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Crosstalk between adiponectin and cytokines (IL-6 and IL-8) during transportation stress in horses

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Summary

According to recent research, during transportation stress, many organs, including skeletal muscles, undergo rapid and extensive adaptive changes, resulting among others in the expression of myokines. This response may evoke systemic inflammation reflected by changes in the cytokine profile. The aim of our study was to assess changes in plasma concentrations of IL-6, IL-8 and adiponectin in response to long- and short-distance transportation of young and middle-aged horses.

The study was conducted on 24 cold-blooded female horses divided into four groups. Six fillies aged 6-18 months and six mares aged 10-12 years were transported over a distance of about 550 km, whereas six fillies aged 6-18 months and six mares aged 10-12 years were transported over a distance of about 50 km. Plasma was obtained from blood samples taken before transportation (T0), immediately after transportation (T1) and at an abattoir during slaughter (T2). Plasma concentrations of IL-6, IL-8 and adiponectin were measured with ELISA kits.

The highest concentration of IL-6 was found in fillies after long-distance transportation during slaughter: it amounted to 978 ± 98 pg/ml, compared with 400 ± 90.60 pg/ml before transportation. The IL-8 level was maximal during slaughter (T2) in all groups, and was higher in older horses and after long-distance transportation than in fillies and after short-distance transportation. A significant change in adiponectin level was found only in the group of fillies after long-distance transportation.

We demonstrated that during transportation stress, the concentrations of IL-6 and IL-8 increased in the blood of horses examined, and this response was more pronounced during slaughter. The concentration of adiponectin, on the other hand, increased significantly in fillies after long-distance transportation.

Keywords: horse, stress, myokines

Transportation stress may aggravate many inflammatory conditions, such as respiratory disorders, laminitis, collitis, and rhabdomyolysis (14). So far, concentration of plasma cortisol, neutrophil:lymphocyte ratio, muscle fatigue, and suppression of body weight in horses were regarded as sensitive markers of transportation stress. According to new concepts, during transportation stress many organs, including skeletal musculature, undergo rapid and extensive adaptive changes, which may also include the expression of myokines. Additionally, it is known that skeletal muscles and adipose tissue could be sources of cytokines, especially in the course of metabolic disorders. The first identified muscle-derived cytokine was IL-6. After prolonged exercise, its level markedly increases in circulation, where it can exert its effect in hormone-like fashion (3, 11, 18). Similarly to IL-6, IL-8 responds to exercise, and its concentration increases during muscle activity. Muscle-derived IL-8 is chemoattractant for neutrophils and macrophages, and it also stimulates angiogenesis (15-18).

Adiponectin, on the other hand, has been shown to have insulin-sensitizing properties and may play a role in metabolic substrate utilization. For these reasons, it is called “adipokine,” as a bioactive molecule involved in the pathogenesis of various obesity-linked disorders (21), and has direct actions in the liver, skeletal muscles, and vasculature (13).

Owing to stress-related disturbances, we suggest that transportation stress may evoke systemic inflammation, manifested at least by changes in the concentration of the above-mentioned cytokines and their interdependence.

In an effort to more fully understand cytokine response to stress in horses, in this study we determined the effects of long- and short-distance transportation on cytokine plasma levels. We also sought to determine the relationship between cytokines before and after transportation and age-dependent responses.

Material and methods

The experimental animals were 24 cold-blooded female horses divided into four groups. Six fillies (Group 1) aged 6-18 months (13 ± 4.05, mean ± SD) weighting 420 ± 20.73 kg and six mares (Group 2) aged 10-12 years (11 ± 0.89) weighting 650 ± 24.00 kg were transported over a distance of about 550 km and then rested for about 24 hours before slaughter. Six fillies (Group 3) aged 6-18 months (14 ± 4.24) weighting 400 ± 19.23 kg and six mares (Group 4) aged 10-12 years (11 ± 1.1) weighting 600 ± 26.00 kg were transported over a distance of about 50 km and rested for about 6 hours before slaughter.

Blood samples were taken from the jugular vein from all horses before transportation (time 0 – T0), immediately after transportation (time 1 – T1), and at an abattoir during slaughter (time 2 – T2) into tubes containing EDTA as an anticoagulant. The blood samples were centrifuged for 15 min at 1500 g and plasma was harvested and stored at –70°C until used for analyses.

