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Behavioral and physiological effects of photoperiod-induced migratory state and leptin on a migratory bird, *Zonotrichia albicollis*: I. Anorectic effects of leptin administration

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ABSTRACT

The hormone leptin is involved in the regulation of energy balance in mammals, mainly by reducing food intake and body adiposity and increasing energy expenditure. During energetically demanding periods, leptin's action is often altered to facilitate fat deposition and maintain high rates of food intake. Despite the present controversy over the existence of an avian leptin, there is evidence that a leptin receptor exists in birds and its activation influences energy intake and metabolism. However, it is unknown whether the effects of the activation of leptin receptor on energy balance are modulated during migration. We manipulated photoperiod to induce migratory behavior in captive white-throated sparrows (*Zonotrichia albicollis*) and injected migratory and wintering sparrows with either murine leptin or PBS for 7 days. We measured food intake, changes in body composition and foraging behavior to test if leptin's effects are altered during migratory state. Leptin decreased foraging behavior, food intake and fat mass in wintering sparrows, but had no effect on foraging behavior or food intake in migratory sparrows. Migratory sparrows injected with leptin maintained fat better than sparrows injected with PBS. Thus, sparrows' responses to leptin changed with migratory state, possibly to aid in the increase and maintenance of rates of food intake and fat deposition. We also found that long-form leptin receptor and SOCS3 were expressed in tissues of sparrows, including the hypothalamus, but their expression did not change with migratory state. Further study of the leptin receptor system and other regulators of energy balance in migratory birds will increase our understanding of the physiological mechanisms that are responsible for their ability to complete energetically demanding journeys.

1. Introduction

Migratory birds are able to exercise at very high aerobic intensities for extended periods [33,58]; some species undergo nonstop flights of over 11,000 km lasting several days [31]. Fat stores constitute most of the energy used to fuel these journeys [39,58]. Consequently, some species double in mass and deposit upwards of 50% of body mass as fat in preparation for migratory periods e.g., [71]. Most species periodically interrupt migratory flights to replenish energy stores, and many of the behavioral decisions during stopover are affected by the amount of lipid and other energy stores [3,12,15,92,94]. The substantial changes that avian migrants undergo in preparation for, and during, migratory journeys require a change in the regulated levels of body mass and fatness. Thus, the ability to monitor and regulate energy stores appropriately clearly provides major benefits for wild birds, and yet we understand little about the physiological mechanisms involved.

The discovery of the hormone leptin was a major advance towards understanding the regulation of energy stores in vertebrates because it represents a direct link between adipose tissue and the central nervous system [97]. In mammals, leptin is synthesized primarily by adipose tissue and its circulating concentration is proportional to total body adiposity [67]. Leptin provides a signal indicating the magnitude of lipid storage to the brain through hypothalamic receptors [2]. Reports of direct effects of leptin and the presence of leptin receptors in peripheral tissues also suggest that some of leptin's control over energy balance occurs locally, but most regulation appears to be centrally mediated (see [9]). The major effect of leptin on behavior is a reduction in food intake, but it can also increase metabolic rate and fat oxidation to reduce fat mass (see [93]). Thus, elevated leptin levels signal an abundance of energy stores, and influence behavior and physiology to increase energy expenditure and decrease energy intake.

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The presence and expression of leptin homologues have been described in several non-mammalian vertebrates, including fish [30,32,40,48] and amphibians [10,19]. Leptin-like compounds have been detected by immunological assays in lepidosaurs e.g., [69]. As in mammals, leptin is involved in the regulation of lipid metabolism, the reduction of food intake and increase in energy expenditure in frogs [19], fish [55,60,70] and lizards [63], although not all of these effects are always observed. For example, leptin does not reduce food intake [55] and may not be involved in long-term regulation of feeding [38] in some fish species.

For birds, leptin could be integral to the control and success of migration by regulating the availability and amount of fuels that are used to power migratory journeys, and thus influencing decisions involved in stopover timing and behavior. However, despite several independent reports of the expression of leptin in the chicken (*Gallus gallus*; [6,7,84]) and mallard (*Anas platyrynchos*; [20]), and the presence of leptin-like immuno-reactivity in a variety of avian species e.g., [5,42,43,73], the validity of these findings is highly debated e.g., [28,72,79,81,82]. Despite multiple efforts, the reported avian leptin gene has not been found in either the genome or EST libraries any avian species (see [26,81]). Thus, avian leptin has yet to be discovered. However, there is an abundance of evidence that (1) a leptin receptor does exist in birds e.g., [37], (2) the receptor is functional [1,36] and (3) its activation reduces food intake [13,21,23,47,53,54,74] and influences fatty acid metabolism [22,24].

