Ornithology

Arctic waders are not capital breeders

Birds prepare their eggs from recently ingested nutrients ('income' breeders) or from body stores ('capital' breeders)[1]. As summers are short at Arctic latitudes, Arctic migrants have been presumed to bring nutrients for egg production from their previous habitats, so that they can start breeding immediately upon arrival[1–3]. But we show here that eggs laid by 10 different wader species from 12 localities in northeast Greenland and Arctic Canada are produced from nutrients originating from tundra habitats, as inferred from carbon stable-isotope ratios in eggs, natal down, and juvenile and adult feathers.

During winter and migration, most Arctic-breeding waders eat estuarine invertebrates, shifting to terrestrial and limnic invertebrates on tundra breeding grounds. Invertebrates from estuarine and tundra habitats have distinctly different carbon-isotope ratios[4]. Diet-based differences in carbon-isotope ratios are expressed in bird tissues, including eggs and feathers[4,5]. We investigated whether Arctic-breeding wader species that use estuarine habitats during the non-breeding season are capital breeders, producing eggs and hatchlings with carbon-isotope ratios that are typical of estuaries.

As expected, the flight feathers produced during early winter show ratios that are typical of estuarine systems (Fig. 1). The same is true for shoulder feathers, which are among the last to be moulted before migration to breeding grounds[6]. However, the carbon-isotope signatures of eggs and natal down are typical of terrestrial and limnic systems. The eggs and resultant hatchlings therefore seem to be produced from local nutrients. This conclusion is supported by the strong resemblance of the carbon-isotope signatures of natal down and juvenile flight feathers, showing that eggs and post-hatch tissues have the same nutritional source.

Our results also indicate that the use of a mixed capital/income breeding strategy by these waders is unlikely. Egg-laying females use a single, local nutrient source, as indicated by the low within-clutch variation compared with the among-clutch variation in carbon-isotope ratios of natal down. (We used a hierarchically nested design in a mixed-model ANOVA, in which brood is nested within species as a random factor, and found significant effects of species ($F_{6,30}=4.8$, $P<0.002$) and brood ($F_{29,,93}=16.8$, $P<0.001$) on the carbon stable-isotope ratios of natal down.)

We conclude that, with respect to egg production, the Arctic-breeding waders investigated here are income, rather than capital, breeders. For these waders, the fitness costs of transporting extra nutrient stores to breeding grounds[7] outweigh the potential benefits[1–3]. The capital strategy may still be used by large species of Arctic breeding migrants, such as geese[1,2] — it is likely that larger species need relatively smaller body stores for egg production. Also, larger species, although constrained by the same fixed period of opportunity as smaller birds, require longer to complete their breeding.

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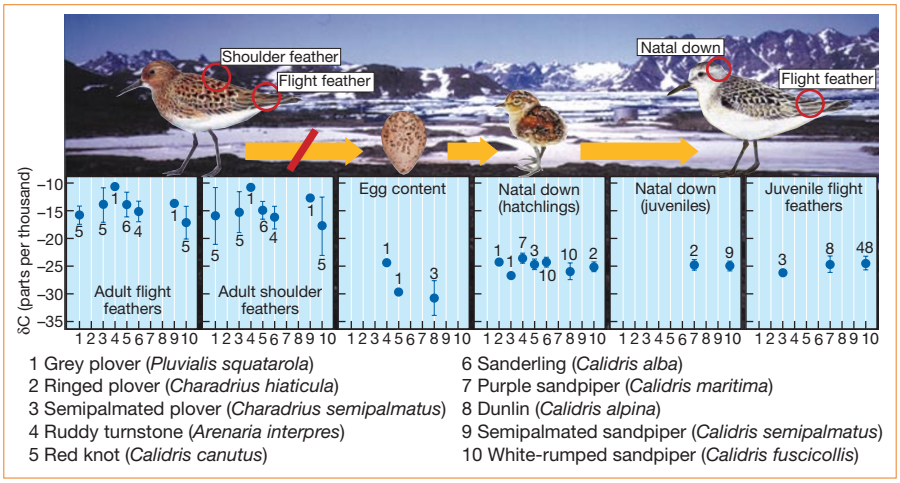

