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Original Research Paper

CIRCULATING LEPTIN CONCENTRATIONS IN LIPIZZAN HORSES AND JEZERSKO-SOLCHAVA SHEEP

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Summary: Leptin is a protein hormone synthesized in adipocytes of the white adipose tissue, whose level of expression and secretion parallels the quantity of body fat. Investigations in different species have identified leptin as an indicator of nutritional status and an important regulator of appetite, energy metabolism and body composition. The aim of our work was to measure circulating leptin levels in two Slovenian autochthonous breeds, Lipizzan horses and Jezersko-Solchava sheep, in order to study the dependence of its concentration on age, body weight and gender.
Serum leptin concentrations in 89 Lipizzan stallions and mares, 47 Jezersko-Solchava lambs of both sexes and 48 Jezersko-Solchava rams were measured with a commercial radioimmunoassay (RIA) kit. The significant elevations paralleled age and weight gain, as reported in other animal species and breeds. In young Lipizzan horses, the lowest leptin concentrations (1.064 ng/ml ± 0.19 ng/ml) were observed in yearling colts and the highest (1.94 ng/ml ± 0.33 ng/ml) in 4-year old fillies. In adult Lipizzan stallions and pregnant mares, the values were 1.99 ng/ml ± 0.20 ng/ml and 1.19 ng/ml ± 0.15 ng/ml, respectively. In Jezersko-Solchava sheep, the lowest concentrations (0.10 ng/ml ± 0.09 ng/ml) were found in lambs weighing 1 - 5 kg and the highest (0.81 ng/ml ± 0.12 ng/ml) in 12-month old rams.
Agreement of these leptin concentrations with values reported in the literature indicates the suitability of the multispecies RIA kit for measuring circulating leptin concentrations in equine and ovine species.

Key words: leptin-blood-physiology; age factors; body weight; sex; radioimmunoassay; horses; sheep

Introduction

The protein hormone leptin is synthesized in white adipose tissue, the levels of its expression and secretion paralleling the amount of body fat. Blood peptide carriers, which bind to it, regulate its biological half-life and activity (1, 2). Investigations in humans, rodents and domestic animals have shown the role of leptin to be an indicator of nutritional status, informing the central nervous system about the status of energy reserves and their balance, and regulating the appetite, energy metabolism and body composition (1, 2, 3). The primary role of leptin is not prevention of obesity, but regulation of physiological adaptation to starvation (4). Leptin receptors have been demonstrated in hypothalamic regions, regulating appetite, growth and reproduction, indicating the influence of leptin on secretion of various neurotransmitters, neuropeptides and hormones (1, 2, 4). Leptin can also act directly on some peripheral tissues like liver, skeletal muscle, pancreas and suprarenal cortex (1, 2, 4, 5, 6) and has an important influence on immunity, angiogenesis and haematopoiesis (1, 2, 7).

Leptin plays a crucial role in controlling reproduction, as demonstrated (2) in animals carrying mutation in Ob gene encoding leptin (ob/ob mice). It acts directly on gonadotropin secretion, but receptors in ovaries, testes and uterus indicate the additional possibility of direct leptin action on reproduction (1, 2, 8). Insufficient nutrition, or starvation, leads to decreased secretion of gonadotropin in most animals, delaying, or even hindering, the onset of puberty or cyclic ovarian activity in females and causing hypogonadotropism and infertility in males (2, 7, 8, 9, 10). Irrespective of energy status, circulating leptin levels are increased during pregnancy in humans, rodents and ewes, decreasing just before parturition (1, 2, 8).

