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Research paper

Changes in serum adipokines during natural extended fasts in female northern elephant seals

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ABSTRACT

Adipose tissue is essential to endotherms for thermoregulation and energy storage as well as functioning as an endocrine organ. Adipose derived hormones, or adipokines, regulate metabolism, energy expenditure, reproduction, and immune function in model systems but are less well studied in wildlife. Female northern elephant seals (NES) achieve high adiposity during foraging and then undergo natural fasts up to five weeks long during haul-outs associated with reproduction and molting, resulting in large changes in adipose reserves. We measured circulating levels of four adipokines: leptin, resistin, adiponectin, and kisspeptin-54, in 196 serum samples from female NES at the beginning and end of their breeding and molting fasts. We examined the relationships between these adipokines and life-history stage, adiposity, mass, cortisol, and an immune cytokine involved in the innate immune response interleukin 6 (IL-6). All four adipokines varied with life-history stage. Leptin concentrations were highest at the beginning of the breeding haul-out. Resistin concentrations were higher throughout the breeding haul-out compared to the molt haul-out. Adiponectin concentrations were highest at the beginning of both haul-outs. Kisspeptin-54 concentrations were highest at the end of the breeding haul-out. Leptin, resistin, and adiponectin were associated with measures of body condition, either adiposity, mass, or both. Resistin, adiponectin, and kisspeptin-54 were associated with circulating cortisol concentrations. Resistin was strongly associated with circulating IL-6, a multifunctional cytokine. Adiponectin was associated with glucose concentrations, suggesting a potential role in tissue-specific insulin sensitivity during life-history stages categorized by high adiposity. Increased cortisol concentrations late in lactation were associated with increased kisspeptin-54, suggesting a link to ovulation initiation in NES. This study suggests dramatic changes in circulating adipokines with life-history and body condition that may exert important regulatory roles in NES. The positive relationship between adiponectin and adiposity as well as the lack of a relationship between leptin and kisspeptin-54 differed from model systems. These differences from biomedical model systems suggest the potential for modifications of expression and function of adipose-derived hormones in species that undergo natural changes in adiposity as part of their life-history.

1. Introduction

All vertebrates store energy in the form of adipose tissue. These body reserves are often used during behaviors fundamental to an animal's life-history such as migration, fasting, and hibernation (Young, 1976; Pond, 2011, 2017). Because adipose tissue is often used to support energy requirements of behaviors essential for survival and reproduction, individual body condition directly influences fitness (Young, 1976). An organism's ability to regulate their adiposity can strongly affect their success in foraging, mating, and fighting (Toïgo et al., 2006). The importance of sufficient energy stores and the influence of body condition on reproductive success has been well documented across taxa (Harcourt, 1989; Drent and Daan, 1980). This paradigm is especially true for capital breeders which rely entirely on accumulated energy stores to support the energetic requirements of breeding (Jönsson, 1997). Animals must optimize their body condition based on life-history

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and environment.

Matching body condition with internal and external variables requires the ability to sense and communicate energy status within the organism. Until the 1990s, adipose tissue was only known for its function of storing lipids. In 1993, the first cytokine produced by adipose tissue was discovered and in 1994, the hormone leptin was identified (Zhang et al., 1994). Adipose tissue is now recognized as an important endocrine organ that produces hormones (adipokines), cytokines, and chemokines (Tsatsanis et al., 2015). Studies on humans and rats have linked a suite of adipokines to regulation of food intake, metabolism, gonadal function, immune function, and stress adaptation (Tsatsanis et al., 2015).

Leptin was identified in mammals as a negative regulator of appetite and obesity (Zhang et al., 1994). It is produced primarily by adipocytes (Zhang et al., 1994), has a positive relationship with energy reserves in model organisms (Frederich et al., 1995; Rosenbaum et al., 1997; Friedman and Halaas, 1998; Ahima and Flier, 2000), and may regulate fasting metabolism in mammals by promoting lipid catabolism and altering insulin sensitivity (Stern et al., 2016). Biomedical studies have found that leptin indirectly regulates gonadotropin-releasing hormone (GnRH) production (Quennell et al., 2009). Leptin may also stimulate immune function indirectly via action on the sympathetic nervous system and directly via effects on lymphoid tissues (Demas and Sakaria, 2005; Wang et al., 2019).

Resistin is expressed by adipocytes, macrophages and the gonads and is a powerful driver of insulin resistance (Steppan et al., 2001a, 2001b). This adipokine is associated with enhanced gluconeogenesis as well as higher levels of insulin and lipids (Rajala et al., 2004). Resistin also increases inflammatory cytokines TNFα and IL-6, thereby directly regulating immune function (Lo et al., 2007). Interestingly, obesity studies found that resistin mRNA in adipose tissue and circulating resistin levels are not associated in obese mice, suggesting the importance of non-adipose production and potential effects of binding proteins or tertiary changes on rates of clearance (Rajala et al., 2004).

Adiponectin is negatively correlated with body fat in most mammals and is known as a driver of adipocyte maturation and triglyceride synthesis and storage (Hotta et al., 2001; Tsatsanis et al., 2015). Adiponectin increases insulin sensitivity and alters both glucose uptake and lipid mobilization, and has been correlated with plasma glucose concentrations in several model systems (Arita et al., 1999; Hotta et al., 2001; Ishioka et al., 2006; Tschritter et al., 2003).

