Proc. 3rd All Africa Conf. Anim. Agric. & 11th Conf. Egyptian Soc. Anim. Prod., Alexandria, Egypt, 6-9 November 2000: 297-300

DETERMINATION OF PLASMA LEPTIN LEVELS IN CHICKEN AND OTHER AVIAN SPECIES

S. Dridi, J. Williams, V. Bruggeman, M. Onagbesan, N. Raver, E. Decuypere, J. Djiane, A. Gertler and M. Taouis

Endocrinologie Moléculaire et Cellulaire du Métabolisme, SRA, INRA Nouzilly 37380, France

SUMMARY

Recombinant chicken leptin was used to produce an antiserum in order to develop a specific and sensitive radioimmunoassay (RIA) for chicken leptin in plasma. Either murine or chicken leptin as tracer and competition curves were performed using recombinant chicken leptin. Variations in leptin plasma levels in different chicken strains and various nutritional states were correlated with the physiological status. Plasma leptin concentrations were regulated by the nutritional states with higher levels in the fed state as compared to the fasted state and being dependent upon the age. Indeed, higher leptin levels were found in 22 week-old as compared to 15 week-old layer chickens. It was demonstrated that this RIA is also able to determine the circulating leptin levels in other avian species. In conclusion the RIA developed in the present study is specific to the chicken and thus may be considered as powerful tool for investigating the physiological significance of leptin in birds.

Keywords: Leptin; chicken; adiposity; RIA; avian species

INTRODUCTION

Leptin has been described as a satiety factor produced exclusively by adipose tissue in rodent and in mammals. Leptin is secreted into the bloodstream as a 16 kDa protein and probably bind to binding proteins and acts on hypothalamic leptin receptors which are most likely to be one of the key signal transducers controlling appetite. Recently, chicken leptin has been cloned by our group (Taouis *et al.*, 1998) and confirmed by others (Ashwell *et al.*, 1999 and Sato *et al.*, 2000). Chicken leptin is expressed not only in adipose tissue but also in liver suggesting the key role of leptin in hepatic lipogenesis. We have recently prepared recombinant chicken leptin and its C4S analog and have demonstrated its inhibitory effect on food intake in two chicken lines (Raver *et al.*, 1998 and Dridi *et al.*, 2000), indicating that the effect of leptin in reducing food intake is similar to that described in mammals. Therefore, the estimation of plasma leptin levels is an important tool for understanding the physiological role of this hormone in the chicken. Here, we report the first homologous RIA for chicken leptin. We have evaluated this assay for accuracy, precision, sensitivity and linearity by comparison with the multispecies leptin RIA kit (LINCO Inc) and also by using radio-labeled murine leptin as tracer.

MATERIALS AND METHODS

1- Production of recombinant chicken leptin

Recombinant chicken leptin was prepared as described previously (Raver *et al.*, 1998).

2- Immunization and preparation of antiserum

One mg recombinant chicken leptin was dissolved in PBS with Freund's complete adjuvant (Sigma, France)(1:1). Four rabbits were injected intradermally at several locations with this mixture (100 µl). Three weeks later the injection was repeated in the same conditions except that Freund's complete adjuvant was replaced by Freund's incomplete adjuvant. The same treatment was continued at one and two weeks later. Serum was tested 10 days following the last injection.

3- Iodination of chicken leptin

Five µg of chicken leptin was radio-iodinated by the chloramine T method (Sugawara *et al.*, 1984). Briefly, leptin (5 µg/10 µl) was diluted with 50 µl of 0.5 M sodium phosphate buffer pH 7.4 in a 1.5 ml polypropylene conical tube. 300 µCi of ^{125}I and 10 µl of chloramine T (3 mg/ml) were introduced and the reaction was allowed to proceed for 20 s then stopped by the addition of 750 µl of sodium metabisulfite (24mg/ml) followed by 200 µl of sodium Iodide (2 mg/ml). The contents of the tube were

transferred to a Sephadex G25 PD-10 column previously equilibrated with 25 ml of incubation buffer. Fractions were normally collected and 10 µl samples counted on a gamma counter allowing the peak of label and the peak of free iodine to be defined.

