Leptin in Chickens - a Review

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Abstract

Leptin, the product of the ob gene, is primarily produced and released from adipocytes and acts on the hypothalamus to decrease food intake and increase energy expenditure. Defect in leptin or leptin receptors results in severe metabolic syndromes such as obesity, diabetes, and hypertension. Evidence suggests that leptin plays beyond a satiety factor; in fact, it is a pluripotent player in regulation of numerous body functions. Although its actions have been relatively well studied in mammals scanty data are available in birds. In this article, recent advances in understanding of the roles of leptin in chicken physiology are reviewed with the focus on the effects on food intake, lipid metabolism, development and reproduction, and stress.

▸ **Key words:** leptin; food intake; immunity; lipid metabolism; development and reproduction; stress.

적요

비만유전자의 산물인 렙틴은 주로 지방세포에서 생성 분비된다. 렙틴은 뇌 시상하부에 작용하여 식이섭취량을 줄이고 에너지발산을 증가시켜 궁극적으로 체중을 감소시킨다. 따라서 렙틴 또는 렙틴 수용체의 이상은 극도의 대사증후군을 유발한다. 현재까지 렙틴에 관한 연구의 대부분은 사람을 포함한 포유류에서 수행되었다. 렙틴의 생리적 작용은 만복인자뿐만이 아니다. 본고에서는 현재까지 발표된 논문을 중심으로 닭에서 식이섭취, 면역, 지방대사, 번식과 발달, 그리고 스트레스에 관한 렙틴의 작용을 검토했다.

Introduction

Discovered by Freedman and associates (1994), leptin, the product of the ob gene, is primarily synthesized in the adipocyte and travels to the central nervous system to act as a key signal that mediates energy status in the periphery. Leptin reduces food intake and body weight in rodent models when injected both systemically and centrally (Choi et al. 2003; Della-Fera et al. 2003). Mutation of the gene of leptin or leptin receptors leads to severe metabolic syndromes such as obesity and type 2 diabetes. Thus, leptin has been suggested to function as part of a signaling pathway that links the adipose tissue to the brain to regulate the size of the body fat depot (Zhang et al. 1994). It is now clear that leptin is more than a satiety signal (Harris 2000; Macajova et al. 2004) and, in fact, is a multi-functional protein that participates in the regulation of body functions, ranging from nutrient transport in the intestine to immunity and reproduction(Harris 2000; Macajova et al. 2004) (see Figure 1). Whereas much of the findings have come from mammalian studies, much less has been investigated about the effects of leptin

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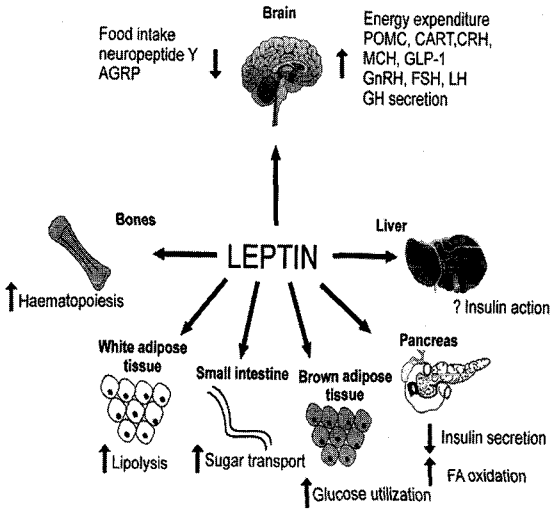

Figure 1. Schematic illustration of central and peripheral actions of leptin.Leptin is secreted by adipocytes and influences energy expenditure and food intake, interacting with almost all neuropeptides in the brain known to be involved in the regulation of energy balance: pro-opiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript (CART), corticotrophin -releasing hormone (CRH), melanin- concentrating hormone (MCH), glucagon-like peptide-1 (GLP-1), neuropeptide Y (NPY) and agouti-related peptide (AGRP). Leptin also influences reproduction and growth, stimulating secretion of gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH) and growth hormone (GH) from the pituitary gland. Leptin inhibits insulin secretion, but in the liver, the role of leptin in insulin action is unclear. Leptin also increases fatty acids(FA) oxidation in the pancreas, glucose utilization in brown adipose tissue, sugar transport in the small intestine, lipolysis in white adipose tissue and haematopoiesis in bones (Macajova et al. 2004).

in chickens. In the current article, recent advances in understanding of the roles of leptin in chicken physiology are reviewed.