Kisspeptin-54 links energy homeostasis and reproduction (Castellano et al., 2010). Originally identified as a modulator of GnRH production by the hypothalamus, kisspeptin gene expression in adipose tissue suggests a role in linking body condition and the HPG axis (Brown et al., 2008). In mammals that breed seasonally, administration of kisspeptin-54 will cause females to ovulate outside of typical breeding season, supporting the idea that this adipokine may be driving reproductive behavior in mammals (Pinilla et al., 2012). Food intake and sex hormones regulate gene expression of kisspeptin-54 in rodents, potentially by mediating the effects of leptin on GnRH neurons (Brown et al., 2008; Greives et al., 2007). It was initially hypothesized that leptin acted directly on the HPG-axis to trigger sexual maturity in humans and mice. However, the lack of leptin receptors on GnRH neurons led to the conclusion that kisspeptin responded to leptin levels and then acted on GnRH neurons to stimulate sexual maturity (Castellano et al., 2010; Smith et al., 2006). Therefore, adipokines play an important role in matching body condition with reproductive activity.

Numerous mammalian adipokine studies have been published in the last two decades using human or domestic animal subjects. A limited number of studies measured leptin and adiponectin in hibernating mammals, including marmots, minks, and bears. These studies found that both adipokines decreased during hibernation and both had a positive relationship with body composition and circulating insulin (Florant et al., 2004; Mustonen et al., 2005; Rigano et al., 2017). Despite the strong evidence in wildlife for impacts of adipose reserves on behavior, metabolism, and survival, with the exception of studies on leptin, most adipokines and their functions have been relatively unstudied in wildlife systems. Their putative roles in carnivores and especially in species that undergo large variations in adiposity as part of their natural life-history are not well understood.

Adipose tissue is especially important for marine mammals, homeotherms adapted to living in a thermally challenging aquatic environment. Cetaceans, pinnipeds, and sirenians are the only taxonomic groups in which almost all adipose tissue is present in the form of subcutaneous blubber (Pond, 2011). Many marine mammals are also capital breeders, meaning that foraging and breeding do not occur concurrently. Because these organisms cannot feed while performing energetically demanding behaviors, they are reliant on stored energy in the form of adipose tissue. This makes capital breeders an intriguing study system for adipokines because their fitness is directly dependent on their body condition.

Northern elephant seals (*Mirounga angustirostris*, NES) have a unique life-history during which animals engage in energetically demanding behaviors including lactation and molting, concurrent with prolonged fasting. Natural fasts result in marked depletion of adipose reserves which are then recovered during foraging, creating an informative paradigm to study natural changes in the production of adipokines. Previous investigations have identified an inherent body condition tradeoff in NES. A thick blubber layer represents substantial energy reserves obtained from foraging, while increasing the animal's buoyancy. This forces it to expend more energy during foraging dives (Webb et al., 1998). Locomotor costs during diving are lowest when seals are neutrally buoyant, suggesting a limitation to blubber content while foraging (Adachi et al., 2014). There have also been numerous studies linking body condition to reproductive effort and success, immune function, fasting metabolism, and oxidative stress in NES (Crocker et al., 2001, 2012, 2014a, 2016; Peck et al., 2016; Sharick et al., 2015). In females, body reserves were the most important determinant of reproductive effort and both maternal mass and body composition influenced energy expended during lactation (Crocker et al., 2001). Body condition was also associated with the magnitude of both innate and adaptive immune responses in adult females (Peck et al., 2016). Lastly, the regulation of both lipid and carbohydrate metabolism was influenced by adiposity in both adult female and weaned pup NES (Fowler et al., 2008; Crocker et al., 2014a), suggesting potential regulatory roles for adipose-derived hormones in reproduction, immunity, and fasting metabolism.

There is limited research regarding the function of adipokines in NES, and more broadly pinnipeds and other marine mammals. However, the presence and function of leptin has been investigated in multiple pinnipeds. Contrary to other mammalian studies, no relationship was found between body composition and leptin levels in Antarctic fur seals or NES weaned pups (Arnould et al., 2002; Ortiz et al., 2003; Viscarra et al., 2011a). In most mammals, leptin is involved in production of lung surfactant during fetal development and is expressed in the lungs until birth when excessive surfactant is no longer needed (Torday and Rehan, 2002). However, adult phocid seals require additional lung surfactant to facilitate the re-inflation of their lungs after diving (Falke et al., 1985; Hammond et al., 2005, 2012), suggesting another role for leptin separate from body condition in phocids.

The role of adiponectin has been investigated in gray seal females and pups (Bennett et al., 2015) as well as NES pups (Suzuki et al., 2013). Both studies found a positive relationship between circulating adiponectin and body mass in pups and suggest a potential role for adiponectin in fat accumulation. There was no association between adiponectin and mass in gray seal adults.

A more recent study found that blubber expression of genes encoding adiponectin, adiponectin receptors, and leptin receptors had a negative relationship with body mass in adult female NES sampled during the breeding and molting fasts (Khudyakov et al., 2019). Levels of transcripts encoding leptin receptors, adiponectin, and resistin genes were positively associated with serum cortisol, suggesting that cortisol may be

a driver of production of some circulating adipokines (Khudyakov et al., 2019).

Our objective was to examine changes in circulating levels of adipokines with depletion of adipose reserves during natural fasting, and their relationships to metabolism, immune function, and reproduction. We examined relationships between adipokines and glucose concentrations measured in this study and IL-6 and cortisol data measured in the same animals during a previous study (Peck et al., 2016). As one of the few studies to investigate multiple circulating adipokines concurrently in wildlife, these results provide insights into how body condition exerts influences on systemic physiology in species that naturally undergo large variations in body condition.

