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Variation in plasma leptin-like immunoreactivity in free-living European starlings (Sturnus vulgaris)

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ABSTRACT

Leptin, a protein hormone secreted by fat cells, is best known for its role as an adiposity signal; however, leptin has diverse physiological roles ranging from regulation of feeding behavior and body weight, to effects on reproduction and immune function. Although leptin has been extensively studied in mammals, the identification and function of leptin in birds remains controversial, and studies have focused on captive or domesticated species. Here, we describe changes in plasma leptin-like immunoreactivity during the reproductive and non-reproductive seasons in free-living female European starlings (Sturnus vulgaris). Plasma leptin-like immunoreactivity was high during egg-laying (27.8 ± 2.4 ng/mL) and clutch completion (23.8 ± 1.6 ng/mL), decreased during incubation (13.0 ± 1.6 ng/mL) and chick-rearing (12.0 ± 1.3 ng/mL), but was elevated again in non-breeders in November (23.7 ± 1.1 ng/mL). Although there was marked and consistent variation in total body mass and body composition with breeding stage and season in this population, plasma leptin-like immunoreactivity did not parallel changes in body mass or body composition. These data suggest that the strong positive relationship between plasma leptin-like immunoreactivity and body mass reported for captive birds and mammals does not hold for free-living birds. Rather, among free-living female European starlings, variation in plasma leptin-like immunoreactivity is associated with breeding stage or seasonal variation per se, and we discuss possible mechanisms underlying this variation, focusing on ovarian function and egg production.

1. Introduction

Leptin, the protein hormone product of the obese (ob) gene secreted by fat cells (adipose tissue), was sequenced and cloned in mice and humans less than 15 years ago (Zhang et al., 1994). Since then, a large amount of research, mainly in mammals, has demonstrated that leptin has diverse physiological roles ranging from regulation of feeding behavior and body weight to effects on reproductive and immune functions (Budak et al., 2006; Friedman and Halaas, 1998; La Cava and Matarese, 2004). Few mammalian studies (excepting human studies) have examined variation in leptin in free-living individuals (but see Kunz et al., 1999), but there is some evidence to suggest that plasma leptin can vary "seasonally" in some captive mammals (Concannon et al., 2001; Hissa et al., 1998; Mustonen et al., 2005; Nieminen et al., 2001, 2002). For example, plasma leptin levels in the blue fox (Alopex lagopus) are relatively low during the summer, increase during the fall when individuals accumulate body fat, and then decrease during the winter and spring when the animals reduce their body fat (Mustonen et al., 2005). In contrast, in woodchucks (Marmota monax), plasma leptin is elevated in late spring and peaks in the summer before decreasing through the winter, following the general trend of body fat gain in spring and summer and loss in fall and winter (Concannon et al., 2001).

Far fewer studies have investigated variation in, or the physiological roles of, leptin in birds or other non-mammalian vertebrates. In part, this is likely due to the ongoing controversy regarding the identification of leptin in birds and, specifically, the putative avian leptin gene sequence which has been deposited in GenBank but which has not been identified in the chicken genome to date (Friedman-Einat et al., 1999; Sharp et al., 2008). However, a chicken leptin receptor gene (CLEPR or chLEPR or cOB-R) has been cloned and the structure of the gene resembles mammalian leptin receptor genes (Horev et al., 2000; Ohkubo et al., 2000). Ohkubo et al. (2000) have shown ovarian and hepatic expression of chicken leptin receptor mRNA, as well as expression in the kidneys, intestines, and brains of hens. Additionally, the chicken leptin receptor is capable of binding mammalian leptin, as well as mediating the leptin signal in vitro (Adachi et al., 2008; reviewed in Ohkubo and Adachi, 2008).

