



Relationship between plasma leptin-like protein levels, begging and provisioning in nestling thin-billed prions *Pachyptila belcheri*

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ABSTRACT

While there have been many studies in various species examining the physiological role of leptin, there are so far no data in free-living seabirds. In the present study, we assess whether leptin is expressed in thin-billed prions (*Pachyptila belcheri*) and we investigate its relationship with feeding-related parameters including body condition, begging intensities and provisioning rates. We showed by Western Blot analysis using leptin-specific antibody that leptin-like protein (14–16 kDa) is expressed in adipose tissue and liver of nestling thin-billed prions. Plasma leptin-like protein levels, determined by RIA, were in the same range (1–3 ng/ml) as in other avian species and increased with age. In two breeding seasons, the plasma leptin-like protein levels were negatively correlated with provisioning rates ($R = -0.67$ and -0.35 in 2003 and 2004, respectively, $P < 0.05$) indicating that endogenous leptin may be an anorexigenic hormone in wild birds. Plasma leptin-like protein levels were positively correlated with begging intensities ($R = 0.43$ and 0.37 in 2003 and 2004, respectively, $P < 0.05$), and this may be because hungry nestling seabird chicks with low body conditions increased their begging intensities. Plasma leptin-like protein levels did not correlate either with plasma triglyceride or glucose levels in thin-billed prions. Overall, these findings show the presence of leptin-like protein in free-living seabirds and provide new insights into its function and its possible role in feeding-associated behaviours.

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1. Introduction

Leptin, a 16-kDa adipocytokine hormone encoded by the ob gene, has been shown to play an important role in food intake regulation (Zhang et al., 1994; Pellemounter et al., 1995; Halaas et al., 1995; Freidman and Halaas, 1998), energy homeostasis (Mistry et al., 1997; Scarpace et al., 1997), and reproduction in mammals (Chehab et al., 1996). Leptin is mainly secreted by mammalian adipose tissue (Zhang et al., 1994) and functions as a hormonal signalling mechanism for fat deposition. Mammalian adipocytes produce and secrete more leptin in the bloodstream as fat storage increases (Maffei et al., 1995) signalling the brain via leptin receptor (Fei et al., 1997) and modulating the hypothalamic feeding-related (an)orexigenic neuropeptides to suppress appetite and increase energy expenditure (Schwartz et al., 1996). Leptin has recently sustained an increasing interest in non-mammalian species. Biochemical studies (immunohistochemistry and

Western blotting) using specific anti-mammalian leptin antibodies revealed immunoreactive bands and cells in various tissues from fish, amphibian, lizard, snake, and birds (Johnson et al., 2000; Niewiarowski et al., 2000; Paolucci et al., 2001; Muruzabal et al., 2002; Bosi et al., 2004; Kochan et al., 2006; Neglia et al., 2007). Several laboratories described the existence of a leptin-like gene in fish (Kurokawa et al., 2005), amphibians (Crespi and Denver, 2006; Boswell et al., 2006), and birds (Taouis et al., 1998; Ashwell et al., 1999). However, there are some controversial reports about the presence of a leptin-like gene in the avian species. In fact, several research groups cloned the coding region of the leptin gene in chicken (Taouis et al., 1998; Ashwell et al., 1999; Sato et al., 2003), turkey (GenBank Accession No. AF082501), and duck (GenBank Accession No. AY555727), however other laboratories have since been unable to amplify the report sequences (Friedman-Einat et al., 1999; Pitel et al., 2000). Furthermore, recent data reported that the leptin gene is missing in the chicken genome (Carre et al., 2006), and a molecular evolutionary analysis suggested that it is unlikely that the chicken leptin cDNA sequence in GenBank is a chicken gene (Sharp et al., 2008). We here use the term “leptin-like protein” to take this controversy into account, by admitting that the mammalian leptin RIA might be measuring either the avian homologue of mammalian leptin in avian plasma, or some other protein with similar immunological characteristics.

Abbreviations: BC, body condition; EDTA, ethylene diamine tetra-acetic acid; EGTA, ethylene glycole tetra-acetic acid; GH, growth hormone; GLM, general linear model; IGF, insulin like growth factor; MAPK, mitogen activated protein kinase; m_{mean} , mean mass; NaCl, sodium chloride; RIA, radioimmunoassay.

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Unlike mammals, a leptin-like protein is expressed in the liver as well as in the adipose tissue of domestic (chicken, *Gallus gallus*) and wild (dunlin, *Calidris alpina*) birds (Taouis et al., 1998; Ashwell et al., 1999; Kochan et al., 2006), and this situation, apparently unique to avian species, undoubtedly reflects a major role for the liver in the regulation of lipogenesis (Leveille et al., 1968). Leptin-like gene expression and plasma leptin-like protein levels have been shown to be altered by nutritional state: decreased by food deprivation and increased after meal feeding in chickens and dunlin (Sato et al., 2003; Dridi et al., 2005a; Kochan et al., 2006). Peripheral or central administration of recombinant (human or chicken) leptin significantly reduced food intake in chickens (Raver et al., 1998; Cassy et al., 2004; Dridi et al., 2000a, 2005b; Denbow et al., 2000; Kuo et al., 2005; Yang and Denbow, 2007) and in great tits (*Parus major*) (Löhmus et al., 2003).