2. Material and methods

2.1. Study site and study animals

This study was conducted at Año Nuevo State Park in San Mateo County, California, USA where 196 blood samples were collected from 127 adult female NES between 2011 and 2014. All procedures were performed under NMFS permit #14636 and approved by the UCSC and SSU IACUC. Adult females were sampled at four life-history stages: early breeding season (EB, n = 58), late breeding season (LB, n = 54), early molt (EM, n = 41), and late molt (LM, n = 43). Of these females, 84 were sampled once and the remaining 43 females were sampled 2–4 times (median = 2). EB samples were collected 5 days post-partum and LB samples were collected 22–23 days post-partum, immediately before weaning and female departure. EM samples were collected as close to arrival on the beach as possible (2 ± 2 days) and LM samples were collected after individuals had completed the molt, approximately 30 days after arrival.

2.2. Field procedures

Individuals were sedated with an intramuscular injection of ~ 1 mg kg^{-1} of telazol and immobilization was maintained with intravenous injections of ketamine and valium (all drugs: Fort Dodge, Fort Collins, CO, USA). Blood was collected from the extradural vein using an 18-gauge, 3.25-inch spinal needle and samples were kept chilled until transported to the lab. Samples were centrifuged at 1500 g for 20 min at 4°C and serum and plasma were collected then stored at −80°C until analysis. Individuals were weighed using a tripod, weighing sling, and hanging scale (±2 kg; MSI, Seattle, WA, USA). Adipose reserves were determined using the truncated cones method with 18 ultrasound measurements and corresponding length and girth measurements at various body locations (Gales and Burton, 1987; Crocker et al., 2001). 134 samples were from 82 known age females based on plastic flipper tags applied at weaning.

2.3. Laboratory procedures

Concentrations of adipokines (leptin, adiponectin, resistin, kisspeptin-54) were measured in duplicate in serum samples using commercially available enzyme-linked immunosorbent assays (ELISA) and radioimmunoassays (RIA). All assay kits were validated for use in NES. For each analyte several high concentration samples were pooled and 4–5 serial dilutions were compared to the standard curve for parallelism and linearity of dilution. Parallelism was assessed using log-logit transform. For each adipokine, 4–5 samples were spiked with kit standards and % recovery of added hormone was calculated. Intra-assay CV% were calculated for all samples. Inter-assay CV% was calculated from > 15 samples repeated across plates for each adipokine (Table 1). Glucose was measured in plasma samples in triplicate using a YSI 2300 glucose autoanalyzer (YSI, Yellow Springs, OH). Serum cortisol and IL-6 concentrations were previously measured in samples from the same animals during a different study (Peck et al., 2016).

Table 1
Adipokines analyzed, assay platforms, and validation results.

Adipokine	Leptin	Resistin	Adiponectin	Kisspeptin-54
N	196	183	182	161
Assay Platform	EMD Millipore Multi-Species RIA	RayBio Canine ELISA	SPI Bio Human ELISA	MyBioSource Canine ELISA
Parallelism	yes	yes	yes	yes
Mean intra-assay CV%	3.18	6.18	3.63	4.51
Mean inter-assay CV%	5.06	7.35	4.87	5.89
Validation slope	0.98	1.01	1.02	0.96
Validation R^2	0.99	0.99	0.99	0.99
Recovery %	102 ± 3.1	98 ± 3.7	101 ± 2.9	103 ± 4.2

2.4. Data analysis

Analyses were performed with JMP Pro 14 (SAS Institute, Cary, NC, USA). Changes in adipokine concentration across life-history stages were examined using linear mixed effects (LME) models with animal ID as a random effect and life-history stage as a fixed effect. Model residuals were visually assessed for normality and to confirm model homoscedascity. The response variables were log-transformed if necessary. If significant differences between life-history stages were present, *post hoc* comparisons were performed using Student's t-tests.

To examine the potential effects of body reserves and cortisol on each adipokine, a LME model was fit with mass, adiposity, and cortisol as fixed effects and animal ID as a random effect. Cortisol was included because of the strong association evident with adipokine gene expression in NES (Khudyakov et al., 2019). When significant effects were found, leverage residual plots were used to visualize the effects of the explanatory factors having controlled for the other factors in the model. Mixed model R^2 were calculated for significant explanatory factors using the F statistics derived from the LME models (Edwards et al., 2008). Due to the potential role of adiponectin and resistin in modulating insulin sensitivity and glucose metabolism in model systems, a LME model was used to examine the variation in glucose concentrations with cortisol, adiponectin, and resistin as fixed effects and animal ID as a random effect. To determine the relationship between IL-6 and adipokines, a LME model was used with adipokines as fixed effects and animal ID as a random effect. Because kisspeptin-54 has been shown to change with puberty and reproductive senescence in some species we also examined the effects of female age on kisspeptin-54 in a LME model that included life-history stage as a fixed effect. Data are expressed ± standard error of the mean. Results were considered significant at $p < 0.05$.

Table 2
Mean ± SE of adipokines across life-history stages in NES. EB = early breeding, LB = late breeding, EM = early molt, LM = late molt. Different superscripts denote significant differences in least square means from the LME model between life-history stages.

