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Hormonal regulation of glucose clearance in lactating northern elephant seals (*Mirounga angustirostris*)

Melinda A. Fowler*, Cory D. Champagne, Dorian S. Houser and Daniel E. Crocker
Sonoma State University, Biology Department, 1801 E. Cotati Ave, Rohnert Park, CA 94928, USA
*Author for correspondence (e-mail: mfowler@biology.ucsc.edu)

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SUMMARY

Northern elephant seals exhibit the rare strategy of fasting and lactating concomitantly. We investigated hormonal regulation of glucose clearance in northern elephant seals using glucose tolerance tests (GTT) performed early in lactation and again just prior to weaning. For comparison, identical measurements were made on separate females late in the molt fast. Serial blood samples were used to assess glucose clearance and hormone responses for 3 h post glucose injection. Plasma glucose remained elevated at the end of the sampling period in all groups. Glucose clearance rates were not significantly different among test groups. A significant insulin response was observed in early lactation, no significant response was observed late in lactation and an intermediate response was observed late in the molt fast. The insulin response to a glucose load decreased with adipose tissue proportions. Plasma glucagon decreased significantly following GTT in early and late lactation, although the magnitude of the depression was small in comparison to other species. Hypoinsulemia may be critical to facilitate net lipolysis late in lactation. Consistently low glucose clearance among test groups suggests insulin insensitivity within peripheral tissues. Glucagon suppression independent of insulin release suggests modification of the typical insulin–glucagon counter-regulation. These findings suggest that metabolic features of diabetic-like conditions may be adaptive in the context of long-term fasting.

Key words: glucose tolerance test, insulin, glucose metabolism, elephant seal, lactation, fasting.

INTRODUCTION

Some species have incorporated long-term fasting as part of their life history strategies. In general, long-term fasting is associated with reductions in metabolic rate and metabolic strategies designed to conserve essential stored body nutrients (Castellini and Rea, 1992). A major challenge of long-term fasting is providing fuel substrates for glucose-dependent tissues while sparing vital body protein. Protein sparing is facilitated by the contribution of non-amino acid precursors (e.g. glycerol and lactate) to gluconeogenesis and the use of ketone bodies as an alternative to glucose oxidation. Phocid seals provide an excellent system within which to investigate fuel partitioning strategies during fasting as they undertake predictable fasts concurrent with life history events that elevate substrate demands (e.g. lactation, development).

Temporal separation of foraging and reproduction has led to simultaneous extended fasting and lactation in several species of phocid seals, including northern elephant seals (*Mirounga angustirostris* Linnaeus). Elephant seals spend the majority of their lives at sea, returning to land only to breed and molt (Le Boeuf et al., 2000). During the molting fast, seals come ashore to grow new skin and fur, a process which takes approximately 1 month (Le Boeuf and Laws, 1994). Females give birth and nurse their young for ~26 days, during which time they transfer enough nutrients to facilitate the tripling of pup mass, while depleting their own mass by up to ~40% (Crocker et al., 2001; Kretzmann et al., 1993). Both milk production and energy expenditure in elephant seals are strongly impacted by body reserves (Crocker et al., 2001).

Of all reproductive costs, lactation demands the most energy (Gittleman and Thompson, 1988). Lactation is characterized by homeorhesis, or a shift in nutrient partitioning for the priorities of a physiological state (Bauman and Currie, 1980). The constraints associated with simultaneous fasting and lactation may require a significant change in the nutrient partitioning relative to either fasting or lactation alone, enabling the mother to provide the necessary nutrients for her offspring. An animal's capacity to sustain the production of large amounts of milk is closely related to its ability to mobilize body reserves (Bauman and Elliot, 1983). The low carbohydrate, high fat composition of pinniped milk has been hypothesized to be driven by the constraints of fasting (Oftedal, 1993). Pinniped milk contains only trace amounts of carbohydrate (Oftedal, 1984), thus there are no carbohydrate demands for milk synthesis and we would expect glucose uptake by the mammary gland to be limited to that used for oxidation.

