Journal of Equine Veterinary Science 33 (2013) 969-976

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Blood Levels of Selected Metabolic Factors, Cytokines, and Lymphocyte Subpopulations in Arabian and Thoroughbred Horses During the Longest and Shortest Days of the Year

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ARTICLE INFO

Article history:
Received 15 October 2012
Received in revised form
30 November 2012
Accepted 5 March 2013
Available online 20 April 2013

Keywords:
Cytokine
Day length
Horse
Leptin
Lymphocyte subpopulation

ABSTRACT

Day length-related alterations of several metabolic factors (glucose, leptin, insulin, and insulin-like growth factor-1 [IGF-1]), cytokines (interleukin-2 [IL-2], IL-4, IL-6, tumor necrosis factor-alpha [TNF-α], interferon-gamma [IFN-γ], and lymphocyte subpopulations [CD2, CD3, CD4, CD8, CD19, natural killer (NK) cells) were evaluated in Arabian and Thoroughbred horses. Plasma glucose, leptin, IGF-1, insulin, and cytokines levels were measured on the longest day of the breeding season and on the shortest day of the nonbreeding season. Determination of lymphocyte subpopulations was performed by flow cytometry. Glucose and IL-2 levels, CD4:CD8 ratio, and NK cells showed variations that depended on the day length. Mean concentrations of plasma leptin were higher in Arabian horses than in Thoroughbred horses, whereas mean concentrations of IGF-1 and IL-2 were lower in Arabian horses. Day length-by-breed-by-gender interaction was found for insulin, IFN-γ, and IL-4 levels. An interaction was also found between day length and gender for the expressions of CD2, CD3, CD8, and CD19. Correlations were detected between expression of $CD8^+$ cells and levels of TNF-α and IFN-γ and between percentages of NK cells and levels of IGF-1, insulin, and glucose. Results suggested that day length and, therefore, season are important determinants or factors in modulating the immune system and could affect lymphocyte subpopulations depending on the sex of the horse. Additionally, it seems that a complex relationship in horses, as in humans and mice, exists between the immune and metabolic system, which changes according to day length, breed, and gender.

1. Introduction

In the adult horse, season and nutrition are two main factors in regulation of annual reproductive activity [1]. When food availability and environmental temperature decrease during winter, physiological adaptations, including changes in reproductive and immune functions, have evolved among animals to cope with this energy restriction. However, the specific mechanisms that mediate reproductive and immune functions remain unclear [2]. Classic knowledge, for example, involving melatonin [3,4] is unable to explain completely all the reproductive and immunological events. Thus, other factors involved in regulation of immune and reproductive functions should be investigated by considering the seasonal modifications of these functions. It has been reported [5] that a number of mares that have good body condition score show ovulation

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http://dx.doi.org/10.1016/j.jevs.2013.03.001

continuously throughout the year, suggesting that nutrition and nutritional factors such as leptin and insulin interact with season to synchronize the annual reproductive activity. For this purpose, investigation of season-related quantitative characterizations of nutritional factors including glucose, leptin, insulin, and insulin-like growth factor-1 (IGF-1) are important in determining physiological mechanisms underlying nutritional status and reproductive activity [1].

Leptin, a hormone-cytokine secreted mainly from adipose tissue, is one of the key mediators regulating metabolic and reproductive processes [6,7]. In the horse, as in other species, fat mass is the main determinant of blood leptin concentration, but the effects of many factors such as fasting, circadian rhythm, diet, age, and sex have been described to modify circulating leptin levels [3,5,8,9]. In addition, a seasonal variation in blood leptin levels has also been reported to exist in the horse [3,10].