Studies of how the functional effects of leptin are influenced by organismal ecology, especially during periods of high energy demand, have produced intriguing results. For example, during energetically demanding periods of the annual cycle, some seasonally breeding and migratory mammals increase fat storage in the face of increased circulating leptin levels; elevated leptin does not reduce food intake or increase energy expenditure [16,41,44,45,61,62,76,88]. This change in response to leptin and the disruption of its role in the control of food intake has been interpreted as a possible adaptive mechanism that allows for the maintenance of high rates of energy intake and storage in spite of high body fatness (i.e., a means to regulate energy storage at a different set point during energy-intensive periods; [85]). The mechanisms responsible for adaptive changes in leptin sensitivity in mammals is focused upon a reduction in signaling capacity downstream of the leptin receptor, mainly through the inhibition of signal transducer and activator of transcription 3 (STAT3) by suppressor of cytokine signaling 3 (SOCS3) [86]. An alternate mechanism to achieve seasonal changes in leptin sensitivity is a modulation in the amount of leptin receptors or in the relative expression of different receptor isoforms. Because short isoforms of the leptin receptor lack full signaling capacity, a relative increase in this isoform could reduce leptin sensitivity, as is suggested from findings in migratory bats [85].

For avian migrants, seasonal changes in the leptin receptor system might provide similar advantages, allowing for hyperphagia, high rates of fat deposition and high fat loads in preparation for migratory journeys. Whereas leptin administration has been shown to decrease food intake and influence fatty acid metabolism in birds, it is unknown whether or not these effects of leptin change seasonally in migratory birds. While SOCS3 and two membrane-bound isoforms of leptin receptor, including the long-form receptor, are found in poultry [37,50,66], their presence and expression in relation to migratory state have not been reported in avian migrants.

In our first experiment, we induced migratory condition in white-throated sparrows (*Zonotrichia albicollis*) by photoperiod manipulation and administered murine leptin to activate the leptin receptor system to test whether its response is altered during migratory periods. We expected migratory sparrows to increase food intake, body fat content and body mass in preparation for migration. If the avian leptin receptor system is modulated to facilitate the maintenance of both high body fat and rates of energy intake in response to the energetic requirements of migration, then we expected leptin administration to be less effective in decreasing body mass, fat mass, food intake and foraging behavior in migratory sparrows than in non-migratory sparrows. In a second experiment we again used photoperiod to manipulate migratory behavior in sparrows and measured the expression of leptin receptor and SOCS3 in sparrow tissues. We then tested if the level of expression of these genes is associated with changes in response to leptin between migratory and non-migratory sparrows.

2. Methods

2.1. Birds (experiment 1)

The white-throated sparrow is a small (25 g) passerine that migrates between wintering areas in central and southern North America to breeding sites throughout sub-arctic Canada [26]. For experiment one, we captured 48 wild sparrows in mist nets during fall migration (06–17 October, 2008) on private land (42°38′20.14″N, 80°34′21.49″W) approximately 10 km north of Long Point, Ontario, Canada. Sparrows were immediately transported to captive facilities at the University of Western Ontario, and singly housed in cages (40 × 45 × 45 cm) at 21 °C. We acclimated birds for 14 days to captive conditions on short-days (Light:-Dark (L:D): 8:16) with *ad libitum* access to water, mixed wild bird seed (Wild Bird Chow™, Purina Mills®, Gray Summit, MO) and millet sprays (Nutriphase®, Petsmart Inc., Phoenix, AZ, USA). Sparrows were then acclimated over 21 days to bird feed (Mazuri® Small Bird Breeder, PMI Nutrition International, Brentwood, MO), which was ground to a powder, and were maintained on *ad libitum* access to the ground feed for the duration of the study. All animal procedures were approved by The University of Western Ontario Institutional Animal Care and Use Sub-Committee (protocol # 2005-060). Birds were captured under a scientific collection permit from the Canadian Wildlife Service (CA 0170).

2.2. Photoperiod conditions (experiment 1)

All birds were initially held on short days (8:16 L:D) to mimic wintering light conditions for 60 days to break photorefractoriness. We then switched the photoperiod for half of the sparrows to long days (16:8 L:D) to induce migratory restlessness [59] and changes in metabolic physiology (see [26,56]), while the remaining half were held on the short-day light regime. We commenced experiments after birds had experienced the long-day photoperiod for 21 days. Mini-infrared cameras (Advanced Security Products, Bellville, IL, USA) and an infrared light source (Sony Digital 8 camcorder with nightvision) were used to monitor nighttime activity following the change in light cycle and throughout the experimental period. All sparrows held on the long-day light regime ("migratory sparrows," hereafter) exhibited migratory restlessness throughout the experimental period, while sparrows kept on short days ("wintering sparrows," hereafter) displayed little to no nocturnal activity (Cerasale et al., unpublished data).

2.3. Experimental protocol (experiment 1)

Following the 21 days of long-day light conditions, we randomly split migratory sparrows into three groups; leptin-injected ($N = 9$), PBS-injected ($N = 9$), and non-injected ($N = 6$). Leptin and PBS-injected sparrows were injected intra-muscularly into the pectoralis major twice daily for 7 days with either murine leptin