4- Radioimmunoassay (RIA)

RIA was performed as described previously (Dridi *et al.*, 2000).

5- Specificity of chicken leptin RIA

To determine the cross-reaction of the chicken leptin RIA, the radio-immunoassay was performed as described previously except that ^{125}I chicken leptin binding was displaced by increasing concentrations of chicken leptin, human IGF-I, human GH or porcine insulin.

6- Administration of recombinant chicken leptin

To validate the developed RIA, changes in circulating leptin levels in five week-old ISA brown cockerels (600g) that have received single intraperitoneal injection of 1 mg leptin/kg (n = 4) were measured. The control group received a placebo. Plasma was collected before and two hours after leptin or placebo treatment.

7- Effect of nutritional or physiological states and age on plasma leptin level

Several chicken lines and various avian species at different nutritional and physiological states were used. To determine the effect of age, broiler breeder hens were grown in standard conditions with free access to water and balanced diet. Breeder hens were divided into three groups that were sacrificed at different ages: 15, 18 and 22 (age of the first egg) weeks old. plasma were collected and stored at –20°C until use.

8- Statistics

Results are expressed as means ± SEM and statistical comparisons were performed using student's t test. For RIA curve analysis Graph pad Software (version 2, 94-95) was used.

RESULTS

1- Comparison of typical standard curves

The standard competition curves for chicken leptin RIA using iodinated recombinant chicken or murine leptin (as tracer), specific anti-chicken leptin antibodies and increasing concentrations of cold recombinant chicken leptin were determined. The chicken specific RIA curve with the multispecies leptin RIA kit (Linco inc) were compared (Fig1). The IC_{50} values, defined as the concentration of unlabeled leptin able to inhibit 50% of specific binding to the leptin antibodies, varied between 8 to 20 ng/ml for the three assays. The specific binding in the three assays varied from 30 to 55%.

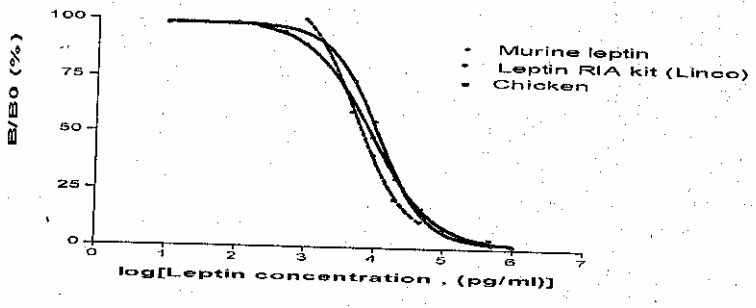

Fig 1. Comparison of typical standard curves

2- Effect of chicken leptin administration on plasma leptin levels

The serial dilution curve from plasma of treated cockerels was parallel to the standard curve and the concentrations were correlated with the dilution factors (d1 = 83; d1/2 = 36 and d1/4 = 19 ng/ml). The plasma leptin levels were significantly increased (about 23 fold) in cockerels two hours after administration of recombinant chicken leptin (Fig 2).

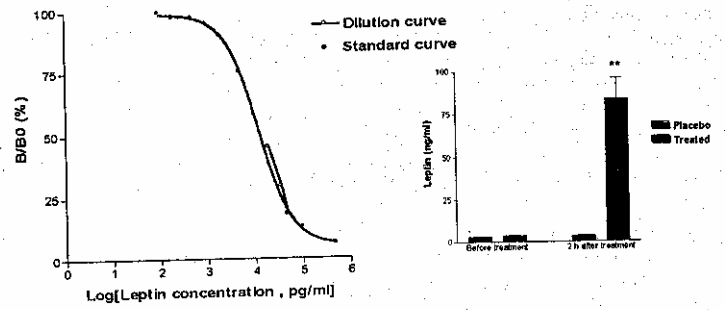

Fig 2. Effect of chicken leptin administration on plasma leptin levels in cockerels

3- Specificity of chicken leptin RIA

Increasing concentrations of hGH, hIGF-1 and porcine insulin were used to displace 125I-chicken leptin binding to chicken leptin antibodies. Fig 3 shows that these hormones did not crossreact with chicken leptin RIA.