Leptin expression

Leptin is produced in primarily adipocytes in mammals (Zhang et al. 1994) and its expression is

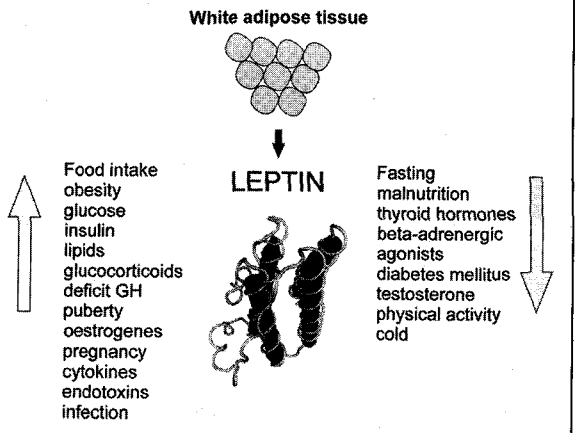

Figure 2. Various factors involved in the regulation of leptin production in white adipose tissue. GH, growth hormone (Macajova et al. 2004).

modulated by varying factors (Figure 2). In contrast, Taouice et al. (1998) demonstrated the presence of a leptin homolog in chickens, not only in adipose tissues but also in the liver. Expression of leptin mRNA in liver tissues of both female and male broiler chickens at 8 weeks of age was nearly twice as high as that observed in abdominal and subcutaneous adipose tissues (Richards et al. 2000). At embryonic stages, leptin was detected to be expressed in whole day 5 chicken embryo, day 17 embryonic liver, and day 17 embryonic yolk sac (Ashwell et al. 1999). This trend that leptin gene expression is higher in the liver than in fat tissues has been consistently shown in several studies (Ashwell et al. 1999a; Ashwell et al. 1999b; Richards et al. 1999; Richards et al. 2000). Leptin expression in the liver (Taiuis et al. 1998; Ashwell et al. 1999) is hypothesized to be due to the role of the avian liver as the primary source of avian lipogenesis (Leveille et al. 1975). The presence of leptin in the liver is supported by in vitro studies demonstrating that leptin is constitutively expressed in a hepatoma cell line derived from a male Leghorn chicken and has a molecular weight of approximately 16kDa (Cassy et al. 2003), corresponding to that of recombinant chicken leptin (Raver et al. 1998). This cell line also expresses a functional leptin receptor that is identical to the one previously described

(Horev et al. 2000; Ohkubo et al. 2000) and that is down-regulated in response to insulin and leptin but not to dexamethasone and triiodothyroxine (Cassy et al. 2003). In contrast to the findings in in-vivo study (Ashwell et al. 1999b), insulin failed to stimulate leptin secretion in this cell line (Cassy et al. 2003)

Chicken leptin has similarity (83~97%) to mammalian leptins and an extra cysteine residue (Taouis et al. 1998). Leptin concentrations in chickens are dependent upon feeding status (fasted and fed) and age (Dridi et al. 2000). Plasma leptin concentrations were significantly increased in5-week old layer chickens injected intraperitoneally with leptin at a dose of 1 mg/kg body weight (80 5.3ng/ml vs. 3.4 0.2ng/ml, 2h after injection). Feeding resulted in increased leptin concentration (Dridi et al. 2000).