Little is known about hormonal regulation of glucose metabolism in phocids. Despite efficient protein sparing, plasma glucose levels in lactating elephant seals have been shown to increase across the fast (Champagne et al., 2006), and are high relative to fasting glucose levels in non-fasting adapted animals of similar body mass (Umminger, 1975). This presents a paradox, as fasting normally results in a decrease in plasma glucose (Cahill et al., 1966; Klein et al., 1990), even during lactation (Chelikani et al., 2004; Neville et al., 1993). The role of increased plasma glucose in seals, simultaneous with the cessation of nutrient input, remains undetermined. Levels of glucose production are typical of that observed in post-absorptive terrestrial mammals and fail to exhibit the suppression with fasting duration seen in non-fasting adapted species (Champagne et al., 2006).

Kirby and Ortiz (Kirby and Ortiz, 1994) found a lack of insulin response to injected glucose in weaned pups and suggested that elephant seal weanlings do not use the typical mammalian insulin–glucagon counter-regulation of glucose metabolism. Champagne et al. (Champagne et al., 2006), however, found

significant relationships between proportional glucose cycle activity and plasma insulin:glucagon ratios (I:G) in adult females, consistent with their typical regulatory roles. Basal glucose, as well as the changes in insulin and glucagon across the fast, differ between adult females (Champagne et al., 2006) and weaned pups (Champagne et al., 2005; Ortiz et al., 2003; Costa and Ortiz, 1982), suggesting that glucose regulation may vary with development and physiological state.

In this study, glucose tolerance tests (GTT) were used to assess the efficiency of glucose disposal, to investigate the insulin response to elevated glucose levels and gain insight into how elephant seals regulate the utilization of nutrient reserves. In most other mammals, insulin secretion increases in response to elevated glucose, enabling tissues to uptake the circulating glucose and reduce glucose levels in the blood. Most species exhibit concurrent dramatic reductions in plasma levels of glucagon in response to an exogenous glucose load (Basu et al., 1996; Butler and Rizza, 1991). The magnitude of the insulin response to glucose is indicative of the pancreatic β cells response to glucose, and the disappearance of glucose over time relative to a given release of insulin is indicative of the sensitivity of peripheral tissues to the hormone. Few previous studies have investigated insulin responses to GTT in pinnipeds (Hochachka et al., 1979; Kirby and Ortiz, 1994; Robin et al., 1981), and none in simultaneously fasting and lactating individuals. We tested the hypothesis that pancreatic cells in lactating elephant seals are insensitive to elevated plasma glucose levels.

MATERIALS AND METHODS

Study site and individual animals

This study was carried out at Año Nuevo State Reserve, San Mateo County, CA, USA during the 2005 breeding season (January–February) and molting period (April–May). Soon after arrival on land, adult female seals were marked with hair dye (Lady Clairol, Stamford, CT, USA) to facilitate identification. Parturition dates were established by daily observations and considered to be the first day a marked female was observed with a pup, provided she had been observed without a pup the previous day. Ten females were sampled in early lactation (day 5 *postpartum*) and eight of these females were recaptured late in lactation (day 22 *postpartum*) and the sampling procedures repeated. Two females from the initial sample were observed without their pups for several days between sampling periods and one female was seen suckling multiple pups intermittently. These three females are included in early lactation means, but excluded from matched pairs comparisons of early and late lactation samples. Molt study females (N=8) were selected on the basis of fully molted pelage, ensuring ~3 weeks of fasting (Le Boeuf and Laws, 1994).

Sample collection and processing

Females were initially immobilized with Telazol (tiletamine/zolazepam HCl, Fort Dodge Labs, Ft Dodge, IA, USA) at a dosage of ~1 mg kg^{-1}, administrated intramuscularly. Continued immobilization was maintained with ~100 mg bolus intravenous injections of ketamine. A blood sample was taken prior to the administration of glucose to ascertain basal glucose and hormone levels. The glucose tolerance test (hereafter referred to as a GTT) was administered intravenously. Females were given a bolus injection of 150 g glucose as a 50% glucose solution. Injections were administered *via* the epidural vein and the duration of the injection averaged ~8 min. Samples were collected into chilled, heparinized blood collection tubes (BD Vacutainer®, Fisher Scientific, Franklin Lakes, NJ, USA) every 10 min for the first 30 min post-injection and every 15 min until 180 min post-injection. Additional serum samples for hormone analysis were collected into chilled blood collection tubes every 10 min for the first 30 min and every 30 min until 180 min. Samples were immediately placed on ice and transported back to laboratory within 2–3 h. Samples were centrifuged at 4°C, and frozen at –80°C until further analysis.