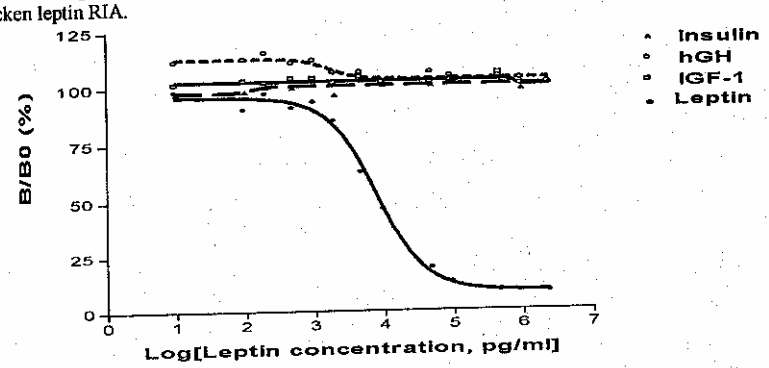

Fig 3. Specificity of chicken leptin RIA

4- Effect of age on plasma leptin levels

Fig. 4 shows that leptin plasma levels are higher in broiler breeder hens at the age of first egg (22 weeks) as compared to 15 and 18 week-old hens.

Fig 4. Effect of age on plasma leptin levels in broiler breeder hens

5- Plasma leptin levels in other avian species.

The developed RIA is able to determine the concentration of circulating leptin in different avian species. Table 1 shows that leptin is influenced by physiological and/or nutritional states and acts as an "adipostat" signal: decreasing with fasting and increasing with feeding.

Table 1. Concentrations of circulating leptin in different avian species

Avian species	Status	Leptin (ng/ml)	n	P
Chicken	Fasted	2.78 ± 0.11	4	<0.01
	Fed	3.36 ± 0.09	4	
Duck	Feed restricted	2.50 ± 0.09	12	<0.0001
	Force fed	3.51 ± 0.11	12	
Turkey	Broody	3.40	Pool	
	Laying	4.94	pool	
Pheasant	Broody	2.72	Pool	
	Laying	3.68	pool	

REFERENCES

Ashwell C.M., S.M. Czerwinski, D.M. Brocht and J.P. McMurtry, 1999. Hormonal regulation of leptin expression in broiler chicken. Am.J.Physiol.276, R226-R232.

Dridi S., N. Raver, M. Derouet, M. Picard, A. Gertler and M. Taouis, 2000. Preparation of C4S analogue of recombinant chicken leptin and comparison of its in vitro and in vivo biological activities with unmodified chicken and ovine leptins. Am.J.Physiol.279, E116-E123.

Dridi S, Williams J, Bruggeman V, Onagbesan M, Raver N, Decuypere E, Djiane J, Gertler A and Taouis M., 2000. A chicken leptin-specific radioimmunoassay. Domestic Animal Endocrinology. 18, 325-335.

Raver N., M. Taouis, S. Dridi, M. Derouet, J. Simon, B. Robinzon, J. Djian and A. Gertler 1998. Large-Scale preparation of biologically active recombinant chicken obese protein (Leptin). Protein Expression and Purification 14, 403-408.

Sato K, M. Nishida, T. Takahashi and Y. Akiba, 2000. Nutritional regulation of leptin expression in adipose tissues of broiler chickens. XXI world's Poultry Congress, Canada.

Sugawara A, N. Morii, M. Sakamoto, M. Suda, M. Shimokura, Y. kiso, M Kihara, Y yamori, K Nishimura, J Soneda, T. Ban and K. Nakao, 1984. Alpha-human atrial natriuretic polypeptide is released from the heart and circulates in the body. Biochem.Biophys.Res.Commun.129, 439-446.

Taouis M., JW Chen, C. Daviaud, J Dupont, M Derouet and J Simon, 1998. Cloning the chicken leptin gene. Gene 208, 239-242.