Aquatic Mammals 2020, *46*(3), 243-253, DOI 10.1578/AM.46.3.2020.243

Insulin and Blubber Deposition in Rehabilitating Harbor Seal (*Phoca vitulina*) Pups

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Abstract

Seals and sea lions (pinnipeds) go through naturally occurring stages of nutrient restriction alternating with rapid weight gain. Recent work has focused on how pinnipeds manage fuel stores during periods of fasting, whereas the physiological management of weight gain has been less well-studied. Pinnipeds undergoing rehabilitation were used in a controlled setting to investigate the relationship of an important metabolic hormone, insulin, to mass gain and lipid deposition. Over two seasons, 16 rehabilitating harbor seal (*Phoca vitulina*) pups were monitored as they were fed a steady caloric intake. Plasma insulin levels, mass gain, and blubber deposition were measured over the 8-week rehabilitation period. Plasma insulin levels were low but increased significantly across the sampling stage and were positively related to blubber deposition and overall mass gain. These results indicate that despite low circulating levels, insulin may play a lipogenic role in seals and that insulin sensitivity may be an important labile physiological variable affecting lipid metabolism during different life history phases. Quantifying insulin sensitivity in blubber as well as additional mediators of lipogenesis such as adiponectin during rapid lipid accumulation will further elucidate the mechanisms by which pinnipeds modulate the relationship between insulin and lipid metabolism.

Key Words: lipid metabolism, insulin, harbor seal, *Phoca vitulina*

Introduction

Appropriately managing fuel stores is critical for all animals. Storing appropriate fuel and then using it in an efficient manner has the potential to impact fitness (Young, 1976). Seals and sea lions (collectively termed *pinnipeds*) experience periods of nutrient restriction (fasting) alternating with rapid weight gain (Champagne et al., 2012) which necessitates close management of ingested fuel and energy stores.

Fuel types can be broken into three general categories—(1) carbohydrates, (2) protein, and (3) lipids (Frayn, 2010)—and each has different properties, requiring different physiological processes to metabolize. Pinnipeds are adept at sparing protein when fasting (Adams et al., 1991; Crocker et al., 1998; Houser & Costa, 2001; Houser et al., 2012; Kelso et al., 2012). Lipid powers the vast majority of pinniped metabolism during periods of fasting (Crocker et al., 2001, 2012; Bennett et al., 2007; Houser et al., 2012; Kelso et al., 2012), representing a very important fuel. Pinnipeds consume very little carbohydrate in their diet, either when foraging as juveniles/adults or as nursing pups (Oftedal, 1993) and, thus, insulin would not play a large role in glucose disposal from dietary input. However, insulin has many other important functions, including effects on lipid metabolism. Phocids (seals) and otariids (fur seals and sea lions) both develop blubber layers of varying thickness. The importance of this blubber layer is 2-fold: (1) it has a thermoregulatory function, and (2) it provides a fuel reserve (Noren & Mangel, 2004). Insulin is a potent stimulator of fat deposition and an inhibitor of lipolysis (Kraemer et al., 1998). In human models, insulin stimulates hepatic lipogenesis from glucose precursors. In adipocytes, lipogenesis is facilitated by insulin through upregulation of transcription factors for lipogenic enzymes (e.g., SREBP-1 and PPARγ; Kersten, 2001; Ito et al., 2013). Despite the lack of carbohydrate in the diet of most marine mammals, insulin may be an important regulator of both lipid deposition and catabolism in pinnipeds.

Most studies to date have focused on fasting phocids (notably grey seals [*Halichoerus grypus*] and northern elephant seals [*Mirounga angustirostris*]), which are mobilizing lipid. Insulin levels are low and drop even further during fasting (Bennett et al., 2015), likely facilitating lipolysis (Fowler

et al., 2008, 2016) in these species. Fasting juvenile elephant seals appear to modulate insulin sensitivity on a tissue-specific basis. Circulating insulin concentration decreases later in the fast, and adipose tissue remains insensitive to this hormone. However, when late fasting seals were administered exogenous insulin, muscle tissue displayed intracellular insulin signaling, indicating maintenance of skeletal muscle insulin sensitivity (Viscarra et al., 2013). Due to insulin's importance in lipogenesis in other mammals, insulin's lipogenic properties would presumably be beneficial to promoting blubber deposition in developing pinnipeds (e.g., in young before weaning as well as in older individuals maintaining a blubber layer). In studies addressing insulin and fuel metabolism in pinnipeds, it appears that different life history stages can modulate insulin secretion and tissue sensitivity (Fowler et al., 2008, 2016, 2018; Viscarra et al., 2011b, 2013; Crocker et al., 2014a, 2014b). Adipose tissue cultures in grey seals suggest that glucocorticoids can have complex effects on lipolysis and lipogenesis. The amount of lipolysis relative to lipogenesis varies by sex, nutritional state, and insulin level and sensitivity (Bennett et al., 2017).

There are currently little data regarding the direct relationship between insulin and blubber deposition in phocids (seals). Our knowledge of fuel metabolism during fasting in pinnipeds has grown, but our understanding of changes in insulin release and/or sensitivity between fasting and feeding is limited. In suckling grey seal pups, circulating insulin concentrations are generally low but still higher than in fasting weaned pups (Bennett et al., 2013). Insulin was associated with an increase in mass gain, but blubber depth was not quantified (Bennett et al., 2015). To our knowledge, insulin sensitivity in blubber tissue has not been quantified in suckling phocids. Thus, much remains to be learned about the dynamics of insulin in feeding vs fasting and the regulation of fuel stores in pinnipeds.

To understand how seals and sea lions manage their fuel stores, it is important to understand the physiological processes regulating blubber deposition. We hypothesized that insulin is a positive regulator of mass gain and blubber deposition. To test this, we repeatedly sampled juvenile harbor seals (*Phoca vitulina*) in a controlled environment with access to adequate nutrition. The longitudinal sampling of rehabilitating harbor seals is compared to adult resident harbor seals and California sea lions (*Zalophus californianus*). Pinnipeds in a rehabilitation or aquarium setting are a good model to further our understanding as nutritional variability can be controlled, thereby removing confounding effects of differing food availability or quality.

Methods

Subjects

Animal samples were obtained from the Mystic Aquarium in Mystic, Connecticut (USA). Permission for sample transfer was obtained from the National Marine Fisheries Service (NMFS) Greater Atlantic Region Marine Mammal Stranding Network. Primary data collection was performed on rehabilitating harbor seal pups. For comparison, adult resident harbor seals and California sea lions were sampled opportunistically over several years, whereas the rehabilitating harbor seal pups were sampled in longitudinal fashion over 8 wks in two different years (2016: $n = 10$ seals; 2017: $n = 11$ seals).