Adipokine	EB (n = 58)	LB (n = 54)	EM (n = 41)	LM (n = 43)
Leptin (ng mL^{-1})	4.5 ± 0.3^{A}	2.5 ± 0.2^{B}	2.9 ± 0.3^{B}	2.4 ± 0.2^{B}
Resistin (pg mL^{-1})	3888.4 ± 395.8^{A}	4553.3 ± 448.0^{A}	1791.1 ± 162.8^{B}	2226.5 ± 306.0^{B}
Adiponectin (ng mL^{-1})	172.5 ± 8.1^{A}	112.3 ± 3.3^{C}	145.8 ± 7.1^{B}	119.3 ± 4.7^{C}
Kisspeptin-54 (pg mL^{-1})	253.8 ± 14.8^{A}	438.6 ± 15.4^{B}	236.5 ± 15.4^{A}	243.9 ± 19.0^{A}

3. Results

3.1. Adipokines and Life-History Stage, Adiposity, and cortisol

Leptin concentrations were highest during the beginning of the breeding haul-out compared to the other stages (Table 2, Fig. 1, $F3_{,143.3} = 20.28$, $p < 0.0001$). Leptin was positively associated with both mass (Fig. 2A, $R^2 = 0.13$, $F_{1,181.3} = 9.26$, $p = 0.027$) and adiposity (Fig. 2B, $R^2 = 0.22$, $F_{1,179.9} = 16.38$, $p < 0.0001$) at all life-history stages.

Circulating levels of resistin were significantly higher throughout the breeding haul-out compared to the molt haul-out (Table 2, Fig. 1, $F_{3,120.6} = 12.24$, $p < 0.0001$). Resistin was positively associated with both mass (Fig. 3A, $R^2 = 0.10$, $F_{1,178.4} = 6.75$, $p = 0.0102$) and cortisol (Fig. 3B, $R^2 = 0.17$, $F_{1,171.3} = 12.02$, $p = 0.0007$).

Adiponectin varied significantly with life-history stage, with highest levels measured in females sampled during both early fasting periods and decreasing throughout the fasts (Table 2, Fig. 1, $F_{3,158.7} = 21.94$, $p < 0.0001$). Adiponectin had a positive association with adiposity (Fig. 4A, $R^2 = 0.35$, $F_{1,177.6} = 31.33$, $p < 0.0001$) and a weak negative association with cortisol (Fig. 4B, $R^2 = 0.09$, $F_{1,176.4} = 5.99$, $p = 0.0153$).

Circulating levels of kisspeptin-54 were significantly higher during the end of the breeding haul-out compared to all other life-history stages (Table 2, Fig. 1, $F_{3,143.1} = 12.13$, $p < 0.0001$). Kisspeptin-54 was not significantly associated with either mass or adiposity ($p > 0.05$). However, it was positively associated with cortisol (Fig. 5A, $R^2 = 0.24$, $F_{3,160} = 16.85$, $p < 0.0001$). Since both cortisol and kisspeptin-54 increased in late breeding, a LME model with a cortisol × stage interaction was also fitted and determined that the interaction best predicted kisspeptin-54 levels ($F_{3,152.2} = 9.73$, $p < 0.0001$). This interaction revealed that the strong association of cortisol with kisspeptin-54 was only present at LB.

Kisspeptin-54 decreased significantly with age (Fig. 5B, $R^2 = 0.23$, $F_{1,33.36} = 5.02$, $p = 0.0319$). There was no significant relationship between other adipokines and age. Among the adipokines, only leptin was weakly positively associated with adiponectin ($R^2 = 0.05$, $F_{1,137.7} = 8.22$, $p = 0.004$).

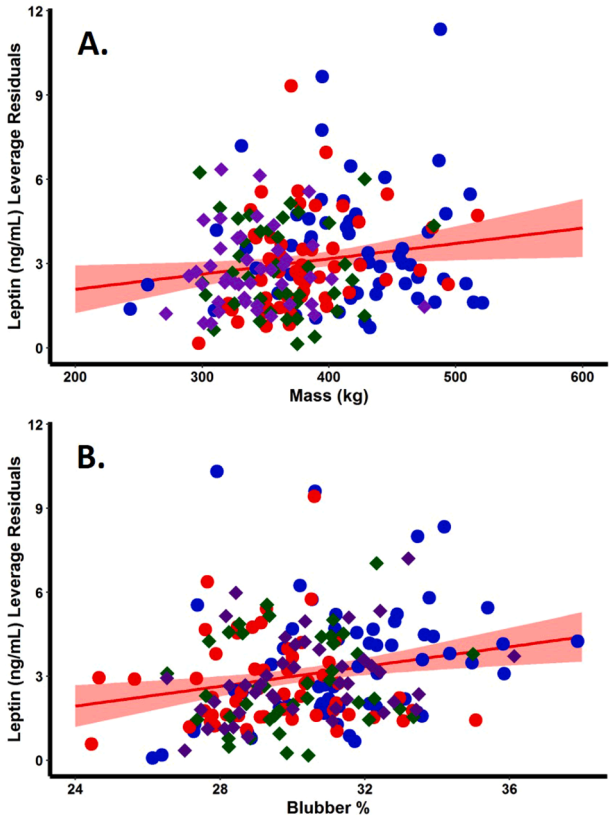

Fig. 2. Leverage plots showing relationships between mass and concentration of leptin (**A**: $R^2 = 0.13$, $F_{1,181.3} = 9.26$, $p = 0.027$) and relationship between percent blubber and leptin in female NES (**B**: $R^2 = 0.22$, $F_{1,179.9} = 16.38$, $p < 0.0001$) in a LME model with mass, blubber, and cortisol as fixed effects. Colors denote breeding stage (blue = early breeding, red = late breeding, green = early molt, purple = late molt).

