Adiponectin and Insulin in Gray Seals during Suckling and Fasting: Relationship with Nutritional State and Body Mass during Nursing in Mothers and Pups

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ABSTRACT

Animals that fast during breeding and/or development, such as phocids, must regulate energy balance carefully to maximize reproductive fitness and survival probability. Adiponectin, produced by adipose tissue, contributes to metabolic regulation by modulating sensitivity to insulin, increasing fatty acid oxidation by liver and muscle, and promoting adipogenesis and lipid storage in fat tissue. We tested the hypotheses that (1) circulating adiponectin, insulin, or relative adiponectin gene expression is related to nutritional state, body mass, and mass gain in wild gray seal pups; (2) plasma adiponectin or insulin is related to maternal lactation duration, body mass, percentage milk fat, or free fatty acid (FFA) concentration; and (3) plasma adiponectin and insulin are correlated with circulating FFA in females and pups. In pups, plasma adiponectin decreased during suckling (linear mixed-effects model [LME]: $T = 4.49$; $P < 0.001$) and the early postweaning fast (LME: $T = 3.39$; $P = 0.004$). In contrast, their blubber adiponectin gene expression was higher during the early postweaning fast than early in suckling (LME: $T = 2.11$; $P = 0.046$). Insulin levels were significantly higher in early (LME: $T = 3.52$; $P = 0.004$) and late (LME: $T = 6.99$; $P < 0.001$) suckling than in fasting and, given the effect of nutritional state, were also positively related to body mass (LME: $T = 3.58$; $P = 0.004$). Adiponectin and insulin levels did not change during lactation and were unrelated to milk FFA or percentage milk fat in adult females. Our data suggest that adiponectin, in conjunction with insulin, may facilitate fat storage in seals and is likely to be particularly important in the development of blubber reserves in pups.

Introduction

The allocation of energy by female capital breeders and their offspring during nursing is important in understanding life-history decisions and lactation strategies (Boggs 1992; Oftedal et al. 1993; Boyd 2000; Crocker et al. 2001). Energy allocation links foraging and reproductive success and, thus, ultimately indicates how individuals and populations may respond to variation in food availability (Boggs 1992; Jönsson 1997; Boyd 2000; Crocker et al. 2001). Capital breeders store energy in advance of reproduction and then utilize those body energy stores to attempt to breed successfully (Jönsson 1997). Careful management of their energy balance is thus central to maximizing their reproductive fitness and survival potential (Calow 1979). Females must possess sufficient energy to "finance" their reproductive expenditure and must allocate reserves judiciously to both sustain their own metabolism and invest in the young at least enough to nurse them to independence (Jönsson 1997). The young must also be able to maximize energy provided by the mother in terms of rapid growth or fat storage (Oftedal et al. 1993; Lindström 1999; Hou et al. 2008).

Fuel allocation must be based, at least in part, on information derived from the availability of metabolic fuel. Endogenous signals that link energy stores with fuel use are well documented in many terrestrial mammals and birds (Woods and Seeley 2000; Benoit et al. 2004). They incorporate a variety of inputs, including circulating levels of metabolites and/or hormones involved in energy balance. Adipose tissue itself is a large endocrine organ and an important regulator of body energy reserves (Trayhurn and Beattie 2001; Kershaw and Flier 2004). It produces an array of physiologically important fat-regulating hormones, termed adipocytokines, which can act centrally to influence appetite and metabolic rate and locally to regulate the balance between lipolysis and lipogenesis (Ahima and Flier 2000; Meier and Gressner 2004; Qi et al. 2004).

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Adiponectin (ACRP30, ApN, or ADIPOQ) is an adipocytokine with key roles in energy balance, acting primarily through powerful insulin-sensitizing effects at the whole-animal and cellular levels (Hu et al. 1996; Berg et al. 2001, 2002; Hotta et al. 2001; Gao et al. 2013). Adiponectin levels and adiponectin messenger RNA (mRNA) abundance negatively correlate with indices of body fat content and insulin sensitivity in dogs, pigs, cows, and primates (Arita et al. 1999; Hotta et al. 2001; Jacobi et al 2004; Ishioka et al. 2006; Taniguchi et al. 2008; Cahill et al. 2013; Gao et al. 2013). Adiponectin mRNA is found exclusively in adipocytes, and its expression is increased up to ~400-fold during their differentiation (Scherer et al. 1995; Körner et al. 2005). In contrast to its effects on muscle and liver, in which it stimulates fatty acid clearance and fatty acid oxidation (Fruebis et al. 2001; Yamauchi et al. 2001; Berg et al. 2002; Thamer et al. 2002; Tomas et al. 2002; Combs et al. 2004), in fat tissue, adiponectin accelerates lipid accumulation during adipogenesis, promoting both proliferation and differentiation of preadipocytes (Fu et al. 2005). Adiponectin has no effect on lipolysis and appears to suppress de novo fatty acid synthesis in adipocytes (Jacobi et al. 2004). However, it enhances insulin-responsive glucose transport and fatty acid uptake and promotes their esterification into triglyceride for fat storage (Combs et al. 2004). Mice with a deletion in the adiponectin gene that causes them to have threefold higher plasma concentrations of the hormone have 30% body fat, compared with only 18% in wild-type littermates (Combs et al. 2004). Adiponectin is thus a powerful driver of adipocyte maturation, triglyceride synthesis, and storage in fat cells. Given the importance of adiponectin in fuel regulation in other species, adiponectin may be a key endocrine signal in energy balance regulation in capital breeders.

Large phocids, such as gray seals (*Halichoerus grypus*), are typical models of capital investment (Boyd 2000). Gray seals possess substantial fat reserves (Reilly and Fedak 1990), rely heavily on fat as a metabolic fuel (Nordøy and Blix 1985; Worthy and Lavigne 1987; Nordøy et al. 1990; Reilly 1991; Bowen et al. 2001; Bennett et al. 2007), and experience large seasonal (Beck et al. 2003) and developmental (Hall and McConnell 2007) changes in the size of fat depots. Female gray seals come ashore to breed at ~33% body fat (Beck et al. 2003) and fast during their annual ~18-d lactation period (Fedak and Anderson 1982), during which they produce milk of up to 60% fat (Iverson et al. 1993). They lose approximately 40% of their initial mass (Pomeroy et al. 1999) and 61%–84% of their fat reserves (Fedak and Anderson 1982; Baker et al. 1995) to raise a single pup. Females must therefore balance use of fuel for maintenance costs with fat allocation to milk and attempt to maximize energy transfer to the pup (Lang et al. 2011). Mass transfer from females to pups varies substantially, from 17% to 89% of females' postpartum mass (Baker et al. 1995; Pomeroy et al. 1999). Larger mothers produce larger, fatter pups (Mellish et al. 1999; Pomeroy et al. 1999; Bowen et al. 2001; Lang et al. 2009). The consequences for fitness are substantial, because first-year survivorship, which is a major driver of population dynamics (Harwood and Prime 1978; Sinclair 1996), is heavily dependent on pups' weaning mass (Hall et al. 2001, 2002, 2009).

Mechanisms that govern energy gain and allocation by gray seal pups are likely to depend on characteristics of and decisions made by the pup (Mellish et al. 1999; Jenssen et al. 2010), as in other pinnipeds (Donohue et al. 2002; McDonald et al. 2012), in addition to the female's quality and investment. While suckling, gray seal pups gain up to 2.5 kg d^{-1} and triple in body mass, largely through the accumulation of subcutaneous blubber (Fedak and Anderson 1982; Anderson and Fedak 1987; Mellish et al. 1999). The pups are weaned abruptly when the mother returns to sea to forage. They then undergo a postweaning fast of 10 d to >4 wk (Reilly 1991; Noren et al. 2008; Bennett et al. 2010) and are completely reliant on the fuel reserves laid down while suckling to sustain them until they begin to feed. They regulate their fuel use during fasting at least in part on the basis of the size of their fuel reserves (Bennett et al. 2007).