Procellariiform seabirds such as albatrosses, petrels, and shearwaters, rear only one chick at a time but may breed many times in their lives (e.g. Brooke, 2004). Parents should thus limit food delivery to the chick to keeping a balance between current and future reproductive output. Despite the limit and irregular energy supply, chicks of seabirds show an extreme growth pattern with a large accumulation of lipid (some times reaching over 190% of adult mass) during nestling development (e.g. Quillfeldt and Peter, 2000). Thus, the term “nestling obesity” has been used to describe the high peak masses of the chicks (e.g. Hamer and Hill, 1997; Phillips and Hamer, 1999; Schultz and Klomp, 2000). Leptin, regarding its key role in energy homeostasis and lipid metabolism in mammals, might be involved in the regulation and sensing of energy reserves and feeding-associated behaviour in free-living seabird chicks. Therefore, the present study aimed to determine firstly whether leptin is expressed in liver and adipose tissue of thin-billed prions, and secondly to investigate the relationship between plasma leptin levels, body conditions, provisioning rate, and begging intensity.

2. Materials and methods

2.1. Field site and study species

The study was carried out at New Island, Falkland Islands, from 8 January to 4 February 2003 and from 8 January to 10 March 2004. The life cycle and basic biology of Thin-billed prions have been described by Strange (1980). More recent studies described sexual dimorphism (Genevois and Bretagnolle, 1995), feeding ecology (Quillfeldt et al., 2007a; Cherel et al., 2002; Chastel and Bried, 1996), parental investment (Quillfeldt et al., 2007b; Quillfeldt et al., 2003; Duriez et al., 2000; Weimerskirch et al., 1995) and hormonal regulation of behaviour (Quillfeldt et al., 2006; Quillfeldt et al., 2007c). Thin-billed prions show the typical procellariiform pattern of a single-egg clutch and slow chick development, with an average fledging period of 50 days (Strange 1980). Thin-billed prions are burrow nesters, and we reached chicks in their nest chambers via short access tunnels in the roof of each burrow, capped with removable stone lids. This system facilitated rapid access to chicks, reducing overall disturbance. Marked nests were monitored for eggs and hatching chicks.

2.2. Chick measurements

On our first visit, we determined the hatching dates of chicks by calibrating their wing length against wing growth in chicks of known age (e.g. Quillfeldt et al., 2006). Chicks were weighed daily at 07.30 and 19.30 h to the nearest 1 g using a digital balance (Kern CM320-1N, Germany). Feeding rates were estimated from the evening and morning weights, by correcting the mass differences

for metabolic mass loss, using the equations given previously (Quillfeldt et al., 2003). An index of chick body condition at 19.30 h each evening was calculated relative to the mean mass for study chicks of each age (m_{mean}), using the following formula: $BC = m * 100 / m_{\text{mean}}$. The index varied between 75 and 122 for mean chick values.

Begging rates were recorded as described previously for burrow-nesting Procellariiformes (Quillfeldt, 2002; Quillfeldt et al., 2004; Quillfeldt and Masello, 2004). Briefly, we placed a portable tape recorder or digital voice recorder outside the nest entrance and an external microphone with a 2 m connection in the nest entrance close to the nest chamber. The recorders were switched on at 23.00 h each night (before the first adults returned) and recorded at low speed until the end of the tape (95 min in tape recorders and 6 h in digital recorders). We included only first begging sessions of each chick and night in the analyses of begging behaviour, and included only chicks from 10 days of age. This way, daily variation in begging behaviour reflected the chick's need at the time of adult arrival. From the recorded begging sessions, we recorded the duration (in min), the total number of calls in the session, the mean call rate (call/min), and the maximum call rate sustained for one minute (calls/min).

2.3. Measurement of plasma triglyceride, glucose and leptin-like protein levels

Blood (0.2–0.4 ml) was sampled during the day (1000–1700 h) by puncture of the wing vein, collected with heparinized capillaries and immediately transferred to 0.5 ml tubes. Tubes were kept on ice until centrifugation, and plasma was stored frozen at -20°C . Blood samples for the analysis of plasma contents were obtained from 63 chicks (20 in 2003 and 43 in 2004, aged between 12 and 51 days). However, sample sizes are lower in some tests according to available plasma volumes, which did not, in all cases, allow all analyses to be carried out.

Plasma glucose and triglyceride levels were determined spectrophotometrically by using commercially available kits from Instrumentation Laboratories (Lexington, KY, USA) with an automatic apparatus (Monarch Chemistry System, Instrumentation Laboratories, Zaventem, Belgium).

Plasma leptin-like protein concentrations were measured by radioimmunoassay using the multi-species leptin RIA kit (Linco Research, St. Charles, Missouri, USA). The parallelism and the specificity of the assay were also performed as described above except that ^{125}I -human leptin binding was displaced by increasing concentrations of human insulin, chicken GH, or chicken IGF-1 (1–50 ng/ml). The coefficient of variation was less than 10%.