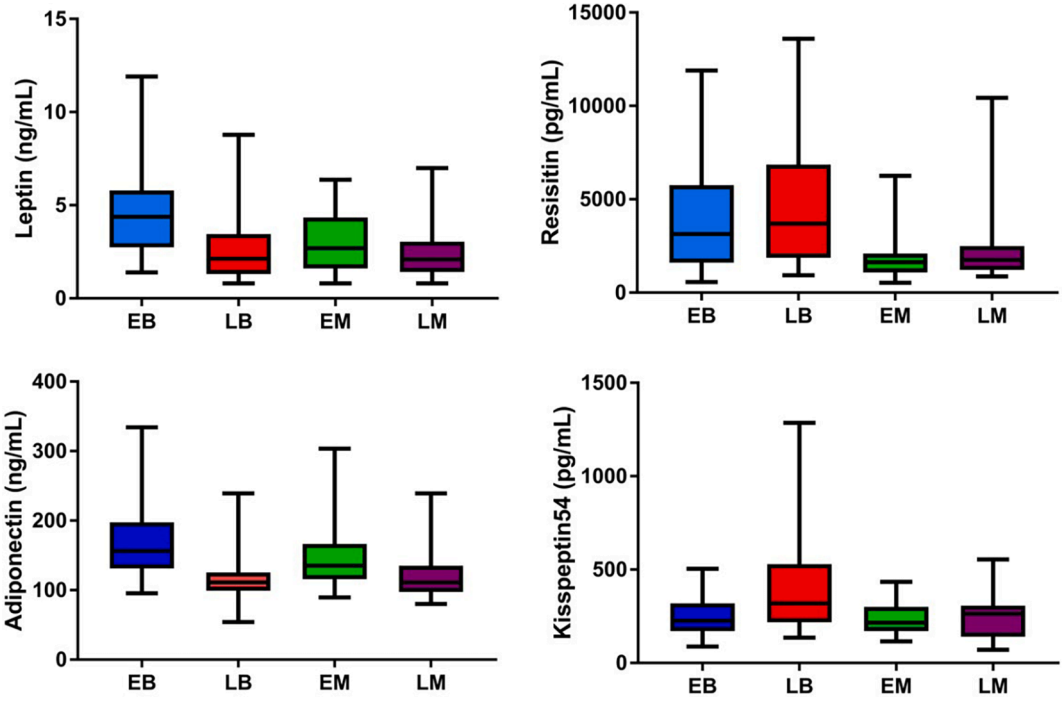

Fig. 1. Box plots of adipokine concentrations across female NES life-history stage. 1st and 3rd quartiles are shown by the box, central line is the median. Whiskers show minimum and maximum values. EB = early breeding, LB = late breeding, EM = early molt, LM = late molt.

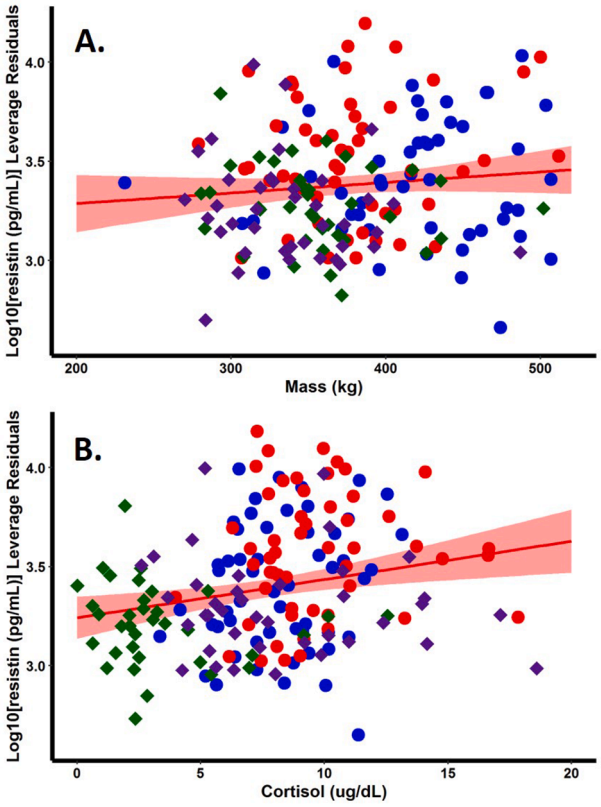

Fig. 3. Leverage plots showing the relationships between mass and concentration of resistin (**A**: $R^2 = 0.10$, $F_{1,178.4} = 6.75$, $p = 0.0102$) and relationship between cortisol and resistin (**B**: $R^2 = 0.17$, $F_{1,171.3} = 12.02$, $p = 0.0007$) in a LME model with mass, blubber, and cortisol as fixed effects. Colors denote breeding stage (blue = early breeding, red = late breeding, green = early molt, purple = late molt).