Figure 1 Carbon stable-isotope ratios (parts per thousand difference from $^{13}C/^{12}C$ ratio in PeeDee limestone[4]; δC) of eggs, natal down and feathers of different species of Arctic-breeding waders at different times during the year. All samples were collected in northeast Greenland and Arctic Canada in 1999 and 2000. Feathers collected from nest-attending adults were grown either in winter (adult flight feathers) or during spring migration (adult shoulder feathers); data are averages across individuals ± s.d. Eggs were collected from deserted nests (egg content); natal down was collected from hatchlings; data are averages across clutches ± s.d. per species. Data for natal down still attached to the tips of head and neck feathers of independent young[8], and for secondary flight feathers from independent young are averages across individuals ± s.d.). Values of δC were determined using a Carlo Erba model 1106 elemental analyser which was coupled online, through a Finnigan conflow 2 interface, to a Finnigan Delta S mass spectrometer. Feathers were washed in chloroform before carbon-isotope analysis. Using a hierarchically nested design in a mixed model ANOVA, in which species was nested within tissue type, which in turn was nested within the tissue groups 'adult feathers' and 'egg, natal down and juvenile feathers', we found significant effects of tissue group ($F_{1,152}=805.3$, $P<0.001$), tissue type ($F_{4,152}=6.7$, $P<0.001$) and species ($F_{9,152}=2.8$, $P<0.005$) on δC.

Intracellular signalling

Key enzyme in leptin-induced anorexia

Leptin is a key hormonal regulator of energy balance that acts upon hypothalamic neurons to reduce food intake, but the intracellular mechanisms involved are incompletely understood. Here we show that systemic administration of leptin in rats activates the enzyme phosphatidylinositol-3-OH kinase (PI(3)K) in the hypothalamus and that intracerebroventricular (i.c.v.) infusion of inhibitors of this enzyme prevents leptin-induced anorexia. Our results indicate that PI(3)K is a crucial enzyme in the signal-transduction pathway that links hypothalamic leptin to reduced food intake.

Leptin, a hormone derived from adipocytes (fat cells), is known to activate PI(3)K in non-neuronal cells and to elicit cellular responses that are blocked by PI(3)K inhibitors *in vitro*[1,2]; it also activates the transcription factor STAT3 (ref. 3). To determine whether leptin activates PI(3)K, as well as STAT3, in the hypothalamus *in vivo*, we observed the effect of systemically administered leptin on their activity in male Wistar rats. As before[3], leptin induced rapid tyrosine-phosphorylation of STAT3

