Adiponectin and leptin are two adipocytokines originally found to be secreted mainly by white adipose tissue (AT) in mammals. They are involved in the control of energy homeostasis, body weight, lipid metabolism, and insulin sensitivity. The interplay between these two hormones was exclusively studied in mammals and yielded conflicting results. In birds, adiponectin and leptin are expressed not only in AT, but also in liver (for leptin) and in a wide range of tissues (for adiponectin). However, their physiological roles and relationship are still unknown. The aim of the present study was to investigate the effect of recombinant chicken leptin on adiponectin gene expression in three metabolically important tissues (liver, hypothalamus, and muscle). The effect of gender and cerulenin, the natural fatty acid synthase inhibitor which has been shown to share some molecular mediators with leptin, were also evaluated. Females exhibited significantly (P<0.05) higher levels of adiponectin mRNA in muscle and the liver, but not in the hypothalamus compared to male broiler chickens. Regardless of gender, muscle was found to contain the highest amount of adiponectin mRNA followed by the liver and hypothalamus. Continuous infusion of leptin (8 μg/kg/h) for 6h in 3-wk-old broiler chickens significantly (P<0.05) increased plasma leptin levels, reduced food intake, and downregulated adiponectin gene expression in liver and muscle compared to the control. Cerulenin treatment (15 mg/ml) at different times significantly (P<0.05) reduced food intake. These changes were accompanied by significant (P<0.05) upregulation of hepatic adiponectin gene expression. Hypothalamic and muscle adiponectin mRNA abundance however were significantly (P<0.05) downregulated by cerulenin treatment compared to the control. Our data showed that adiponectin gene expression is regulated by gender, leptin, and cerulenin in a tissue-specific manner. It also suggests that cerulenin does not mimic leptin by downregulating hepatic adiponectin, however it does mimic it by reducing food intake.

**Keywords:** Adiponectin; Leptin; Cerulenin; Gender; Chickens

**Abbreviations:** Acrp: adipocyte complement related protein; Adipo-R: adiponectin receptor; AMPK: AMP-activated protein kinase; ANOVA: analyse of variance; ApM: adipose most abundant gene transcript; DMSO: dimethyl sulfoxide; GBP: gelatin-binding protein; GLUT: glucose transporter; QPCR: real-time quantitative PCR; RIA: radioimmunoassay; RPMI: Roswell park memorial institute medium

**Abstract**

Adiponectin and leptin are two adipocytokines originally found to be secreted mainly by white adipose tissue (AT) in mammals. They are involved in the control of energy homeostasis, body weight, lipid metabolism, and insulin sensitivity. The interplay between these two hormones was exclusively studied in mammals and yielded conflicting results. In birds, adiponectin and leptin are expressed not only in AT, but also in liver (for leptin) and in a wide range of tissues (for adiponectin). However, their physiological roles and relationship are still unknown. The aim of the present study was to investigate the effect of recombinant chicken leptin on adiponectin gene expression in three metabolically important tissues (liver, hypothalamus, and muscle). The effect of gender and cerulenin, the natural fatty acid synthase inhibitor which has been shown to share some molecular mediators with leptin, were also evaluated. Females exhibited significantly (P<0.05) higher levels of adiponectin mRNA in muscle and the liver, but not in the hypothalamus compared to male broiler chickens. Regardless of gender, muscle was found to contain the highest amount of adiponectin mRNA followed by the liver and hypothalamus. Continuous infusion of leptin (8 μg/kg/h) for 6h in 3-wk-old broiler chickens significantly (P<0.05) increased plasma leptin levels, reduced food intake, and downregulated adiponectin gene expression in liver and muscle compared to the control. Cerulenin treatment (15 mg/ml) at different times significantly (P<0.05) reduced food intake. These changes were accompanied by significant (P<0.05) upregulation of hepatic adiponectin gene expression. Hypothalamic and muscle adiponectin mRNA abundance however were significantly (P<0.05) downregulated by cerulenin treatment compared to the control. Our data showed that adiponectin gene expression is regulated by gender, leptin, and cerulenin in a tissue-specific manner. It also suggests that cerulenin does not mimic leptin by downregulating hepatic adiponectin, however it does mimic it by reducing food intake.