Leptin Receptor

Ohkubo et al. (2000) showed expression of chicken leptin receptor, with the most abundant expression in the brain and ovary of both 5- and 22-week-old female chickens, suggesting that age does not affect expression patterns of the leptin receptor in chickens. Horev et al. (2000) observed a similar expression pattern of the leptin receptor. Expression pattern of leptin receptor in the brain is consistent with that observed in mammal (Ghilardi et al. 1996). In contrast to mammals, the soluble form of leptin receptors appears to be absent in chicken plasma as the attempt to find circulating leptin binding proteins was not successful (McMurtry et al. 2004). Significance of this absence remains to be determined.

Leptin clearance

In 4-week old male broiler chickens infused intravenously with 125I-labled mouse leptin, McMurtry et al (2004) demonstrated that leptin was cleared from blood by the kidney, with a half-life of 23 min. This result is similar to the half-life of leptin (25 min) observed in humans (Klein et al. 1996), but shorter than those for rhesus monkeys (96.4 min) and mice (49.5 min) (Ahren et al. 2000).

Leptin on food intake

Leptin'saction on food intake in chickens appears to vary depending upon age and birds that were used in studies. An early study by Bungo et al. (1999) using neonatal chicks demonstrated that intracerebroventricular (ICV) injection of mouse leptin (up to 5 g)failed to decrease food intake in both egg-type and meat-type neonatal chicks fasted for 3 hours. Raver et al. (1998), however, demonstrated that both intraperitoneal and intravenous injections of recombinant chicken leptin significantly reduced food intake in 9-day old broiler chickens adapted to a daily 2-h fasting regime (Raver et al. 1998). When intraperitoneal injection of recombinant chicken leptin was given to 9- and 56-day old layer and 9-day old broiler chickens, however, food intake was greatly reduced in the older layer chickens but was not modified in broiler chickens (Cassy et al. 2004).

The reason for this failure is not clear but might be due to different experimental conditions such as different leptin and neonatal chicks. Food intake was significantly reduced, however, when recombinant human leptin was administered ICV into about 5-week old broiler and 8-week old Single Comb White Leghorn chickens (Denbow et al. 2000). Consistent with these findings is that intravenous injection of chicken leptin and its mutant (C4S) at a dose of 1 mg/kg BW significantly decreased food intake in 9-day old broiler chickens previously adapted to a 2-h fasting for 3 days (Dridi et al. 2000a).

More recently, Denbow and associates (Kuo et al. 2005) demonstrated that ICV injection of recombinant human leptin resulted in reduced food intake in chickens selected for low (LWS) but not high (HWS) body weight at 8 weeks of age for more than 45 generations from a common White Rock base population. Continuous infusion of recombinant chicken leptin into 3-week old broiler chickens via osmotic mini pumps at a rate of 8g/kg/h significantly decreased food intake over 6h (Dridi et al. 2005b). These findings suggest that chickens selected for a fast growth rate may be less sensitive to leptin than those for a low growth rate and that age may be a factor that influences chickens' sensitivity to leptin.

Leptin and immune system

Leptin concentrations in the circulation rapidly decrease during fasting and increase after re-feeding, and are positively related to the amount of body fat mass. Fasting and stress are well known to modify endocrine and immune functions in mammals (Ahima et al. 1996 ; Faggioni et al. 2000 ; Lord et al. 1998). Moreover, leptin reversed the immune suppression observed during fasting in mice (Ahima et al. 1996), suggesting a direct link between leptin and immune function (Lord et al. 1998). In fact, animals lacking leptin or functional leptin receptors exhibited a compromised immune function in mammals (Busso et al. 2002 ; Madiehe et al. 2003 ; Mancuso et al. 2002 ; Takahashi et al. 1999). Moreover, leptin concentrations were acutely increased during infection and inflammation (Finck et al. 1998; Moshyedi et al. 1998). In domestic birds, results from both in vitro and in-vivo studies showed that leptin enhances mitogen-stimulated T-cell proliferation (Lohmus et al. 2004). Although paucity data are currently available on the effect of leptin on immune system in chickens, it is plausible to postulate that leptin plays a role in chickens in a sense that the protein has a key impact on mammalian immunity.