Harbor seal pups were brought in to the Mystic Aquarium rehabilitation program and sampled as part of the routine rehabilitation program to facilitate release back into the wild. Pups were estimated to be 5 to 10 d old upon admission. Pups were released into the wild after determination by the veterinarian that they were healthy by both physical exam and diagnostic testing; that they had a minimum body condition of 20% (as calculated by the animals' weight/length, multiplied by 100); and that they were off systemic medications for no less than 2 wks. Rehabilitating seals were maintained on a diet of formula consisting of Zoologic Milk Matrix, water, and salmon oil. This formula also contained various supplements (multivitamin, fortiflora probiotic, lactase enzyme tablet, and soy lecithin). Pups were blood sampled as part of routine health screenings by veterinarians. Blood samples were obtained from the extradural vein and were taken ~30 min prior to their first meal of the day. Mass and blubber depth were also measured at the time of the blood sampling. Mass was measured on an SR Instruments' SRV712 digital scale, and blubber depth was measured with the GE Logiqbook ultrasound from the left caudal axillary region.

As part of routine blood work checkups, rehabilitating harbor seal pups were blood sampled at Weeks 1, 4, and 8, hereafter referred to as Stages 1, 2, and 3, respectively. Two seals were sampled at Week 9 instead of Week 8, but their mass gain was lower than the average mass gain of the other seals sampled at Week 8. Thus, since they would not be disproportionately increasing mass (because they had more time to gain weight), they were included as Stage 3 samples. For some individual seals, samples from all stages were unavailable; sample size for each stage is noted in Table 1.

Resident, adult harbor seals and California sea lions were maintained on a diet of capelin, herring, squid, lake smelt, sardines, and anchovies. Blood samples were obtained from the plantar plexus spun at 4,000 rpms for 20 min in

Table 1. Insulin, mass, and blubber thickness in rehabilitating harbor seals (*Phoca vitulina*) across sample weeks (stages) and year

	Mean insulin ($ng \cdot ml^{-1}$) (SD)	Mean blubber thickness (cm) (SD) 2016	Mean blubber thickness (cm) (SD) 2017	Mean mass (kg) (SD) 2016	Mean mass (kg) (SD) 2017
Stage 1 (Week 1)	0.27 (0.05) n= 20[a]	0.14 (0.04) n = 7[a#]	0.61 (0.13) n = 2[a#]	8.1 (1.4)[a]	8.5 (1.2)[a]
Stage 2 (Week 4)	0.34 (0.1) n = 18[b]	0.26 (0.14) n = 8[b#]	0.73 (0.15) n = 9[b#]	9.9 (1.3)[b]	11.2 (1.8)[b]
Stage 3 (Week 8)	0.41 (0.08) n = 16[c]	0.44 (0.07) n = 8[c#]	1.49 (0.4) n = 8[c#]	12.7 (1.7)[c#]	17.4 (2.4)[c#]

SD = standard deviation; # = significant difference between 2016 and 2017; and within columns, different superscript letters = significantly different

a centrifuge (Fisherbrand Model #225A; Fisher Scientific, Hampshire, NH, USA). The serum was then removed and frozen at -80ºC. Samples were transported on dry ice to Springfield College and maintained at -80ºC until the time of assay. Adult resident harbor seal and California sea lion sampling was opportunistic; therefore, at times there were years between samples, and insulin was measured in banked serum samples. Two adult male and two adult female harbor seals were sampled between 2013 and 2016 (total samples = 8). Four adult female sea lions and one adult male were sampled between 2009 and 2015 (total samples = 9). Mass is available for these harbor seals but not blubber depth. Due to these limitations, we report seasonal trends and correlations only between insulin and mass.

Sample Analysis

Insulin was quantified via the Crystal Chem Ultra Sensitive Rat Insulin ELISA kit (Catalog #90060). This kit has been previously validated for grey seals (Bennett et al., 2015). We performed a validation with pooled harbor seal and pooled California sea lion serum. The assay demonstrated parallelism of the standard curve to both serially diluted harbor seal and serially diluted sea lion serum samples. The standard curve is $R^2 = 0.99$, y - 0.0764x - 0.00295. Serially diluted sea lion plasma is $R^2 = 0.99$, y - 0.0765x - 0.00298, and serially diluted harbor seal plasma is $R^2 = 0.99$, y - 0.0757x - 0.00292 (data not shown). The mean intra-assay %CV was 11.6%, and the inter-assay %CV was 4.9%.

Statistical Analyses

Statistical analyses were performed in *R*, Version 3.5.1. For rehabilitating harbor seal pups, differences due to sex and year within stage (week) were assessed with *t* tests. Relationships between variables within a stage were assessed with linear regression. Mass and blubber were $\log_{10}$ transformed to attain normality. Linear mixed effects models in the 'nlme' package (Pinheiro et al., 2009) were used to assess relationships across stages, both within and between variables. When changes across stages were assessed, Tukey's post-hoc test was applied following a significant result, comparing the means of the groups and providing an adjusted p value using the 'multcomp' package (Hothorn et al., 2008). Insulin, mass, and blubber all increased over time. Including "sampling stage" as a fixed effect removes all explanatory power because "stage" explains the vast majority of the changes in blubber and mass across time (i.e., mass and blubber are increasing, but the explanatory power of insulin in this relationship is swamped by the variance due to temporal changes if stage is included). Instead of including "stage" as a fixed effect, we chose to instead account for the autocorrelation of temporal samples and the repeated, longitudinal nature of the data with a mixed effects model with "seal ID" as random and a compound symmetry covariance structure. We applied the AR1 correction for autocorrelation. We also explore options of changes in variables between stages as a separate variable (e.g., Δ insulin, Δ mass, and Δ blubber depth) with linear regression. We note that there is substantial overlap between variables among the stages (we are not connecting three completely disparate groups of data over time), and individual differences that are not related to the explanatory variables would be accounted for by the random ID effect and would not be included in the slope estimate.

Adult harbor seal and California sea lion samples were spread out across several years and seasons. Due to the low samples sizes, we were unable to test for differences between seasons. Thus, a similar linear mixed effects model was run, testing for the relationship between insulin and mass, with "individual" as random (due to repeated sampling among some individuals across

years). Marginal R^2 (mR^2) for the mixed effects models were extracted with the 'piecewiseSEM' package (Lefcheck, 2016).