**Introduction**

Adipose tissue secretes a large number of physiologically active peptides that often share structural properties of cytokines and are therefore referred to collectively as “adipocytokines” or “adipokines” [1,2]. The discovery of one of these peptides, leptin, which is the product of the obese gene [3], along with the characterization of adiponectin [4], has renewed interest in the hormonal regulation of energy homeostasis.

Leptin is a 16 kDa glycosylated protein of 146 amino acids produced predominantly by adipose tissue in mammals [3] and functions as a hormonal signaling mechanism for fat deposition. Mammalian adipocytes produce and secrete more leptin in the bloodstream as fat storage increases [5], signaling the brain via leptin receptor [6] and modulating the hypothalamic neuropeptide systems to suppress appetite and increase energy expenditure [6,7]. Mutations in the genes encoding leptin or the leptin receptor result in increased obesity and a hormonal deficit [6]. Leptin treatment decreases serum glucose and lipogenesis and stimulates lipid oxidation in rodents [8,9]. These actions are mediated primarily through neuronal targets in the hypothalamus and peripheral tissues [10].

Adiponectin, also named gelatin-binding protein 28 (GBP-28), adipocyte complement related protein 30 (Acrp30), adipose most abundant gene transcript 1 (apM1), or adipQ [4,11-13], is a 30 kDa adipocytokine hormone of 244 amino acids exclusively secreted from adipose tissue in mammals. Adiponectin consists of an N-terminal collagenous domain and a C-terminal globular domain [14]. It circulates as low-molecular-weight dimers or trimers and high-molecular-weight complexes in mammalian plasma [14]. Adiponectin acts, via two specific receptors adipo-R1 and adipo-R2 [15], as an insulin-sensitizing hormone whose blood concentrations are reduced in obesity and type-2 diabetes [16]. Administration of recombinant adiponectin to rodents increases glucose uptake and fat oxidation in muscle, reduces fatty acid uptake and hepatic glucose production in liver, and improves whole-body insulin resistance [17]. Moreover, intracerebroventricular administration of adiponectin decreases body weight and increases energy expenditure in rodents [18].

Altogether, these data showed that leptin and adiponectin exert...
major metabolic properties and suggest a potential interplay between these adipocytokine hormones. These hypotheses drew the attention of many scientists and represented the focus of intense research efforts exclusively in several mammalian species [19,20]. Acute leptin treatment did not modify plasma adiponectin levels in humans [21]. Longer administration of leptin yielded conflicting results in rodents. Indeed, leptin treatment did not affect plasma adiponectin levels in lipotropic A-ZIP/F-1 mice [22] however, it increased adiponectin gene expression in normal rats [23] and adiponectin plasma levels in ob/ob mice [19]. Such studies are currently lacking in non-mammalian species. Therefore, the present study primary aimed to investigate the effect of recombinant chicken leptin on adiponectin gene expression in broiler chickens.

Natural fatty acid synthase inhibitor cerulentin and its synthetic analog C75 have been shown to affect food intake and energy expenditure in mammals similarly to leptin and adiponectin [24,25]. These products were found to share some molecular mediators with adipocytokine hormones, such as feeding-related hypothalamic neuropeptides for leptin [26,27], and AMPK signaling cascades for both leptin and adiponectin [28-32]. Thus, the secondary aim of this study was to explore the effect of cerulentin on the expression of adiponectin gene, in light of its similarities to leptin.

Materials and Methods

Animals

Experiments were conducted in accordance with the directives of the European community (86/609/EEC) on the care and use of laboratory animals and the experimental protocols were approved by the K.U. Leuven Ethical Committee for Animal Experiments.

Experiment 1. Adiponectin gene expression in male and female broiler chickens: Male and female broiler chickens (Avibel, Halle-Zoersel, Belgium) of 6 weeks of age (1765 and 2665g for female and male, respectively) were kept on a floor pen under a 14:10-h light-dark cycle. Chickens were allowed food (12.6 MJ/kg; 21.4% protein) and water ad libitum. Chickens were killed by cervical dislocation and three anatomical sites (hypothalamus, liver and leg muscle) were removed and snap frozen in liquid nitrogen and stored at -80°C until use.