The role of adiponectin in seals is poorly understood. Because adiponectin is a powerful insulin sensitizer in other animals, it may work in conjunction with insulin to influence energy balance in these animals. However, phocids have low circulating levels of insulin (Fowler et al. 2008; Viscarra et al. 2011*a*, 2011*b*, 2013; Bennett et al. 2013). Elephant seals (*Mirounga* species) show a limited insulin response to glucose challenge and a high degree of insulin resistance during fasting, which increases with fast duration (Fowler et al. 2008; Viscarra et al. 2011*a*, 2011*b*, 2013). Adiponectin levels in the circulation and adiponectin gene expression and protein abundance in blubber all decrease during the 7 wk of the postweaning fast in northern elephant seals (*Mirounga angustirostris*; Viscarra et al. 2011*a*, 2011*b*; Suzuki et al. 2013).

No data on adiponectin during the intensive nursing period have been documented in seals, despite large changes in body mass and fat content in both mothers and pups and the potential importance of the hormone in modulating energy transfer. In addition, links between plasma insulin and adiponectin levels have not been explored in gray seals. Given the importance of high-fat milk in mass transfer from mother to pup (Iverson et al. 1993) and the ability of insulin to have an impact on milk fat content in ruminants (Bequette et al. 2001; Corl et al. 2006; Winkelman and Overton 2013), both adiponectin and insulin could influence mass transfer efficiency in seals by acting on mammary gland function. To better understand the role of adiponectin and insulin in mass transfer efficiency and control of fat reserves in gray seals, here we investigated (1) whether circulating adiponectin and insulin levels or blubber adiponectin gene expression change during suckling and fasting in gray seal pups, (2) the relationship between plasma adiponectin and insulin in mothers and pups, (3) the relationship between both hormones and body mass and circulating free fatty acid (FFA) in both females and pups, and (4) the relationship between circulating adiponectin and insulin levels and milk fat content and FFA concentration in females.

Material and Methods

Study Animals and Capture Regime

All animals in this study were part of a long-term population-monitoring program undertaken by the Sea Mammal Research Unit in the United Kingdom. Gray seal females, identified as part of the study program from either brands or flipper tags, were observed daily throughout lactation on the Isle of May, Scotland (56°12′N, 2°32′W), in November 2011 and 2012, from the time they were first sighted. The date of birth of their pups was recorded when possible. A total of 24 and 10 mother-pup pairs were captured for this study in 2011 and 2012, respectively. In each year, animals were captured early (day 7 [SD, ±1.5 d] in 2011 [$n = 21$]; day 5 in 2012 [$n = 10$]) and late (day 17.5 [SD, ±1.9 d] in 2011 [$n = 21$]; day 15 in 2012 [$n = 10$]) in the nursing period. In 2011, three mother-pup pairs were captured only at early nursing, because they were not seen again on the colony, and three pairs were not sighted until late in the nursing period.

Each time they were captured, adult females were anesthetized using a 1 mL 100 kg^{-1} intramuscular dose of $zoletil_{100}$ (Virbac, Carros, France) delivered using a pressurized dart and weighed (±0.2 kg) in a stretcher net using a load cell and tripod (Pomeroy et al. 1999). Pups were weighed (±0.2 kg), sex was recorded, and, at first capture, a cattle ear tag (Rototag; Dalton ID Systems, Henley on Thames, Oxforshire, UK) bearing a unique five-digit identifier was attached in the interdigital webbing of one rear flipper (Fedak and Anderson 1982; Bennett et al. 2007) to allow subsequent identification for this study and in the future.

Pups were assumed to have weaned when the female was not seen in attendance for a full day (Bennett et al. 2007). An accurate weaning date was determined for 16 of the pairs (7 male pups; 9 female pups) in 2011 and all 10 animals in 2012. An additional 14 pups (7 male; 7 female) that were not sampled for this study during suckling were sampled after weaning in 2011. Weaned pups were recaptured when resighted on the colony up to 11 d after they had weaned (mean ± SD, 2 ± 2 d postweaning) in 2011 and on day 5 (early fasting) and day 15 (late fasting) postweaning in 2012. All capture and handling procedures were performed under Home Office project licence 60/4009 and conformed to the Animals (Scientific Procedures) Act 1986.

Maternal Postpartum Mass, Pup Weaning Mass, and Expenditure Estimates

Maternal postpartum mass (MPPM), pup weaning mass, percentage maternal expenditure (percentage of MPPM utilized by weaning), and percentage mass transfer efficiency (total mass gain of pup/total mass loss of female × 100) were estimated as described elsewhere (Pomeroy et al. 1999) in mother-pup pairs from both years that had been captured early and late in nursing and for which birth and weaning dates were known. This provided pup mass gain rates during suckling for 18 of the animals in 2011 and all of the animals in 2012 (total $n = 28$). MPPM, weaning mass, and maternal expenditure and mass transfer efficiency could be calculated for all animals in 2012 and for 14 animals in 2011 (total $n = 24$). Daily rates of mass loss were calculated for postweaned, fasting pups in 2012.

Blood and Milk Sampling

Blood samples were obtained from six females and their pups (one male and five females) at the early-nursing capture and 21 mothers and pups (8 males; 13 female) at the late-nursing capture in 2011. Although this first blood sample set was primarily cross-sectional, three of the mother-pup pairs were sampled early and late in the nursing period. Blood samples were taken from the 10 pups in 2012 (4 males; 6 females) at both captures during suckling and after weaning to explore the relationship between blubber adiponectin gene expression and plasma adiponectin in longitudinal samples across nutritional states (early and late suckling and fasting).

Blood samples were taken from the extradural vein into heparin-coated (all animals) or plain (mothers and suckling pups in 2011) sterile 10-mL vacutainer (Becton Dickinson, Cowley, Oxfordshire, UK) using either 19-gauge, 2-inch, or spinal needles, whichever was most appropriate for the size of the animal. All puncture sites were disinfected with a 1:15 solution of the disinfectant Savlon (3% w/v cetrimide; 0.3% w/v chlorhexidine gluconate; Novartis, Horsham, UK) before sampling and were sprayed with topical terramycin (oxytetracycline; Pfizer, Sandwich, Kent, UK) immediately afterward.

After blood sampling, during the cross-sectional study in 2011, females were given a 1-mL intravenous dose of oxytocin (10 IU mL^{-1}; Intervet, Milton Keynes, Bucks, UK) to facilitate milk release from the mammary glands. A modified 20-mL syringe with the luer adaptor end removed was used to take 50 mL of milk from the nipple of the female (Habran et al. 2013). Blood and milk samples were stored on cold packs until processing.