Results

Rehabilitating Harbor Seal Pups: Inter-Annual and Inter-Stage Differences

There were no differences in any of the variables (mass, body composition, and insulin) due to sex (either within or across stages). Initial mass was not different between years, but blubber depth and insulin were: seals in 2017 started with higher insulin levels—0.29 ± 0.04 ng·ml^{-1} vs 0.23 ± 0.05 ng·ml^{-1} (t = -2.8, df = 10.5, p = 0.02). Although baseline insulin was different between years, when all stages were assessed together and year was included in a mixed effects model with "seal ID" as random, year was not significant. Insulin was significantly different across all stages ($F_{2, 31}$ = 14.8, p < 0.001; Table 1). The change in insulin across the sampling stage was not significant between years (Table 2).

Average blubber depth was different between years (t = -8.6, df = 39.7, p < 0.001), with seals in 2017 starting with thicker blubber (t = -7.9, df = 2.3, p = 0.01; Table 1), as well as gaining more overall (t = -3.9, df = 7.7, p = 0.005; Table 2). When blubber was assessed relative to sampling weeks, each stage differed between years, with 2017 consistently emerging with thicker blubber (Table 1). Average mass between 2016 and 2017 was equal. When the change in mass across the sampling stages was assessed relative to year, seals in 2017 gained more (t = -3.7, df = 25.4, p = 0.001; Table 2). There was a significant year by sampling stage interaction for mass. Thus, mass was separated by year to look at differences between sampling stages. In 2016, mass was significantly different among sampling stages ($F_{2,13}$ = 95.4, p < 0.001). Similarly, in 2017, mass was significantly different among sampling stages ($F_{2,16}$ = 180.6, p < 0.001; Table 1).

Within each stage (sampling time 1, 2, or 3), a linear model was used to explore associations between insulin levels and blubber or mass, and "year" was included as a covariate due to the above described differences between years. No significant relationships within any stage were found. The change in insulin between subsequent stages (Δ) was not related to either the change in blubber or mass (p > 0.05; Table 2).The overall change in insulin across all sampling stages was also not predictive of blubber or mass (p > 0.05).

Insulin Affects Mass and Body Condition in Rehabilitating Harbor Seals

We included "insulin" and "year" as fixed effects in the model predicting blubber, with "seal ID" as random and temporal autocorrelation due to the sample sequence correction included. There was no interaction between insulin and year, so the interaction term was removed. Insulin was significantly associated with blubber depth ($F_{(1,22)}$ = 8.8, mR^2 = 0.69, p = 0.0069; Figure 1). Using the same model construction as above, only with "mass" as the response variable ("insulin" and "year" as fixed effects, "seal ID" as random, and sample sequence temporal autocorrelation accounted for), we found that the year X insulin was a significant interaction and so kept it in the model. Insulin was a significant predictor of mass ($F_{(1,31)}$ = 6.1, mR^2 = 0.43, p < 0.02; Figure 2).

Comparison Between Rehabilitating Seals and Adult Resident Seals and Sea Lions

Mass varied considerably across the years, even with repeated individuals. The mean mass across all adult harbor seals was 40 ± 9.9 kg, with one seal fluctuating 15 kg from the fall of 2013 to the spring of 2014, and another individual gaining 26 kg over 21 mo. Mean insulin for adult harbor seals was 0.44 ± 0.15 ng·ml^{-1}, comparable to the mean insulin values for the Stage 3 rehabilitating harbor seal pups (0.41 ± 0.08 ng·ml^{-1}). A linear mixed effects model, with "individual" as random, revealed a positive relationship between mass and insulin level ($F_{(1,3)}$ = 12.2, mR^2 = 0.64, p = 0.04; Figure 3).

California sea lion mass likewise fluctuated between years, with mean mass 55.4 ± 23.1 kg over the sampling time frame from 2009 to 2015.

Table 2. Mean change (Δ) in mass, blubber depth, and insulin in rehabilitating harbor seals across the sampling stage by year

	2016	2017	
Mass (kg)	2.47 (0.92)^a	4.51 (2.0)^b	t = -3.7, p = 0.001
Blubber (cm)	0.14 (0.13)^a	0.84 (0.51)^b	t = -3.9, p = 0.005
Insulin (ng·ml^{-1})	0.07 (0.15)	0.1 (0.14)	NS

Note: Different superscripts within rows = significantly different; p < 0.05; and NS = not significant

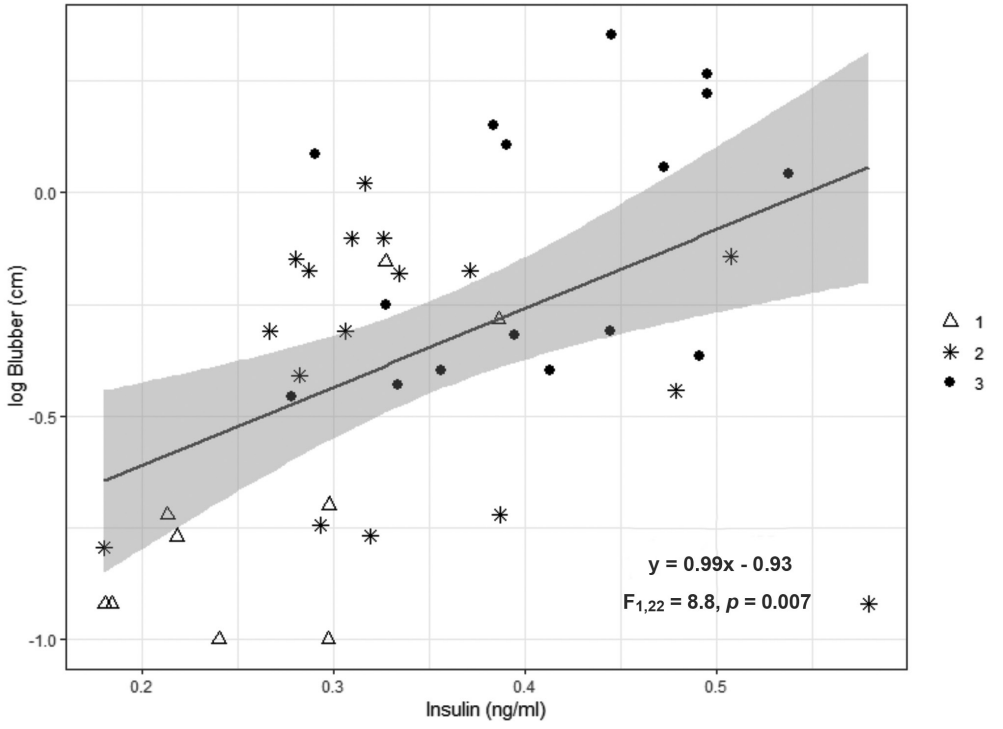

Figure 1. Insulin levels are positively related to blubber depth in rehabilitating harbor seals (*Phoca vitulina*) over 8 wks of sampling. Numbers correspond to sampling stages 1, 2, and 3. See "Methods" for description of weekly time scale for sample stage.