Experiment 2. Leptin treatment: One-day-old male broiler chicks (Avibel, Halle-Zoersel, Belgium) were reared on a floor pen until two weeks of age, at which time the birds were transferred to individual cages and were fed ad libitum (12 MJ/kg; 22% protein). After three days of adaptation, birds were weighed and cannulated in the brachial artery [66] under local anaesthesia (xylocaine). The chickens were allowed to recover and to adapt during four more days. Before the infusion experiment, the chickens were divided into two homogeneous weight Matched groups (n = 5, mean of body weight was 1000g) and food deprived for two hours in order to increase their appetite. Each bird received an intravenous (IV) injection (at 0, 4 and 24h) of 15 mg/kg cerulentin (Sigma, Belgium) or equal volume of vehicle (10% DMSO in RPMI 1640 medium). Cumulative food intake was measured after 28 hours (hypothalamus, liver and leg muscle) were removed, frozen in liquid nitrogen and stored at -80°C until use.

Experiment 3. Cerulentin administration: One-day-old broiler chickens (Avibel, Halle-Zoersel, Belgium) were reared on a floor pen until one week of age, at which time the birds were transferred to individual cages and provided with individual feeders and drinking nipples. Food (12 MJ/kg; 22% protein) and water were consumed ad libitum and the lighting schedule provided 14h of light per day. After one week of adaptation, birds were divided into two homogenous weight (267g) and food intake matched groups (n = 4), and food deprived for two hours in order to increase their appetite. Each bird received an intravenous (IV) injection (at 0, 4 and 24h) of 15 mg/kg cerulentin (Sigma, Belgium) and equal volume of vehicle (10% DMSO in RPMI 1640 medium). Cumulative food intake was measured after 28 hours and tissues (hypothalamus, liver and leg muscle) were removed, frozen in liquid nitrogen and stored at -80°C until use.

Real-time Quantitative PCR (QPCR)

Total RNA was extracted from 100 mg of tissue using the Trizol reagent (Invitrogen, Belgium) according to the manufacturer’s protocol and was treated with RQI RNAase-free DNase (Promega, Belgium). RNA integrity and quality were assessed by using 1% agarose gel electrophoresis and RNA concentrations and purity were determined for each sample spectrophotometrically by using UV absorbance (260/280). Total RNA (1 µg) was reverse transcribed as previously described [56] and subjected to QPCR. QPCR analyses were performed in ABI PRISM 7000 Sequence Detector apparatus (Applied Biosystems, Belgium) in the presence of 25 µL master mix containing 25 ng template cDNA, 2X Power SYBR GREEN PCR Master Mix (Applied Biosystems, Belgium), and 300 nM forward and reverse primers (Invitrogen, Belgium). Oligonucleotide primers specific for chicken adiponectin [33] and standard housekeeping genes β-actin and ribosomal 18S as internal controls were used (Table 1). The primers were selected and designed for optimal hybridization kinetics using the Primer Express Software (version 2, Applied Biosystems, CA, USA). The QPCR cycling conditions were: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of two-step amplification program (95°C for 15s, and 59°C for 1 min). At the end of amplification, melting curve analysis was applied to exclude the contamination of unspecific PCR products such as primer dimers using the dissociation protocol from the Sequence Detection System. Each sample was run in triplicate and the average threshold cycle (Ct) values were determined for adiponectin, -actin and 18S. For negative controls, no RT products were used as template in the QPCR. Relative quantities of target adiponectin mRNA were estimated by the 2-ΔΔCt method [67]. For experiments 2 (leptin) and 3 (cerulentin), the untreated groups were chosen as the calibrators. For the first experiment (gender), male liver was arbitrarily chosen as the calibrator.

Plasma leptin measurement

Circulating leptin concentrations were determined by RIA (multi-species leptin RIA kit, Linco Research Co). The RIA has been validated.

Table 1: Oligonucleotide QPCR primers.
for chicken leptin [68]. Samples were assayed in a single assay and the intra-assay coefficient of variation was 6.3%.

Statistical analysis

The data were analyzed by using the student’s unpaired t-test, except for the data from experiment 1, which were analyzed by two-factor ANOVA with tissue and gender as classification variables. If ANOVA revealed significant effects, the means were compared by Student-Newman-Keuls (SNK)’s multiple range test using the Graph pad software (version 3.02, 2000, San Diego, USA). Differences were considered significant at P<0.05.

