The Veterinary Journal 202 (2014) 372–377

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Influence of racing on the serum concentrations of acute-phase proteins and bone metabolism biomarkers in racing greyhounds

M. Tharwat [a,b], F. Al-Sobayil [a], S. Buczinski [c,*]

[a] Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia
[b] Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt
[c] Bovine Ambulatory Clinic, Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, Canada

ARTICLE INFO

Article history:
Accepted 26 August 2014

Keywords:
Acute-phase proteins
Biomarkers
Bone metabolism
Greyhounds
Racing
Stress

ABSTRACT

This study was designed to evaluate the influence of racing on the serum concentrations of the acute-phase proteins (APPs) C-reactive protein (CRP), haptoglobin (Hp) and serum amyloid A (SAA) in 32 endurance-racing greyhounds. The study also aimed to investigate the effect of a 7 km race on the bone biomarkers osteocalcin (OC), bone-specific alkaline phosphatase (b-ALP) and pyridinoline cross-links (PYD). Total white blood cell (WBC) count, and the serum concentrations of cortisol, tumour necrosis factor-α (TNF-α), vitamin D and testosterone were also determined. Blood samples were collected 24 h prior to (T0) and within 2 h of completion of the race (T1).

Compared to baseline values, WBC count did not change significantly ($P = 0.2300$), serum cortisol, Hp and SAA increased, while TNF-α and CRP decreased ($P < 0.0001$ for each). There were no significant differences between the pre- and post-race serum concentrations of OC and PYD ($P = 0.9500$ and $P = 0.2600$, respectively), but serum b-ALP increased significantly ($P = 0.0004$). Serum concentrations of vitamin D and testosterone increased after racing ($P = 0.0100$ and $P < 0.0001$, respectively). In this study, a 7 km race stimulated an acute-phase response, demonstrated by significant increases in the serum concentrations Hp and SAA in racing greyhounds. Increased serum b-ALP post-race probably indicates a change in bone metabolism and deserves further study.

Introduction

In Saudi Arabia, the population of endurance-racing greyhounds has greatly increased over the last several years, as has their enrolment in endurance competitions. Endurance-racing greyhounds have developed many adaptive physiological traits that distinguish them from other breeds, including a unique musculoskeletal conformation and increased myocardial muscle, both of which increase exercise efficiency. In addition, they are subject to a variety of physical and biochemical changes resulting from racing (LaVecchio et al., 2009; Tharwat et al., 2013).

Acute-phase proteins (APPs) are a group of blood proteins that decrease or increase in concentration in response to external or internal challenges (Eckersall and Bell, 2010). The acute-phase response (APR) is a rapid, nonspecific, systemic response occurring secondary to many types of tissue injury and might be a protective physiological mechanism during inflammatory events (Yazwinski et al., 2013). The origin of APR can be attributed to infection, inflammation, surgical trauma, or other causes (Petersen et al., 2004; Ceron et al., 2005), and the purpose of the response is not only to restore homeostasis, but to remove the cause of the homeostatic disturbance (Ebersole and Cappelli, 2000; Ceron et al., 2005).

In domestic animals, a critical mass of knowledge on the use of APPs as biomarkers of inflammatory conditions has accumulated over recent years, so there is now sufficient understanding of the pathophysiology of the response to support the use of these compounds as diagnostic tools in clinical settings (Eckersall and Bell, 2010). In dogs, APPs can be specifically classified, according to the magnitude of their response to stimuli, as positive (major and moderate) or negative reactants (Martínez-Subiela et al., 2003; Ceron et al., 2005). Positive APPs that increase their rates of synthesis and release during inflammation include C-reactive protein (CRP), haptoglobin (Hp) and serum amyloid A (SAA; Eckersall, 2000; Ceron et al., 2005). They can be used in dogs as a screening test for systemic response to an inflammatory stimulus and are considered to be the most accurate biomarkers of inflammation (Ceron et al., 2008).