Circulating leptin concentrations can be measured by enzyme (EIA) or radio immunoassays (RIA) 1). Only one commercial multispecies RIA kit is currently available, based on antibodies raised against human leptin that display broad crossreactivity to leptin molecules of several species. It is recommended by the manufacturer that investigators test the suitability of the assay for the

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Table 1: Serum leptin concentrations in Lipizzan horses of various age groups

	Stallions		Mares	
Age group	**Number (n)**	**Leptin (ng/ml)**	**Number (n)**	**Leptin (ng/ml)**
foals*	7	1.064 ± 0.19	18	1.18 ± 0.08
1 year	6	0.90 0.16	6	1.09 ± 0.06
2 years	6	1.12 0.19	6	1.41 ± 0.14
3 years	6	0.92 0.13	6	1.19 ± 0.12
4 years	6	1.69 0.32	6	1.94 ± 0.33
adult**	8	1.99 0.20	8***	1.19 ± 0.15

** 1 - 4 months; **7-19 years; ***pregnant*

Table 2: Serum leptin concentrations in lambs of various body weights

Body weight	**Number (n)**	**Leptin (ng/mL)**
1 - 5 kg	8	0.10 ± 0.09
6 - 10 kg	14	0.924 ± 0.06
11 - 15 kg	10	0.76 ± 0.09
16 - 20 kg	11	0.69 ± 0.06
21 kg and more	10	0.855 ± 0.16

Table 3: Serum leptin concentrations (ng/mL) in rams of various ages

Age (months)	**Number (n)**	**Leptin (ng/mL)**
1	22	0.97 ± 0.05
2	9	0.66 ± 0.026
3	17	0.78 ± 0.08
4 - 5	12	1.278 ± 0.06
6	12	1.42 ± 0.08
7	12	1.488 ± 0.10
12	12	1.808 ± 0.12

species of interest and establish their own reference values. The aim of this work was to determine variations in leptin levels in Lipizzan horses and Jezersko-Solchava sheep, having first established the assay for these species.

Materials and methods

89 Lipizzan horses of both sexes and various ages were selected at random in a stud farm, and 48 Jezersko-Solchava rams and 53 lambs of both sexes from a sheep farm. All animals were kept under the standard breeding conditions specific for each species. Categorisation of animals is shown in the Results section (see Tables 1, 2 and 3).

Blood was sampled, by jugular venipuncture, on the same day from all animals kept at the same location. Sampling was performed in accordance to animal welfare legislation and approved by the Veterinary administration of the Republic of Slovenia.

Blood serum was prepared using commercial evacuated tubes (Vacutainer®, Becton Dickinson, Heidelberg) and kept frozen (-20 °C ± 2 °C) until analysed. Serum leptin concentrations were measured by a commercial radioimmunoassay kit (Multi-Species Leptin RIA Kit, Linco, USA). Intra- and inter-assay coefficients of variability (CV) were for high values (X = 28.16 ng/ml) 11.3 % and 10.3 %, respectively, and for low values (X = 4.35 ng/ml) 13,2 % and 8.3 %, respectively. Statistical analysis was performed by SPSS for Windows, Release 12.01 (SPSS Inc.) with subprograms Paired Samples T-Test and Analysis of variance. Correlations are reported as Pearson correlation coefficients. Results were evaluated as statistically significant on the level of $P < 0.05$, and presented as mean standard error of mean (X ± s.e.m.).

Results

Leptin concentrations in Lipizzan horses are listed in Table 1. The mean value was 1.23 ± 0.52 ng/mL. The lowest leptin concentrations for both sexes were observed in yearlings, the highest in 4 year old mares and adult stallions ($P < 0.05$). A positive, statistically significant correlation was established between leptin concentration and age of horses ($r = 0.7878$, $P<0.01$). Serum leptin concentration in all age groups of young horses was insignificantly higher in fillies than in colts, but significantly lower in pregnant mares than in stallions ($P<0.01$).

Discussion

Although the serum leptin concentrations in Lipizzans were in the lower range of normal values reported for horses (11, 12), the variations and correlations depending on age and gender, determined in other breeds of horses (2, 11), were also confirmed in this breed. Lower serum leptin concentrations in younger, growing animals indicate their lower body fat mass and greater nutritional needs than those of adult horses. The latter have greater body fat mass and lower nutritional needs, depending on their level of activity (11). In foals and young Lipizzan horses (1 to 4 years), leptin concentrations in females were higher than in males, as reported for other breeds (11). On the other hand, leptin concentrations in adult Lipizzans were higher in stallions than in pregnant mares, indicating higher energy demands during late pregnancy (11).