2.4. Western Blot analysis

Liver and adipose tissue (1 g) were taken from a thin-billed prion fledgling found freshly dead after predation. Tissues were homogenized in lysis buffer (10 mM Tris base, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 0.5% NP-40, protease inhibitor cocktail (Sigma, Belgium)). Homogenates were centrifuged at 600g for 20 min at 4°C , and supernatants were then ultra centrifuged for 45 min at 45,000 rpm. Protein concentrations were determined using a Bradford assay kit (Bio-Rad, Belgium) with bovine serum albumin as a standard. Proteins (20 g) were run on 4–12% Novex Bis–Tris gels (Invitrogen life technologies, Belgium). The transferred membranes were blocked in ECL blocking agent for 1 h at room temperature, and incubated with rabbit polyclonal anti-leptin antibody (PA1-051; 1:1000) (Affinity BioReagents, Golden, USA) at 4°C overnight. The peroxidase-conjugated goat anti-rabbit secondary antibody was used (1:2000) for 1 h at room temperature. The signal was visualized by enhanced chemilumi-

nescence (ECL plus) (Amersham Biosciences, Belgium). The SeeBlue Plus2 pre-stained standard (Invitrogen, Belgium) was used as molecular weight of the protein bands.

2.5. Statistical analyses

Statistical tests were conducted using SPSS 12.0.1. Normality was tested using Kolmogorov–Smirnov tests and non-normally distributed data (plasma triglyceride levels and begging call number and duration) were ln-transformed to achieve normal distribution for parametric tests. We tested between-chick effects with correlations performed on mean data for individual chicks. In order to control for multiple testing, we calculated *P*-values corrected for the number of tests (*P*_{corr}), using a conversion of the Dunn–Šidák equation (Sokal and Rohlf, 1994, see also Quillfeldt et al., 2006). Mean values for chicks were also used to test differences between years. Within-chick effects were tested using General Linear Models (GLM). In order to avoid unreliable assessments of the effects of covariates, we included only those chicks into the General Linear Models of within-chick effects of which we had obtained 3–5 blood samples (6 chicks in 2003, 12 chicks in 2004, no nest was repeatedly used in both years). For plasma triglycerides and leptin-like protein levels, we tested some within-chick effect by identifying

elevated values (plasma triglycerides >400 mg/dl, plasma leptin-like protein >3.2 ng/ml, defined by visual inspection of the distribution as separated from symmetric distribution). We compared the body condition at the first day we measured elevated triglyceride values with the average body condition of each chick by paired *t*-tests.

3. Results

3.1. Leptin-like protein expression in liver and adipose tissue of thin-billed prions

An immunoreactive band located at approximately 14–16 kDa was revealed in both liver and adipose tissue of thin-billed prion (Fig. 1) as well as in the liver of chicken, which was chosen as the positive control.

3.2. Relationship between leptin-like protein and plasma metabolite levels in thin-billed prions

The level of leptin-like protein in the plasma of thin-billed prion chicks varied between 1 and 3 ng/ml, which is in the same range as that of chickens. Increasing concentrations of human insulin, chicken GH, or chicken IGF-1 did not crossreact with multi-species leptin RIA when compared to recombinant human leptin (Fig. 2A). To determine the parallelism of the assay, a serial dilution of plasma were used. The dilution curve is parallel to that of standard and proportional to the dilution factor (Fig. 2B).

Chicks differed significantly (*P* < 0.05) in plasma triglyceride and leptin-like protein levels, but not in plasma glucose levels. Plasma triglyceride and glucose levels were highly and positively correlated (*P* = 0.002), however plasma leptin-like protein levels did not correlate with either plasma triglyceride or glucose levels.

3.3. Relationship between plasma parameter levels, provisioning rate and body condition

Plasma triglyceride levels were positively correlated to provisioning rates and chick body condition (Table 1, Fig. 3) and this

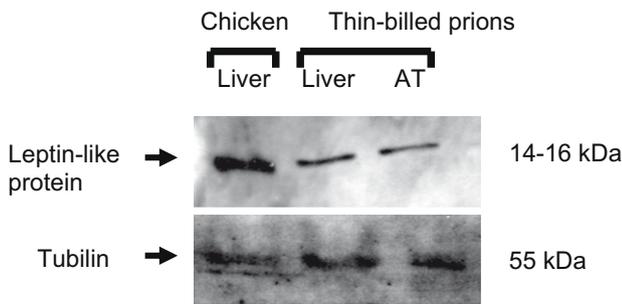


Fig. 1. Expression of leptin-like protein in the liver and adipose tissue (AT) of thin-billed prions. Liver and adipose tissue lysates were subjected to Western Blot as described in Section 2. Blot was incubated with anti-leptin antibody and revealed by ECL. Chicken liver was used as a positive control.

