson et al., 2001
Herbivores		
Cow (Holstein) (*Bos taurus*)	3.8	Gama et al., 2008
Goat (Saanen) (*Capra hircus*)	3.0	Baldi et al., 2002
Asian elephant (*Elephas maximus*)	17.0	Abbondanza et al., 2013
Companion animals		
Dog (beagle) (*Canis familiaris*)	9.5	Oftedal, 1984
Cat (domestic shorthair) (*Felis catus*)	13.0	Jacobsen et al., 2004
Primates		
Human (*Homo sapiens*)	4.0	Koletzko et al., 2011
Mountain gorilla (*Gorilla beringei*)	1.8	Whittier et al., 2011
Bonnet macaque (*Macaca radiata*)	7.5	Laudenslager et al., 2010

Breeds indicated where appropriate. The values shown are the highest reported mean value throughout lactation.

reported between these phocids that deliver exceptionally lipid-rich milk while fasting, these groups seem to have arrived at the same endpoint through slightly different regulatory avenues. The significant advantage conferred by the ability to produce high-fat milk while fasting would likely be under strong selection, potentially via multiple mechanisms.

Metabolic research in bears (Ursidae), another group that displays simultaneous fasting and lactation, has revealed some interesting similarities. Polar (*Ursus maritimus*), grizzly (*Ursus arctos*) and black bear (*Ursus americanus*) milk is high in lipid, but not as high as NES (Farley and Robbins, 1995; Iverson et al., 2001; Hedberg et al., 2011) (Table 2). Bears go through periods of hyperphagia before hibernation and during this time exhibit an increased insulin response to a glucose tolerance test, as well as increased insulin sensitivity (Jessen et al., 2016; Kamine et al., 2012). The dramatic switch from lipogenesis to lipolysis may be mediated not only through modulating inhibitors of insulin signaling (e.g. the phosphatase and tensin homolog PTEN) but also through the expression of proteins that mediate the activity of lipolytic enzymes (e.g. GS02, see above) (Jessen et al., 2016). PTEN and GS02 could be avenues for future research on the regulation of lipogenesis and lipolysis in fasting, lactating seals. Thus, while several other species show simultaneous fasting and lactation, much remains to be learned about the regulation of lipid mobilization and delivery.

In mammals that feed throughout lactation, insulin decreases but insulin sensitivity increases (Tigas et al., 2002; Hatfield et al., 1999; Sartin et al., 1985; Debras et al., 1989; Burnol et al., 1986), which is counter to the pattern exhibited by elephant seals. In humans, obesity is linked to delayed lactogenesis and a detrimental reduction of lactation duration; with strong implications that impaired insulin sensitivity is a driving force behind these characteristics (Nommsen-Rivers, 2016). In non-fasting mammals, the mammary gland becomes highly insulin sensitive – not to increase glucose transport, but for cell proliferation and to promote milk lipid, protein and lactose synthesis by mammary tissue (Berlato and Doppler, 2009; Neville et al., 2013). In NES, these processes are minimal – milk contains almost no carbohydrate, and *de novo* lipid synthesis is minimal; insulin may, therefore, play less of a role in the mammary gland than other species, although tissue-level insulin sensitivity has not been quantified.

Conclusions

How do fasting, lactating NES mobilize the significant amounts of lipid required to support both maternal metabolism and milk production, without adverse consequences? Female NES accumulate large adipose stores at pelagic foraging grounds. These reserves are then used to fuel terrestrial reproduction, enabling them to exploit distant, rich, food sources while still using terrestrial breeding grounds. The importance of lipid stores to elephant seals is evident in the strong impacts of variation in adiposity on reproductive effort, parental investment, milk composition and the ability to spare body protein during fasting. Alterations in protein, glucose and lipid metabolism are linked through hormonal and enzymatic regulation. NES compress lactation duration and reduce activity to lower metabolic overheads and direct massive quantities of mobilized lipids to the mammary gland. The mammary gland synthesizes minimal lipid and carbohydrate, instead depositing circulating fatty acids liberated from blubber. Adipose tissue may display complementary but differing mechanisms to regulate lipolysis and re-esterification rates for the purpose of increasing fatty acid availability for milk synthesis. NES maintain high rates of lipolysis without ketoacidosis, in part, by maintaining high rates of glucose recycling concomitant with high rates of TCA cycle activity and associated anaplerotic pathways, which facilitate reduced protein catabolism, one of the hallmarks of efficient fasting. Differential regulation of hormone secretion and tissue sensitivity, primarily for insulin and cortisol, helps to facilitate these processes. Together, these features result in one of the highest rates of milk energy synthesis found in nature despite a complete lack of nutrient input during lactation. Several unresolved mechanisms remain to be determined. In NES, the regulatory mechanism of the primary lipase ATGL is unknown; however, several potential signaling agents have been identified, e.g. CGI-58, GS02, perilipin and AMPK. Changes in tissue-level insulin sensitivity may play an important role in partitioning fuel appropriately during fasting. We also know little about the lipid transport mechanisms in phocids required to support the high rate of lipid mobilization and delivery to the mammary gland.

Acknowledgements

Much of the research investigating reproductive metabolism in northern elephant seals included in this Review has been conducted over many years at Año Nuevo State Reserve, in San Mateo County, CA, USA. We wish to extend our thanks to the Rangers and staff of Año Nuevo Reserve for their long-standing support of this research site. We are indebted to graduate students and undergraduate volunteers in Dr Daniel Costa's lab at UC Santa Cruz, especially Gitte McDonald, Nicole Teutschel, Luis Huckstadt, Patrick Robinson and Pat Morris.

Competing interests

The authors declare no competing or financial interests.

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