

## Assessment of neutrophil functional activity following prolonged endurance exercise

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### ABSTRACT

**Introduction:** Neutrophils not only play an important role in host defense by migrating to the site of infection and producing reactive oxygen species (ROS), but also mediate pathological process in inflammatory tissue damage. Therefore, it is important not only to assess but also modulate neutrophil activities for disease prevention. We have reported that exhaustive exercise causes neutrophil priming (Suzuki et al. *J. Appl. Physiol.*, 81, 1213-1222, 1996) which might be associated with muscle damage (Suzuki et al. *J. Appl. Physiol.*, 87, 1360-1367, 1999), but antioxidant capacity is also activated following exercise (Suzuki et al. *Med. Sci. Sports Exerc.*, 35, 348-355, 2003). In the present study, we examined the effects of endurance exercise on neutrophil activation in relation with muscle damage (Sugama et al. *EIR*, 18, 115-126, 2012) and report the *ex vivo* findings based on a newly-developed neutrophil activity measurement system (Suzuki et al. *Anticancer Res*, 32, 2369-2376, 2012).

**Methods:** Fourteen male triathletes participated in a duathlon race consisted of 5 km of running, 40 km of cycling and 5 km of running. Venous blood samples were collected before, immediately after, 1.5 h and 3 h after the race. A mixture of blood and luminol was layered on hydrogel (Mebiol Co., Kanagawa, Japan) in each tube and incubated for 60 min. The cell counts in the hydrogel were quantified as the migratory activity of neutrophils, whereas the ROS production was measured by luminol-dependent chemiluminescence. Serum concentration of myoglobin (Mb) as a marker of muscle damage and plasma reactive oxygen metabolites (d-ROMs) as a marker of oxidative stress were also measured.

**Results:** The migratory activity of neutrophils was significantly elevated immediately after exercise, further increased 1.5 h, and slightly decreased but remained significantly elevated 3 h after the race. ROS production of neutrophils was significantly elevated 3 h after the race. Serum Mb concentration increased significantly after exercise and correlated positively with the migratory activity of neutrophils, suggesting that neutrophils could infiltrate into the injured muscle. On the other hand, plasma d-ROMs tended to correlate with ROS production, indicating that exercise-induced oxidative stress can be explained at least in part by the ROS production from neutrophils.

**Conclusions:** This new method for measuring neutrophil activities can be applied not only for assessing the status of inflammation and oxidative stress in exhaustive exercise, but also as a method for evaluating the efficacy of antioxidant and anti-inflammatory substances for reducing muscle damage.