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Inflammatory responses associated with cortisol and CK after intensive endurance exercise

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Objective The aim of this study was to investigate the effect of acute intensive endurance exercise on circulating leucocyte and cytokine levels in trained kayakers, and to explore inflammatory responses associated with stress hormone and muscle damage.

Methods Nine male trained kayakers (average age 21.00 ± 3.94 years, average training history 5.56 ± 1.88 years, average height 187.70 ± 4.69 cm, average weight 89.25 ± 8.66 kg, average body fat% $10.96 \pm 4.15\%$, and average $VO_2\max$ 4.33 ± 0.62 l/min) participated in this study. All participants were well informed with the procedures and gave written informed consent. All participants completed a 3000m running test 2 h after a normal breakfast. Participants were rested for 24 h prior to the exercise test, and kept fasting in 90 min after running test, but drunk water ad arbitrium. Mean heart rate was recorded by a Polar heart rate meter, the earlobe capillary blood lactate was tested with a portable lactate analyzer 3 min after running. Venous blood samples were taken from antecubital vein before (T0), 15 min (T1) and 90 min (T2) after exercise. Whole blood samples were used for leucocyte counting and its subpopulation counting immediately by Coulter Counter, CD3+, CD4+, CD8+, CD4+/CD8+, natural killer cells (NK) was evaluated by flow cytometry (Beckman Coulter), T cells were defined as CD3+ lymphocytes and NK was defined as CD3-CD16+CD56+ lymphocyte. Plasma and serum were isolated at 4°C. Plasma ACTH and serum testosterone, cortisol were assayed by CLIA (Immulite 2000), Serum IL-4, IL-6 were detected by ELISA (ST-360). Serum creatine kinase (CK) was assayed by autoanalyzer (Beckman Coulter AU680). Post-exercise concentration of parameters in plasma and serum was corrected according to the formula by Dill. The data was analysed by IBM SPSS Statistics for Windows, Version 21.0. Continuous variables with normal distribution were presented as mean \pm standard deviation. Differences between the 3 time points were tested using repeated measures analysis of variance. To classify immune-endocrine relationships and immune-muscle damage relationships, changes of inflammatory parameters were analysed in relation to testosterone, cortisol, ACTH and CK using Pearson's correlation coefficient respectively. Correlation between changes of testosterone, cortisol, ACTH and inflammatory parameters was tested to verify stress hormone related inflammation response, correlation between changes of CK and inflammatory parameters was tested to evaluate muscle damage related inflammation response. Only changes which from T1 or T2 differ significantly from T0, and from T1 differ significantly from T2 were included in the correlation analysis. The level of significance was set at $p \leq 0.05$.

Results Participants finished 3000m running with an average time of 715 ± 33.0 Sec, and the average heart rate was 167.00 ± 12.88 b/min, Blood lactate concentration was 12.31 ± 1.91 mmol/l. The result showed significant increases for serum cortisol, serum CK, NK, neutrophile granulocyte% (GR%), and significant decreases for testosterone, CD4+, CD4+/CD8+, lymphocyte% (LY%) immediately after intensive running. Change of leucocyte count was not significant as a result of increased NK, GR and decreased CD4+, LY. Compared with result of T1, significant increases of CD4+/CD8+, CD4+, leucocyte count, GR%, and significant decreases of ACTH, cortisol, CD8+, CD3+, LY% were observed at T2, leucocyte count increased with a trend for GR. Furthermore, compared the results of T2 with that of T0, participants presented increased CD4+/CD8+, CK, leucocyte, GR% and decreased testosterone, cortisol, ACTH, CD8+, CD3+, LY%, monocyte% (MO%) at T2. Compared all the levels of IL-4 and IL-6 at T0, T1 and T2 with each other, differences between any two were not significant. The time course of

the changes revealed that significantly increased cortisol, NK and decreased CD4+ from T0 to T1, which recovered at T2 compared with T0 were fast response and fast recovery (FR-FR) parameters; Significantly increased CK, CD4+/CD8+, GR%, MO% and decreased testosterone, LY% from T0 to T1, which didn't recover at T2 compared with T0 were fast response and slow recovery (FR-SR) parameters; Significantly increased leucocyte from T0 to T2 and decreased ACTH, CD3+, CD8+ from T1 to T2 were slow response and slow recovery (SR-SR) parameters; IL-4 and IL-6 were nonsensitive response (NR) parameters. Significant correlation was observed between $\Delta\text{cortisol}_{T1-T0}$ and ΔNK_{T1-T0} ($r=0.78$, $p=0.04$), $\Delta\text{cortisol}_{T2-T1}$ and $\Delta\text{leucocyte}_{T2-T1}$ ($r=-0.70$, $p=0.04$), $\Delta\text{ACTH}_{T2-T1}$ and $\Delta\text{leucocyte}_{T2-T1}$ ($r=-0.76$, $p=0.02$), $\Delta\text{ACTH}_{T2-T0}$ and $\Delta\text{CD8}^{+}_{T2-T0}$ ($r=-0.79$, $p=0.03$). No changes (Δ_{T1-T0} , Δ_{T2-T1} , Δ_{T2-T0}) of inflammatory parameters correlated significantly to changes of testosterone and CK. But the correlation analysis revealed a significant correlation between $\Delta\text{CK}_{\text{post-exercise}}$ and $\Delta\text{cortisol}_{\text{post-exercise}}$ (Δ_{T2-T1} , $r=0.90$, $p=0.001$; Δ_{T2-T0} , $r=0.78$, $p=0.01$).

Conclusions Findings demonstrate different time-course responses and recovery of inflammatory parameters to intensive endurance exercise, athletes and coaches should consider these different recovery time-courses in the subsequent training session after intensive endurance training. Correlation analysis between cortisol, CK and inflammatory parameters indicates that inflammatory response is a stress hormone other than a muscle damage tuned process, and on the contrary, the correlation between post-exercise changes of CK and cortisol suggests a hint that inflammatory response caused by cortisol may contribute to the post-exercise muscle damage but not that during exercising. Correlation analysis and time-course analysis reveal that cortisol exerting an acute effect on NK, but maybe a false time-lag effect on leucocyte. This research investigated the inflammatory responses to intensive endurance exercise by correlation analysis, but inflammatory response is a multifactor process, and the conclusion is still challenging, further research is necessary to understand the underlying mechanism.