Leptin and lipid metabolism

Adipose tissue functions as a primary energy depot that stores triglycerides during a time of energy surplus and provides fatty acids and glycerols when fasting. Feeding results in increased release of leptin into blood. Leptin concentrations in the circulation are increased proportionally with the increase of mass of the adipose tissue. Expression of leptin, leptin receptor, and fatty acid synthase was detected in the chicken liver that is responsible for >90% of de novo fatty acid synthesis and is a lipid storage (Leveille et al. 1975).

In fat (FL)- and lean (LL)- broiler chicken lines, which were developed for abdominal fatness-to-body weight ratio via a long term divergent selection, with the former having about 1.52-fold higher abdominal fat weight than the latter at 9 weeks of age, mRNA expression of both fatty acid synthase and leptin in the liver was significantly higher in FL than in LL chickens (Dridi et al. 2005). Consistently, plasma leptin concentrations were higher in the FL than LL chickens. Feeding state (overnight fasting or feeding) did not affect these trends.

In an attempt to figure out the effect of leptin on lipogenesis in chickens, Dridi et al (2005) infused recombinant chicken leptin into the brachial artery of broiler chickens. Three-week-old broiler chickens, administered continuously with recombinant chicken leptin at 8 g/kg/h for 6 h, had increased plasma leptin concentrations by 23-fold compared with control, reduced food intake by 51%, increased FAS mRNA levels in the liver, and down-regulated its receptors by 18.5 and 23.2% of Ob-R1 (extracellular region) and Ob-R2 (intracellular region), respectively. However, leptin treatment failed to alter SREBP-1 gene expression, the main transcription factor that controls the expression of lipogenic pathway as well as the concentrationsof glucose, non-esterified fatty acids, T3, triglyceride, and uric acid (Dridi et al. 2005). These results are consistent with the hypothesis that leptin plays a potential role in lipogenesis in the chicken liver,but conflicts with the findings in mammals (Soukas et al. 2000).

Leptin and Development and Reproduction

Leptin appears to alter embryonic development. Leptin enhanced in vitro proliferation and proteinsynthesis of muscle cells and hepatocytes isolated from 11- or 19-day old chicken embryos, with those from the younger being more sensitive to leptin (Lamosova and Zeman. 2001). Lamosova et al. (2003) demonstrated that eggs of Japanese Quails, treated with leptin on day 5 of incubation, hatched earlier with higher body weight of chicks.

It is well documented that leptin is an important regulator of reproductive functions in mammals, including farm animals (Barb and Kraeling. 2004). Almog et al. (2001) demonstrated leptin attenuates follicular apoptosis by elevating expression of Bcl-2 and accelerates the onset of puberty in immature

rats. Likewise, leptin appears to be involved in regulation of ovarian functions in chickens. Paczoska-Eliasiewicz et al.(2003) showed that administrations of leptin during fasting delayed cessation of egg laying in brown laying hens, in addition to attenuating regression of yellow hierarchical follicles and altering ovarian steroidogenesis, implying that leptin is able to alleviate the negative effects of fasting on ovarian functions. In contrast to the findings in laying hens, leptin seems to be involved in ovarian dysfunctions in broiler breeder hens fed ad libitum, whose reproductive functions are poor after years of genetic selection for a high growth rate (Cassy et al. 2004). It seems likely that leptin playsdual roles in regulation of ovarian functions in chickens.

Leptin and Stress

A solid line of evidence suggests that leptin is a key regulator in stress. Heiman et al. (1997), for example, demonstrated that intraperitoneal administration of leptin inhibited activation of the hypothalamic-pituitary-adrenal axis in response to restraint stress in mice. Furthermore, leptin appears to be a neuronal survival factor which prevents neurons from going through apoptosis under severe energy restriction (Dagon et al. 2005), which activates the AMP-activated protein kinase (AMPK) system in the central nervous system whereas leptin inhibits its activity (Minokoshi et al. 2004). Lepin treatments suppressed hypeactivity associated with metabolic stress in rats under a limited feeding regime (Exner et al. 2000; Ruffin and Nicolaidis. 2000).

