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BIOTECHNOLOGY APPLICATION IN ANIMAL PRODUCTION: MOLECULAR AND CELLULAR ASPECTS IN POULTRY INTAKE REGULATION

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SUMMARY

During the growth, the chicken rapidly develops breast and leg muscles which is essential for meat producers. However, the fast development induces increased adiposity which affects the nutritional quality and economic yield. Therefore it is necessary to understand the mechanisms controlling food intake, nutrient deposition and energy expenditure in order to direct the nutrients toward muscle and to reduce adipose tissue development. This is actually an important issue for molecular biologist and biotechnologist to identify genes involved in these regulations. These genes may be used as molecular markers for animal genetic selection. Nutrient distribution, food intake control and energy expenditure are regulated by insulin, leptin and UCP (uncoupling proteins), respectively. Furthermore, these proteins interact with each other and act in synergy or in an antagonist manner depending upon nutritional state, age and genetic origin. In the five last years, we have studied the impact of nutritional state and genetic origin on the early steps of insulin signaling in chicken. The activation of these steps are essential for muscle development and hepatic metabolism (mainly the control of lipogenesis). The major finding is that in the chicken, liver is highly sensitive to insulin whereas muscle shows a relative insulin resistance. This indicates that both tissues behave differently toward food intake and changes in circulating insulin. We have also showed that chicken liver expresses leptin. Leptin is an hormone secreted by liver and adipose tissue in avian species and by adipose tissue in mammals which inhibits food intake. Using recombinant chicken leptin , we have clearly showed that leptin reduced food intake. Furthermore, liver leptin expression is stimulated in the fed state which is characterized by a high plasma insulin level. Interestingly, in cultured hepatocyte insulin induced the over-expression of leptin. This indicates that nutrient deposition controls food intake through the activation of leptin expression. In addition to these two proteins, the uncoupling proteins play a major role in controlling the transformation of nutrients to intracellular lipids or proteins or to heat production (diet-induced thermogenesis).

Keywords: Food intake, gene expression, insulin, leptin

INTRODUCTION

The recent progress in the poultry production have reach an optimum with only 48 days necessary from hatching to meat commercialization. This progress has been achieved with a very stringent genetic selection. However, these fast growing chickens develop beside breast and leg muscle, adipose tissue. The increased adiposity alters the meat quality but also mirrors a weak food intake efficiency. The main goal of poultry biotechnologist or/ and molecular biologist is to maintain the chicken growth curve but without over-development of the adiposity by driving nutrients towards muscle instead of the adipose tissue fat deposition. To accomplish this ambitious program it is necessary to understand the mechanisms regulating food intake, nutrient distribution and energy expenditure, in order to identify gene (s) that control these regulatory processes. These genes may be used as molecular markers for future genetic selection.

In the present paper, I will present three groups of candidate genes that may play major role in controlling food intake, tissue nutrient up-take and utilization, and energy expenditure which are leptin, insulin and the uncoupling proteins, respectively.

Leptin

In mammals, leptin has been described as a satiety hormone produced almost exclusively by the adipose tissue (Zhang *et al.*, 1994; Tsuro, 1996). Recent studies have showed that this hormone is also secreted by other tissues such as stomach and placenta. Leptin is released in bloodstream as a 16 kDa protein and acts on the hypothalamic located receptors, (Campfield, 1995) in order to reduce food intake.

Recently, our group and others have cloned and sequenced the chicken leptin and showed that this gene is highly conserved (Taouis *et al.*, 1998; Ashwell *et al.*, 1999). However, chicken leptin is characterized by the presence of an unpaired cystein residue at position 3 of the sequence and this may contribute to significant changes in the secondary and ternary organization of the hormone. In fact this modification may explain the significant differences between chicken and mammalian leptins in activating the mitotic activity of cells over-expressing the human leptin receptor (Raver, 1998). Furthermore, chicken leptin is expressed by adipose tissue but also by liver, which is the main location for lipogenesis in avian species. This finding indicates the potential role of hepatic leptin in controlling lipogenesis or vice versa.

To further investigate the mechanism of leptin action it was necessary to develop new tools such : production of recombinant chicken leptin, development of a specific radioimmunoassay for circulating chicken leptin, and routine measurement of leptin expression.