The common biomarkers of bone formation include osteocalcin (OC), bone-specific alkaline phosphatase (b-ALP) and amino and carboxy propeptides of collagen type I. The most common biomarkers of bone resorption include pyridinoline cross-links (PYD), deoxypyridinoline enzyme tartrate resistant acid phosphatase and amino and carboxy telopeptides of collagen type I. Biochemical markers of bone turnover are widely used in human clinical practice, mainly for non-invasive monitoring of bone metabolism and

* Corresponding author. Tel.: +1 450 7738521.
E-mail address: s.buczinski@umontreal.ca (S. Buczinski).

http://dx.doi.org/10.1016/j.tvjl.2014.08.027
1090-0233/

to assess the response to therapy in certain musculoskeletal and bone disorders (Swaminathan, 2001; Watts et al., 2001; Kanakis et al., 2004; Sabour et al., 2014). In veterinary medicine, bone biomarkers are mostly used in preclinical and clinical studies to rapidly and sensitively assess bone response to medical treatment and surgical interventions and for the detection of musculoskeletal injuries (Allen, 2003; DeLaurier et al., 2004; Frisbie et al., 2008, 2010).

In humans and animals, the effect of exercise on inflammation and bone biomarkers has not been widely evaluated, despite its importance (Al-Sobayil, 2008; Cywinska et al., 2012; Shin et al., 2012; Casella et al., 2013; Yazwinski et al., 2013; Sabour et al., 2014). The aim of the current study was to investigate the effect of a 7 km race on the serum concentrations of APPs (CRP, Hp and SAA) and biomarkers of bone formation (OC, b-ALP) and bone resorption (PYD) in endurance-racing greyhounds. Understanding the effects of physical exercise on stimulation of the inflammatory response and bone remodelling is important for the development of strategies to control inflammation and to increase and maintain bone mass and could prove increasingly useful for the future health and welfare of racing greyhounds.

Materials and methods

Animals

This study was approved by the Animal Ethical Committee, Deanship for Scientific Research, Qassim University, Saudi Arabia (Approval number 1860; 3 March 2012).

The experimental design has been recently reported by Tharwat et al. (2013). Briefly, 32 male racing greyhounds (mean age 5.8 ± 2.6 years; mean bodyweight 23.9 ± 7.2 kg) that had participated in 7 km races were enrolled. On three successive weeks during March 2012, between 11.00 and 12.00 h, the greyhounds participated in races in the Qassim region, Saudi Arabia. Enrolment was based on owner consent, lack of abnormalities on physical examination and cardiac auscultation, and complete blood cell count, biochemistry panel, electrocardiography and echocardiography results within the reference range. The greyhounds were treated according to the Laboratory Animal Control Guidelines of Qassim University, which basically conform to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health in the USA (NIH publications Numbers 86 to 23, revised 1996).

Blood sampling

Six millilitres of blood were drawn from the jugular vein of each dog via vacutainer and collected in plain tubes. The blood samples were collected at rest 24 h prior to the race (T0) and within 2 h of completion of the race (T1). Within 2 h of collection, blood samples were centrifuged at 1200 *g* for 10 min and serum was obtained. Sera were stored at –20 °C and analysed within 2 weeks of collection.

White blood cell count, calcium, inorganic phosphorus, magnesium, cortisol and tumour necrosis factor assays

White blood cell counts (WBCs) were carried out using an automated analyser (VetScan HM5). The serum concentrations of calcium (Ca), inorganic phosphorus (Pi) and magnesium (Mg) were measured in serum samples using an automated biochemical analyser (Biosystems A15). Serum cortisol concentrations were measured with an electrochemiluminescent immunoassay kit (Cortisol, Roche Diagnostics). The intra- and inter-assay coefficients of variations (CVs) were 1.22 and 1.54%, respectively, and the minimal detectable concentration was 0.018 µg/dL. The serum concentration of tumour necrosis factor (TNF)-α was determined using a commercially available ELISA kit (Canine TNF-α ELISA Kit, MyBioSource). The sensitivity of this kit was 2.0 pg/mL, and the detection range was 15.6–500.0 pg/mL. Both the intra- and inter-assay CVs were <15%.