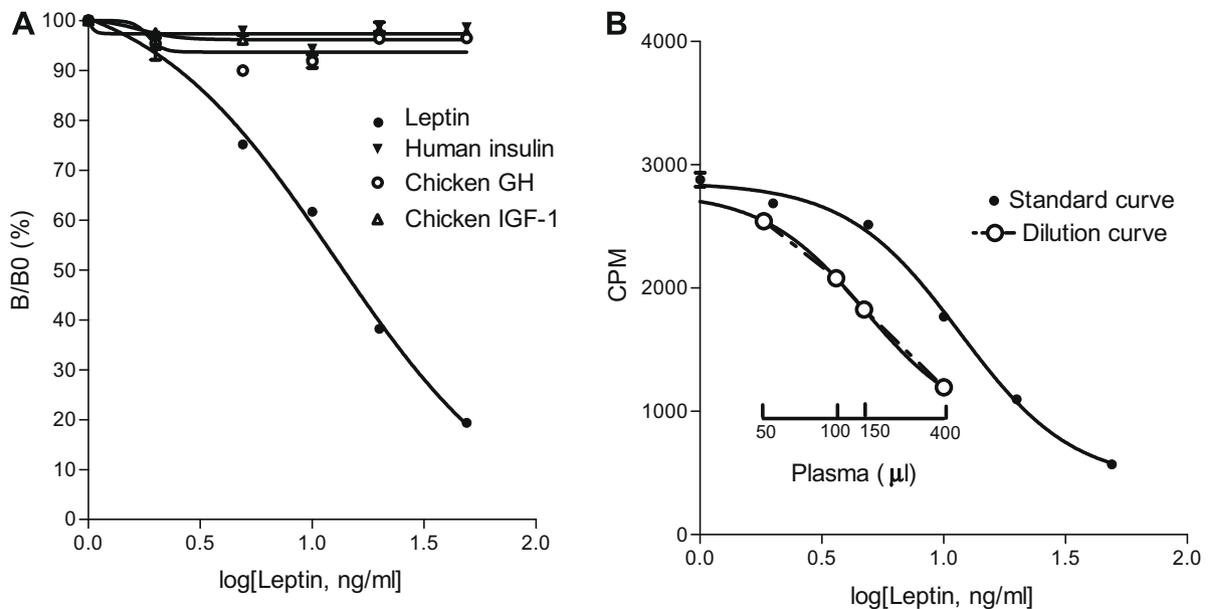


Fig. 2. Determination of specificity of Thin-billed prion (*Pachyptila belcheri*) leptin RIA. (A) Cross-reactivity was determined by adding increasing concentrations of human insulin, chicken GH and IGF-1 to displace ¹²⁵I-human leptin binding to leptin antibody (linco kit). (B) Typical standard curve (Linco kit) and dilution curve of Thin-billed prion plasma. Each point of the curve represents the mean of triplicate measurements.

Table 1
Between chick correlations of plasma triglycerides and leptin with measures of body condition and begging intensity in Thin-billed prions at New Island, Falkland Islands. Parameters marked (*) are ln-transformed.

Parameter	Plasma triglycerides*	Plasma glucose	Plasma leptin
Feeding rate (g/day)	$R = 0.377$, $df = 59$, $P = 0.003$, $P_{\text{corr}} = 0.006$	$R = 0.181$, $df = 59$, $P = 0.167$	$R = -0.353$, $df = 55$, $P = 0.007$, $P_{\text{corr}} = 0.014$
Body condition	$R = 0.494$, $df = 59$, $P < 0.001$, $P_{\text{corr}} < 0.001$	$R = 0.170$, $df = 59$, $P = 0.194$	$R = -0.261$, $df = 55$, $P = 0.050$, $P_{\text{corr}} = 0.100$
Number of begging calls*	$R = -0.377$, $df = 51$, $P = 0.006$, $P_{\text{corr}} = 0.012$	$R = -0.161$, $df = 51$, $P = 0.260$	$R = 0.387$, $df = 48$, $P = 0.011$, $P_{\text{corr}} = 0.022$
Duration of begging session*	$R = -0.370$, $df = 51$, $P = 0.007$, $P_{\text{corr}} = 0.014$	$R = -0.107$, $df = 51$, $P = 0.457$	$R = 0.324$, $df = 48$, $P = 0.036$, $P_{\text{corr}} = 0.072$

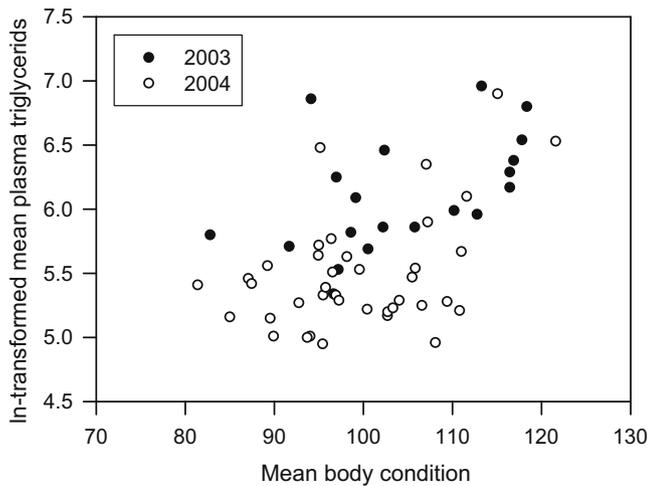


Fig. 3. Relationship between the mean body condition of chicks of Thin-billed prions (*Pachyptila belcheri*) and their plasma triglyceride levels for two breeding seasons at New Island, Falkland Islands.

correlation was maintained in both seasons ($R = 0.498$, $df = 19$, $P = 0.025$ in 2003 and $R = 0.296$, $df = 40$, $P = 0.060$ in 2004).