Blubber Sampling

To investigate developmental changes in adiponectin levels in more detail and relate them to relative adiponectin gene expression in blubber tissue in pups, a blubber biopsy was taken at each capture in the longitudinal study in 2012. Pups were given 0.01 mL intravenous $zoletil_{100}$ and 2 mL 2% w/v subcutaneous lignocaine (Lignol, Dechra, Northwich, UK) by injection in the dorsal midpelvic region. A small incision was made in the skin using a scalpel, and a full-depth blubber core was removed using a 6-mm-diameter biopsy punch (Acu-Punch, Acuderm, Fort Lauderdale, FL). Pups were then given an intramuscular dose of terramycin (1 mL 10 kg^{-1}; Pfizer). Any adhering muscle tissue or skin was removed from the blubber, and the core was divided into inner and outer portions, because the inner portion is thought to be more

metabolically active (Strandberg et al. 2008). The inner portion was immersed immediately in five times the volume (~1 mL) of RNAlater (Ambion, Life Technologies, Paisley, UK) and kept on a cold pack until return to the laboratory. The blubber was stored overnight at 4°C to allow penetration of the RNAlater into the tissue and then transferred to storage at −20°C. A minimum 10-d interval separated sampling events to allow healing of the biopsy site. On each subsequent sampling, a core was taken from the opposite side of the body from the last sample, and the previous biopsy sites were checked for healing and were avoided.

Blood and Milk Sample Processing

As described elsewhere (Bennett et al. 2012), blood samples were centrifuged in a swing-out benchtop centrifuge at 2,000 *g* for 15 min within 10 h after sample collection. Aliquots of plasma (heparin tubes) and serum (plain tubes) were transferred to 500-μL microtubes using glass Pasteur pipettes and stored at −80°C until analysis. A 4-mL subsample of milk was removed for this study and stored at −80°C until analysis.

Adiponectin and Insulin Measurements

Samples, standards, and quality controls were assayed in duplicate for both hormones within 12 mo of collection. Adiponectin concentrations in plasma samples were measured using an enzyme-linked immunosorbent assay (ELISA) intended for quantification of human adiponectin (product KHP0041; Life Technologies) in accordance with the manufacturer's instructions, with the exception that the incubation steps were performed at room temperature. Intra-assay coefficient of variation (CV) was 10.3% ± 5%. An eightfold serial dilution of seven samples in assay buffer showed that samples were parallel with the standard curve to a dilution factor of four. Recovery of human adiponectin standard from seal plasma samples was 98.47% ± 3.85%.

Circulating insulin was measured in plasma from 20 of the mother-pup pairs from the cross-sectional study in 2011. A commercially available ultrasensitive ELISA for determination of low insulin concentrations in rat plasma was used with a canine insulin standard according to the manufacturer's instructions (Crystal Chem, Downers Grove, IL). This kit has previously been validated for use in gray seal plasma (Bennett et al. 2013).

Fatty Acid Concentration and Milk Fat Content

The FFA concentrations were measured in duplicate within 10 mo of collection in milk and serum from the cross-sectional samples from 2011. Serum was used to avoid any activation of serum lipoprotein lipase by heparin (Tan 1978; Riemersma et al. 1982; De Sanctis et al. 1994). An enzymatic colorimetric assay (SFA-5; Zenbio, Durham, NC), which uses acyl-CoA synthase to generate fatty acyl-CoA thiol esters, was used. The thiol esters react with oxygen in the presence of acyl-CoA oxidase to produce hydrogen peroxide (H_2O_2). The H_2O_2 facilitates oxidative condensation of 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline with 4-aminoantipyrine, catalyzed by peroxidase. The absorbance of the purple product of this reaction at 550 nm is proportional to FFA in the initial sample. The absorbance was read in a VERSAmax plate reader (Molecular Devices, Sunnyvale, CA), and a standard curve was constructed for calculation of sample concentrations.

Milk creamatocrit was used as a proxy for milk fat content (Lucas et al. 1978) and was measured in triplicate. In brief, milk samples were vortexed, and 75 μL was drawn up into capillary tubes and sealed at each end with putty. Samples were centrifuged at 12,000 *g* for 5–20 mins in a hematocrit centrifuge to determine the duration at which there was no further change in creamatocrit. The cream layer was then expressed as a percentage of the length of the tube, using a hematocrit scale, to give creamatocrit.

Primer Design

Primer pairs to amplify potential reference genes and adiponectin were designed to have a melting temperature of 60°C using Primer3 software (Koressaar and Remm 2007; Untergrasser et al. 2012). Primers were designed on the basis of mammalian sequences available through the National Center for Biotechnology Information (NCBI), focusing on regions with a high degree of conservation between the carnivores aligned using ClustalW (https://www.ebi.ac.uk/Tools/msa/clustalw2/). We used published primers for reference genes (glyceraldehyde 3-phosphate dehydrogenase; Beineke et al. 2004) and ribosomal protein L8 (Tabuchi et al. 2006). We designed primers for four additional potential reference genes: tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta, cyclin A, ubiquitously expressed prefoldin-like chaperone, and ribosomal protein S9. All primers (table 1) were synthesized by MWG Eurofins Operon (Ebersberg, Germany).

RNA Extraction and Complementary DNA Synthesis

To extract total RNA, the blubber samples were thawed in 1 mL of Trireagent (Ambion, Life Technologies), homogenized on ice using a mechanical homogenizer, and incubated at −80°C for 5 min, followed by addition of 200 μL chloroform (Sigma-Aldrich, Dorset, UK). The mixture was vortexed for 30 s and incubated at room temperature for 5 min and then centrifuged at 13,000 rpm in a fixed-angle benchtop centrifuge (Eppendorf 5415R, Stevenage, UK) at 4°C for 15 min. The aqueous phase was retained, and 500 μL of cold isopropanol was added to precipitate RNA, mixed by inversion, incubated at −20°C for 1 h, and centrifuged at 13,000 rpm at 4°C for 15 min. The supernatant was removed, and the RNA pellet was washed twice with 400 μL cold 75% ethanol. The RNA pellet was air-dried on the bench for 5 min and resuspended in 40 μL of molecular-grade water. The RNA was quantified using a Nanodrop 2000 spectrophotometer (Wilmington, DE), integrity was assessed by 1% agarose gel electrophore-

Table 1: Forward (F) and reverse (R) sequences for the primer pair, predicted amplicon size, efficiency, and R^2 for each target gene in gray seal blubber tissue

Target	Sequences	Size	Efficiency (%)	R^2
GAPDH	F: GCCAAAAGGGTCATCATCTC; R: GGGGCCATCCACAGTCTTCT	232	123.21	.96
L8	F: GGTGTGGCTATGAATCCTGT; R: ACGACGAGCAGCAATAAGAC	126	113.26	1
S9	F: ACATCCCGTCCTTCATTGTC; R: CAATCCTCCTCCTCGTCATC	157	101.44	.99
CycA	F: TCATCTGCACCGCCAAGAC; R: AAGCGCTCCATGGCTTCCAC	260	93.20	.99
UXT	F: CTCACAGAGCTCAGCGACAG; R: AGGTGTCTCCGGGAAATTCT	117	118.41	.99
YWHAZ	F: GAGGTTGCTGCTGGTGATGA; R: TCCGGGGAGTTCAGAATTTCG	170	91.61	.99
Adiponectin	F: TATGATGGCACCACTGGAAA; R: GCCTGGTCCACATTCTTCTC	164	93.38	.99

Note. CycA = cyclin A; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; L8 = ribosomal protein L8; S9 = ribosomal protein S9; UXT = ubiquitously expressed prefoldin-like chaperone; YWHAZ = tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta.

sis in Tris-acetate edetic acid buffer, and RNA was stored at −80°C until use. Complementary DNA (cDNA) was synthesized from 250 ng of total DNA-free RNA after an initial genomic DNA elimination reaction of 42°C for 2 min, using the QuantiTect Reverse Transcription kit (QIAgen, Manchester, UK) following the manufacturer's instructions.