Acute-phase protein assays

Serum CRP concentration was determined using a commercially available human kit (Minineph Hunan CRP kit, Binding Site Group), according to the manufacturer's instructions. The determination of soluble antigen concentration by the nephelometric method involves a reaction with the antibody bound to a latex particle to form insoluble complexes. When light is passed through the suspension, a portion of the light is scattered by a photodiode. The amount of light scattered is directly proportional to the CRP concentration in the serum samples. The analytical sensitivity of the assay was 0.44 mg/L, and intra- and inter-assay CVs were 3.6–6.6% and 3.6–6.0%, respectively.

Serum Hp concentration was determined using a colorimetric assay (Haptoglobin kit, second generation, Tridelta), according to the manufacturer's instructions. Free haemoglobin (Hb) exhibits peroxidase activity, which is inhibited at a low pH. Haptoglobin present in the specimen combines with Hb and at a low pH preserves the peroxidase activity of the bound Hb. Preservation of the peroxidase activity of Hb is directly proportional to the amount of Hp present in the specimen. The analytical sensitivity of the assay was 0.0005 mg/mL, and intra- and inter-assay CVs were 5–6% and 4–6%, respectively. Serum SAA concentration was measured using a commercially available ELISA kit (Multispecies SAA ELISA kit, Tridelta) according to the manufacturer's instructions. A monoclonal antibody specific for SAA was coated onto the wells of microtitre strips. The analytical sensitivity of the assay was 0.15 µg/mL and intra- and inter-assay CVs were 4.5% and 6%, respectively.

The bone biomarker assays

The bone biomarkers OC, b-ALP and PYD serum concentrations were determined using commercial human immunoassay kits (Metra Biosystems, Quidel). All three human assays had been shown to have a good cross-reactivity with canine OC, b-ALP and PYD (Allen et al., 2000; Breur et al., 2004; Belić et al., 2012). The limit of quantification of OC ranged from 2 to 32 ng/mL, and precision CVs within and between runs were 5–10%. The dynamic range of BAP was 2–140 U/L, and precision CVs within and between runs were 4–6% and 5–8%, respectively. The dynamic range of PYD was 15–750 nM/L, and precision CVs within and between runs were 6–10% and 3–11%, respectively.

Testosterone and vitamin D assays

Serum testosterone (17β-hydroxyandrostenone) concentration was determined using an electrochemiluminescence immunoassay kit (Roche Diagnostics), with a measurement range of 0.025–15 ng/mL. The intra- and inter-assay CVs were 4.7 and 8.4%, respectively. The serum concentration of 25-hydroxyvitamin D (25-OH vitamin D) was determined using a chemiluminescence microparticle immunoassay kit (Abbott), with a measurement range of 8–160 ng/mL. The intra- and inter-assay CVs were 3.1 and 4.0%, respectively.

In this study, the CVs of the assays were captured from data information sheets provided by the manufacturers.

Statistical analysis

A data normality assessment was performed using the D'Agostino Pearson test. The data were presented as means ± standard deviation (SD), since no significant deviation from normality was observed. Data were analysed statistically using a commercially available statistical package (SAS 9.2). The graphical representation of the results was performed using MedCalc Software (Mariakereke). A paired *t* test for repeated samples was used for comparisons between pre- and post-race values. Statistical significance was set at $P < 0.05$.

Results

Compared to baseline pre-race values, WBC count and serum concentrations of Ca, Pi and Mg did not differ significantly ($P = 0.2300$, 0.7700, 0.1900, 0.4700, respectively) after racing. Serum cortisol increased significantly ($P < 0.0001$; Fig. 1), while that of TNF-α decreased significantly post-race ($P < 0.0001$; Fig. 2).

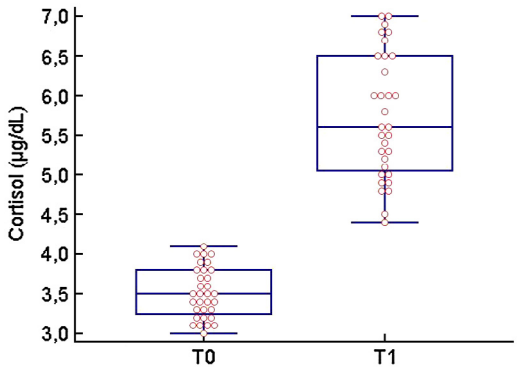

Fig. 1. Serum concentration of cortisol in racing greyhounds before (T0) and after (T1) racing.