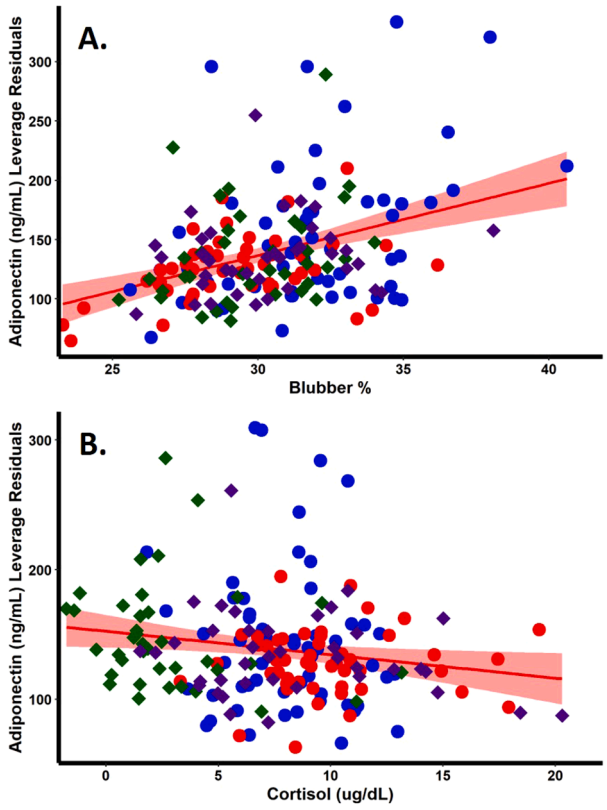

Fig. 4. Leverage plots showing relationships between percent fat and concentration of adiponectin (**A**: $R^2 = 0.35$, $F_{1,177.6} = 31.11$, $p < 0.0001$) and concentration of cortisol and concentration of adiponectin (**B**: $R^2 = 0.09$, $F_{1,176.4} = 5.99$, $p = 0.0153$) in a LME model with mass, blubber, and cortisol as fixed effects. Colors denote breeding stage (blue = early breeding, red = late breeding, green = early molt, purple = late molt).

3.2. Adipokines and immune function and metabolism

Resistin had a significant positive association with IL-6 (Fig. 6, $R^2 = 0.38$, $F_{1,160.2} = 99.11$, $p < 0.0001$). Leptin had a weaker positive association with IL-6 ($R^2 = 0.09$, $F_{1,44.9} = 4.33$, $p = 0.04$). No other adipokines had significant relationships with IL-6 ($p > 0.05$). Adiponectin had a strong negative association with glucose concentrations (Fig. 7A, $R^2 = 0.27$, $F_{1,169.9} = 31.57$, $p < 0.0001$). Cortisol had a positive association with glucose concentrations (Fig. 7B, $R^2 = 0.09$, $F_{1,169.5} = 8.78$, $p = 0.004$). Resistin was not associated with plasma glucose ($p > 0.05$).

4. Discussion

Northern elephant seals provide an informative system for studying adipokines in a free-ranging mammal as they experience natural depletion of adipose reserves due to extended fasting. Our data demonstrate that in female NES, circulating adipokines change over fasting periods and life-history stages and are associated with body mass, body composition, cortisol, levels of circulating glucose, and immune marker IL-6.

Serum leptin concentrations were highest during the beginning of the breeding season, just after females return from their longest foraging trip and prior to the major component of reproductive effort, at which point individuals are typically in their best body condition. Both mass and adiposity were drivers of leptin and this result supports the association between leptin and adiposity found in numerous other mammalian studies. This is the first study to confirm that this relationship exists in NES, as previous studies on other demographic groups (pups and males) did not find a significant relationship between leptin and body composition (Viscarra et al., 2011a, Crocker et al., 2012). Due to significant differences in fasting metabolism between sexes and ages, it is possible that regulation of leptin production in adult females differ from that in males or pups. While blubber mRNA expression of leptin did not change in female NES over fasting, expression of leptin receptor increased across the breeding haul-out (Khudyakov et al. 2019). This suggests a potential mechanism by which seals may maintain tissue sensitivity to leptin as adiposity and leptin production decrease over the course of fasting. This, in turn, may help to sustain the high rates of lipid catabolism characteristic of fasting elephant seals (Reidy and Weber, 2000; Crocker et al., 2014a), as well as maintain immune capacity during colonial haul-outs.

Resistin, although produced by adipocytes, is also produced by immune macrophages residing in adipose tissue (Curat et al., 2006). This adipokine was elevated throughout the breeding haul-out when females are giving birth and nursing in a pathogenic environment. Previous studies have shown that females mount both innate and adaptive immune responses throughout the breeding season (Peck et al., 2016). When macrophages are recruited during an innate immune response, they produce resistin, increasing its circulating levels. The strong positive association between circulating resistin and IL-6 levels in the current study suggests that resistin may play a role in upregulation of the innate inflammatory response in seals, especially during breeding. The positive relationship of resistin with body mass is consistent with the previously described impacts of body reserves on the innate immune response (Peck et al., 2016). Resistin also had a positive relationship with cortisol, possibly due to the fact that IL-6 and inflammation in general have been found to increase circulating cortisol (Steensberg et al., 2003). Resistin gene expression in adipose was also positively