Body composition measurements were made using the truncated cones method (Crocker et al., 2001; Gales and Burton, 1987). This method allows the proportion of adipose and lean tissue masses to be calculated and has been validated in elephant seals using isotopic methods (Webb et al., 1998). Dorsal, lateral and ventral blubber depth measurements were made using a portable ultrasound scanner (Ithaca Scanprobe, Ithaca, NY, USA) at each of six locations along the seal. Lengths and girths were taken at these six points, as well as total curved length. These measurements allowed the seal to be modeled as a series of truncated cones. Mass was measured using a tripod, canvas sling and scale (±2 kg) (MSI, Seattle, WA, USA).

Sample analysis

Plasma glucose was measured in duplicate using an YSI 2300 glucose autoanalyzer (YSI, Yellow Springs, OH, USA). Serum glucagon and insulin levels were measured by radioimmunoassay (all kits from Linco, St Louis, MO, USA). Glucagon (# GL-32K) and insulin (# SRI-13K) kits have been previously validated for use in elephant seals (Champagne et al., 2005; Ortiz et al., 2003). The average intra-assay coefficients of variation for insulin and glucagon were 10.2% and 12.3%, respectively.

Statistical analysis

Differences between matched early and late lactation samples were examined using a paired t-test. Differences between molt and late lactation samples were examined using a Student's t-test. We used a linear mixed model with individual as a random effect subject to examine the effects of body composition on the insulin response. Hormonal responses were assessed using a repeated measures analysis of variance (RM ANOVA), with a multivariate approach to detect differences within a time series. Significant RM ANOVAs were analyzed by looking for significant differences from basal hormone values. All data are expressed ± standard error of the mean (s.e.m.). Results were considered significant at P<0.05.

A glucose tolerance index (K) was calculated assuming that the 20 min post-glucose-injection sample represented complete dilution of the injected glucose within the total body pool (Champagne et al., 2006). K was then calculated using least-squares linear regression as the negative slope of the natural log of glucose concentrations from 20 min to 180 min post-injection (Alder et al., 1997; Bergman et al., 1981; Chen and Nyomba, 2004).

Average glucose present was calculated using the area under the curve (AUC) for glucose divided by the total duration of the sampling period. The area under the curve was calculated using the trapezoid rule from 20–180 min post-injection and after subtraction of basal levels (Chen and Nyomba, 2004; Grottoli et al., 1997; Hatfield et al., 1999). Area under the curve (AUC) for insulin and glucagon were likewise calculated using the trapezoid rule, after subtracting basal values (Chen and Nyomba, 2004) from 10 min post-injection until the final sample. Average secretion per minute of each hormone was calculated by dividing the AUC by the duration of the sampling period, in minutes.

There are numerous methods available to assess insulin sensitivity (Avignon et al., 1999; Bergman et al., 1987; Bonora et al., 2000; Ciampelli et al., 2005; Katz et al., 2000). These insulin sensitivity indices are based on basal insulin and glucose levels and have been

developed in human subjects who display either normal or abnormally high basal insulin levels. The insulin:glucose ratio may not maintain the same relationship in individuals who lack endogenous insulin secretion (Avignon et al., 1999; Katz et al., 2000). Elephant seals display low basal insulin, therefore, the indices were inappropriate in this case and we developed an I_s index unique to this study. Insulin sensitivity indices (I_s) were calculated by dividing the area under the insulin curve (AUC_I) by the area under the glucose curve (AUC_G).