Plasma concentrations of IL-6 and IL-8 were measured with commercial enzyme-linked immunosorbent assay (ELISA) kits (Genorise Equine IL-6 and IL-8 kits). Adiponectin level was determined with an Adiponectin kit (Millipore). Absorbances were read with Absorbance Microplate Reader ELx800 (BioTek Instruments, Inc).

Data were presented as mean ± SD. Statistical analysis was carried out by Student's t-test. The level of significance was set at $P < 0.05$. The relations between concentrations of cytokines (IL-6, IL-8) and adiponectin were evaluated using a regression coefficient.

Results and discussion

Our study revealed an increase in IL-6 plasma level in both measurements after transportation (T1 and T2) in all groups of horses in comparison with values before transportation (T0). Marked augmentation of IL-6 concentration in plasma from both groups of horses was noted especially after long-distance transportation (groups 1 and 2). The highest plasma concentration of IL-6 was found in fillies after long-distance transportation (group 1) during slaughter (T2) (978 ± 98 pg/ml), whereas before transportation (T0) it was 400 ± 90.60 pg/ml. In the group of middle-aged mares (group 2) the concentration of IL-6 was 970 ± 90.10 pg/ml during slaughter, and increased significantly ($p < 0.05$) from 428.50 ± 76.00 pg/ml before transportation. Elevated concentrations of this cytokine were also observed in both groups after short-distance transportation, and the response to stress was higher in older horses at all time points (Group 4) (Fig. 1).

Elevation of IL-8 plasma concentration during slaughter in comparison with values before transportation was seen in all groups, whereas immediately after transportation it was not observed in group 3. The IL-8

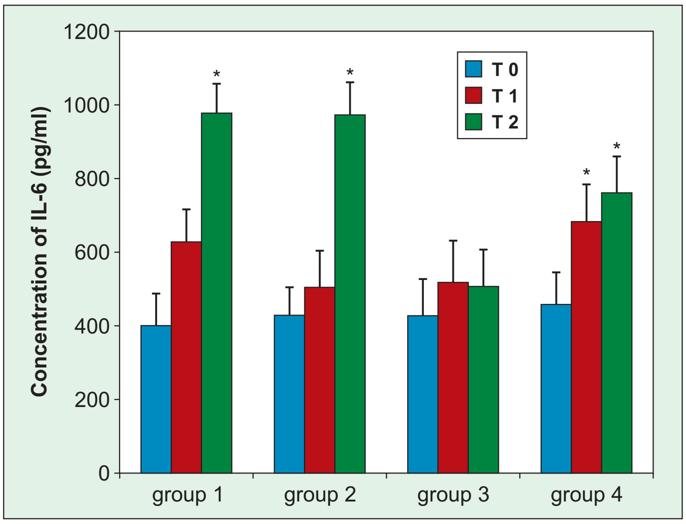

Fig. 1. The concentration of IL-6 (in pg/ml) in the blood of horses before transportation, after transportation, and during slaughter. * p < 0.05 compared to the level before transportation (mean ± SD)

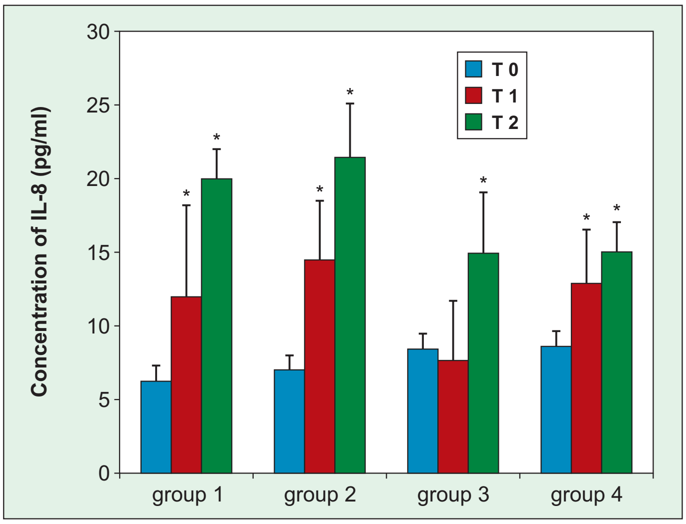

Fig. 2. The concentration of IL-8 (in pg/ml) in the blood of horses before transportation, after transportation, and during slaughter. * p < 0.05 compared to the level before transportation (mean ± SD)

plasma level during slaughter (T2) was higher in older horses and after long-distance transportation than in fillies and after short-distance transportation. Also immediately after transportation (T1), IL-8 plasma concentrations were significantly higher ($P < 0.05$) in groups after long-distance transportation and in older horses, than in fillies and groups after short-distance transportation, in which response was less pronounced and without statistical significance (Fig. 2).