Plasma glucose levels were not correlated to provisioning rates or body condition for both years analysed separately or in combination (Table 1).

Plasma leptin-like protein levels were negatively correlated to provisioning rates among chicks (Table 1, Fig. 4), and this relationship was also observed when each year was analysed independently ($R = -0.675$, $df = 14$, $P = 0.006$ in 2003 and $R = -0.354$, $df = 40$, $P = 0.023$ in 2004). However, there was no relationship be-

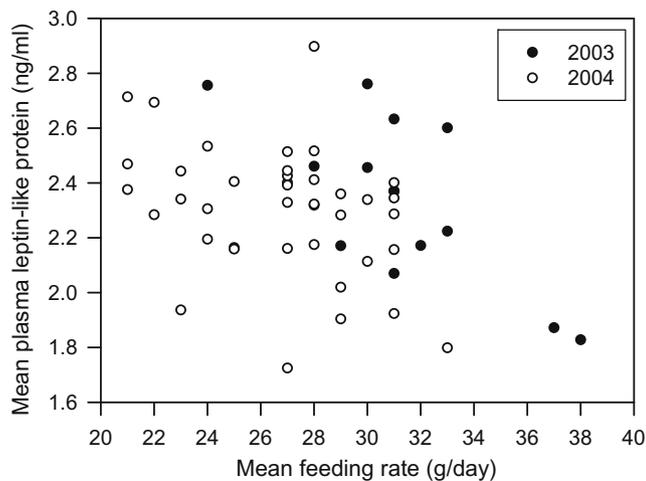


Fig. 4. Relationship between plasma leptin-like protein levels and provisioning rate in Thin-billed prions (*Pachyptila belcheri*) for two breeding seasons at New Island, Falkland Islands.

tween plasma leptin-like protein levels and body condition within chicks (GLM with leptin as dependent variable, chick as factor and body condition and age as covariates; effect of age: $F_{17,39} = 2.84$, $P = 0.100$, $\eta^2 = 0.070$, $t = 1.69$; effect of body condition: $F_{17,39} = 0.79$, $P = 0.379$, $\eta^2 = 0.020$, $t = -0.89$, effect of chick: $F_{17,39} = 2.05$, $P = 0.033$, $\eta^2 = 0.478$).

Few cases of high plasma leptin-like protein concentrations were recorded [2 cases (5% of measurements, 3.3 and 10.1 ng/ml) in 2003 and 1 case (1% of measurements, 4 ng/ml) in 2004] (Fig. 5). In 2003, one case of 3.3 ng/ml was registered in a chick of near-average mean bc (102%) after a large single meal (49 g), when the chick increased its body condition by 13% and had the highest registered plasma triglyceride value of the season (1867 mg/dl). The second case (10.1 ng/ml leptin-like protein) was a well-fed chick (mean bc 116%), which received a double meal of 90 g, and had a body condition increase of 35% and also elevated triglyceride concentration in plasma (788 mg/dl). In 2004, the case of elevated plasma leptin-like protein (4.0 ng/ml) was registered in the chick with the highest measurement of body condition (161%) and triglycerides (1570 mg/dl) of the season, but the previous feeding had not been measured.

Although all cases of elevated plasma leptin-like protein were associated with elevated plasma triglyceride values, the opposite was not the case (Fig. 5).

3.4. Relationship between plasma parameter levels and begging intensities in thin-billed prions

Plasma leptin-like protein levels were positively correlated to the begging call number among chicks (Table 1), and this relationship was maintained when each year was analysed independently ($R = 0.439$, $df = 14$, $P = 0.102$ in 2003 and $R = 0.379$, $df = 33$, $P = 0.033$ in 2004).

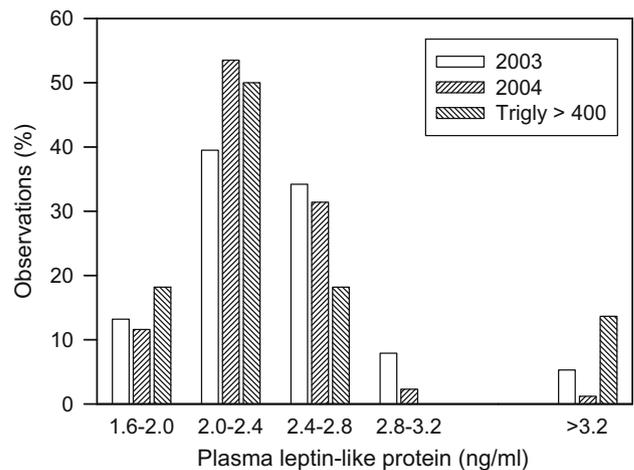


Fig. 5. Distribution of plasma leptin-like protein concentrations in two seasons and in samples with elevated triglyceride concentrations (>400 mg/dL), indicating a positive energy balance. Three cases were separated from the symmetric distribution and thus, defined as elevated plasma leptin-like protein concentrations (>3.2 ng/ml). These had leptin-like protein concentrations of 3.3, 4.0 and 10.1 ng/ml.