RESULTS

Lactating females lost 26.6±1.0% of their mass between measurements (Table 1). Body composition varied significantly between all groups (P<0.01) with lactating females depleting an average of 21% of their initial blubber reserves between samples.

Basal glucose and hormone levels

Glucose levels (125.2±3.1 mg dl^{-1}) did not vary between classes (P>0.05). I:G ratio decreased significantly across lactation (paired t=–3.76, P=0.01; Table 1). Insulin, glucagon or I:G ratio were not related to plasma glucose in any group (P>0.05). Basal insulin levels decreased significantly across the fast (paired t=–3.0, P=0.02; Table 1). Late in the molt insulin values were significantly different from those of the late lactation females (84.2±5.5 pg ml^{-1}; t=–3.6, P=0.005) but not early lactation insulin values (t=–0.08, P=0.93; Table 1). Basal glucagon levels did not change significantly across the fast (P=0.34; Table 1).

Responses to glucose tolerance test

Glucose levels post glucose injection are shown in Fig. 1A. Glucose levels 20 min post-injection increased by 144.0±5.8% in early lactation, 158.2±5.3% in late lactation and 130.8±10.4% in the molt. These differences were probably due to body mass differences among subjects. Despite the differences in percentage increase among groups, there were no relationships between percentage increase and rate of glucose clearance (P>0.05). By 180 min post-injection, plasma glucose levels remained high in all classes (61.1±7.0% above basal in early lactation, 90.0±8.1% above basal in late lactation and 58.9±10.4% above basal late in the molt). Glucose tolerance indices (K) were not different among classes (P>0.05; Table 2).

Peak insulin response occurred at 10 min post-injection (Fig. 1B). The 10 min response values and mean insulin secreted are shown for all groups in Table 2. Early lactation females showed a significant insulin response (viewed as the percentage increase 10 min post-injection; RM ANOVA $F_{7,63}$=5.49, P=0.001). The 10 min sample showed a 129.0±24.4% increase above basal in early lactation (paired t=4.88, P=0.001). There was no insulin response to exogenous glucose in late lactation (P>0.05). Insulin levels deviated only –4.8±21.6% from basal 10 min post-injection and never deviated more than 55.4±47.8%. The molted female insulin response to the GTT was significant ($F_{7,49}$=8.04, P=0.001), with a 61.6±11.6% increase above basal at 10 min post-injection (paired t=7.64, P=0.0001). The response of the molted females was intermediate to responses observed during early (t=2.49, P=0.03) and late lactation (t=–2.71, P=0.03; Table 2). Mean insulin secreted per minute did not predict K in early or late lactation or after the molt. There were no relationships between mean insulin secreted per minute and mean glucose present per minute in early or late lactation, or post-molt, respectively. The percentage increase in insulin at 10 min post-injection was significantly affected by adipose tissue proportions ($F_{1,4}$=13.0, P=0.02; Fig. 2).

Mean calculated I_s for all groups is shown in Table 2. I_s declined significantly from early to late lactation (paired t=2.47, P=0.005). The early lactation I_s was not significantly different from the post-molt I_s (t=0.83, P=0.42). Late lactation I_s was significantly lower than the post-molt I_s (t=–2.67, P=0.02).