Results

Adiponectin gene expression in male and female broiler chickens

Female broiler chickens exhibited significantly (P<0.05) higher levels of adiponectin mRNA in the liver (1.6-fold) and muscle (2.4-fold) compared to males (Figure 1). In contrast, hypothalamic adiponectin gene expression was similar for both genders. Independent of sex, muscle was found to contain the highest amount of adiponectin mRNA, followed by the liver and hypothalamus.

Effect of leptin on food intake, leptinemia, and adiponectin gene expression in broiler chickens

Continuous infusion of recombinant chicken leptin significantly (P<0.05) increased plasma leptin levels (23-fold, Figure 2A), and reduced food intake by 50% (Figure 2B) compared to the vehicle-treated group. Recombinant chicken leptin significantly downregulated adiponectin gene expression in chicken liver and muscle by 81% and 63.5%, respectively (P<0.05) compared to the vehicle-treated group (Figure 3). The slight decrease (9%) of hypothalamic adiponectin mRNA levels after leptin treatment was not statistically discernable at the 5% level.

Effect of cerulenin on food intake, leptinemia, and adiponectin gene expression in broiler chickens

Cerulenin treatment slightly decreased plasma leptin levels (Figure 4A) and significantly reduced food intake (22%, P<0.05) compared to the vehicle-treated group (Figure 4B). These changes were accompanied by a significant upregulation (2-fold, P<0.05) of hepatic adiponectin gene expression, whereas hypothalamic and muscle adiponectin mRNA levels were significantly (P<0.05) decreased by 82% and 74%, respectively, compared to the vehicle-treated group (Figure 5).

Discussion

Although adiponectin is expressed in a wide range of tissues in chickens [33], its hormonal regulation and physiological roles are still unknown. In the present study, we investigated the regulation of adiponectin gene expression by gender, leptin, and cerulenin in three metabolically important tissues; the hypothalamus (the main site for energy homeostasis control [34], the liver (the main site for de novo fatty acid synthesis, lipogenesis [35], and muscle (the main site for thermogenesis [36]).

Muscle was found to contain the highest amount of adiponectin mRNA, followed by the liver and hypothalamus in 6-wk-old male and female broiler chickens. This result is quite different from that reported...
by Maddineni et al. [33] who have shown that adiponectin gene was highly expressed in the liver followed by the hypothalamus and muscle in 35-wk-old laying hens. It is not clear why such disparate results have been observed, but the use of tissues from different chicken strains (layer vs broiler) at different ages (6 vs 35 weeks) could contribute to these discrepancies. In fact, about 70 years of extensive selection for different performances, high meat yield for broilers and efficient egg production for layers, have resulted in two dramatically different strains of obese and lean phenotypes, respectively. These strains differ not only in food intake [37], growth rate [38] and fat accumulation, but also in behavioral [39] and neuroendocrine systems [40-43].

Interestingly, the hepatic and muscle expression of adiponectin gene is gender-dependent with higher levels in female compared to male chickens (n = 4) were intravenously treated with cerulenin or RPMI medium as a control. Birds were killed and total RNA was isolated from liver, hypothalamus, and muscle and subjected to RT-QPCR. Data are presented as mean ± SEM. * P<0.05 indicates a significant difference between cerulenin-treated group and the control.  

Figure 4: Effect of cerulenin treatment on plasma leptin levels (A) and cumulative food intake (B) in broiler chickens: Two homogenous weight-matched groups of 2-wk-old broiler chickens (n = 4) were intravenously treated with cerulenin (15 mg/kg at 0, 4, and 24h) or RPMI medium as a control. Cumulative food intake was monitored after 28h and plasma leptin levels were measured by RIA as described in materials and methods. Data are presented as mean ± SEM and * different from the control, P<0.05.

Figure 5: Effect of cerulenin treatment on adiponectin gene expression in broiler chickens: Two homogenous weight-matched groups of 2-wk-old broiler chickens (n = 4) were intravenously treated with cerulenin or RPMI medium as a control. Birds were killed and total RNA was isolated from liver, hypothalamus, and muscle and subjected to RT-QPCR. Data are presented as mean ± SEM. * P<0.05 indicates a significant difference between cerulenin-treated group and the control.