Primer Testing and Optimization

A Taq polymerase chain reaction (PCR) Core kit (QIAgen) was used with each primer pair to amplify from a pool of cDNA in a standard thermal cycler (Applied Biosystems 2720, Life Technologies) to ensure that they produced a single amplicon of predicted size. Cycling conditions were 3 min at 94°C for an initial denaturation; followed by 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 60°C, and 1 min extension at 72°C; and ending with a final extension of 10 min at 72°C. The PCR products were visualized on a 1.5% agarose gel in Tris-borate edetic acid buffer, and amplicon identity was verified by Sanger sequencing. Primer annealing temperature and performance of primer pairs that had produced a single correct amplicon in standard PCR conditions were tested by melt curve analysis in real-time quantitative PCR (qPCR) using StepOne software (Applied Biosystems) under the following cycling conditions: initial denaturation of 94°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C annealing for 1 min, and a final step at 95°C for 15 s. Each primer pair was run in triplicate over a four-point log serial dilution of pooled cDNA template (1∶10, 1∶100, 1∶1,000, 1∶10,000) to determine amplification efficiency. Reference gene primer pairs with amplification efficiencies between 90% and 110% were run in triplicate for all samples, and the average cycle threshold (C_T values) were input into NormFinder (ver. 0.953) and Bestkeeper (Pfaffl et al. 2004) to identify the most suitable reference gene. The S9 gene was identified as the only invariant normalizer between the four nutritional states (early and late suckling and early and late fasting).

The cDNA from each pup at each time point was diluted 1∶10 in RNAse-free water and used as the template for qPCR using iTaq Universal Master Mix (BioRad, Hertfordshire, UK) and 0.5 μL of each of the forward and reverse primers. The cDNA from all four time points was run in triplicate on the same plate for both S9 and adiponectin primer pairs for each animal. The average C_T values for adiponectin were normalized to S9 and were expressed as fold differences in mRNA abundance relative to the early-suckling sample for each animal using the Pfaffl (2001) method.

Statistical Analysis

Statistical analyses were performed using R Studio (R 2.13.1; Ihaka and Gentleman 1996; R Development Core Team 2003). Percentage values were arcsine transformed before use as dependent variables in analysis (Sokal and Rolfe 1981). Linear mixed-effects models (LMEs) that included animal identification as a random effect (Chatfield 1989; Crawley 2002) were used to investigate changes in plasma adiponectin in both pups and adult females and changes in relative gene expression in pups. For pups, each year was analyzed separately as a result of differences in sampling regime. Within each year, fixed effects included nutritional state (early or late suckling and early or late fasting) or age, sex, and body mass to determine the best model to explain variability in circulating adiponectin. In addition, insulin levels and adiponectin gene expression were included as fixed effects in 2011 and 2012, respectively. Insulin and relative adiponectin gene expression

values were log transformed before inclusion as dependent variables in the analysis, because these data did not have a normal distribution ($P > 0.05$ by Anderson-Darling test). Nutritional state, sex, body mass, and age were tested to determine which of these fixed effects best explained insulin levels and log-relative adiponectin gene expression levels. For adult females, body mass and day of lactation were tested to determine which of these fixed effects best explained variability in plasma adiponectin and insulin levels. LMEs were used to examine the relationship between circulating adiponectin, insulin, and FFA concentration for both mothers and pups and the relationship between adiponectin, insulin and FFA concentration in milk, and milk fat content in mothers. LMEs were fitted using maximum likelihood.

Linear models (LMs) were used to investigate the relationship between adiponectin and MPPM, daily mass loss, and percentage expenditure in females and pup birth mass, daily mass gain, and weaning mass in pups from both years together, given known effects of maternal size and condition (Pomeroy et al. 1999). The relationship between circulating adiponectin levels and daily rate of mass loss during the postweaning fast was investigated using LM in pups from 2012.

Forward and reverse stepwise model selection was performed, and residual plots were examined to assess model fit. Models were compared as described previously (Bennett et al. 2013) using the anova function in R (Chatfield 1989; Crawley 2002; Fox 2002). This produces a likelihood ratio (L ratio) and probability that the expanded model explains more of the variability in the response variable. In all cases (LMEs and model comparisons), P was considered significant at <0.05. For LMEs, $P < 0.05$ for any given coefficient indicates that it is significantly greater than 0. For model comparison, $P < 0.05$ indicates that the expanded model explains significantly more of the variability in the response variable than the simpler model.

Results

Adiponectin and Insulin during Feeding and Fasting

In the cross-sectional sampling regime in 2011, there was a significant decrease in circulating adiponectin in pups over the study period. The decrease was best explained by nutritional state (fig. 1; LME: Akaike information criterion [AIC] = 244.78; log likelihood = 117.39; n observations = 55; n individuals = 39). There was a significant decrease from early to late suckling (LME: $T = 4.49$; $P < 0.001$) and again from late suckling to weaning (LME: $T = 3.39$; $P = 0.004$). For pups in which insulin had also been measured, the model that contained nutritional state was significantly improved by the inclusion of insulin levels as an additional explanatory variable (L ratio = 3.911; $P = 0.048$). Given the effect of nutritional state, there was a positive relationship between circulating adiponectin and insulin values that approached significance (LME: adiponectin = 0.11 [insulin] + 11.82; $T = 2.13$; $P = 0.054$; fig. 2). In turn, log insulin levels were best explained by nutritional state and body mass (LME: AIC = 0.10; log likelihood = 6.05; n observations = 36; n individuals = 21). Insulin levels were not different between early and late suckling ($T = 1.71$; $P = 0.112$), but they were higher during both early ($T = 3.52$; $P = 0.004$) and late ($T = 6.99$; $P < 0.001$) suckling than after weaning and, given the effect of nutritional state, were positively related to body mass (log insulin = 0.03 [mass] + 0.79; $T = 3.58$; $P = 0.004$; fig. 3).

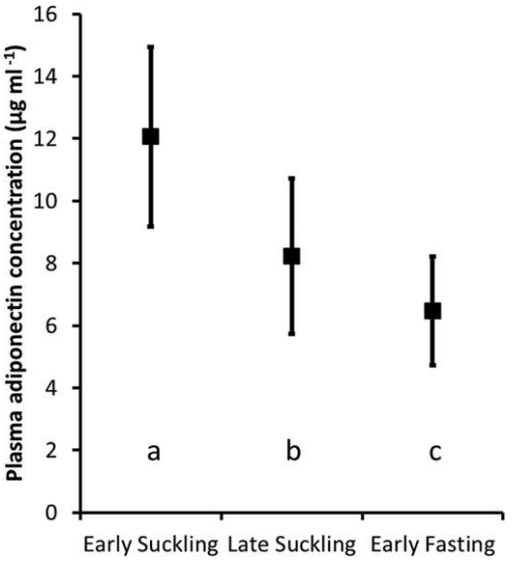

Figure 1. Mean ± SD plasma adiponectin in healthy wild gray seal pups at early (7 ± 1.5 d after birth) and late (17 ± 1.5 d after birth) suckling and early fasting (2 ± 2 d after weaning) in a cross-sectional study in 2011. Points with different letters are significantly different from each other (linear mixed-effects model: $P < 0.05$).