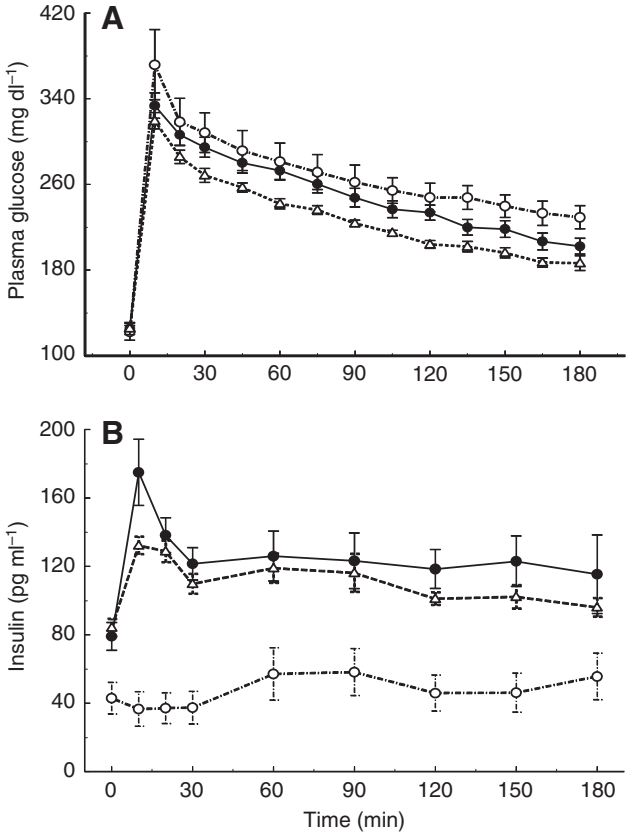

Fig. 1. (A) Plasma glucose levels in adult female elephant seals from time 0 to 180 min post glucose injection. Samples at 180 min post-injection are significantly elevated; early lactation: paired t=8.93; P<0.0001; late lactation: paired t=17.57, P<0.0001; molted females: paired t=5.07, P=0.002. (B) Serum insulin values of adult elephant seals in response to 150 g exogenous glucose from time 0 to 180 min post-injection in early and late lactation and post-molt. Closed circles, early in lactation (5 days *postpartum*); open circles, late in lactation (22 days *postpartum*); triangles, fully molted females, late in the molt fast (>20 days fasting). See text for RM ANOVA statistics. Error bars are ± s.e.m.

Table 1. Mass, body composition, basal insulin, glucagon and glucose levels in early and late lactation and post molt adult female elephant seals

	Early lactation	Late lactation	Post-molt
Glucose (mg dl^{-1})	125.6±2.8	122.5±8.6	125.5±6.2
Insulin (pg ml^{-1})	83.3±8.8^a	43.0±10.1^b	84.2±5.5^a
Glucagon (pg ml^{-1})	29.8±2.1	36.6±5.5	NA
I:G ratio	1.72±0.19^a	0.68± 0.12^b	NA
Mass (kg)	432.7±26.6^a	317.0±20.4^b	344.4±15.8^b
% Blubber	38.5±0.7^a	30.6±0.9^b	35.7±0.6^c

Early and late samples are from 5 and 22 days *postpartum*, respectively; I:G ratio, molar ratio of insulin to glucagon. NA, not available.

Data are ± s.e.m. Different superscript letters within rows indicate a significant difference at P<0.05.

Table 2. Response of adult northern female elephant seals to 150 g exogenous glucose in early and late lactation and post-molt

	Early lactation	Late lactation	Post-molt
Insulin levels 10 min post GTT (pg ml^{-1})	175.0±20.4^{a}	36.6±11.9^{b}	132.3±5.5^{a}
Insulin response (% increase in 10 min)	129.0±24.4^{a}	–4.8±21.6^{b}	61.6±11.6^{c}
Mean insulin secreted (pg ml^{-1})	0.045±0.012^{a,*}	0.009±0.006^{b}	0.026±0.005^{a,*}
Insulin sensitivity index (I_s)	$3.4\times10^{-4}\pm9.4\times10^{-5a}$	$6.1\times10^{-5}\pm4.4\times10^{-5b}$	$2.5\times10^{-4}\pm5.4\times10^{-5a}$
Glucose tolerance index (K; % min^{-1})	0.27±0.02	0.19±0.05	0.27±0.03
Mean glucose present (mg dl^{-1})	123.0±6.0	140.7±8.1	117.4±15.6

Early and late samples are from 5 and 22 days *postpartum*, respectively.

K, glucose tolerance index (see Materials and methods for calculation). Mean glucose present was calculated as the area under the curve $(AUC)_{glucose}$ divided by the total duration (in min) of the sampling period. Mean hormone secreted was calculated as $AUC_{hormone}$ divided by the total duration (in min) of the sampling period. See Materials and methods for calculation of I_s. Different superscript letters within rows indicate a significantly difference at $P<0.05$; asterisks within rows indicate a significant response to the glucose tolerance test (GTT; RM ANOVA $P<0.05$).