Plasma triglyceride levels were negatively correlated to begging intensities, (Table 1, Fig. 6). Plasma glucose levels were not related to measures of begging among chicks. Begging intensity did not correlate with the mean provisioning rate or body condition in nestling thin-billed prions.

3.5. Effect of age and seasons on plasma parameter levels and feeding-associated behaviour

Feeding rates were higher in 2003 than in 2004 (31.7 ± 0.69 g/night in January 2003 vs. 27.6 ± 0.58 g/night in January 2004; $t = -4.68$, $df = 58$, $P < 0.001$). Correspondingly, higher mean body conditions were observed in 2003 than in 2004 (109.5 ± 3.3 vs. 98.6 ± 1.6 ; $t = 3.1$, $df = 44$, $P = 0.003$). A substantial part of the variation in chick body conditions was explained by individual differences among chicks, while body condition was independent of chick age (GLM of body condition as dependent variable, with chick as factor and age as covariate; effect of chick: $F_{17,62} = 1.89$, $P = 0.046$, $\eta^2 = 0.421$; effect of age: $F_{1,53} = 1.76$, $P = 0.188$, $\eta^2 = 0.039$).

There was a large difference between the median plasma triglyceride values of chicks in the two seasons (420.5 vs. 206.7 mg/dl in 2003 and 2004, respectively, Mann–Whitney U test: $U_{21,46} = 111$, $P < 0.001$), and this was related to the difference in body condition (GLM with ln of triglycerides as dependent variable, using the first sample of each individual, with year as factor and body condition and age as covariates; effect of year: $F_{1,53} = 0.77$, $P = 0.384$, $\eta^2 = 0.015$; effect of age: $F_{1,53} = 0.39$, $P = 0.534$, $\eta^2 = 0.008$; effect of body condition: $F_{1,53} = 15.06$, $P < 0.001$, $\eta^2 = 0.231$, $t = 7.34$). Plasma triglyceride levels were not affected by age (see age effect in GLM and Fig. 7).

Plasma glucose also differed between years (223.2 ± 5.0 vs. 200.6 ± 3.1 mg/dl in 2003 and 2004, respectively; $t = 3.9$, $df = 60$, $P < 0.001$), and this was related to the difference in body condition (GLM with glucose as dependent variable, using the first sample of each individual, with year as factor and body condition and age as covariates; effect of year: $F_{1,53} = 1.01$, $P = 0.320$, $\eta^2 = 0.020$; effect of age: $F_{1,53} = 0.02$, $P = 0.904$, $\eta^2 < 0.001$; effect of body condition: $F_{1,53} = 9.77$, $P = 0.003$, $\eta^2 = 0.163$, $t = 3.13$). There was no relationship between plasma glucose and age (see age effect in GLM and Fig. 7).

In contrast, there was no difference in plasma leptin-like protein levels among years (2.34 ± 0.07 vs. 2.31 ± 0.04 ng/ml in 2003 and 2004, respectively; $t = 0.4$, $df = 56$, $P = 0.724$). Plasma leptin-

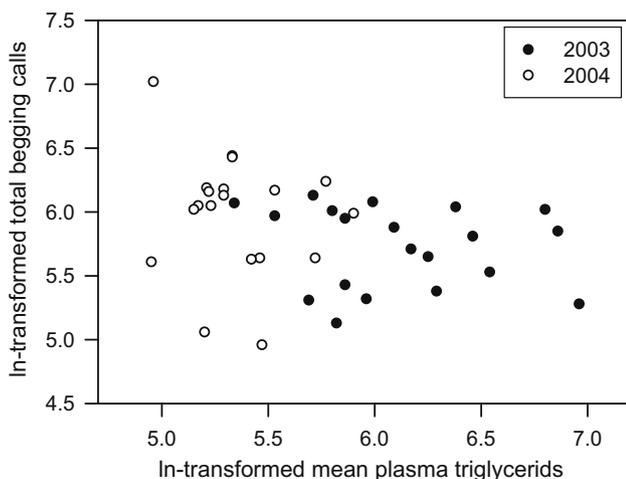


Fig. 6. Relationship between the mean concentration of triglycerides in plasma of chicks of Thin-billed prions *Pachyptila belcheri* and their mean begging intensities for two breeding seasons at New Island, Falkland Islands. Begging intensity was measured as the total number of begging calls during the feeding session.