The alterations of lymphocyte subpopulations and cytokines play a key role regarding the immune response of the host. This may be shown in the characterization of specific immune functions such as cellular and humoral immunity [11]. Binding of antigen by T cells initiates production of cytokines including interleukin-4 (IL-4), IL-6, and interferon-gamma (IFN-γ) by T-helper cells. T-helper cells then develop into different subsets such as Th1 and Th2, which produce different cytokines and activate effector functions of lymphocytes. Activation of Th1 cells, which express IFN-γ, promotes cytotoxic T-cell activity and action of macrophage. Th2 cells, which express IL-4 and IL-5, induce B-cell differentiation and antibody secretion [11-13]. In horse, many studies have focused on characterizing lymphocyte subpopulations in certain disease-related conditions to immune system pathologies [14-18]. However, a limited number of studies have been reported about normal [13,19] and seasonal characterizations of lymphocyte subpopulations in the horse [20,21]. It has been reported that additional studies were necessary to determine whether the immune system, including cellular immunity, was mediated by photoperiod and photoperiodic changes of leptin or other metabolic hormones/factors concentrations [2]. Interactions between metabolic and immune functions throughout the season are important for understanding of susceptibility to diseases. Therefore, assessment and/or relationship between season-related cell or humoral immune mechanisms and metabolism will provide important information in respect to determination of seasonal susceptibility to infection in the horse. Thus, we hypothesized that in horse, alterations of some basic metabolic and immune factors play a central role in regulating host defense in summer/winter solstice.

The primary aim of this study was to evaluate the quantitative characterizations of plasma leptin, several metabolic factors and cytokine levels, and lymphocyte subpopulations in Arabian and Thoroughbred horses on the longest (summer solstice) and shortest days (winter solstice) throughout the year. Additionally, we evaluated the relationship between selected metabolic factors and lymphocyte subpopulations.

2. Materials and Methods

2.1. Animals

Forty-nine purebred Arabian horses (28 mares, 21 stallions) and 39 Thoroughbred horses (22 mares, 17 stallions), from 4 to 8 years old, were involved in the study. The horses were routinely maintained on pasture together and were fed good quality grass hay and alfalfa hay (at a ratio of 4:1) twice a day according to their requirement levels before and after pasture feeding. They were housed in a semiopen stable only in the dark period of day and kept separately according to gender at General Directorate of Agricultural Enterprises and at the Jockey Club of Turkey. All horses were subjected to periodic clinical examination, including whole-blood and biochemistry analysis as well as parasitic control. Respiratory system auscultation was also included in the clinical examination. The horses were weighed, and body condition scores of all horses were considered good (mean 5.65 $\pm$ 0.09 SEM) [22]. The experiment was conducted under natural photoperiodic conditions at 890 meters above sea level, at longitude 30:32 E and latitude 39:46 N in the northern hemisphere.

2.2. Experimental Procedures

Peripheral blood samples were collected in heparinized containers (Vacuette; Greiner Labortechnik, Frickenhausen, Germany) from each horse via jugular venipuncture, before feeding in the early morning of the longest day of the breeding season and the shortest day of the nonbreeding season. Mares were checked for estrous behavior with a vigorous stallion before the study started. Only nonpregnant mares that had demonstrated typical cyclic patterns of estrus during the breeding season were used. In the shortest day of the nonbreeding season, noncyclic and also nonpregnant mares were used. Glucose, hormones (leptin, insulin, IGF-1) and cytokines (tumor necrosis factor-alpha [TNF-α], IFN-γ, IL-2, IL-4, IL-6) levels and expressions of lymphocyte subpopulations were evaluated on both the longest day of the breeding season and the shortest day of the nonbreeding season. Blood samples were centrifuged at 1,500 $\times$ g for 10 min. Plasma samples were stored at -70°C until assayed for glucose, hormones, and cytokines.

2.3. Glucose, Hormone, and Cytokine Analysis

Plasma glucose levels were analyzed by colorimetric procedures (glucose [HK] assay kit; Sigma-Aldrich, St. Louis, MO) [23]. Leptin and IGF-1 assays were performed using radioimmunoassay (RIA) kits (multispecies leptin RIA kit; Linco Research Inc., St. Charles, MO; IGF-1 RIA kit, Diagnostic Systems Laboratories, Inc., Webster, TX). Insulin, TNF-α, IFN-γ, IL-2, IL-4, and IL-6 assays were performed using suitable enzyme-linked immunosorbent assay (ELISA) kits for the equine proteins according to manufacturers' specifications (bovine insulin ELISA kit, DRG International, Mountainside, NJ; equine TNF-α screening set, porcine IFN-γ ELISA kit, human IL-2, IL-4, and IL-6 ELISA kits were from Endogen, Pierce Biotechnology, Rockford, IL). Intra-assays of coefficients of