One sea lion sampled three times in this time frame increased from 29 kg in 2009 to 70 kg in 2015. Mean insulin was 0.45 ± 0.12 ng·ml^{-1}. While the mean insulin values were very similar to the adult harbor seal values, the relationship between insulin and mass was not significant in adult California sea lions ($p > 0.05$).

Discussion

Mass gain and blubber depth across the 8-wk rehabilitation period varied with increasing insulin. Insulin levels were quite low but increased significantly across the sampling weeks. Mass and blubber thickness varied between years, possibly reflecting differences in the pups prior to stranding. We included year in our analysis to control for this annual variation. However, despite different mass and blubber depth between years, the overall relationship between insulin and blubber and mass over the rehabilitation period remained. Comparison to adult feeding harbor seals showed similar insulin values to the final sampling of the suckling pups as well as a similar positive relationship between circulating insulin and mass gain. Adult California sea lion insulin values were in a comparable range to adult harbor seal insulin values, but the lack of relationship to mass gain contrasted with the harbor seals and may be an area for more specific investigation in a future study.

These results indicate that despite low circulating levels, insulin may play a lipogenic role in phocid seals. Insulin values reported for grey seal pups range from 0.28 to 0.51 ng·ml^{-1} (Bennett et al., 2013). A similar study (Bennett et al., 2015) citing the same assay reports values as high as ~2.3 ng·ml^{-1} in suckling grey seal pups. In comparison, in this study, insulin ranged from 0.18 to 0.58 ng·ml^{-1}. The 2015 study in grey seals is the only prior study to demonstrate an increase in insulin for feeding seals that is related to mass gain. Our results and conclusions concur with the assertion of Bennett et al. (2015) that circulating insulin and blubber insulin sensitivity are likely important to adipogenesis in growing pinnipeds. Insulin is often thought of as being secreted only in response to a glucose load, but high fat loads (Cen et al., 2016), as well as amino acids (Newsholme et al., 2007), can also stimulate insulin release. Thus, the nutritional signal of high

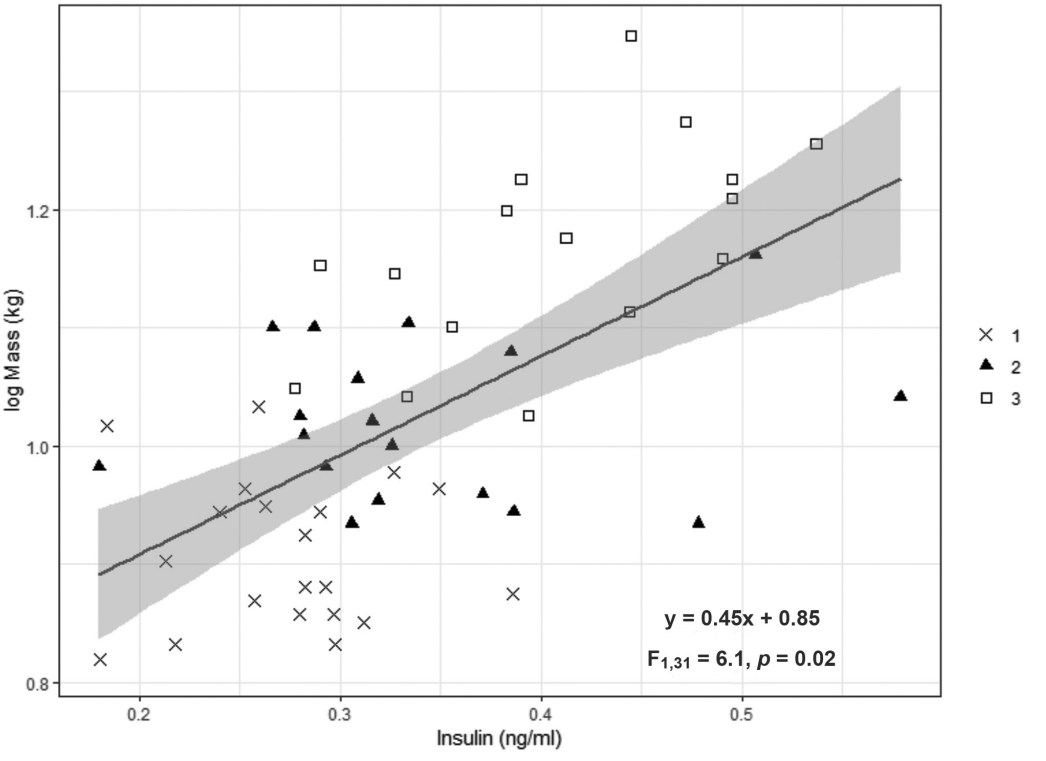

Figure 2. Insulin levels are positively related to mass in rehabilitating harbor seals over 8 wks of sampling. Numbers correspond to sampling stages 1, 2, and 3. See "Methods" for description of weekly time scale for sample stage.

fat, high protein milk (Crocker et al., 2001) does appear to stimulate insulin release from feeding seal pup pancreatic islets increasingly over time. While insulin signaling activity at the tissue level is not known for suckling phocid pups, and circulating levels are low, insulin is related to mass gain and blubber deposition. The molecular signaling mechanisms linking insulin release and adipose insulin signaling require further investigation.

Both pancreatic function as well as cellular response to circulating insulin are important for understanding metabolic changes to insulin. Most research in insulin secretion and sensitivity in marine mammals has been conducted in fasting pinnipeds. For example, in northern elephant seals, the pancreatic response to an exogenous glucose load decreases during fasting (Fowler et al., 2008; Viscarra et al., 2011b), suggesting that insulin release from the pancreas varies with nutritional state. Administration of exogenous glucagon in both fasting, lactating adult females and fasting, weaned elephant seals stimulated insulin release, indicating that the pancreatic islets produce insulin due to a stimulus other than glucose (Crocker et al., 2014b). This response varied by adiposity, suggesting that although the pancreas remains capable of releasing insulin, it is less responsive with lower fat stores, and glucose failed to stimulate insulin secretion at later fasting stages (Fowler et al., 2008; Viscarra et al., 2011a). Further, adipocyte-derived signaling factors may influence the responsiveness of the pancreas (Crocker et al., 2014b). While detailed insulin secretion data in feeding adult seals is not published, and much remains to be learned about the regulation of pancreatic function, it appears that insulin does play a role in depositing lipid.