Recombinant chicken leptin have been produced as described by Raver *et al.* (1999). Briefly, the coding sequence of chicken leptin was cloned in an expression vector and leptin was produced by E. Coli as cell protein. The biological activity of recombinant leptin was tested in vitro and in vivo. In vitro, its effect was tested in cells expressing human leptin receptor where it acts as activator of cell proliferation. In vivo, the intraperitoneal or intravenous injection of 1 mg/kg of leptin significantly (up to 35% for 2 first hour after the treatment) reduced chicken food intake. This has been demonstrated in broiler and layer chickens of 3 to 5 week of age (Dridi, 2000a). However, the impact of recombinant chicken in young chickens (9 day-old) on food intake is dependent upon genetic line (Dridi, 2000a). These data clearly demonstrated that leptin reduced food intake in an age and genetic line dependent manner. We have also developed an radioimmunoassay (RIA) specific for chicken leptin (Dridi, 2000b). The plasma leptin level is dependent upon genetic line and age. At 5 week of age, plasma leptin level is higher in broiler chickens (3.15 ± 0.44 ng/mL) than in layer chickens (2.2 ± 0.4 ng/mL). Plasma leptin level also varies during the sexual maturation. In broiler breeder hens of 18 weeks of age leptinemia is significantly lower (1.48 ± 0.05 ng/mL) than in 22 weeks old (at the first egg) (2.7 ± 0.17 ng/mL).

In addition, plasma leptin levels and liver leptin expression are increased at the fed state and reduced in fasted chickens. In isolated hepatocyte, insulin stimulates leptin expression. These data indicate a probable link between leptin expression and insulin secretion. Thus, a possible cross-talk between leptin and insulin is most-likely important for the equilibrium between food intake and nutrient distribution, and satiety. In addition, it has been demonstrated in mammals that leptin inhibits insulin secretion by the pancreas (Kieffen *et al.*, 2000).

Insulin

Chicken is characterized by its glucose metabolism with a glycemia of 2 g/L and with a relative insulin-resistance to exogenous insulin injection (Simon and Touis, 1993). Insulin acts trough binding to a heterotetramere receptor that undergoes autophosphorylation (Touis *et al.*, 1998). The activated receptors phosphorylate several substrates such as Insulin Receptor Substrate 1 and Src Homology and Collagen protein (Shc). We have recently cloned chicken IRS-1 cDNA (Touis, 1996) and partially Shc cDNA (Dupont *et al.*, 1998a). These two substrates activate the PI 3 kinase, enzyme involved in metabolic actions of insulin such glucose, amino acid transports and in protein, lipids and protein metabolism. The insulin receptor, IRS-1, Shc and the PI 3 kinase activity are the early steps of insulin signaling. We have showed that the activation of the insulin receptor cascade is dependent upon nutritional state and genetic line (Dupont *et al.*, 1998b; Dupont *et al.*, 1999). In the fed state insulin receptor, IRS-1, Shc phosphorylation and PI 3 kinase activity are significantly higher as compared to the fasted state. The activation of the insulin receptor pathway is dependent upon plasma insulin level. We have also showed that the activation of this pathway is tissue-specific with a high sensitivity in liver as compared to muscle which shows a high basal activity with a relative insulin-resistance. However, the translocation the insulin-dependent glucose transporter (Glut 4) in chicken muscle is highly sensitive to changes in nutritional status. The measurement of Glut 4 expression in muscle may reflect the level nutrient uptake by this tissue and consequently the muscle growth.

Uncoupling proteins

We have recently identified complementary DNA from chickens that encodes a bird uncoupling protein, referred as ggUCP. The ggUCP is exclusively expressed in muscle. We have used Rhode Island Red chicken lines divergently selected for their low (R+) and high (R-) efficiency of food utilization (Laloi, 1997). The R+ animals consume 30 to 40 % more food than the R- line. We have demonstrated that ggUCP is significantly increased in R+ muscle as compared to R-. This suggests a possible role of ggUCP in and mitochondrial oxidation in the increased energy dissipation in chicken. Therefore, this

protein and its gene may be an important index for energy expenditure, where the high efficiency of food utilization corresponds to low expression of ggUCP and vice versa. Further investigations are needed to study the impact of nutritional state on ggUCP expression.

CONCLUSION

In this paper we have reported the latest data concerning the molecular regulation of food intake, nutrient utilization and energy expenditure in chicken. Theoretically, to obtain efficient chickens they should secrete leptin (to reduce food intake), express more muscle Glut 4 (which is dependent upon circulating insulin) increasing then muscle protein deposition and should express less ggUCP to reduce energy dissipation. To obtain such animals a very stringent selection is necessary and it is more complex because of the number of parameters. Furthermore, the fast progress of molecular biology will help to reach this ambitious goal.

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