There was no significant change in adiponectin level, except for the group of fillies after long-distance transportation (group 1), in which an increase was observed after transportation (T1) and during slaughter (T2) in comparison with the level measured before transportation (T0) (Fig. 3). Moreover, we found that plasma adiponectin level in the group of fillies after long-distance transportation (group 1) was positively

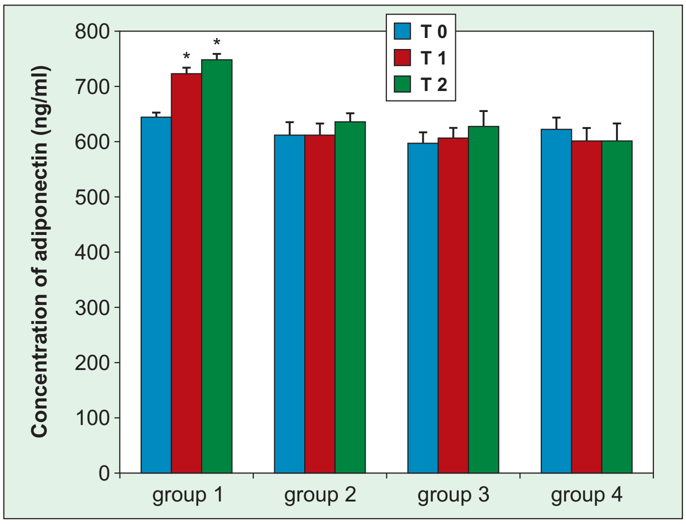

Fig. 3. The concentration of adiponectin (in ng/ml) in the blood of horses before transportation, after transportation, and during slaughter. * p < 0.05 compared to the level before transportation (mean ± SD)

correlated (r = 0.64) with the plasma concentration of IL-6 during slaughter (Fig. 4) and with the plasma concentration of IL-8 (r = 0.78) at the same time (Fig. 5).

The levels of both cytokines analyzed (IL-6 and IL-8) increased immediately after long-distance transportation (T1) and during slaughter (T2) in comparison with the values before transportation (T0). After short-distance transportation, changes in both cytokines were less pronounced. According to findings presented especially by Pedersen et al. an elevated level of cytokines (IL-6 and IL-8) may originate from skeletal musculature. These authors argue that contracting skeletal muscles release the kind of cytokines named myokines, which exert specific endocrine effects or act locally on muscles (16-18).

According to Welc et al. (22), the expression of IL-6 begins when skeletal muscles are exposed to a variety of "internal" and "external" stress stimuli. These internal stresses include hypoxia, mechanical stress, thermal stress, osmotic stress, and oxidative stress. Furthermore, muscles are also exposed and sensitive to blood-borne mediators of stress, such as catecholamines, bacterial toxins, and oxidized lipids. These may be considered as "external" stressors that threaten the entire organism. IL-6 expressed in skeletal muscles may therefore be regarded as an acute sensor of both external and internal stress signals (11, 22).

According to Lankveld et al. (10), chronic or excessive exposure of horses to IL-6 can promote or even cause serious illnesses, such as endotoxaemia, during gastrointestinal disorders. Therefore, IL-6, as a stress hormone, acts in a delicate balance between its life-saving and life-threatening impacts on whole-body homeostasis (22).

The number of publications on IL-8 response to stress in horses is very limited. Cappelli et al. (4) noted an increase in gene expression for IL-8 in exercise-induced stress in horses during endurance exercise.