In the longitudinal sampling regime in 2012, the significant decrease in circulating adiponectin in pups over time was best explained by nutritional state (early/late suckling and early/late weaning) and mass (LME: AIC = 123.78; log likelihood = 54.89; n observations = 38; n individuals = 10; fig. 4). There was a significant reduction in pups' adiponectin from early to late suckling ($T = 2.22$; $P = 0.040$) and again from late suckling to early postweaning ($T = 4.18$; $P < 0.001$), and levels did not change during the postweaning fast ($T = 0.25$; $P = 0.806$), such that both early ($T = 4.30$; $P < 0.001$) and late ($T = 5.12$; $P < 0.001$) fasting values were significantly lower than at early suckling. Given the effect of the nutritional state of the animal, body mass significantly improved the model fit (L ratio = 4.20; $P = 0.041$) and showed a positive relationship with adiponectin levels that closely approached significance (adiponectin = 0.11 [mass] + 0.44; $T = 1.98$; $P = 0.058$). Blubber adiponectin expression did not significantly improve the model that contained nutritional state and body mass (L ratio = 1.255; $P = 0.262$). Circulating adiponectin concentration was not related to blubber adiponectin gene expression levels (LME: $T = 0.48$; $P = 0.962$; AIC = 151.56; log likelihood = 71.78; n observations = 37; n individuals = 10).

Relative log adiponectin gene expression in blubber changed with nutritional state (LME: AIC = 72.07; log likelihood =

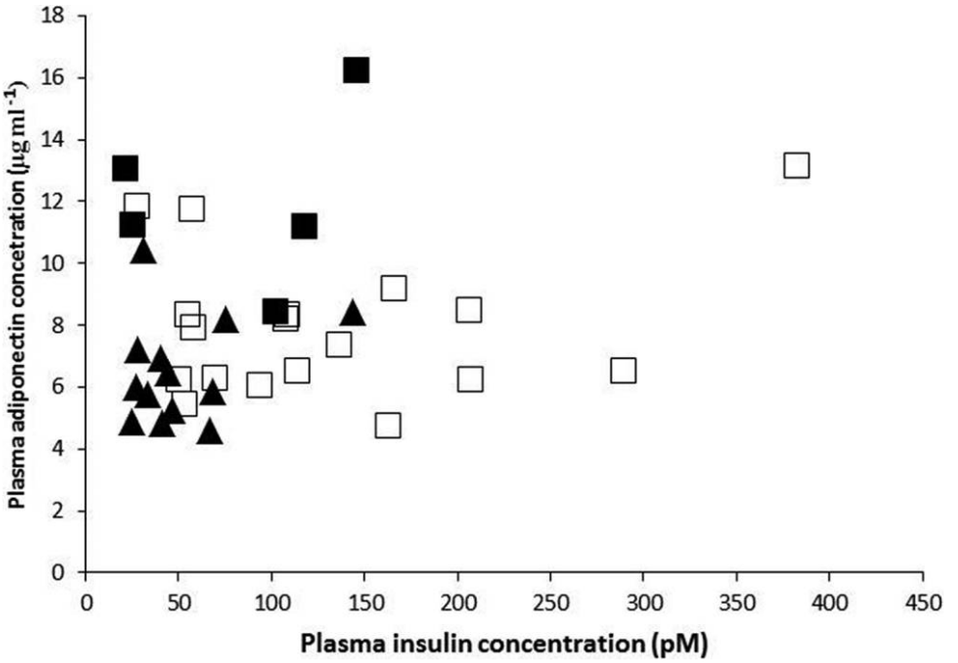

Figure 2. Relationship between plasma insulin and adiponectin in healthy wild gray seal pups at early (filled squares) and late (open squares) suckling and early fasting (filled triangles) in a cross-sectional study in 2011. Linear mixed-effects model: Akaike information criterion = 159.90; log likelihood = 73.95; *n* observations = 36; *n* individuals = 21.

30.04; *n* observations = 37; *n* individuals = 10; fig. 5). The increase from early to late suckling approached significance (LME: $T = 1.80$; $P = 0.085$). Relative adiponectin gene expression remained higher early in the postweaning fast than early in suckling (LME: $T = 2.11$; $P = 0.046$) and then returned to levels that were not different from those in early suckling (LME: $T = 1.33$; $P = 0.196$).

In contrast to the decrease in circulating adiponectin levels in pups, plasma adiponectin concentration was not related to days of lactation in adult females in the cross-sectional

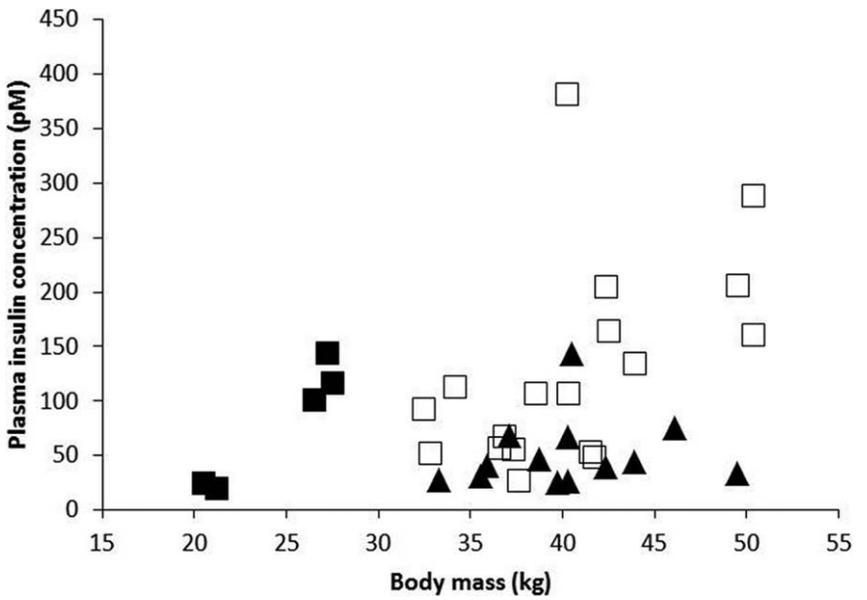

Figure 3. Relationship between plasma insulin and body mass in healthy wild gray seal pups at early (filled squares) and late (open squares) suckling and early fasting (filled triangles) in a cross-sectional study in 2011. Linear mixed-effects model: Akaike information criteria = 0.10; log likelihood = 6.05; *n* observations = 36; *n* individuals = 21.

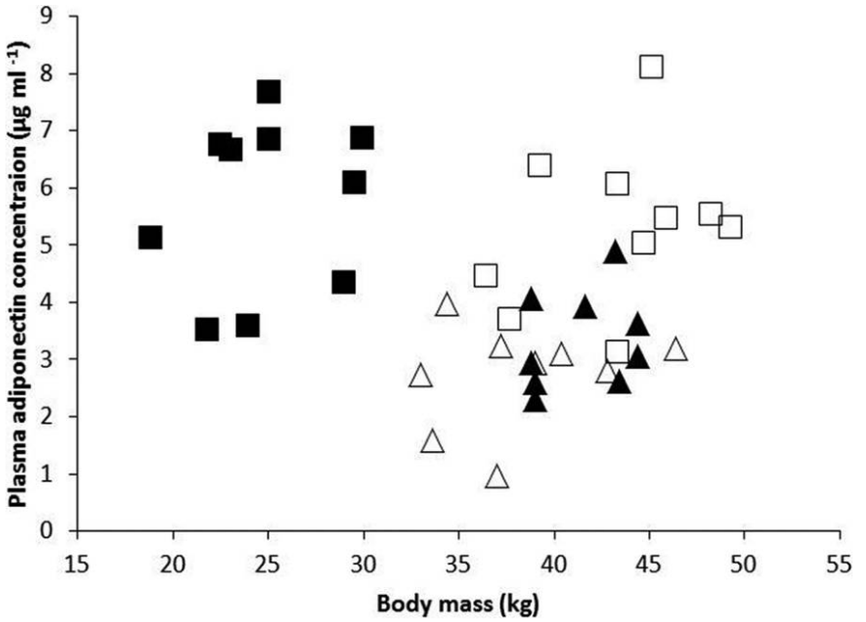

Figure 4. Relationship between body mass and plasma adiponectin in healthy wild gray seal pups at early (filled squares) and late (open squares) suckling and early (filled triangles) and late (open triangles) in the postweaning fast in a longitudinal study in 2012. Linear mixed-effects model: Akaike information criterion = 123.78; log likelihood = 54.89; *n* observations = 38; *n* individuals = 10.