In chickens, stress seems to impair posthatch growth, perhaps via increased release of corticosterone. Indeed, Lay at al. (2002) demonstrated that body weight 100 days post-hatch was decreased in chicks hatched from eggs exposed to heat stress during the last 6 days of incubation. McIlroy et al (1999) demonstrated that corticosterone concentrations in vitro were enhanced in adrenal steroidogenic cells that were derived from adrenal tissue of protein-restricted birds and then incubated with either ACTH or cholera toxin.

Although little is available about the effects of leptin on stress in chickens, there is indirect evidence suggesting that leptin might participate in regulation of stress in chickens. Alonso-Vale et al. (2005) showed that there is a cross-talk between leptin and melatonin in rats. The latter is involved in alleviating stress responses associated with heat and isolation in chickens (Rozenboim et al. 1998 Saito et al. in press). We recently showed that both isolation and ICV injection of corticotropin- releasing factor enhanced

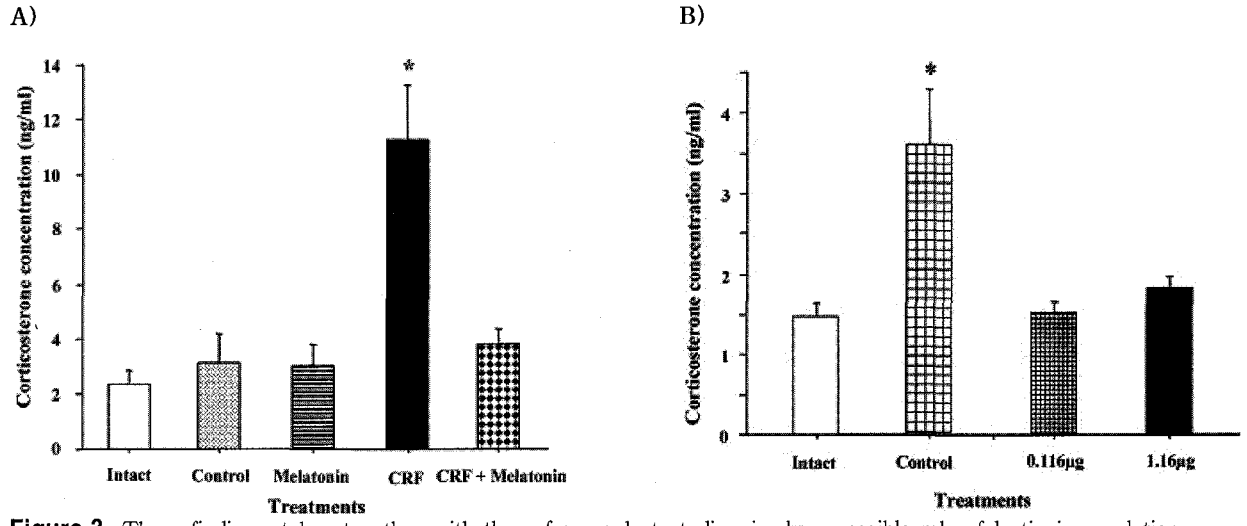

Figure 3. These findings, taken together with those from rodent studies, imply a possible role of leptin in regulation of stress in chickens.

corticosterone concentrations in plasma of neonatal chickens (Saito et al. 2005; Saito et al. in press), which was reversed following ICV administreation of melatonin (Saito et al. 2005; Saito et al. in press) (Figure 3). These findings, taken together with those from rodent studies, imply a possible role of leptin in regulation of stress in chickens.

Concluding Remarks

Studies in mammalian species indicate that leptin plays as a pluripotent signal beyond a satiety factor which affects many functions of the body. A few studies, however, have been so far done to elucidate its effects on chicken physiology. Available data have begun to shed light on its effects in chickens, indicating that there are similarities and differences in leptin's actions between chicken strains and between developmental stages, in addition to those between mammals and birds. Leptin's effect on stress remains to be determined. Leptin expression in the liver is of interest, for example, and further investigation is warranted for its significance in chickens.

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