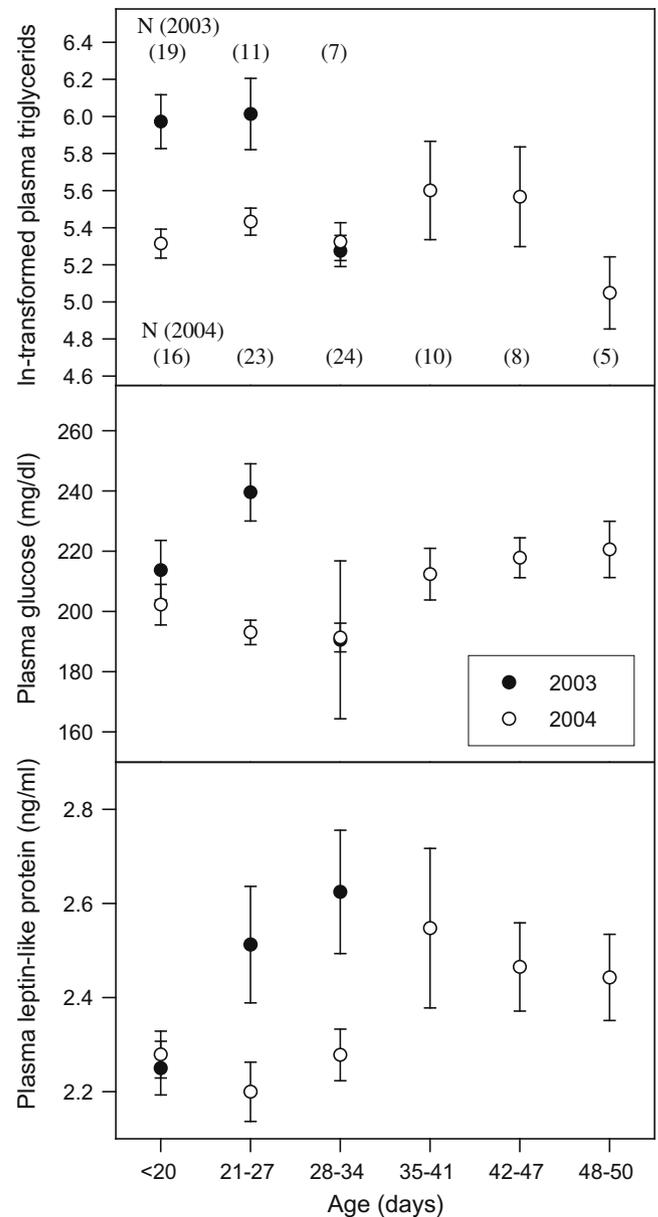


Fig. 7. Triglycerides, glucose and leptin-like protein of chicks of thin-billed prions *Pachyptila belcheri* at different ages during two breeding seasons at New Island, Falkland Islands.

like protein levels had no relationship with body condition, but increased with age (GLM with leptin-like protein as dependent variable, using the first sample of each individual, with year as factor and body condition and age as covariates; effect of year: $F_{1,53} = 1.19$, $P = 0.281$, $\eta^2 = 0.022$; effect of age: $F_{1,53} = 10.97$, $P = 0.002$, $\eta^2 = 0.171$; effect of body condition: $F_{1,53} < 0.001$, $P = 0.992$, $\eta^2 < 0.001$, $t = 0.01$). When chick means were calculated using only samples collected during the age represented in both years (12–32 days, Fig. 7), there was still no difference in plasma leptin-like protein concentrations between the two years (2.34 ± 0.07 vs. 2.27 ± 0.04 ng/ml in 2003 and 2004, respectively; $t = 0.8$, $df = 56$, $P = 0.433$).

4. Discussion

Chicks of tubenosed seabirds are infrequently fed and are therefore subject to large and variable (timing/intensity) cycles of food deprivation and feeding. Despite the limited and irregular food

supply, chicks of seabirds show an extreme growth pattern with a large accumulation of lipid during their nestling development referred to a “nestling obesity”. Since leptin, the adipocytokine hormone, was found to play a key role in the control of energy homeostasis (for review, see Freidman and Halaas, 1998) and lipid metabolism (for review see, Heynes and Jones, 2001) in mammals, we predicted that a leptin-like protein may be also involved in sensing of body energy reserves and feeding-associated behaviour in free-living seabirds.

We showed firstly that leptin-like protein is expressed in liver and adipose tissue of thin-billed prions by using a Western immunoblotting method with a specific primary antibody. The anti-leptin antibody (PA1-051, Affinity BioReagents, Golden, USA) was raised against a synthetic peptide corresponding to amino acids 25–44 of mouse leptin (QKVQDDTKLIKTIVTRIND). This sequence is highly conserved in several species including human, mouse, rat, carp, duck, sheep, turkey, and chicken (but see Sharp et al., 2008). The PA1-051 immunizing peptide has shown to detect a leptin-like protein from tissues of several species and more recently from wild migratory birds (dunlin) (Kochan et al., 2006). Our data corroborate previous findings in chickens and dunlin and suggest that the particular hepatic expression of leptin-like protein may be related to the major role of liver in the control of lipogenesis in birds (Leveille et al., 1968). Recent studies showed that leptin gene/protein is expressed also in the liver of various non-mammalian species including fish (pufferfish: Kurokawa et al., 2005, green sunfish, largemouth bass, bluegill, and white crappie: Johnson et al., 2000), amphibians (Crespi and Denver, 2006), and reptiles (Paolucci et al., 2001), indicating that the hepatic expression of leptin is not limited only to avian but it also extended to oviparous species.

