Nutritional Modulation of Leptin Messenger RNA in Broiler Chickens

Kan Sato, Miyako Nishida and Yukio Akiba

Animal Nutrition, Life Science, Graduate School of Agriculture, Tohoku University, Aoba-ku, Sendai-shi, Japan

Three sets of experiments were conducted to investigate the nutritional modulation of leptin mRNA expression in chicken adipose tissue and liver. Chicken leptin expression in adipose tissue and liver decreased following 24 h starvation, and gradually increased on refeeding, attaining a level comparable to that of the pre-fasting level at 72 h post-refeeding. Expression of leptin mRNA in adipose tissue and liver in chickens fed for 7 days on a triolein-rich (18 : 1) diet was significantly higher than that in chickens fed trilinolein-rich (18 : 2) or trilinolenin-rich (18 : 3) diets. The adipose leptin expression in chickens force-fed for 12 h with trilinolenin (18 : 3) emulsion after 48 h starvation was comparable to that prior to starvation, while the expression in chickens force-fed with triolein (18 : 1) or trilinolein (18 : 2) emulsions remained low even at 12 h after refeeding. The hepatic expression of leptin was not modified by feeding triacylglycerol emulsions irrespective of the fatty acid composition. These findings suggest that chicken leptin mRNA expression, in both adipose tissue and liver, is modulated by nutritional treatments and is closely associated with changes in lipid metabolism and adipose fat accumulation.

Key words: leptin mRNA, dietary fat, fast-refeeding, fat accumulation, broiler chickens

Introduction

Leptin, mainly released from adipose tissue, has been shown to play an important role in the regulation of food intake, energy metabolism and body fat stores in mammals. Recently, Taouis and colleagues cloned chicken leptin, which shows a high degree of sequence conservation with that of other species (Taouis et al., 1998). In addition, the chicken leptin receptor, which is highly expressed in brain and ovary, has been cloned by Ohkubo et al. (2000). These findings suggest that the function and/or metabolism of chicken leptin may be similar to that of mammals.

The lipogenic activity of the liver is much greater than that of adipose tissue in chickens and almost all of the fat accumulation in the adipose tissues may be accounted for by incorporation of triacylglycerols from plasma lipoproteins which are either synthesized in the liver or provided from dietary fats (Griffin and Hermier, 1988). During fat accumulation, lipoprotein lipase (LPL)-catalyzed hydrolysis of triacylglycerols in adipose tissues is the rate limiting step; inhibition of LPL activity by
anti-LPL monoclonal antibody has been found to cause lipemia and decreased fat deposition (Sato et al., 1999a). Therefore, in chickens, body fat stores and accompanying energy metabolism are dependent on the regulatory mechanisms occurring in both liver and adipose tissue, whereas fat accumulation is mainly regulated by adipose tissue in mammals (Weinstock et al., 1997). Ashwell et al. (1999) reported that chicken leptin was expressed in both liver and adipose tissue and that the expression in liver was regulated by several hormones, whereas, in mammals, it was produced almost exclusively by adipose tissue (Bado et al., 1998). Therefore, it is likely that food intake, energy metabolism and body fat stores in chickens might be regulated by leptin secreted from both liver and adipose tissue.

It has been shown in mammals that leptin mRNA expression is modified by fasting and refeeding (Mizuno et al., 1996; Kolaczynski et al., 1996). Takahashi and Ide (2000) reported that the expression level of leptin mRNA in white adipose tissue was affected by dietary fat sources in rats. However, the modulation of leptin expression by nutritional protocols in broiler chickens has not yet been studied. In this paper we investigate whether the manipulation of nutritional state can be used to modulate leptin mRNA expression in broiler chickens.

Materials and Methods

Animals

Male broiler chickens (Ross strain) were housed in an electrically heated battery brooder and fed on a commercial broiler starter diet (crude protein 22%, crude fat 4%, metabolizable energy 3,200 kcal/kg diet) until 3 week of age, when each experiment started. The chickens in all experiments were housed individually in wire cages in a temperature-controlled room (25 ± 3 °C).

RNA isolation and semi-quantitative RT-PCR

Tissues were homogenized with TRIzol®-regents (Invitrogen, Corp., Carlsbad, CA.), and total RNAs were isolated. Tissue mRNAs were prepared using oligotex-dT super (Takara), resuspended in 10 mM Tris-HCl pH 8.0 and stored at -70 °C until use. A 1μl aliquot of the mRNA suspension was used in each RT-PCR. The SuperScript™ One-Step RT-PCR System (Invitrogen Corp., Carlsbad, CA) was used for the RT-PCR. Temperature cycles were as follows : 50°C for 30 min, 94°C for 3 min followed by 28 cycles of 94°C for 30 s, 63°C for 1 min and 72°C for 1 min.