Serum leptin concentrations in Jezersko-Solchava lambs and growing rams are in general accordance with the values in other breeds of sheep (3, 13), but in lambs the levels are lower than reported (14). The deviations in these lambs could be attributed to the breed differences, the variety of sampling protocols or the use of different assay systems (15). Sexual dimorphism, as determined for children, horses and sheep (12, 15), with females having higher leptin concentrations than males, was not evident in these lambs, as reported for twin lambs of different sexes (16). Our further calculations were performed without considering the sex of lambs. Serum leptin concentration in Jezersko-Solchava lambs decreased insignificantly from the group weighing 1 - 5 kg to that of 16 - 20 kg, followed by a slight, insignificant increase in the group of 21 kg and over. The decrease in circulating leptin concentration in lambs after the 5th day of age probably ensures elevated appetite and deposition of fat (14). With approaching puberty, circulating leptin levels gradually rise (16). Significant increases in circulating leptin levels were found in Jezersko-Solchava rams from the 1st to 12th month of age. Progressive elevation of leptin concentrations can be attributed to the elevation of body fat supplies, as leptin concentrations in rams parallel the amount of body fat (1, 15).

In conclusion, circulating levels of leptin in Lipizzan horses and in Jezersko-Solchava sheep were consistent with values reported by other authors (3, 11, 12, 13), as were the significant elevations of circulating leptin concentration, which correlated with age and weight (10, 11, 14, 16). The sexual dimorphism in leptin levels, confirmed in adult horses, is most probably the consequence of feedback action of sexual steroid hormones on leptin secretion (11).

Agreement of leptin concentrations determined in the presently examined species with values reported in the literature indicates the suitability of the multispecies RIA kit used for measurement of circulating leptin concentrations in equine and ovine species.

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KONCENTRACIJA LEPTINA V KRVI LIPICANSKIH KONJ IN OVC JEZERSKO-SOLČAVSKE PASME

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Povzetek: Leptin je beljakovinski hormon, ki nastaja v adipocitih belega maščobnega tkiva. Njegovi ekspresija in sekrecija sta premo sorazmerni količini telesnih maščob. Raziskave pri številnih vrstah živali kažejo, da je leptin indikator prehrambenega stanja in pomemben regulator apetita, energetskega metabolizma in sestave telesa. Namen našega dela je bil izmeriti koncentracijo leptina v krvi dveh slovenskih avtohtonih pasem živali, lipicanskih konj in jezersko-solčavskih ovc, da bi proučili razlike v koncentracijah, ki so odvisne od starosti, telesne mase in spola.

Koncentracije leptina v serumu 89 lipicanskih žrebcev in kobil, 47 jezersko-solčavskih jagnjet obeh spolov in 48 jezersko-solčavskih ovnov so bile izmerjene s komercialnim radioimunskim testom (RIA). Značilen dvig koncentracije leptina v krvnem serumu je bil premo sorazmeren z naraščanjem starosti ali telesne mase, kot je že bilo ugotovljeno pri drugih vrstah oziroma pasmah živali. Pri mladih, rastočih lipicanskih konjih je bila najnižja koncentracija leptina (1.064 ng/ml 0.19 ng/ml) izmerjena pri enoletnih žrebičkih in najvišja (1.94 ng/ml 0.33 ng/ml) pa pri žrebicah v 4. letu življenja. Tudi pri odraslih lipicancskih žrebcih in brejih kobilah sta bili vrednosti 1.99 ng/ml 0.20 ng/ml oziroma 1.19 ng/ml 0.15 ng/ml. Pri jezersko-solčavski pasmi je bila najnižja koncentracija leptina izmerjena pri jagnjetih s telesno maso 1 - 5 kg (0.10 ng/ml 0.09 ng/ml) in najvišja (0.81 ng/ml 0.12 ng/ml) pri 12 mesecev starih ovnih.

Izmerjene vrednosti pri obeh preiskovanih vrstah živali in njihova skladnost s podatki iz literature potrjujejo primernost uporabljenega RIA testa za merjenje koncentracije leptina pri konjih in ovcah.

Ključne besede: leptin-kri-fiziologija; starostni faktorji; telesna teža; spol; radioimunski test; konji; ovce