Adiponectin mRNA expression (% change)

Liver Hypothalamus Muscle


The effect of leptin on the adiponectin system has recently been reported and the bulk of data were exclusively obtained from mammals [19,20]. To our knowledge, however, there is a paucity of information in non-mammalian species. The present study is the first to provide novel data on the regulation of adiponectin gene expression by leptin in broiler chickens. These strains were selected for rapid growth and high food intake, and are prone to obesity [53]. Chicken leptin is expressed not only in adipose tissue but also in the liver [54,55], and adiponectin is ubiquitously expressed in chickens [33], while these two adipocytokine genes are predominantly expressed in adipose tissues in mammals [3,4]. The mechanism of central action of leptin on food intake regulation in chickens is different from that described in mammals [56]. Furthermore, chickens are characterized by hyperglycemia, insulin resistance [57,58] and are lacking glucose transporter GLUT4 [59]. Taken together, these peculiarities suggest that chicken liver-derived leptin may exert different effects on adiponectin gene expression than that described in mammals. Our data showed that leptin regulates adiponectin gene expression in a tissue-specific manner with a down regulation in the liver and muscle, but not in the hypothalamus. This result confirms previous findings in human placenta [60], but it contrasts with previous data observed in rat adipose tissue [23]. The mechanism(s) behind these differences are not clear and may be related to many factors such as dose and time of leptin treatment, and tissue- or species-specific effects.

Although leptin has long been known to play roles in the regulation of food intake and energy homeostasis, the potential role of fatty acid synthesis in this process has been considered only recently. Inhibition of fatty acid synthesis by cerulenin reduces food intake and induces profound reversible weight loss [24]. Centrally, this compound was hypothesized to alter, like leptin, the expression profiles of feeding-related neuropeptides, often inhibiting the orexigenic and inducing the anorexigenic neuropeptide gene expression [27]. Therefore, in this study we sought to assess whether cerulenin affects, as does leptin, the avian adiponectin gene expression. Our data showed that in contrast to leptin, cerulenin treatment, at a dose that produces similar effects on food intake, upregulated adiponectin gene expression in chicken liver. However, the hypothalamic and muscle adiponectin mRNA levels were decreased by this treatment. Again, this result supports the notion of tissue-specific regulation of adiponectin gene and indicates that cerulenin does not act as leptin in the hepatic adiponectin system, although it does mimic some effects of leptin in the hypothalamus to reduce food intake [61]. The underlying molecular mechanism(s) of the divergent effects of leptin and cerulenin on the hepatic adiponectin gene expression is unclear. We speculate that it could be related to...
the leptin system itself since it has been suggested that leptin and adiponectin levels are inversely associated in mammals [62]. In our experimental conditions, cerulein administration decreased plasma leptin levels to nearly 1 ng/ml, however leptin treatment increased circulating leptin levels to 60 ng/ml. Additionally, we have previously shown that in contrast to cerulein, leptin treatment induces the hepatic FAS gene expression [63], the key enzyme in fatty acid synthesis which catalyzes the synthesis of long-chain fatty acid synthesis through the condensation of acetyl-CoA and malonyl-CoA in a complex seven-step reaction [for review see [64]]. Moreover, the avian liver is responsible for more than 90% of de novo fatty acid synthesis and is also a site of lipid storage in birds [35]. The negative association between adiponectin mRNA and fat pad size in rodents [65] supports our hypothesis, however further studies are clearly required to fully characterize the relationship between leptin, adiponectin and fatty acid synthesis in birds and their physiological roles in different avian tissues.

In rodents, it has been shown that changes in adiponectin gene expression were not always accompanied by concomitant changes in plasma adiponectin levels [19]. Because of limited quantities of tissues and unavailability of specific chicken adiponectin antibody we were not yet able to measure circulating adiponectin levels and protein content in different tissues in the present study.

In summary, we showed a sexually dimorphic expression of chicken adiponectin gene. Leptin and cerulein differently regulate adiponectin in a tissue-specific manner. Further studies are warranted to fully understand the physiological roles of adiponectin and to shed light on its interaction with leptin and fatty acid synthesis in birds.

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References