Although there are controversial reports about the presence of a leptin-like gene in birds as described in the introduction, there is however evidence that a leptin-like signalling system is present in avian species because the leptin receptor gene has been cloned in chicken (Ohkubo et al., 2000; Horev et al., 2000; Dunn et al., 2000; Liu and Sharp, 2007; Liu et al., 2007), turkey (Richards and Poch, 2003), and duck (GenBank Accession No. EU049612) that shows conservation of the key motifs and predicted exon boundaries found in the long isoform of the mammalian leptin receptor (ob-Rb) (Tartaglia et al., 1995). Additionally, a chicken leptin receptor has been shown to be functional in activating JAK-STAT pathway (Adachi et al., 2008).

Our data support the existence of a leptin-like molecule in a seabird, the Thin-billed prion, however the characterization of prion leptin cDNA is necessary for comparative sequence analysis and expression studies.

The level of leptin-like protein, determined by multi-species leptin RIA, in the plasma of thin-billed prions was in the same range as that of chickens (Dridi et al., 2000b) except for very few cases where leptin was found to be higher. Plasma leptin-like protein levels increased with age in free-living thin-billed prions corroborating previous studies in mammals (Ahima et al., 1998) and chickens (Dridi et al., 2000b; Ashwell et al., 2001).

The effect of leptin-like protein on food intake in avian species was widely studied however its effect (particularly endogenous leptin-like protein) on feeding-associated behaviour was poorly documented. Exogenous administration of recombinant (chicken or human) leptin at pharmacological doses reduced food intake in domestic chickens (Raver et al., 1998; Cassy et al., 2004; Dridi et al., 2000a, 2005b; Denbow et al., 2000; Kuo et al., 2005; Yang and Denbow, 2007) as well as in great tits (*Parus major*) (Löhmus et al., 2003). Monitoring food behaviour revealed that the attenuated food intake in chickens resulted not from a decreased number of approaches to the feeders but from a decrease in the average

time spent eating during each approach (Dridi et al., 2000a). Löhmus and Sundström (2004) have shown that leptin-treated asian blue quail (*Coturnix chinensis*) spent also less time feeding compared to the controls.

In nestling birds, begging behaviour is used to honestly advertise offspring condition and determine food provisioning rates by parents (e.g. Quillfeldt, 2002; Quillfeldt and Masello, 2004; Goodship and Buchanan, 2006). Thus, the lower body condition (the hungrier the bird), the more intense is its begging and parents respond with a high provisioning rate (Quillfeldt et al., 2006). We expect therefore that animals with higher leptin-like protein levels should feel more satiated and beg less. Our data showed that seabirds with low mean body conditions increased begging intensities, but surprisingly they are characterized by high mean plasma leptin-like protein levels.

The negative correlations between plasma leptin-like protein levels and body conditions on one hand and between the concentrations of leptin-like protein and triglycerids on the other hand are intriguing and differ from that observed in mammals where the circulating leptin levels are generally proportional to the amount of body fat content. Such an inverse relationship between fat contents and plasma leptin levels was previously reported in broiler breeder female chickens (Bruggeman et al., 2000). These disparate data may be related to the particular synthesis of leptin-like protein by the avian liver and therefore to substantial species-specific role of leptin-like protein in lipid metabolism. Such discordant data have been reported previously for several hormones such as ghrelin (Furuse et al., 2001) and MAPK-dependent mechanism of leptin action in ovarian cells (Sirotkin and Grossmann, 2007).

The increase in begging intensities, despite the high levels of plasma leptin-like protein, is unclear at this time. It might be achieved by high energy requirement following increased energy expenditure by leptin. When released into the bloodstream, leptin is transported through the blood–brain barrier via a saturable transport system (Banks et al., 1996) and functions via its receptor as a signal between peripheral lipid stores and the central nervous system where it orchestrate neural events for dissipation of appetite and to determine feeding (Freidman and Halaas, 1998). Interruption of one of these upstream events (transport, signalling, and/or effectors) could alter the leptin action. For ethical reasons during our work with these wild birds, the expression of the leptin receptor gene and hypothalamic neuropeptides as well as the correlation between fat content, liver weight and leptinaemia could not be addressed in this study. A further limitation of the present study is that being a single time point measurement of plasma leptin-like protein concentrations we do not take into account diurnal and ultradian rhythm of plasma leptin (Saad et al., 1998; Kousta et al., 1998). To fully assess the physiological relevance of these studies, the identity of the protein measured with the leptin RIA remains to be established (Sharp et al., 2008), in order to design experimental studies including manipulation of leptin-like protein levels. Further characterization of the prion ob gene and the full avian leptin cDNA are necessary for comparative sequence analysis and expression studies.

Taken together, the present observation is the first to demonstrate, to our knowledge, the expression of leptin-like protein in liver and adipose tissue of seabird chicks. Furthermore, it suggests that endogenous leptin-like protein might be involved in feeding-associated behaviour in natural free-living seabirds.

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