Insulin has many physiological roles. Although pinnipeds consume minimal carbohydrates, several life stages have been shown to have high plasma glucose as well as high endogenous glucose production (EGP) (Nordøy & Blix, 1991; Champagne et al., 2005, 2006; Houser et al., 2012). EGP may provide a mechanism to provide glucose to glucose-dependent tissues, as well as a mechanism to process high levels of fatty acid oxidation while minimizing ketosis (Houser et al., 2012). EGP in suckling pups has not been quantified, but the rate-limiting gluconeogenic enzyme (Glucose-6-Phosphatase [G6Pase]) is elevated in suckling grey seal pup hepatocytes (the liver being

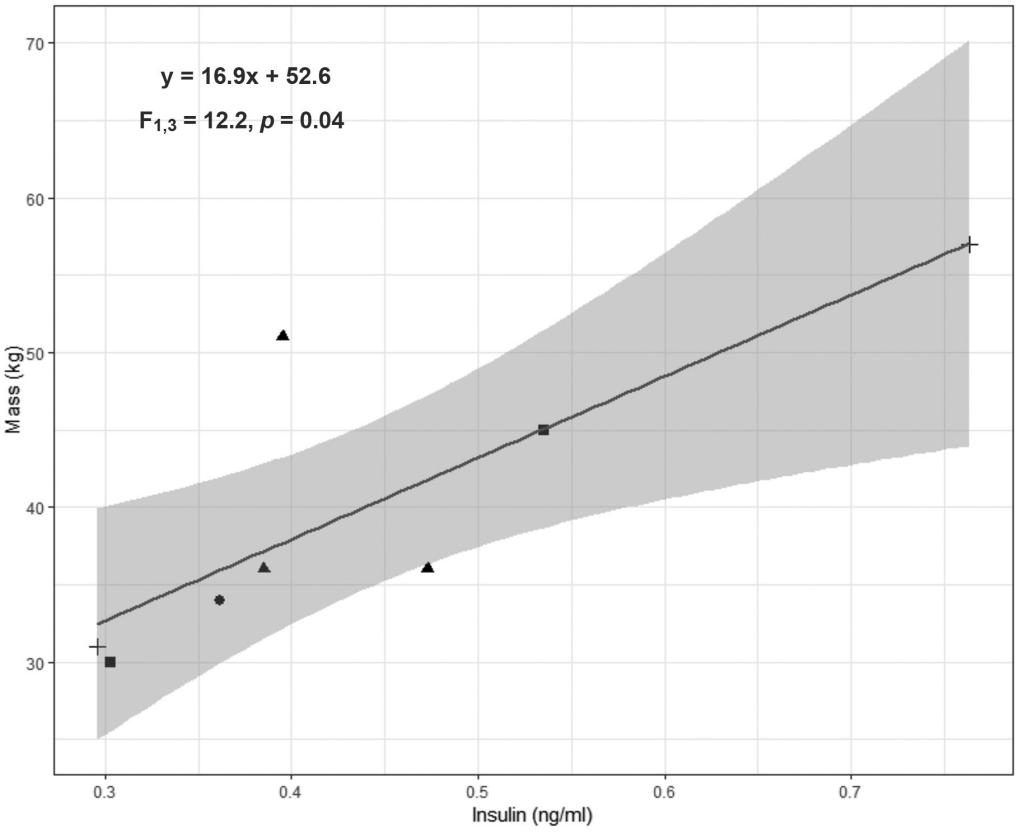

Figure 3. Adult resident harbor seal insulin is positively related to mass gain over 3 y. Different symbols represent the four different individual seals.

the primary site of gluconeogenesis; Bennett et al., 2013), suggesting the potential for high EGP. Insulin suppresses EGP (Bavenholm et al., 2001). If high EGP does facilitate the ability to process elevated fatty acid oxidation (Houser et al., 2012) and is, in fact, high in suckling pups to support the fat metabolism necessary during milk consumption, then maintaining relatively low circulating insulin would facilitate this. For insulin to function in lipogenesis in adipocytes, then, a change in insulin sensitivity of blubber could account for large gains in blubber thickness in the face of low circulating insulin. Tissue-level changes in insulin sensitivity have been documented in fasting northern elephant seal weanlings (Viscarra et al., 2013) and may vary in suckling pups as well.

Although insulin resistance in modern human populations is associated with a host of pathological conditions (e.g., metabolic syndrome and type 2 diabetes), in an evolutionary sense, insulin resistance may have once provided a benefit (Houser et al., 2013; Tsatsoulis et al., 2013). Insulin resistance promotes mobilization of lipid stores and direction of glucose stores to nervous tissue—metabolic aspects that would provide a selective advantage in times of stress or nutrient deficiency. In animals that routinely undergo periods of nutrient restriction, such as pinnipeds, this aspect would allow them to mobilize and manage lipid and glucose stores during fasting. Human pathological conditions likely stem from the dysfunction of excess lipid stores, with multiple signaling molecules disrupted, resulting in inflammation and insulin resistant pathologies (Tsatsoulis et al., 2013).

The lipid storage and mobilization mechanisms in pinnipeds are likely contributing factors to the difference in pathological states between pinnipeds and humans. Although pinnipeds store considerable fat, often in excess of humans, the location of the fat storage in primarily subcutaneous depots may contribute to the lack of pathologies observed in "obese" pinnipeds (Houser et al., 2013). Thyroid hormone signaling effects on

lipolysis also appear to diverge from human pathological models (Martinez et al., 2017a, 2017b).

The rehabilitation and resident status of the subjects in this study allowed us to control nutritional intake and eliminate environmental variability in caloric availability that would be found in the wild. There are several factors that would improve this study. As always, an increased sample size would be beneficial, especially for the adult pinnipeds maintaining their mass rather than growing. Additionally, our study was observational, not experimental, and we recognize that our findings are correlative. More molecular insulin signaling data are necessary for a clearer picture of tissue response to insulin and to characterize the mechanisms that mediate lipogenesis in response to insulin.