samples (LME: $T = 0.92$; $P = 0.453$; AIC = 74.42; log likelihood = 33.21; *n* observations = 26; *n* individuals = 23; table 2). The positive relationship between adiponectin and body mass at parturition was not significant (LM: adiponectin = 0.02 (MPPM) + 1.16; $F_{1,16} = 3.68$; $P = 0.073$; $r^2 = 0.14$). Adiponectin was not related to body mass in females during lactation (LME: $T = 0.074$; $P = 0.948$; AIC = 75.32; log likelihood = 33.66; *n* observations = 26; *n* individuals = 23). Insulin levels were not related to MPPM (LM: $F_{1,14} = 0.03$; $P = 0.859$; $r^2 = 0$). There was no relationship between insulin and adiponectin levels (LME: $T = 0.32$; $P = 0.780$; AIC = 62.27; log likelihood = 30.13; *n* observations = 23; *n* individuals = 20) and no change in insulin during lactation in adult females (LME: $T = 2.55$; $P = 0.125$; AIC = 58.76;

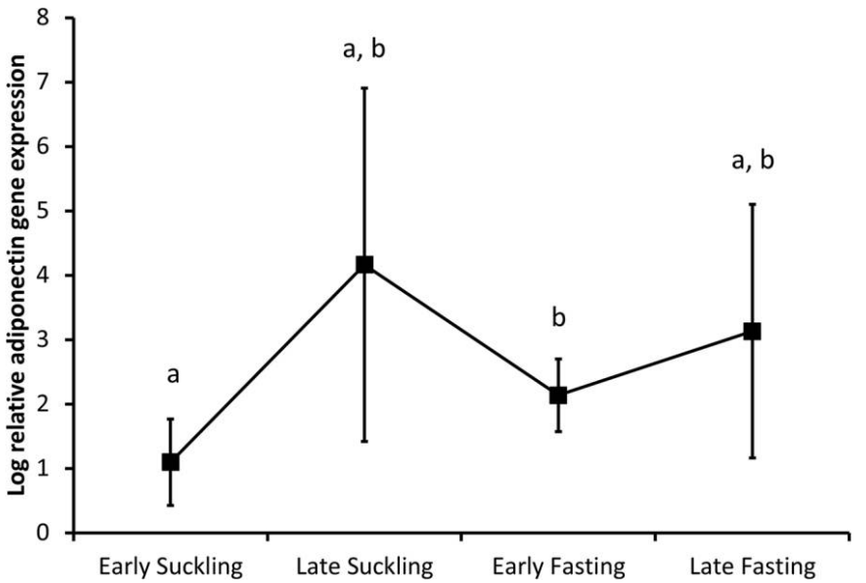

Figure 5. Mean ± SD log relative adiponectin messenger RNA abundance in gray seal pup blubber from 2012 at early suckling (5 d after birth), late suckling (15 d after birth), early fasting (5 d postweaning), and late fasting (15 d postweaning). Points with different letters are significantly different from each other (linear mixed-effects model: $P < 0.05$).

Table 2: Body mass, plasma adiponectin and insulin concentrations, serum and milk free fatty acid (FFA) concentrations, and milk creamatocrit (an index of milk total fat content) for lactating adult female gray seals early and late in lactation in a cross-sectional study in 2011

	Mean ± SD (*n*)	
Variable	Early	Late
Mass (kg)	169.4 ± 27 (21)	133.3 ± 21 (21)
Adiponectin (μg mL^{-1})	3.64 ± 1.14 (6)	4.14 ± 1.23 (21)
Insulin (pM)	157.12 ± 233.10 (5)	83.47 ± 166.59 (21)
Serum FFA (mM)	1.57 ± .57 (6)	1.63 ± .48 (21)
Milk FFA (mM)	3.545 ± 1.67 (6)	5.90 ± 2.36 (21)
Creamatocrit (%)	58.15 ± 6.26 (6)	53 ± 10.98 (21)

log likelihood = 25. 83; *n* observations = 23; *n* individuals = 20; table 2).

Adiponectin, Insulin, and Mass Transfer

Maternal and pup mass at birth and weaning and details of mass transfer are given in table 3 for each year. Pup daily rate of mass gain was positively related to maternal mass loss rate, was negatively related to pup birth mass, and tended to be higher in 2012 than 2011 (table 4; LM: $F_{3,24} = 51.67$, $P < 0.001$; $r^2 = 0.849$). Pups' adiponectin levels during suckling did not explain any additional variation in their daily rate of mass gain (ANOVA: $F = 0.13$, $P = 0.895$). In 2011, pups' insulin levels during suckling did not explain any additional variation in their daily rate of mass gain (ANOVA: $F = 0.04$, $P = 0.849$).

Weaning mass was positively related to MPPM, percentage expenditure, and pup rate of mass gain. Given these relationships, weaning mass was negatively related to maternal rate of mass loss and was significantly higher in 2012 than 2011 (LM: $F_{5,18} = 37.43$, $P = 0.001$; $r^2 = 0.888$; table 4). The inclusion of either pup adiponectin levels (ANOVA: $F = 0.94$, $P = 0.347$) or insulin (ANOVA: $F = 2.51$, $P = 0.144$) did not improve the model. Pups' fasting rate of mass loss in 2012 was not related to adiponectin levels (LM: $F_{1,7} = 0.25$, $P = 0.635$; $r^2 = 0$).

Daily rates of mass loss tended to be higher in females that were heavier at parturition (LM: daily rate of mass loss = 0.01 [MPPM] + 1.52; $F_{1,16} = 4.43$, $P = 0.051$; $r^2 = 0.17$). Circulating adiponectin levels could not explain any additional variation in maternal daily mass loss (ANOVA: $F = 0.01$, $P = 0.947$) or percentage of MPPM expended by females during lactation (ANOVA: $F = 0.16$, $P = 0.695$). In 2011, circulating insulin levels could not explain any additional variation in maternal daily mass loss (ANOVA: $F = 2.22$, $P = 0.160$) and were not related to percentage of MPPM expended by females during lactation (ANOVA: $F = 2.05$, $P = 0.177$).

Adiponectin, Insulin, and Circulating FFA

There was no change in serum FFA with age (LME: $T = 3.00$; $P = 0.205$; AIC = 78.81; log likelihood = 35.40; *n* observations = 25; *n* individuals = 23), time during suckling (LME: $T = 2.67$; $P = 0.228$; AIC = 80.31; log likelihood = 36.15; *n* observations = 25; *n* individuals = 23), adiponectin (LME: $T = 0.98$; $P = 0.507$; AIC = 86.05; log likelihood = 39.03; *n* observations = 25; *n* individuals = 23), or insulin levels (LME: $T = 0.69$; $P = 0.614$; AIC = 75.11; log likelihood = 33.55; *n* observations = 21; *n* individuals = 19) in suckling pups from the cross-sectional study in 2011. Serum FFA concentration did not change in adult females in the cross-sectional study in response to days of lactation (LME: $T = 0.43$; $P = 0.708$; AIC = 41.42; log likelihood = 16.71; *n* observations = 26; *n* individuals = 23; table 2), mass (LME: $T = 0.20$; $P = 0.860$; AIC = 41.54; log likelihood = 16.77; *n* observations = 26; *n* individuals = 23), circulating adiponectin (LME: $T = 0.81$; $P = 0.504$; AIC = 40.87; log likelihood = 16.44; *n* observations = 26; *n* individuals = 23), or insulin levels (LME: $T = 1.57$; $P = 0.255$; AIC = 30.59; log likelihood = 11.27; *n* observations = 22; *n* individuals = 19).