Furthermore, several studies in birds have used either mammalian leptin or putative recombinant chicken leptin in manipulation studies, or have measured variation in plasma leptin-like immunoreactivity using heterologous leptin radioimmunoassays (RIAs;

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(males have more ir(andcent)-342(plumage)-342(than)-349(females))-340(()
10, 2007 between 11:00 and 13:30 PST. Wpla sampled
breeding birds within 3 min of capturedu).Sowmeichutthe complete(42)postrids,
(mass/tarsus), or i

based on recombinant leptin protein derived from the reported gene sequence). These studies strongly suggest that a leptin-like hormone does exist in birds (Simon et al., 2009) with wide ranging physiological effects similar to those reported in mammals, including involvement in feeding behavior, regulation of body mass, and effects on reproduction and immune function (Denbow et al., 2000; Kuo et al., 2005; Lohmus et al., 2004, 2003, 2006; Sirotkin and Grossmann, 2007; Sirotkin et al., 2007). For example, Lohmus et al. (2006) demonstrated that exogenous recombinant chicken leptin administration decreased feeding behavior and body mass in Asian blue quail (Coturnix chinensis). In chickens, both injections of leptin and culturing of ovarian cell walls or granulosa cells with human recombinant leptin changes cell proliferation and apoptosis and affects secretion of several reproductive steroid hormones (Sirotkin and Grossmann, 2007; Sirotkin et al., 2007). Recombinant chicken leptin has also been shown to increase the immune response in Asian blue quail (Lohmus et al., 2004) and recombinant murine leptin has been shown to 'rescue' zebra finches (Taeniopygia guttata) from immunosuppressive effects of testosterone (Alonso-Alvarez et al., 2007).

The vast majority of published studies on leptin-like function in birds to date have involved captive birds (excepting Kochan et al., 2006; Quillfeldt et al., 2009), and most studies have involved domesticated species, such as chicken and quail. Therefore, little is known about leptin in free-living birds, and in particular it is not known whether plasma leptin-like immunoreactivity varies seasonally, as has been suggested in some studies of captive mammals. Studies in free-living birds may inform the debate, and perhaps help resolve the controversy, about the role of leptin-like hormones in non-mammalian taxa (Friedman-Einat et al., 1999; Scanes, 2008; Taouis et al., 1998). In this paper we describe changes in plasma leptin-like immunoreactivity (terminology following Scanes, 2008) during the reproductive and non-reproductive seasons in free-living female European starlings (Sturnus vulgaris). The specific objectives of our paper were (a) to describe variation in total body mass, fat mass and non-reproductive mass in female starlings at different stages of breeding (egg-laying, clutch completion, incubation, and chick-rearing) and in non-breeders in November; (b) to describe seasonal and breeding-stage-specific variation in plasma leptin-like immunoreactivity; and (c) to compare leptin data to the mass data to test the hypothesis that changes in plasma leptin-like immunoreactivity reflect a predominant effect of variation in body or fat mass (see for example, Considine et al., 1996; Halaas et al., 1995; Lohmus et al., 2006; Shi et al., 2006).

2. Methods

2.1. Field site and sample collection

We opportunistically sampled female European starlings at Davistead Dairy Farm in Langley, British Columbia (49°10′N, 122°50′W) during the breeding season (April–June 2007), and we sampled non-breeders in Fall (November 10, 2007). At the site, there are 190 nest boxes attached to wooden stakes or 9(pla)-235(and)-J/T1≥12((April90)-381(nuildings,)-459(between)-463(5)-470(and)-472(15)
Canadian Councillaof Animal Care (CCAC) gu(anlines)-335(in)-341(accordance)-J/T1≥12((Aprilwith)-289(Simon)-292(Fraser)-294(University)-289(A
and color bands (Environment Canada permit # 10646), and a(Aprilblood sample was taken. All blood samples were collected from
the right or left vei−20 °C until assay analysis.d and(Aprilma was frozen at

Co.) was used for dilution of primary and secondary antibodies. Standard hormone (putative recombinant chicken leptin) and tracer (radiolabeled rcleptin) were dissolved, diluted and stored in the phosphate buffer containing 1% BSA. To enhance the sensitivity of the RIA, the assay was conducted under non-equilibrium conditions. On day 1, RIA diluent (100 μL) plus a similar volume of standard or plasma unknown were added to plastic tubes containing 100 μL of first antibody (rabbit anti-chicken leptin; 1:1600 working dilution), vortexed and incubated overnight at 4 °C. On day 2, 100 μL tracer containing 6000 c.p.m. of I-125-labeled rcleptin was added to each tube, vortexed and incubated overnight at 4 °C. On day 3, second antibody (100 μL of a 1:10 dilution of sheep anti-rabbit gamma globulin) and carrier (100 μL normal rabbit ser-

continuous variation in plasma leptin-like immunoreactivity in relation to residual (stage-specific) body mass, body condition,

November was significantly higher than in incubating and chick-rearing birds (P