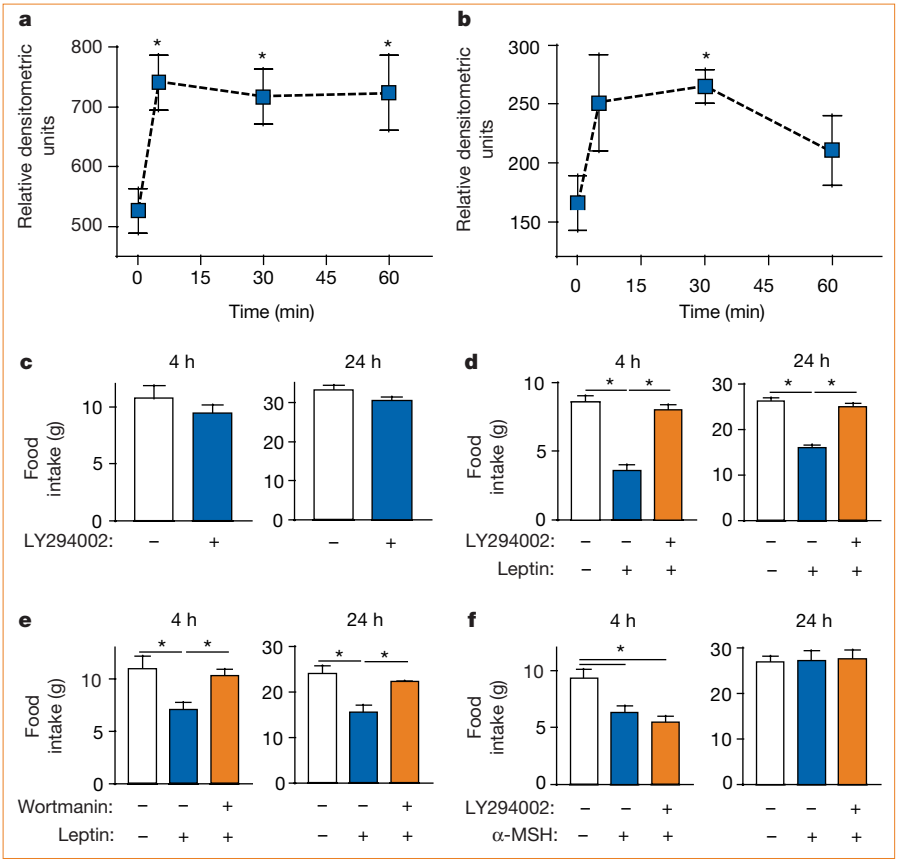

Figure 1 Role of PI(3)K signalling in the anorectic response to leptin. Time course of **a,** tyrosine-phosphorylation of STAT3, and **b,** IRS-2-associated PI(3)K activity[12] in mediobasal hypothalamic extracts after intraperitoneal injection of leptin (1 μg g^{-1}; provided by A. F. Parlow) into male Wistar rats. **c,** LY294002 (1 nmol) injected intracerebroventricularly (i.c.v.) at the onset of the dark cycle does not alter food intake at 4 h or 24 h. **d–f,** Pretreatment with LY294002 (1 nmol i.c.v.; **d, f**), wortmannin (0.01 nmol; **e**) or vehicle (**d–f**) was followed 1 h later, just before the onset of the dark cycle, by i.c.v. administration of leptin (3 μg; **d, e**), α-MSH (25 μg; **f**) or vehicle (**d–f**). Pretreatment with i.c.v. LY294002 (**d**) or wortmannin (**e**) blocks leptin-induced anorexia, whereas LY294002 does not affect α-MSH-induced anorexia (**f**). $n = 6$–8 rats per group; asterisk denotes $P < 0.05$ relative to controls (Student's *t*-test or one-way ANOVA).

in mediobasal hypothalamic extracts, an effect that was sustained for up to 1 h (Fig. 1a). This response was paralleled by a comparable increase in hypothalamic PI(3)K activity associated with the insulin-receptor substrate IRS-2, peaking at 30 min (Fig. 1b) (IRS proteins couple activated cell-surface receptors of the tyrosine-kinase type to PI(3)K and other signalling molecules).

Pharmacological inhibitors of PI(3)K activity, such as LY294002 and wortmannin, can block leptin activity *in vitro*[1,2], suggesting that PI(3)K-dependent signalling is needed for certain intracellular responses to leptin. We tested whether the inhibitory effects of leptin on food intake depend on PI(3)K, by i.c.v. infusion of PI(3)K inhibitors into rats. We first identified a dose of LY294002 (1.0 nanomol i.c.v.; results not shown) that did not alter food intake when administered to male Wistar rats at the onset of the dark cycle (Fig. 1c) — the period of the day during which rats consume the most food — and then evaluated the effect of i.c.v. pretreatment with this dose of inhibitor or its vehicle on the anorexic response to i.c.v. leptin.

Treatment with leptin markedly reduced food intake at both 4 h and 24 h in rats pretreated with vehicle, but we did not detect leptin-induced anorexia after pretreatment with LY294002 (Fig. 1d). By using wortmannin instead of LY294002 in a different group of rats, we confirmed that infusion of PI(3)K inhibitors into the third cerebral ventricle prevents the reduction in food intake induced by i.c.v. administration of leptin (Fig. 1e). As PI(3)K is the only enzyme that is known to be inhibited by both LY294002 and wortmannin[4], these findings indicate that neuronal PI(3)K is important for the effects of leptin on food intake.

Melanocortin peptides such as melanocyte-stimulating hormone (α-MSH) also reduce food intake, but act through a different pathway from that used by leptin, involving intracellular cyclic AMP and protein kinase A[5]. If the effect of PI(3)K inhibitors on leptin-induced anorexia depends only on PI(3)K, then they should not affect the anorectic response to melanocortins. We therefore tested the effect on animals pretreated with i.c.v. LY294002 of injecting i.c.v. α-MSH and found that food intake was still reduced 4 h later (Fig. 1f). As the inhibitory effect of α-MSH on food consumption is short-lived[6], there was no difference between groups after 24 h.

These findings show that leptin activates hypothalamic PI(3)K signalling *in vivo* and that leptin, but not melanocortins, reduces food intake through a PI(3)K-sensitive mechanism. Our results are consistent with a model in which binding of leptin to its receptor on hypothalamic neurons stimulates intracellular signalling through PI(3)K, as occurs in non-neuronal cells[1,2], although leptin-induced activation of hypothalamic PI(3)K may also occur in neurons further 'downstream' in a leptin-activated pathway.

Like leptin, the pancreatic hormone insulin may function as an afferent signal to the hypothalamus in the regulation of body-fat levels[7]. As PI(3)K is an intracellular mediator of insulin activity in peripheral tissues[8] and hypothalamic neurons[9], our results raise the possibility that insulin and leptin can both activate neuronal IRS–PI(3)K signalling. This would not only explain the overlapping hypothalamic activities of these two hormones[7,9], but may also have implications for the development of hypothalamic leptin resistance, which is associated with obesity.

Insulin-stimulated activation of PI(3)K is impaired in the peripheral tissues of obese individuals, which may contribute to the pathogenesis of obesity-induced insulin resistance[8]. If the mechanism used by leptin to reduce food intake is PI(3)K-dependent, as our results suggest, defective activation of PI(3)K in hypothalamic neurons may reduce the ability of leptin to promote weight loss in obese rodents[10] and humans[11].

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