Adiponectin, Insulin, and Milk FFA and Fat Content

There was a positive relationship between milk FFA concentration and days of lactation that approached statistical significance (LME: milk FFA = 0.28 [days of lactation] + 1.19; $T = 3.21$; $P = 0.085$; AIC = 119.89; log likelihood = 55.94; *n* observations = 26; *n* individuals = 23; table 2). Milk FFA concentration was unrelated to body mass (LME: $T = 2.05$; $P = 0.177$; AIC = 124.13; log likelihood = 58.06; *n* observations = 26; *n* individuals = 23), plasma adiponectin concentration (LME: $T = 0.99$; $P = 0.427$; AIC = 126.97; log likelihood = 59.49; *n* observations = 26; *n* individuals = 23), or insulin levels (LME: $T = 1.94$; $P = 0.193$; AIC = 106.64; log likelihood = 49.32; *n* observations = 22; *n* individuals = 19).

Creamatocrit, an index of milk percentage fat (Lucas et al. 1978), was not related to duration of lactation (LME: $T = 0.76$; $P = 0.528$; AIC = 37.01; log likelihood = 22.50; *n* observations = 26; *n* individuals = 23; table 2), body mass (LME: $T = 2.45$; $P = 0.134$; AIC = 42.20; log likelihood = 25.10; *n* observations = 26; *n* individuals = 23), milk FFA concentration (LME: $T = 0.04$; $P = 0.972$; AIC = 36.40; log likelihood = 22.20; *n* observations = 26; *n* individuals = 23), circulating adiponectin levels (LME: $T = 0.21$; $P = 0.856$; AIC = 36.44; log likelihood = 22.22; *n* observations = 26; *n* individuals = 23), or plasma insulin (LME: $T = 0.70$; $P = 0.554$; AIC = 28.45; log likelihood = 18.22; *n* observations = 22; *n* individuals = 19).

Discussion

Adiponectin and Insulin during Feeding and Fasting

Circulating adiponectin decreased markedly in gray seal pups over the suckling period and early postweaning fast. Given the effect of nutritional state, adiponectin levels were positively

Table 3: Maternal postpartum mass (MPPM), mass loss rate during lactation, and total mass loss expressed as a percentage of MPPM (percentage maternal expenditure) for female gray seals and birth mass, rate of mass gain during suckling, weaning mass, percentage of maternal mass loss gained by the pup during suckling (percentage mass transfer efficiency), and postweaning mass loss rate for their pups during 2011 and 2012

	Mean ± SD (*n*)	
Variable	2011	2012
Mother:		
MPPM (kg)	192.18 ± 25.6 (18)	198.45 ± 14.43 (10)
Maternal mass loss rate (kg d^{-1})	3.49 ± .56 (18)	3.87 ± .46 (10)
Percentage maternal expenditure	36.92 ± 5.59 (18)	40.88 ± 4.59 (10)
Pup:		
Birth mass (kg)	14.43 ± 1.76 (18)	13.41 ± 1.99 (10)
Rate of mass gain (kg d^{-1})	1.56 ± .36 (18)	1.92 ± .27 (10)
Weaning mass (kg)	43.69 ± 6.11 (14)	50.99 ± 6.35 (10)
Percentage mass transfer efficiency	40.50 ± 5.81 (14)	46.05 ± 4.32 (10)
Postweaning mass loss rate (kg d^{-1})	…[a]	.58 ± .24 (9)

[a]Postweaning mass loss rate in 2011 is not reported, because animals were sampled at or close to weaning in most cases.

related to body mass and insulin levels. The pattern of change in circulating adiponectin is consistent with a number of potential roles of the hormone. First, high adiponectin levels during suckling may help to minimize inflammatory processes that would otherwise be initiated by high levels of fuel availability, as in other mammals (Meier and Gressner 2004). Second, adiponectin acts centrally to increase food intake in rodents (Qi et al. 2004; Kubota et al. 2007) and may be involved in stimulating appetite in suckling pups. Third, adiponectin promotes oxygen consumption and thermogenesis (Qi et al. 2004; Kubota et al. 2007), actions that may be particularly beneficial in early suckling, when pups require a greater capacity for metabolic heat production in the absence of thick blubber (McCafferty et al. 2005). A decrease in adiponectin levels in pups after weaning may facilitate the reduction in energy expenditure during fasting (Reilly 1991). Finally, high circulating adiponectin in suckling pups and its positive relationship with body mass is consistent with a role for adiponectin in fat deposition and adipogenesis (Combs et al. 2004; Fu et al. 2005).

Adiponectin works in conjunction with insulin. In addition to facilitating rapid adipogenesis and lipid retention directly, it acts indirectly by increasing sensitivity to insulin (Berg et al. 2001, 2002; Fu et al. 2005; Cahill et al. 2013), which in turn promotes fat deposition (Hausberger and Hausberger 1958). The elevation in adiponectin levels seen in humans during short-term overfeeding, which occurs independently of adiposity, has been suggested to maintain insulin sensitivity during rapid weight gain (Brons et al. 2009; Cahill et al. 2013). As in previous studies involving gray seals (Bennett et al. 2013), insulin was higher in suckling pups than in fasting individuals, which is a pattern typical of other mammals (Trenkle

Table 4: Model output for the linear model that best explains variability in pup rate of mass gain during suckling and pup weaning mass in healthy wild gray seal pups from 2011 and 2012

Dependent variable, explanatory variable	β	*T*	*P*	*n*	R^2
Daily pup mass gain (kg d^{-1}):					
Intercept	−233.606	1.906	.0688	28	.85
Daily maternal mass loss (kg d^{-1})	.3745	6.684	<.0001		
Pup birth mass (kg)	−.0961	5.969	<.0001		
Year	.1169	1.919	.067		
Weaning mass (kg):					
Intercept	−5,059.332	2.104	.04967	24	.89
Daily pup mass gain (kg d^{-1})	8.6045	3.531	.00234		
Daily maternal mass loss (kg d^{-1})	−6.5035	3.228	.00467		
Percentage expenditure	.9356	6.526	<.0001		
MPPM (kg)	.1510	5.412	<.0001		
Year	2.5103	2.099	.0502		

Note. MPPM = maternal postpartum mass; *P* = probability that *T* exceeds the critical value; percentage expenditure = 100 × (MPPM − maternal departure mass/MPPM).

1970; Pégorier et al. 1981). Within any given nutritional state, pups with higher insulin levels were heavier, which suggests that insulin is also important in mass and fat gain in seals, as it is in other animals (Hausberger and Hausberger 1958). Our data suggest that adiponectin could facilitate insulin action in seal pups.

Interestingly, there was a positive relationship between insulin and adiponectin levels in pups once the effects of nutritional state and mass were accounted for. This contrasts with the inverse relationship between the two hormones found in humans and macaques (Hotta et al. 2000, 2001; Haque et al. 2002; Cahill et al. 2013). However, it is consistent with the observation that circulating adiponectin decreases by 30% within 10 min of a bolus dose of glucose, accompanied by a decrease in insulin over the same period in fasting northern elephant seal pups (Viscarra et al. 2011*a*). In adult female gray seals, there was no relationship between adiponectin and insulin levels. The regulation of the two hormones and their relationship with each other may depend on nutritional state and/or age in seals. For example, in humans, insulin increases plasma adiponectin and stimulates adiponectin release in lean subjects but not in insulin-resistant obese subjects (Hajri et al. 2011).