Oligonucleotide sequences of sense and antisense primers were as follows :

Chicken leptin, estimated product size 362 bp (nucleotides from 54 to 415, AF012727, Taouis et al., 1998) :
5’-GGCTTTGGCCCTATCTTTTC-3’, 5’-GGATAAGGTCAGGATGGGGT-3’

GAPDH, estimated product size 543 bp (nucleotides from 400 to 942, AF047874) 5’-AAGCGTGTATCATCTCAGCTCC-3’, 5’-GCATCAAAAGGTGGAGGAATGGC -3’
The GAPDH cDNA product was used as control to normalize the mRNA loading in the analysis. PCR products were analyzed on a 1% agarose gel, and the cDNA bands were stained by ethidium bromide. These bands were scanned with an image analyzer (Fluor-S Multimager, Bio-Rad Laboratories, Hercules, CA). Relative abundance of the different mRNAs was calculated as the ratio between the PCR products of the leptin gene and GAPDH gene for each sample, and presented as percentage of control.

**Experiments 1, 2 and 3**

In Experiment 1, chickens were starved for 24 h after feeding, and then refed a commercial starter diet for 72 h. Livers and adipose tissues were collected from chickens that were first fed, then starved for 24 h and refed for 24, 48 and 72 h for analysis of leptin mRNA expression.

In Experiment 2, chickens received daily semipurified diets differing in the fat source (80 g/kg diet): olive oil (triolein-rich), safflower oil (trilinolein-rich) or linseed oil (trilinolenin-rich) as described in our previous report (Sato et al., 1999b). After 7 days of feeding, chickens were killed for collection of livers and adipose tissues.

In Experiment 3, chickens were starved for 48 h, and then force-fed (10 g/kg body weight) with an emulsion containing monoacid triacylglycerols: triolein (18:1, n-9), trilinolein (18:2, n-6) or trilinolenin (18:3, n-3), three times during the subsequent 12 h. Livers and adipose tissues were collected at 12 h after the start of force-feeding.

**Statistical Analysis**

SAS® applications package was used for statistical calculations (SAS Version 6.03, SAS Institute Inc., Cary, NC). Group data for multiple comparisons were analyzed by ANOVA using a general linear models procedure followed by Duncan’s multiple range test to test for differences. The level of significance used in all studies was P<0.05.

**Results**

*Leptin mRNA expression in chickens fed, starved and refed on a commercial starter diet (Experiment 1)*

Leptin mRNA expression in adipose tissues of chickens starved for 24 h was significantly lower than that of chickens fed on a commercial diet. Following refeeding of the diet, leptin mRNA expression in adipose tissue gradually increased and attained a level comparable to that of fed chickens at 72 h post-refeeding, while the expression at 24 h post-refeeding remained low as compared to that in chickens fed *ad libitum* (Fig. 1A).

Expression of leptin mRNA in chicken liver following feed deprivation and refeeding varied in a similar manner to that seen in adipose tissue (Fig. 1B).

*Leptin mRNA expression in chickens fed on mono acid-rich diet (Experiment 2)*

Expression of leptin mRNA in the adipose tissue of chickens fed for 7 days on a trioilein-rich (olive oil) diet was significantly higher than that in chickens fed a trilinolein (safflower oil)- or trilinolenin (linseed oil)-rich diet (Fig. 2A).
Leptin mRNA expression in the liver of chickens fed on a trilinolenin (olive oil)-rich diet was significantly lower than that in chickens fed on diets that were triolein (safflower oil) or trilinolein (linseed oil)-enriched (Fig. 2B).

**Leptin mRNA expression in chickens force-fed with mono acid-triacylglycerols (Experiment 3)**

Fig. 1. Leptin mRNA expression in adipose tissue (A) and liver (B) of chickens fed, starved (24 h) or refed (24, 48, and 72 h) on a broiler grower diet.

RT-PCR amplified mRNAs of leptin were analyzed. Bars indicate SD of the mean values (n=4). Different letters indicate significant difference, P<0.05.

Fig. 2. Leptin mRNA expression in adipose tissue (A) and liver (B) of chickens fed on oleic acid (18 : 1)-, linoleic acid (18 : 2)- or linolenic acid (18 : 3)-rich diet for 7 days.

RT-PCR amplified mRNAs of leptin were analyzed. Bars indicate SD of the mean values (n=4). Different letters indicate significant difference, P<0.05.
Force-feeding for 12 h with triacylglycerol emulsions containing triolein (18 : 1, n−9) or trilinolein (18 : 2, n−6) did not change the leptin mRNA expression in adipose tissue compared to the expression in chickens starved for 48 h prior to the feeding. However, abdominal adipose tissue leptin mRNA expression in chickens force-fed for 12 h with triacylglycerol emulsions containing trilinolenin (18 : 3, n−3) was significantly higher than starved chickens and was comparable to that in chickens fed ad libitum (Fig. 3A).

Force-feeding for 12h with n−9, n−6 and n−3 triacylglycerol emulsions had no significant effects on liver leptin mRNA expression in chickens (Fig. 3B).