I_s was not related to body composition in early or late lactation, or in molted females. There were no relationships between body composition and average glucose present per minute in any sample group, nor were there relationships between body composition and K in any sample group.

The mean level of glucagon secreted per minute (pg ml^{-1}) in early lactation was –0.14±1.06 pg ml^{-1} and in late lactation was –2.44±1.42 pg ml^{-1}. There was no significant difference in levels between early and late lactation (paired t=–1.13, $P>0.05$). There was a significant decrease in glucagon following exogenous glucose in both early (RM ANOVA, $F_{7,63}$=6.65, $P<0.001$) and late ($F_{6,36}$=3.2, P=0.013) lactation.

DISCUSSION

Fasting and lactating simultaneously imposes considerable metabolic conflicts. To facilitate the maximal output of maternal energy as milk, while preserving endogenous nutrients for subsequent reproductive attempts, females must optimize fuel partitioning for milk synthesis and metabolism. In lactating elephant seals, protein degradation represents a potential limiting factor to the duration of fasting and lactation (Crocker et al., 1998). Lipid catabolism powers ~90% of maternal metabolism (Crocker et al., 2001). Although it is not possible to separate the effects of lactation and body reserve depletion in the current study (i.e. lactating females deplete reserves to greater levels than molting females over similar fasting durations), our findings suggest that efficient mechanisms of maximizing the energy obtained from adipose reserves while simultaneously protecting protein stores may be the selective force behind variations in hormone action relative to that observed in non-fasting-adapted mammals.

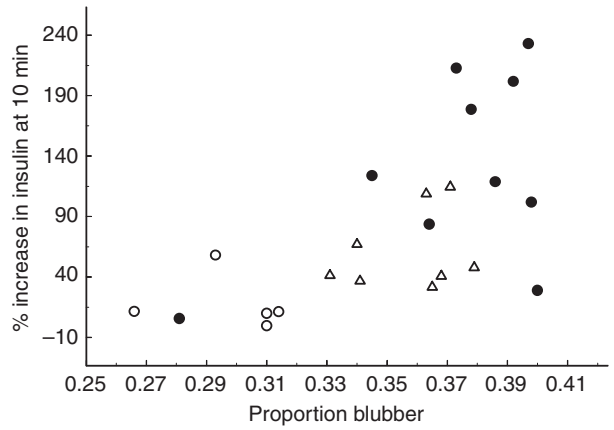

Fig. 2. Percentage increase in plasma insulin over basal values 10 min after glucose injection in relation to the proportion of adipose tissue. $F_{1,4}$=13.0, P=0.02. Closed circles, early in lactation; open circles, late in lactation; triangles are from late in the molt fast.

Suppression of insulin response to glucose tolerance test

Elephant seals showed marked differences in insulin secretion in response to exogenous glucose among different fasting states. Early lactation females displayed the largest insulin response, whereas late lactation females showed no insulin response to exogenous glucose. Molted females showed an intermediate insulin response to the two lactation groups. This variation in insulin response was directly related to variation in adipose tissue proportions, suggesting functional suppression of insulin response as adipose tissue stores are depleted during fasting. Rates of lipolysis are consistent across lactation despite dramatic reductions in lipid stores (Houser et al., 2007). The need to maintain high net rates of lipolysis may be one of the driving forces behind insulin suppression late in lactation, as fatty acid demands for milk synthesis increase relative to body reserves. This is the first quantification of insulin response to glucose administration in a naturally fasting and lactating animal. Only two other groups that utilize fasting as a natural stage in their life history have shown similar responses. In fasting elephant seal pups (Kirby, 1992) and fasting polar bears (Cattet, 2000), fasting duration and/or body composition have been shown to have a significant relationship to insulin suppression.