Insulin stimulates adiponectin expression and/or secretion from adipocytes or adipose tissue explants in vitro (Halleux et al. 2001; Körner et al. 2005; Pereira and Draznin 2005; Blümer et al. 2008). A stimulatory effect of insulin on adiponectin production by blubber could partially explain higher levels of adiponectin in suckling pups. Adiponectin has no effect on insulin secretion in normal-weight, insulin-sensitive mice, but in mice that are made insulin resistant by high-fat feeding, it inhibits insulin secretion when glucose is <3 mM and activates insulin secretion when glucose is >15 mM (Winzell et al. 2004). A similar mechanism operating in seals would allow adiponectin to facilitate insulin-mediated mass gain during high fuel availability, such as occurs in suckling, and could explain differences between pups and fasting adults in the relationship between insulin and adiponectin.

The decrease in circulating adiponectin levels during suckling and the early postweaning fast was not mirrored in the pattern of change in relative adiponectin gene expression in blubber. The change in blubber adiponectin gene expression in seal pups tracked changes in fat stores and may thus indicate adipocyte maturation (Viscarra et al. 2011*b*) or fat content, as in other animals (Scherer et al. 1995; Körner et al. 2005; Ramsay and Caperna 2009). Adiponectin may be transcriptionally regulated by cellular energy state and/or insulin in seals, as in other animals (Kliewer et al. 1997; Iwaki et al. 2003; Körner et al. 2005; Giorgiadi and Kersten 2012; Viscarra and Ortiz 2013), which could explain the reduction in gene expression in blubber after weaning in seal pups.

The lack of a relationship between circulating adiponectin and adipose expression has been reported in other animals (Hotta et al. 2001) and is likely a result of the high degree of posttranslational modification of adiponectin in which it is folded and assembled into oligomers of different sizes (Pajvani et al. 2003; Liu and Liu 2012, 2014; Tsao 2014) and thiol-mediated retention of adiponectin in the endoplasmic reticulum (Wang et al. 2007). This leads to a decoupling of secretion from gene expression (Pereira and Draznin 2005). The degree of adiponectin oligomerization and the secretion of different oligomers is influenced by insulin, cellular redox state, and energy sensing, which change in response to metabolic condition (Wang et al. 2007; Long et al. 2010; Briggs et al. 2011; Liu and Liu 2012). Here, we measured total adiponectin; thus, a key next step in understanding the role of adiponectin in seals is to determine the relative composition and control of secretion of the circulating oligomers.

Adiponectin, Insulin, and Mass Transfer

As in previous work involving gray seals and other phocids (Arnbom et al. 1993; Mellish et al. 1999; Pomeroy et al. 1999; Bowen et al. 2001; Lang et al. 2009), the size of the mother at delivery and the rate of mass gain by the pup were the primary drivers of weaning mass, and pup mass gain was greater for mothers that lost more weight. Adiponectin and insulin levels in either mother or pup had no additional explanatory power in these relationships, but the high levels of both hormones in suckling pups, their positive relationship with mass, and the tendency for higher maternal adiponectin in females that were larger at delivery suggest a role for the hormone in fat deposition. Indeed, if adiponectin facilitates lipogenesis, maternal adiponectin levels could contribute indirectly to the dynamics of mass transfer in seals by influencing postpartum mass, an important determinant of mass transfer efficiency and pup mass gain (Arnbom et al. 1993; Mellish et al. 1999; Pomeroy et al. 1999; Bowen et al. 2001; Lang et al. 2009). The small sample size of early-lactation females, sampling 5–7 d into lactation, and the difficulty of obtaining plasma from animals feeding at sea preclude additional investigation of the hypothesis that adiponectin and insulin facilitate fat deposition before breeding in females in our study. Clearly, experimental data on the effect of insulin and adiponectin on fat tissue are required.

Adiponectin levels in other mammals are more strongly associated with insulin sensitivity than with body fat content or mass itself (Hotta et al. 2001; Weyer et al. 2001; Berg et al. 2002; Gil-Campos et al. 2004; Cahill et al. 2013; Gao et al. 2013; Viscarra et al. 2011*a*, 2011*b*, 2013). Although there was insufficient tissue to measure insulin signalling pathways here, in gray seal liver, there is no evidence of a difference between suckling and fasting pups in phosphorylated insulin receptor (Bennett et al. 2013), a marker of tissue insulin sensitivity. If adiponectin is coupled with insulin sensitivity in gray seals, our results suggest either that they do not alter insulin sensitivity during fasting or that the changes occur early during the fasting period. Clearly, measures of insulin sensitivity in adipose tissue and at the whole-animal level are needed in gray seals to explore the relationship between adiponectin, insulin levels, and insulin sensitivity and understand the roles of these key hormones in energy balance in this species.

Adiponectin, Insulin, and FFA Concentration and Milk Fat

We saw no changes in FFA in pups or females during lactation, which contrasts with previous findings in gray seals, in which plasma FFA concentration doubled in females in the first 10 d of lactation and then began to decrease up to day 15 (Mellish and Iverson 2001). We also observed a trend toward increased milk FFA concentration but no change in milk fat content with lactation duration. In previous studies, milk fat content increased from 35%–38% to 54%–58% over the first 10 d of lactation in gray seals and did not change thereafter (Iverson et al. 1995; Mellish et al. 1999). The discrepancy is likely a result of small sample size in early lactation and the sampling of the females at approximately day 7 and day 17 here, which may have missed the initial increases and the peak in FFA concentrations and milk fat content.

Because both adiponectin and insulin increase clearance of FFA from the plasma and promote their uptake by adipocytes, muscle, and liver (Fruebis et al. 2001; Combs et al. 2004), we expected that both hormones would be negatively related to FFA in serum in seals. We also expected that insulin levels would be negatively related to milk fat content and FFA concentration, as in lactating goats and cows (Bequette et al. 2001; Corl et al. 2006). The lack of a relationship between insulin, adiponectin, and FFA in fasting and suckling pups and in lactating females may be indicative of relative insulin insensitivity of seal tissues, as in other seal species (Fowler et al. 2008; Viscarra et al. 2011*a*, 2011*b*, 2013). Alternatively, hormones that were not measured here may exert a greater level of control over FFA concentrations (Saleh et al. 1999). Insulin and adiponectin do not appear to influence mass transfer through control of milk fat content or fatty acid concentration in gray seals, but they could have an impact on other aspects of milk yield or composition, as in ruminants (Bequette et al. 2001; Corl et al. 2006; Winkelman and Overton 2013).

Summary

In summary, adiponectin shows marked developmental changes in gray seal pups, which are governed by the nutritional state of the animal, correlated with insulin levels and decoupled from gene expression in blubber tissue. Together, our observations suggest that adiponectin, perhaps in combination with insulin, could facilitate adipogenesis and fat accumulation in seals, particularly in suckling pups. It may also be involved in regulation of insulin sensitivity, appetite, and energy expenditure and could protect against inflammation in developing fat tissue. Neither adiponectin nor insulin appears to regulate milk fat content or FFA concentration, which suggests that they do not influence mammary gland lipid turnover. Gene expression of adiponectin in blubber may be a better index of adipocyte maturation than are plasma levels. Because adiponectin appears to contribute to energy balance, it is important to elucidate the control of its secretion and its metabolic role in seals, particularly in mediating insulin sensitivity, at the cellular, molecular, and whole-animal levels.

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