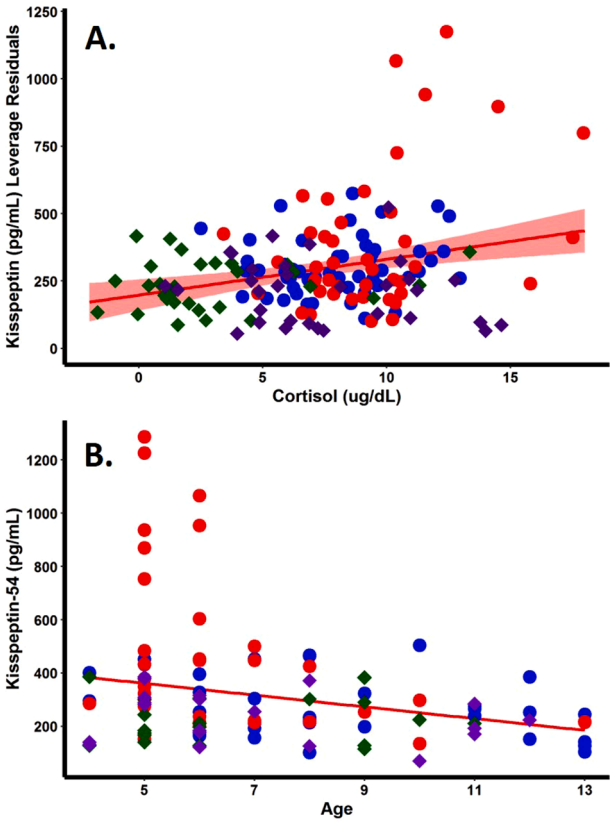

Fig. 5. Leverage plot showing the relationship between concentration of cortisol and concentration of kisspeptin-54 in a LME model with mass, blubber, and cortisol as fixed effects (**A**: $R^2 = 0.24$, $F_{3,160} = 8.25$, $p = 0.0046$). Regression plot showing the relationship between age and concentration of kisspeptin-54 (**B**: $R^2 = 0.23$, $F_{1,33.36} = 5.02$, $p = 0.0319$). Fitted line is from the mixed model parameter estimates. Colors denote breeding stage (blue = early breeding, red = late breeding, green = early molt, purple = late molt).

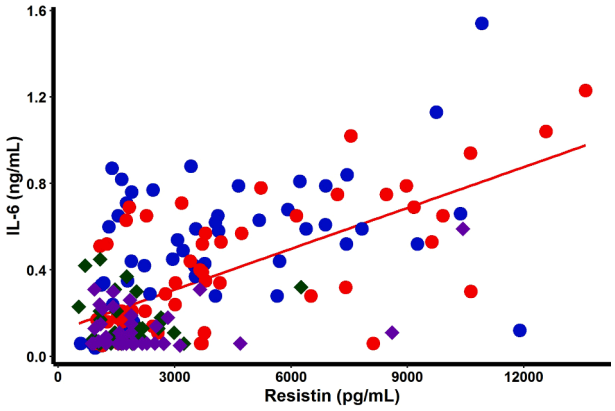

Fig. 6. Relationship between concentration of IL-6 and concentration of resistin ($R^2 = 0.38$, $F_{1,174.3} = 106.39$, $p < 0.0001$). Fitted line is from the mixed model parameter estimates. Colors denote breeding stage (blue = early breeding, red = late breeding, green = early molt, purple = late molt).

correlated with circulating cortisol levels (Khudyakov et al., 2019). While resistin has been shown to promote insulin resistance in biomedical models, we found no relationship between resistin and plasma glucose levels., This suggests resistin may have more of a role in immune function, rather than metabolic regulation in fasting NES. Measuring resistin throughout a glucose tolerance test could help elucidate this relationship.

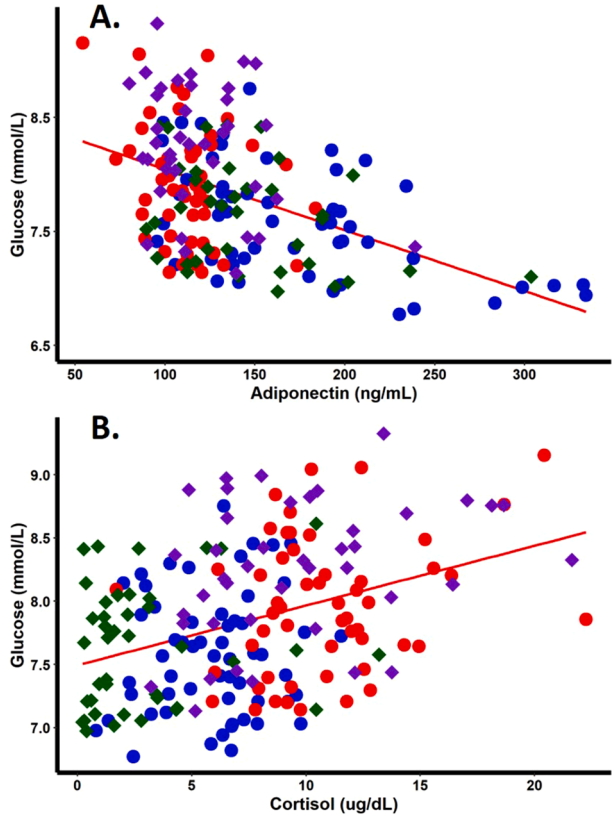

Fig. 7. Relationship between concentration of adiponectin and concentration of glucose (**A**: $R^2 = 0.27$, $F_{1,169.9} = 31.57$, $p < 0.0001$) and concentration of cortisol and concentration of glucose (**B**: $R^2 = 0.09$, $F_{1,169.5} = 8.78$, $p = 0.004$). Fitted lines are from the mixed model parameter estimates. Colors denote breeding stage (blue = early breeding, red = late breeding, green = early molt, purple = late molt).

In both the breeding and molting haul-outs, adiponectin was higher during the beginning of the haul-outs and decreased across each fast, the opposite of what has been observed in most mammals, including humans (Arita et al., 1999; Hotta et al., 2001; Ishioka et al., 2006). However, this positive relationship was also shown in gray seal pups (Bennett et al., 2015) and fasting NES weaned pups (Suzuki et al., 2013). The discrepancy between our data and that in the biomedical literature is consistent with the impact of adiposity on metabolism seen in previous studies on NES. In NES, high adiposity is associated with increased insulin sensitivity and glucose clearance (Fowler et al., 2008) as well as typical gluconeogenic responses to glucagon (Crocker et al., 2014b). NES pups develop insulin insensitivity in adipose as fasting proceeds but retain limited insulin sensitivity in muscle (Viscarra et al., 2013; Suzuki et al., 2013). Further, several studies have suggested that the decoupling of adiposity from adiponectin production in obese humans stems from low production by visceral fat stores that increased with obesity (Drolet et al., 2009). In NES, visceral fat depots are small and the vast majority of adipose stores are subcutaneous (blubber) (Crocker et al., 2014a). Therefore, some of the adipose depot-specific function of adipokines identified in laboratory rodents and obese humans may not be applicable to marine mammals.