The results of this study suggest many exciting avenues to investigate further. It will be important to gather data on adipose tissue insulin signaling or receptor density relative to circulating insulin and blubber deposition to fully understand the relationship between blubber deposition and insulin action. The measurement of additional hormones could also be informative as cortisol was associated with lipogenesis in blubber explants of grey seals (Bennett et al., 2017). Transcriptomic and metabolomic research in fasting seals has identified several patterns of genes and metabolites associated with lipogenesis, insulin signaling, and ketone disposal (Khudyakov et al., 2015, 2017; Martinez et al., 2017b; Olmstead et al., 2017). The application of these techniques to feeding seals would improve our understanding of insulin's involvement in lipogenesis.

Adipokines (signaling molecules released from adipose tissue) represent additional candidates for modulation of the insulin/lipid relationship in phocids during fasting/feeding. An adipokine called adiponectin is typically associated with increased insulin sensitivity and is inversely related to fat mass (Hotta et al., 2001; Okamoto et al., 2008). Interestingly, in both fasting elephant seal pups and suckling grey seal pups, plasma adiponectin decreases with both increasing and decreasing fat mass (Viscarra et al., 2011a, 2011b, 2012; Suzuki et al., 2013; Bennett et al., 2015), and there may be a disconnect between circulating levels and the potential insulin sensitizing effects on the adipocyte locally (Bennett et al., 2015). These local changes and cell-specific sensitivity may facilitate insulin stimulation of lipid deposition even in the face of low circulating levels of insulin. Further investigation of the relationship between tissue-level insulin sensitivity and signaling, as well as the relationship to local regulation by adipokines, will be important as we continue to understand how phocids modulate rapid changes in lipid metabolism.

Conclusion

Circulating insulin is positively associated with blubber deposition and mass gain in suckling harbor seal pups. This is an important aspect of fuel metabolism. Understanding the acquisition of fuel through a foraging ecology is one aspect of clear concern as we seek to manage human interactions with wildlife (Robinson et al., 2012; Harrison et al., 2018). Knowledge about the metabolic processing and the physiological implications of that fuel is important to link foraging ecology and physiology, and to fully understand organisms in their environment. Additionally, the metabolism of fuel, particularly the deposition and mobilization of lipid, has important links to how animals process toxins and contaminants (Ross, 2000; Regnier & Sargis, 2014; Weijs & Zaccaroni, 2016) and how stress may affect their ability to manage and maintain fuel reserves (Khudyakov et al., 2017). Further understanding how animals manage their fuel stores is a valuable tool as we seek to understand the connection between their foraging ecology and how they physiologically process the food they acquire.

Acknowledgments

We would like to thank the husbandry staff at Mystic Aquarium for their hard work and efficiency. Additional thanks to S. Zagarins and C. Champagne for helpful comments throughout the analysis and writing process. Springfield College provided funding for the assays. This constitutes scientific manuscript contribution No. 307 to the Sea Research Foundation, Inc.

Literature Cited

Adams, N. J., Abrams, R. W., Siegfried, W. R., Nagy, K. A., & Kaplan, I. R. (1991). Energy expenditure and food consumption by breeding Cape gannets *Morus capensis*. *Marine Ecology Progress Series*, *70*, 1-9. https://doi.org/10.3354/meps070001

Bavenholm, P. N., Pigon, J., Ostenson, C-G., & Efendic, S. (2001). Insulin sensitivity of suppression of endogenous glucose production is the single most important determinant of glucose tolerance (Statistical data included). *Diabetes*, *50*(6), 1449-1454. https://doi.org/10.2337/diabetes.50.6.1449

Bennett, K. A., Hammill, M., & Currie, S. (2013). Liver glucose-6-phosphatase proteins in suckling and weaned grey seal pups: Structural similarities to other mammals and relationship to nutrition, insulin signalling and metabolite levels. *Journal of Comparative Physiology B: Biochemical, Systems, and Environmental Physiology*, *183*(8), 1075-1088. https://doi.org/10.1007/s00360-013-0768-x

Bennett, K. A., Robinson, K. J., Moss, S. E. W., Millward, S., & Hall, A. J. (2017). Using blubber explants to

investigate adipose function in grey seals: Glycolytic, lipolytic and gene expression responses to glucose and hydrocortisone. *Scientific Reports*, *7*(1), 7731. https://doi.org/10.1038/s41598-017-06037-x

Bennett, K. A., Speakman, J. R., Moss, S. E. W., Pomeroy, P., & Fedak, M. A. (2007). Effects of mass and body composition on fasting fuel utilisation in grey seal pups (*Halichoerus grypus* Fabricius): An experimental study using supplementary feeding. *Journal of Experimental Biology*, *210*(17), 3043-3053. https://doi.org/10.1242/jeb.009381

Bennett, K. A., Hughes, J., Stamatas, S., Brand, S., Foster, N. L., Moss, S. E. W., & Pomeroy, P. P. (2015). Adiponectin and insulin in gray seals during suckling and fasting: Relationship with nutritional state and body mass during nursing in mothers and pups. *Physiological and Biochemical Zoology*, *88*(3), 295-310. https://doi.org/10.1086/680862

Cen, J., Sargsyan, E., & Bergsten, P. (2016). Fatty acids stimulate insulin secretion from human pancreatic islets at fasting glucose concentrations via mitochondria-dependent and -independent mechanisms. *Nutrition & Metabolism*, *13*(1), 1-9. https://doi.org/10.1186/s12986-016-0119-5

Champagne, C. D., Houser, D. S., & Crocker, D. E. (2005). Glucose production and substrate cycle activity in a fasting adapted animal, the northern elephant seal. *Journal of Experimental Biology*, *208*, 859-868. https://doi.org/10.1242/jeb.01476

Champagne, C. D., Houser, D. S., & Crocker, D. E. (2006). Glucose metabolism during lactation in a fasting animal, the northern elephant seal. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, *291*(4), R1129-R1137. https://doi.org/10.1152/ajpregu.00570.2005

Champagne, C. D., Crocker, D. E., Fowler, M. A., & Houser, D. S. (2012). Fasting physiology of the pinnipeds: The challenges of fasting while maintaining high energy expenditure and nutrient delivery for lactation. In M. D. McCue (Ed.), *Comparative physiology of fasting, starvation and food limitation* (pp. 309-336). Springer-Verlag. https://doi.org/10.1007/978-3-642-29056-5_19

