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STRENOUS EXERCISE IN ATHLETE HORSES: qRT-PCR OPTIMIZATION AND STRESS RELATED GENES EXPRESSION PROFILING

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Stress response is a critical factor during training of athlete horses and is critical for obtaining better performance and safeguarding the animal's welfare.

In order to investigate the molecular mechanisms underlying this process, peripheral blood mononuclear cells (PBMCs) were chosen as the candidate cell type to study the physiological changes connected to exhaustive exercise.

Quantitative real-time PCR (qRT-PCR) is the technique of choice to detect modifications in transcription levels of specific genes in a reliable and reproducible manner; however, for an appropriate application are required reference genes whose level of expression is not affected by the test, general physiological conditions or inter-individual variability.

For this reason the expression of nine potential reference genes was evaluated in ten endurance horses during strenuous exercise. These genes were tested by qRT-PCR and ranked according to the stability of their expression using three different algorithms (implemented in *geNorm*, *NormFinder* and *BestKeeper*). Succinate dehydrogenase complex (*SDHA*) and hypoxantine phosphoribosyl-transferase (*HPRT*) always ranked as the two most stably expressed genes.

Subsequently quantitative real-time PCR (qRT-PCR) was performed to detect modifications in transcription levels of Matrix Metalloproteinase-1 (*MMP-1*) and Interleukin 8 (*IL-8*) genes using *SDHA* and *HPRT* as house-keeping genes. The regulation trend of these two genes - revealed among others with a gene discovery approach (cDNA-AFLP) - was confirmed in a larger sample (10 horses) and statistical significance was determined for each assay.

IL-8 expression increased in the race samples (P<0.001) and after 24h is was still greater than the basal value (P<0.05) even if was lower than the race level (P<0.001). *MMP-1* expression increased greatly after the race (P<0.001), and after 24h was still higher than the basal one (P<0.05), but was lower than the race level (P<0.001). *IL-8* showed a similar trend of expression to *MMP-1*, up-regulated immediately after the race, confirming our previous findings with cDNA-AFLP, enhancing the hypothesis that *MMP-1* and *IL-8* are both involved in exercise induced stress.

An attractive hypothesis about their role in the PBMC response to physical stress, besides their relation with inflammatory response, is the involvement in lymphocyte trafficking and in the recruitment of stem progenitor cells from bone marrow, processes that have been recently shown to be related to exercise-induced stress.

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