Bauman and Elliott (Bauman and Elliott, 1983) reported that a marked decrease in pancreatic release of insulin in response to glucose is a normal homeorhetic change in lactating ruminants. Despite this generalization, insulin responses during lactation in ruminants varied widely (Sano et al., 1991; Sartin et al., 1985). Elephant seals are part of a very small subset of mammalian carnivores that fast and lactate. Although discussions about the similarities and differences between ruminants and elephant seals are instructive, any such comparisons should be made with caution. There is a dearth of literature on lactation and glucose metabolism in free-ranging carnivores and more research is required to better understand how insulin responses vary in this group of animals.

Sustained insulin resistance

Elephant seals demonstrate some degree of insulin resistance, regardless of fasting state, as indicated by the low K values and high levels of glucose remaining 3 h post-GTT. Normal glucose tolerance indices in humans was shown to drop from 2.1% min^{-1} to 0.63% min^{-1} after 8 days of fasting (Cahill et al., 1966). Glucose tolerance indices as high as 3.94% min^{-1} have been reported in non-fasting baboons (Ensinck et al., 1997) and 3.66% min^{-1} in rats (Chen

and Nyomba, 2004). Glucose clearance is significantly impaired and similar in all classes of fasting female elephant seals (0.19% min^{-1} to 0.27% min^{-1}), despite variation in insulin response. Impaired clearance combined with the lack of relationship between glucose clearance and insulin secretion suggests insulin resistance in fasting elephant seals. In addition, insulin sensitivity declined slightly late in lactation relative to the other samples.

Decreased sensitivity to insulin has been shown in response to a variety of conditions. Diabetes is notable among these conditions, but insulin resistance is demonstrated in pregnancy (Bauman and Bell, 1997; Ryan, 2003), as well as during prolonged fasting in humans (Cahill et al., 1966). Although a review by Bauman and Bell (Bauman and Bell, 1997) suggests that lactating ruminants experience reduced tissue sensitivity to insulin, the data on insulin resistance during lactation in other species appear equivocal (Burnol et al., 1986; Debras et al., 1989; Hoffman et al., 2003; Sano et al., 1991; Tigas et al., 2002). Performing an insulin tolerance test would give a clearer picture of the level of insulin resistance in adult lactating elephant seals. Kirby and Ortiz (Kirby and Ortiz, 1994) found that glucose levels were suppressed after the injection of exogenous insulin in weaned elephant seals, suggesting some level of tissue response to insulin.

Mechanisms for altering insulin secretion and sensitivity

Elevated nonesterified free fatty acids (NEFA) could directly impact the ability of the islets of Langerhans to express insulin. Free fatty acids stimulate insulin secretion, but chronically high levels of NEFA result in a decrease of insulin content in pancreas cells by decreasing the expression of insulin (Bollheimer et al., 1998; Ritz-Laser et al., 1999). High NEFA levels may also be implicated in the reduction of insulin sensitivity. Although short-term elevations of NEFA are known to stimulate insulin secretion (Bollheimer et al., 1998), a considerable amount of research has shown that chronically elevated NEFA are the initiating cause of an impairment in insulin signal transduction (Boden and Laakso, 2004; Yu et al., 2002). Previous research has shown that fasting elephant seals have elevated NEFA (1.0–3.2 mmol l^{-1}) (Castellini et al., 1987; Houser et al., 2007; McDonald and Crocker, 2006) in comparison to other non-fasting adapted species (e.g. 0.14 mmol l^{-1} in humans) (Fery et al., 1990).

Glucagon response

Insulin inhibits pancreatic release of glucagon (Aronoff et al., 2004). Non-fasting, non-obese, non-diabetic individuals in other mammalian species display depression of glucagon levels following a GTT, even when lactating (Aronoff et al., 2004; Fery et al., 1990; Sartin et al., 1985). Individuals with reduced insulin secretion (e.g. diabetics or obese individuals) do not suppress glucagon (Greenbaum et al., 2002; Staehr et al., 2001; Velliquette et al., 2002) exacerbating the hyperglycemia following a glucose tolerance test, due to continued hepatic glucose production. Elephant seals in the present study exhibited an equivalent depression of glucagon in response to a glucose load during both stages of lactation despite a lack of insulin secretion late in lactation. This disconnection between the insulin and glucagon responses suggests alterations in the typical counter-regulatory responses of the pancreatic hormones. Late lactation seals exhibited low glucose clearance despite a biphasic insulin response coupled with glucagon depression. Although the response was statistically significant, the magnitude of the glucagon depression is smaller than that found in other studies that have documented glucagon depressions following a GTT. Adult females do not appear to regulate glucose metabolism with the normal mammalian insulin–glucagon push-pull model, in agreement with measurements made on weaned elephant seal pups (Kirby and Ortiz, 1994).