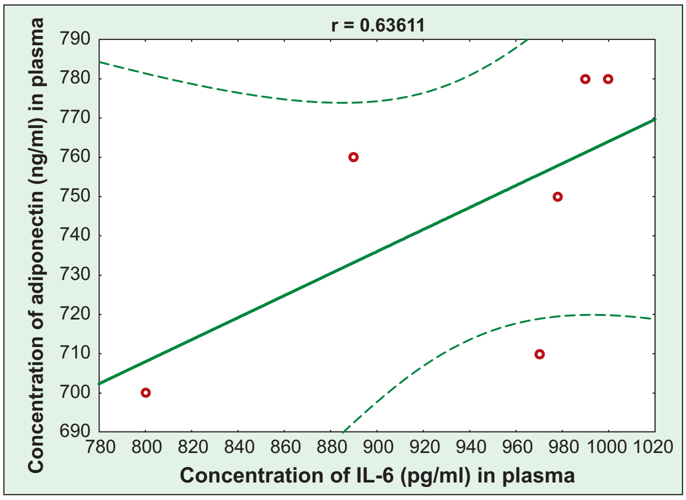

Fig. 4. Correlation between the plasma levels of IL-6 and adiponectin in fillies during slaughter after long-distance transportation

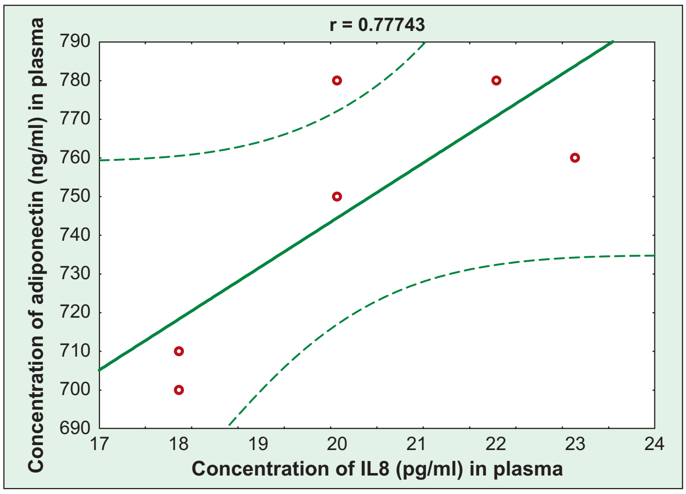

Fig. 5. Correlation between the plasma levels of IL-8 and adiponectin in fillies during slaughter after long-distance transportation

Elevation of IL-8 has also been found in recurrent airway obstruction in horses (2) and during systemic inflammation in the development of equine laminitis (19).

In our experiment, the level of IL-6 was higher in older horses after short-distance transportation than in the group of fillies. The concentration of IL-8, on the other hand, was higher in older horses both after transportation and during slaughter. Some studies discuss an age-related increase in cytokine levels in blood as a complex process named "inflam-aging" (1, 6, 12). Hansen et al. (6) detected differences in the concentration of IL-8 in the blood of horses aged between 5 and 27 years. In a report by Adams et al. (1) the age-dependent elevation of cytokine level in horses was clearly observed, but mostly between 20 and 30 years of age. Also according to McFarlane and Holbrook (12) aged healthy horses showed an increased expression of IL-6 and IL-8.

We found that adiponectin level increased significantly only in young horses after long-distance transportation, whereas in other groups it remained unchanged. This cytokine has not been previously assessed in horses exposed to transportation stress, but some authors studied adiponectin changes during exercise. In earlier studies, adiponectin concentration in horses did not change as a result of exercise (5, 8). However, Kriketos et al. (9) claim that the plasma adiponectin level may increase depending on the duration of exercise and its intensity. Thus, the level of this adipokin cannot be a relevant marker of response to muscle loading, although its level is influenced by the duration and intensity of exercise.

In our study, we found positive correlations between cytokine (IL-6 and IL-8) and adiponectin plasma levels in fillies after long-distance transportation. According to Jortay et al., the production of adiponectin may be induced by chronic and low-grade metabolic inflammation, and this local production is needed to counteract potential muscular damage. Thus, adiponectin production seems to be crucial for controlling inflammatory damage, oxidative stress, and apoptosis. Adiponectin is therefore critical in maintaining the inflammatory/immune balance of myocyte (7).

Our study revealed that, during transportation, stress cytokines IL-6 and IL-8 are released into the circulation, and this response is more pronounced in older horses. In young horses after long-distance transportation, adiponectin concentration is related to IL-6 and IL-8 plasma levels.

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