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Endocrine, morphometric, and ultrasonographic characterization of neck adiposity in Andalusian horses

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ABSTRACT

Equine metabolic syndrome (EMS) can be diagnosed by hormonal measurements; however, it would be important to find simpler measurements that allow easy identification of affected or at risk individuals. In horses, the dorsal neck region is one of the most frequent anatomical sites for fat deposition and neck obesity has been linked to EMS. The aim of this study was to evaluate the association of hormonal markers of obesity (leptin) and insulin resistance (insulin) with morphometric and ultrasonographic neck measurements in Andalusian horses. Plasma leptin and insulin concentrations were measured by RIA in 127 Andalusian horses. Neck circumferences (NC) were measured at 3 equidistant locations at 25%, 50%, and 75% of neck length (NC-25%, NC-50%, and NC-75%). At the same 3 locations, subcutaneous fat thickness (SFT-25%, SFT-50%, and SFT-75%) was measured ultrasonographically. In the population under study, a tendency to adiposity was confirmed by the elevated plasma leptin levels (7.47 ± 5.03 ng/mL). However, plasma insulin concentrations (4.05 ± 3.74 μIU/mL) were within normal range in most horses. Our results indicate that NC showed significant sexual dimorphism and did not correlate well with hormonal measurements. Ultrasonographic assessment of fat thickness at the base of the neck (SFT-75%) was significantly correlated with both plasma leptin and insulin and did not show differences between males and females. Thus, in the search for a single objective parameter which can be used in large populations, SFT-75% is a potential candidate and may be a meaningful parameter to predict EMS.

1. Introduction

Equine metabolic syndrome (EMS) is an important and expanding endocrine disease characterized by 2 main features: obesity and insulin resistance (IR), with laminitis as a frequent complication [1]. From an endocrine perspective, plasma levels of leptin, an adipokine produced by fat tissue, represent a good indicator of adiposity [2], whereas IR can be identified by elevated plasma levels of insulin [3].

Because endocrine studies are complex and expensive, there is an epidemiologic need for diagnostic methods that allow an easy identification of affected or at risk individuals. In this context, simple measurements that can be carried out in large populations have demonstrated to be useful for predicting metabolic syndrome in humans [4]. One additional advantage of using waist circumference is that, in humans, regional fat accumulation in visceral tissue is more closely linked to disease risk than generalized obesity [5]. In equids, the dorsal neck region delineated by the nuchal ligament is one of the most frequent anatomic sites for fat deposition [6]. Furthermore, fat deposition in the neck has been associated with IR and

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increased risk for laminitis [3,7–9] and is considered a phenotypic indicator of EMS [1,10]. Fat accumulation in the neck can be evaluated subjectively using a cresty neck score system; however, a recent study has shown that subjective assessment of body fat is not an accurate predictor of IR [11]. Objective methods for evaluation of neck adiposity include morphometric [2,3,9,12–14] and ultrasonographic [15] measurements.

Because of their phenotype, Andalusian horses have been proposed as an example of a breed predisposed to EMS [16], thus, they represent a good model to evaluate neck measurements and their relationship with hormonal levels. Currently, there are no objective data evaluating neck thickness in this breed. Therefore, the aim of this study was to compare hormonal markers of obesity (leptin) and IR (insulin) with morphometric and ultrasonographic neck measurements in Andalusian horses.

2. Materials and methods

2.1. Animals

In this cross-sectional study sample, 127 Andalusian horses were randomly selected from a population of 1722 Andalusian horses using a stratified probabilistic method (Microsoft Excel 2010 Windows, IBM Corp, Armonk, NY, USA). The sample included a representative proportion of both genders (78 stallions and 49 mares) and different ages (2 to 15 yr). To be included in the study, horses had to be healthy (based on physical examination, complete blood count, and plasma biochemistry). Pregnant and lactating mares, as well as horses receiving any drugs, were excluded from the study. All horses were current on deworming and vaccination and were fed a balanced diet (0.5–1 kg of concentrate/100 kg BW, and at least, double quantity of forage). Owners were contacted by telephone and asked if they were willing to take part in the study, informed consent was obtained from owners. The University of Murcia Committee on the Ethical use of Animals approved all procedures. Four set of data were obtained from each horse: BCS, plasma hormone measurements, and morphometric and ultrasonographic studies of the neck.