Basal glucose and hormone levels

Plasma glucose levels did not change across lactation. The present findings of insulin decrease across lactation and fasting duration are in agreement with previous measurements in fasting and lactating elephant seals (Champagne et al., 2006; McDonald, 2003). Many species exhibit a decrease in insulin levels as they transition to lactation (Burnol et al., 1983; Hatfield et al., 1999; Komatsu et al., 2005), while insulin then increases throughout lactation (Chelikani et al., 2003; Debras et al., 1989; Hoffman et al., 2003). In humans, the anti-lipolytic properties of insulin remain evident even at concentrations so low that glucose transport is not stimulated (Kahn and Flier, 2000). Despite a similar fasting duration to late lactation, molted females' insulin levels were similar to early lactation values. This pattern may arise from the need to mobilize lipids late in lactation and insulin suppression across the fast may facilitate lactation.

Glucagon levels in fasting and lactating elephant seals remain stable (Champagne et al., 2006; McDonald, 2003), in contrast to weanling elephant seals, which exhibit an increase in glucagon across the fasting period (Champagne et al., 2005; Ortiz et al., 2003). Basal glucagon levels are lower in fasting elephant seals than in other species during fasting (Fery et al., 1990) and lactation (Burnol et al., 1983; Tigas et al., 2002). While glucagon has been shown to increase with fasting duration in humans (Boyle et al., 1989; Fery et al., 1990), humans that are fasting and lactating simultaneously have stable glucagon levels (Tigas et al., 2002). Glucagon is stable across lactation in cattle, rats and sheep (Burnol et al., 1983; Sartin et al., 1985; Vernon and Pond, 1997). Glucagon stimulates both gluconeogenesis and lipolysis (Perea et al., 1995). Low levels of glucagon are puzzling in light of high levels of lipolysis and gluconeogenesis but probably contribute to protein sparing.

Studies of carnivore glucose metabolism in the context of lactation are rare. We would expect that the lower carbohydrate diet of carnivores would have important impacts on glucose metabolism; however, some studies do suggest that the high protein diet of carnivores may be associated with impaired ability for glucose clearance. Penguins (Chieri et al., 1972), barn owls (Myers and Klasing, 1999), rainbow trout (Palmer and Ryman, 1972), white sturgeon (Hung, 1991) and American alligators (Coulson and Hernandez, 1983) exhibited reduced glucose clearance when compared to omnivores. We are aware of no similar studies in wild mammalian carnivores.

Conclusions

The combination of fasting with lactation creates a conflict in metabolic processes with respect to the fate of nutrient stores. Simultaneously fasting and lactating northern elephant seals exhibit suppression of insulin response to exogenous glucose that varies across differing metabolic states. Elephant seals also demonstrate some degree of insulin resistance, regardless of fasting state. Additionally, the typical mammalian counter-regulatory push-pull insulin–glucagon model appears to be modified in fasting and lactating elephant seals. Carbohydrate, lipid and protein stores must be tightly regulated during times of nutritional decrement concomitant with high fat milk synthesis. These conflicts have apparently led to novel features of carbohydrate regulation in some fasting adapted animals, including maintenance of fasting blood

glucose, avoidance of ketoacidosis, protein sparing despite high rates of glucose production, impaired glucose clearance and insulin resistance.

LIST OF ABBREVIATIONS

AUC	area under the curve
GTT	glucose tolerance test
I:G	molar ratio of insulin to glucagon
I_s	insulin sensitivity index
K	glucose tolerance index
NEFA	nonesterified free fatty acids

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