Discussion

We have presented here data indicating the possible nutritional modulation of chicken leptin mRNA expression in both liver and adipose tissue. Leptin mRNA levels in abdominal adipose tissue and liver in starved chickens were lower than those seen in chickens fed on a diet. Moreover, leptin mRNA expression varied according to the fatty acid species predominant in the dietary fat source. Feeding for 7 days with an n−3 fatty acid-enriched diet decreased the leptin mRNA expression compared with the expression seen in chickens fed n−9 fatty acid- and n−6 fatty acid-enriched diets. On the other hand, the refeeding of an n−3 monoacid triacylglycerol emulsion for only 12 h stimulated increased leptin mRNA levels in chicken adipose tissues.

In humans, Kolaczynski et al (1996) observed a mild decline in subcutaneous adipocyte ob gene mRNA after 36 and 60 h of fasting. Ishii et al (2000) demonstrated that the expression of leptin in epididymal adipose tissue was reduced by 24 h feed deprivation in rats. In our present study with chickens, the leptin mRNA expression in

Fig. 3. Leptin mRNA expression in adipose tissue (A) and liver (B) of chickens force-fed for 12 h with triolein (n-9), trilinolein (n-6) or trilinolenin (n-3) following 48 h starvation. RT-PCR amplified mRNAs of leptin were analyzed. Bars indicate SD of the mean values (n=4). Different letters indicate significant difference, P<0.05.
abdominal adipose tissue and liver was reduced by 24 h of fasting. These findings suggest that chicken leptin expression responds to starvation in a similar manner to that seen in mammals.

In the present study, the decreased leptin mRNA expression induced by starvation returned to control levels between 48 and 72 h post-refeeding. In growing rats, on the other hand, the expression of leptin mRNA recovered to the control levels within 24 h of refeeding (Ishii et al., 2000). These findings suggest that leptin expression recovers at a slower rate following refeeding in chickens. Since the leptin mRNA level in aged rats (24–months old) remained depressed even after 24 h of refeeding (Ishii et al., 2000), it is possible that the retarded response of leptin mRNA expression seen in growing chickens upon refeeding may be similar to that seen in aged mammals. This may partly explain the increased adiposity characteristic of both broiler chickens and older mammals.

Cha and Jones (1998) found that polyunsaturated fatty acid-enriched diets gave higher plasma leptin levels than diets rich in saturated fat, and that the serum leptin level was normalized by mild energy restriction in rats. Reseland et al. (2001) reported that the leptin mRNA level in epididymal adipose tissue in rats fed n-3 fatty acids for 3 weeks was lower than in lard-fed rats. In our study, leptin mRNA expression in chickens fed on an n-3 fatty acid-enriched diet for 7 days was lower than that in chickens fed on n-6 and n-9 fatty acid-enriched diets. These results suggest that chicken leptin mRNA expression responds to dietary fatty acids via a similar mechanism to that seen in mammals. It has been also shown in mammals that n-3 fatty acids regulate transcription of leptin via the activation of the peroxysomal proliferator activated receptor (PPAR) (De Vos et al., 1996).

Short-term (12 h) feeding of emulsions with n-3 fatty acids had diverse effects on adipose tissue leptin mRNA in chickens while having no effect on liver expression. Gondret et al. (2001) reported that adipocyte determination and differentiation-dependent factor 1/sterol regulatory element-binding protein (ADD-1/SREBP-1) mRNA levels in chicken adipose tissue were considerably lower than that found in other species, whereas liver expression was higher. Xu et al. (1999) showed that polyunsaturated fatty acids (PUFA) suppress the expression of SREBP-1 and regulate SREBP-1 at the post-transcriptional level. These results lead us to speculate that the n-3 fatty acid-induced effect on leptin expression may involve changes in mature SREBP-1 in adipose tissues.

Chicken leptin was expressed in both liver and adipose tissue, whereas mammalian leptin is not expressed in the liver. The presence of leptin mRNAs in liver and adipose tissues suggests different mechanisms of leptin expression and production in chickens as compared to mammals (Taouis et al., 2001). Our previous findings that chicken LPL-mediated hydrolysis of lipoprotein negatively correlated with the level of unsaturation of dietary lipids (Sato et al., 1999b) are compatible with the present findings that an 18 : 1-rich diet significantly increased the leptin expression compared to 18 : 2- or 18 : 3-rich diets in both liver and adipose tissue. These data suggest that leptin expression in liver and adipose tissue is correlated with adipose tissue LPL activity, a
determinant of adiposity in chickens.

In conclusion, chicken leptin expression in adipose tissue and liver is under nutritional modulation, as evidenced during fasting and for feeding with fats differing in their fatty acid composition. This modulation probably occurs in a similar manner, though at a different rate, to that seen in mammals. It is likely that chicken leptin expression is partly associated with the extent of hepatic lipid synthesis and adipose fat stores in chickens.

Acknowledgments

This work was supported in part by a Grant-in-Aid for the Encouragement of Young Scientists (No.12760184 and 14760175) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. The author appreciates to the Japanese Poultry Science Association for providing the financial support to present part of this study at the 21st World’s Poultry Congress held in Montreal, Canada, September, 2000.

References

Sato K, Akiba Y, Chida Y and Takahashi K. Lipoprotein hydrolysis and fat accumulation in chicken adipose tissues are reduced by chronic administration of lipoprotein lipase mono-