2.2. Body condition score

Two independent and trained evaluators (TM and FM) determined the BCS [17] on each horse. The average of both scores was used in the statistics.

2.3. Plasma hormone measurements

Whole blood samples were drawn between 6:00 and 12:00 AM. Concentrated feed and forage was withheld 12 h before the samples were collected. Blood samples were obtained by left jugular venipuncture into EDTA and sodium-heparinized 10-mL Vacutainer tubes. The tubes were immediately placed in ice water and centrifuged at 3,000 $\times g$ for 10 min within 15 min of collection. Plasma was frozen in 1.5-mL aliquots and stored at -20°C until analysis. Plasma was analyzed for insulin (Coat-A Count Insulin, Diagnostic Products Corporation, Los Angeles, CA, USA) and leptin (Multi-species Leptin RIA, Linco Research Inc, St Charles, MO, USA) concentrations by RIAs, previously validated for horses [2,9,13,16,18–22]. Hyperinsulinemia was defined as basal insulin $\geq$20 μIU/mL, according to the cutoff value established by the 2010 American College of Veterinary Internal Medicine consensus statement [1]. Hyperleptinemia was defined as basal leptin $\geq$7 ng/mL, after the cutoff value proposed by Carter et al [9], as a predictor of laminitis.

2.4. Morphometric and ultrasonographic studies of the neck

These studies were conducted immediately after blood collection. Using a plastic measuring tape (Kruuse, Langeskov, Denmark) demarcated in centimeters and with the neck maintained in a normal upright position, neck length was measured as a straight line from the poll to the highest point of the withers [3,14]. Neck circumferences (NCs) were measured perpendicular to this line at 3 equidistant locations (NC-25%, NC-50%, and NC-75%) as previously described [3,14]. Following the technique described by Carter et al [14], crest height was measured at 50% of neck length (Fig. 1).

At the same 3 locations described previously, subcutaneous fat thickness (SFT-25%, SFT-50%, and SFT-75%) was measured ultrasonographically (HS-1500V, Honda Electronics, Aichi, Japan) using a 7.5-MHz linear transducer (Fig. 2). The probe was positioned perpendicular to the floor over the interface between the crest and neck musculature, identified by palpation and visual assessment. All morphometric and ultrasonographic measurements were repeated in triplicate in the same day by the same researcher in all the horses. Reliability of repeated measurements was assessed with intraclass correlation coefficient. Intraobserver repeatability for the morphometric and ultrasonographic measurements were 99.3% for neck length, 99.2% for crest height, 98.4% for NC-25%, 98.2% for NC-50%, 98.5% for NC-75%, 96.8% for STF-25%, 96.6% for STF-50%, and 97.2% for STF-75%. Because the agreement between different measurements was good [23], mean values of the 3 measurements were used for statistical analyses.

2.5. Statistical analysis

Quantitative variables were described using mean, standard deviation (SD), coefficient of variation, and range (minimum and maximum). In the text, all results are presented as means $\pm$ SD. Normality of each variable was evaluated with Kolmogorov–Smirnov test. Student's t or Mann–Whitney U tests were used to compare morphometric measurements, ultrasonographic measurements, and biochemical variables. Correlations between variables were evaluated using Pearson or Spearman coefficients. Significance was set at $P < 0.05$. Statistical analyses were performed using commercial statistical software (IBM SPSS 19.0 for Windows, IBM Corp).

3. Results

The mean, range, SD, and coefficient of variation for the parameters under study are presented in Table 1. Mean BCS of the sample was 6.06 $\pm$ 1.04. Mean plasma insulin