Crocker, D. E., Houser, D. S., & Webb, P. M. (2012). Impact of body reserves on energy expenditure, water flux, and mating success in breeding male northern elephant seals. *Physiological and Biochemical Zoology*, *85*(1), 11-20. https://doi.org/10.1086/663634

Crocker, D. E., Champagne, C., Fowler, M., & Houser, D. S. (2014a). Adiposity and fat metabolism in lactating and fasting northern elephant seals. *Annual Review of Nutrition*, *5*, 57-64. https://doi.org/10.3945/an.113.004663

Crocker, D. E., Webb, P. M., Costa, D. P., & Le Boeuf, B. J. (1998). Protein catabolism and renal function in lactating northern elephant seals. *Physiological Zoology*, *71*(5), 485-491. https://doi.org/10.1086/515971

Crocker, D. E., Williams, J. D., Costa, D. P., & Le Boeuf, B. J. (2001). Maternal traits and reproductive effort in northern elephant seals. *Ecology*, *82*(12), 3541-3555. https://doi.org/10.1890/0012-9658(2001)082[3541:MTAREI]2.0.CO;2

Crocker, D. E., Fowler, M., Champagne, C., Vanderlugt, A., & Houser, D. S. (2014b). Metabolic response to a glucagon challenge varies with adiposity and life history stage in fasting northern elephant seals. *General and Comparative Endocrinology*, *195*, 99-106. https://doi.org/10.1016/j.ygcen.2013.11.005

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

Fowler, M. A., Champagne, C. D., Houser, D. S., & Crocker, D. E. (2008). Hormonal regulation of glucose clearance in lactating northern elephant seals (*Mirounga angustirostris*). *Journal of Experimental Biology*, *211*(18), 2943-2949. https://doi.org/10.1242/jeb.018176

Fowler, M. A., Debier, C., Champagne, C. D., Crocker, D. E., & Costa, D. P. (2016). The demands of lactation promote differential regulation of lipid stores in fasting elephant seals. *General and Comparative Endocrinology*, *225*, 125-132. https://doi.org/10.1016/j.ygcen.2015.09.024

Frayn, K. N. (2010). *Metabolic regulation: A human perspective*: Wiley-Blackwell.

Harrison, A. L., Costa, D. P., Winship, A. J., Benson, S. R., Bograd, S. J., Antolos, M., Carlisle, A. B., Dewar, H., Dutton, P. H., Jorgensen, S. J., Kohin, S., Mate, B. R., Robinson, P. W., Schaefer, K. M., Shaffer, S. A., Shillinger, G. L., Simmons, S. E., Weng, K. C., Gjerde, K. M., & Block, B. A. (2018). The political biogeography of migratory marine predators. *Nature Ecology & Evolution*, *2*(10), 1571-1578. https://doi.org/10.1038/s41559-018-0646-8

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biomedical Journal*, *50*(3), 346-363. https://doi.org/10.1002/bimj.200810425

Hotta, K., Funahashi, T., Bodkin, N. L., Ortmeyer, H. K., Arita, Y., Hansen, B. C., & Matsuzawa, Y. (2001). Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel. *Diabetes*, *50*(5), 1126-1133. https://doi.org/10.2337/diabetes.50.5.1126

Houser, D. S., & Costa, D. P. (2001). Protein catabolism in suckling and fasting northern elephant seal pups (*Mirounga angustirostris*). *Journal of Comparative Physiology B: Biochemical, Systems, and Environmental Physiology*, *171*(8), 635-642. https://doi.org/10.1007/s003600100214

Houser, D. S., Champagne, C. D., & Crocker, D. E. (2013). A non-traditional model of the metabolic syndrome: The adaptive significance of insulin resistance in fasting-adapted seals. *Frontiers in Endocrinology*, *4*. https://doi.org/10.3389/fendo.2013.00164

Houser, D. S., Crocker, D. E., Tift, M. S., & Champagne, C. (2012). Glucose oxidation and non-oxidative glucose disposal during prolonged fasts of the northern elephant seal pup (*Mirounga angustirostris*). *American Journal of Physiology: Endocrinology and Metabolism*, *303*, R562-R570. https://doi.org/10.1152/ajpregu.00101.2012

Ito, M., Nagasawa, M., Omae, N., Tsunoda, M., Ishiyama, J., Ide, T., Akasaka, Y., & Murakami, K. (2013). A

novel JNK2/SREBP-1c pathway involved in insulin-induced fatty acid synthesis in human adipocytes. *Journal of Lipid Research*, *54*(6), 1531-1540. https://doi.org/10.1194/jlr.M031591

Kelso, E. J., Champagne, C. D., Tift, M. S., Houser, D. S., & Crocker, D. E. (2012). Sex differences in fuel use and metabolism during development in fasting juvenile northern elephant seals. *Journal of Experimental Biology*, *215*(15), 2637-2645. https://doi.org/10.1242/jeb.068833

Kersten, S. (2001). Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO Reports*, *2*(4), 282-286. https://doi.org/10.1093/embo-reports/kve071

Khudyakov, J. I., Champagne, C. D., Meneghetti, L. M., & Crocker, D. E. (2017). Blubber transcriptome response to acute stress axis activation involves transient changes in adipogenesis and lipolysis in a fasting-adapted marine mammal. *Scientific Reports*, *7*(1). https://doi.org/10.1038/srep42110

Khudyakov, J. I., Champagne, C. D., Preeyanon, L., Ortiz, R. M., & Crocker, D. E. (2015). Muscle transcriptome response to ACTH administration in a free-ranging marine mammal. *Physiological Genomics*, *47*(8), 318-330. https://doi.org/10.1152/physiolgenomics.00030.2015

Kraemer, F. B., Takeda, D., Natu, V., & Sztalryd, C. (1998). Insulin regulates lipoprotein lipase activity in rat adipose cells via wortmannin- and rapamycin-sensitive pathways. *Metabolism*, *47*(5), 555-559. https://doi.org/10.1016/S0026-0495(98)90239-6

Lefcheck, J. S. (2016). piecewiseSEM: Piecewise structural equation modeling in *R* for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, *7*(5), 573-579. https://doi.org/10.1111/2041-210X.12512

Martinez, B., Scheibner, M., Soñanez-Organis, J. G., Jaques, J. T., Crocker, D. E., & Ortiz, R. M. (2017a). Increased sensitivity of thyroid hormone-mediated signaling despite prolonged fasting. *General and Comparative Endocrinology*, *252*, 36-47. https://doi.org/10.1016/j.ygcen.2017.07.023