In contrast to circulating adiponectin levels, adiponectin gene expression in adipose of female NES increased ~ 1.6 fold over the breeding fast, while blubber content declined ~ 20% over the same period (Khudyakov et al., 2019). A mismatch between circulating adiponectin levels and adiponectin gene expression has been documented in other species, (Stuber et al., 2013) including gray seals (Bennett et al., 2015), and there are several potential explanations for this. Adiponectin gene expression may be increasing to compensate for the decreased

protein production caused by significant weight loss. Alternately, increased gene expression measured at the end of the fast could reflect preparation for the onset of feeding and accompanying metabolic changes after the individual leaves the beach to forage. Lastly, it could be explained by changes in post-translational modification, expression of coactivators or corepressors of adiponectin, or rates of clearance of this adipokine over fasting (Liu and Liu, 2012).

Adiponectin increases insulin sensitivity in many species. Studies in other systems have shown that adiponectin decreases hepatic gluconeogenesis and increases utilization of glucose in skeletal muscle (Dridi and Taouis, 2009). As predicted, increased adiponectin levels were associated with lower glucose levels in this study, which is consistent with the insulin-independent reduction in blood glucose in this species (Viscarra et al., 2011b). The strong negative association of adiponectin levels with glucose suggest it may be important in regulating tissue-specific differences in insulin sensitivity during fasting in NES (Viscarra et al., 2013; Suzuki et al., 2013). The metabolic effects of adiponectin could be modulated by cortisol. We found a negative relationship between circulating levels of adiponectin and cortisol independent of life-history stage. Previous studies have demonstrated that experimentally increasing cortisol leads to increased levels of circulating glucose in fasting NES, suggesting increased gluconeogenesis (Ensminger et al., 2014; Champagne et al., 2015). While an association between cortisol and adiponectin has been described in other species, there is little consensus on the nature of this relationship (Sukumaran et al., 2012). Our findings indicate that production of adiponectin, which may be negatively regulated by cortisol, may play a role in the adipose regulation of sensitivity to insulin and glucagon in elephant seals (Fowler et al., 2008; Crocker et al., 2014b).

Kisspeptin-54 links body composition and reproduction in the biomedical literature, as kisspeptin-54 activation of neurons can trigger ovulation in mammals (Kauffman et al., 2007). Kisspeptin-54 was not associated with leptin or adiposity in NES. Kisspeptin-54 significantly increased at the end of the breeding haul-out when mating occurs. Onset of behavioral estrous suggests that female NES ovulation occurs during late breeding season (Le Boeuf, 1972), suggesting that kisspeptin may regulate ovulation in this species as well.

Neither adiposity nor body mass had any significant effect on kisspeptin-54 and in contrast to biomedical studies there was no relationship between kisspeptin-54 and leptin. Cortisol and kisspeptin-54 concentrations at late breeding were high and variable. Previous studies have shown that cortisol levels increase as lactation progresses (Crocker et al., 2014a). Cortisol was found to have a significant association with kisspeptin-54 but only at the end of lactation, suggesting that the high cortisol at the end of the breeding season could be related to the timing of the onset of estrous via interaction with kisspeptin-54.

Kisspeptin-54 was also inversely associated with age, with younger females having higher circulating levels of the adipokine. In both primates and rats, kisspeptin levels increase before the onset of puberty but as they reach middle age, reduced kisspeptin gene expression and changes in circulating levels have been suggested to contribute to reproductive decline (Funes et al., 2003; de Roux et al., 2003; Mayer et al., 2010; Lederman et al., 2010; Kriegsfeld, 2013). While reproductive senescence is not evident in elephant seals, this change may be important in influencing the age of primiparity.

Adipokines in adult female NES were associated with numerous markers of important physiological processes, suggesting potential important regulatory roles in mediating transitions between feeding and fasting metabolism and linking energy status with the immune and reproductive axes. The regulation of processes like glucose metabolism and insulin sensitivity are critical to capital breeders where an individual's fitness depends on its ability to manage energy stores and avoid lean tissue catabolism during extended fasts. These data provide insights into how adipokine function differs in wildlife systems compared to biomedical models. In this capital breeding marine mammal system, resistin was mainly associated with a marker of the innate immune response and showed no relationship with glucose levels, and thus potentially insulin sensitivity. Adiponectin was positively associated with adiposity and circulating kisspeptin-54 levels were not associated with circulating leptin levels, key differences from biomedical studies. Adipose stores are frequently used as a measure of condition and are especially important for species that use stored body reserves for reproduction. Our findings demonstrate that adipokines are important to understanding body condition and it's link to physiological function in a mammal adapted for extended high energy fasting.

CRediT authorship contribution statement

Caroline L. Rzucidlo: Conceptualization, Investigation, Writing - original draft. **Emily S. Sperou:** Investigation. **Rachel R. Holser:** Resources, Visualization. **Jane I. Khudyakov:** Investigation, Resources. **Daniel P. Costa:** Funding acquisition, Resources. **Daniel E. Crocker:** Funding acquisition, Supervision, Resources, Writing - review & editing.

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