Martinez, B., Soñanez-Organis, J. G., Arquimides Godoy-Lugo, J., Horin, L. J., Crocker, D. E., & Ortiz, R. M. (2017b). Thyroid hormone-stimulated increases in PGC-1α and UCP2 promote life history-specific endocrine changes and maintain a lipid-based metabolism. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, *312*(2), R189-R196. https://doi.org/10.1152/ajpregu.00395.2016

Newsholme, P., Bender, K., Kiely, A., & Brennan, L. (2007). Amino acid metabolism, insulin secretion and diabetes. *Biochemical Society Transactions*, *35*(5), 1180-1186. https://doi.org/10.1042/BST0351180

Nordøy, E. S., & Blix, A. S. (1991). Glucose and ketone body turnover in fasting grey seal pups. *Acta Physiologica Scandinavia*, *141*, 563-571. https://doi.org/10.1111/j.1748-1716.1991.tb09118.x

Noren, D. P., & Mangel, M. (2004). Energy reserve allocation in fasting northern elephant seal pups: Interrelationships between body condition and fasting duration. *Functional Ecology*, *18*, 233-242. https://doi.org/10.1111/j.0269-8463.2004.00840.x

Oftedal, O. T. (1993). The adaptation of milk secretion to the constraints of fasting in bears, seals, and baleen whales. *Journal of Dairy Science*, *76*(10), 3234-3246. https://doi.org/10.3168/jds.S0022-0302(93)77660-2

Okamoto, M., Ohara-Imaizumi, M., Kubota, N., Hashimoto, S., Eto, K., Kanno, T., Kubota, T., Wakui, M., Nagai, R., Noda, M., Nagamatsu, S., & Kadowaki, T. (2008). Adiponectin induces insulin secretion in vitro and in vivo at a low glucose concentration. *Diabetologia*, *51*(5), 827-835. https://doi.org/10.1007/s00125-008-0944-9

Olmstead, K. I., La Frano, M. R., Fahrmann, J., Grapov, D., Viscarra, J. A., Newman, J. W., Fiehn, O., Crocker, D. E., Filipp, F. V., & Ortiz, R. M. (2017). Insulin induces a shift in lipid and primary carbon metabolites in a model of fasting-induced insulin resistance. *Metabolomics: An Official Journal of the Metabolomics Society*, *13*(5), 1-16. https://doi.org/10.1007/s11306-017-1186-y

Pinheiro, J., Bates, D., DebRoy, S., & Sarkar, D. (2009). nlme: Linear and nonlinear mixed effects models. R *package version 3.1-145*. https://CRAN.R-project.org/package=nlme

Regnier, S. M., & Sargis, R. M. (2014). Adipocytes under assault: Environmental disruption of adipose physiology. *BBA: Molecular Basis of Disease*, *1842*(3), 520-533. https://doi.org/10.1016/j.bbadis.2013.05.028

Robinson, P. W., Costa, D. P., Crocker, D. E., Gallo-Reynoso, J. P., Champagne, C. D., Fowler, M. A., Goetsch, C., Goetz, K. T., Hassrick, J. L., Hückstädt, L. A., Kuhn, C. E., Maresh, J. L., Maxwell, S. M., McDonald, B. I., Peterson, S. H., Simmons, S. E., Teutschel, N. M., Villegas-Amtmann, S., & Yoda, K. (2012). Foraging behavior and success of a mesopelagic predator in the Northeast Pacific Ocean: Insights from a data-rich species, the northern elephant seal. *PLOS ONE*, *7*(5), e36728. https://doi.org/10.1371/journal.pone.0036728

Ross, P. S. (2000). Marine mammals as sentinels in ecological risk assessment. *Human and Ecological Risk Assessment*, *6*(1), 29-46. https://doi.org/10.1080/10807030091124437

Suzuki, M., Vázquez-Medina, J. P., Viscarra, J. A., Sonanez-Organis, J. G., Crocker, D. E., & Ortiz, R. M. (2013). Activation of systemic, but not local, renin-angiotensin system is associated with upregulation of TNF-a during prolonged fasting in northern elephant seal pups. *Journal of Experimental Biology*, *216*(17), 3215-3221. https://doi.org/10.1242/jeb.085225

Tsatsoulis, A., Mantzaris, M. D., Bellou, S., & Andrikoula, M. (2013). Insulin resistance: An adaptive mechanism becomes maladaptive in the current environment—An evolutionary perspective. *Metabolism*, *62*(5), 622-633. https://doi.org/10.1016/j.metabol.2012.11.004

Viscarra, J. A., Champagne, C. D., Crocker, D. E., & Ortiz, R. M. (2011a). 5-AMP-activated protein kinase activity is increased in adipose tissue of northern elephant seal pups during prolonged fasting-induced insulin resistance. *Journal of Endocrinology*, *209*(3), 317-325. https://doi.org/10.1530/JOE-11-0017

Viscarra, J. A., Vázquez-Medina, J. P., Crocker, D. E., & Ortiz, R. M. (2011b). Glut4 is upregulated despite decreased insulin signaling during prolonged fasting in northern elephant seal pups. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, *300*(1), R150-R154. https://doi.org/10.1152/ajpregu.00478.2010

Viscarra, J. A., Vázquez-Medina, J. P., Rodriguez, R., Champagne, C. D., Adams, S. H., Crocker, D. E., & Ortiz, R. M. (2012). Decreased expression of adipose CD36 and FATP1 are associated with increased plasma non-esterified fatty acids during prolonged fasting in northern elephant seal pups (*Mirounga angustirostris*). *Journal of Experimental Biology*, *215*(14), 2455-2464. https://doi.org/10.1242/jeb.069070

Viscarra, J. A., Rodriguez, R., Vázquez-Medina, J. P., Lee, A., Tift, M. S., Tavoni, S., Crocker, D. E., & Ortiz, R. M. (2013). Insulin and GLP-1 infusions demonstrate the onset of adipose-specific insulin resistance and suggest a glucogenic role for GLP-1 in a large fasting mammal. *Physiological Reports*, *1*(2), e00023. https://doi.org/10.1002/phy2.23

Weijs, L., & Zaccaroni, A. (2016). Toxicology of marine mammals: New developments and opportunities. *Archives of Environmental Contamination and Toxicology*, *70*(1), 1-8. https://doi.org/10.1007/s00244-015-0233-9

Young, R. A. (1976). Fat, energy and mammalian survival. *American Zoologist*, *16*(4), 699-710. https://doi.org/10.1093/icb/16.4.699