

## Exhaustive Exercise-Induced Neutrophil-Associated Tissue Damage and Possibility of its Prevention

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### Abstract

Neutrophils and inflammatory cytokines that accumulate in tissues often cause organ damage/dysfunctions. Herein, background information on some research findings of exertional effects on systemic inflammation centered on neutrophils is introduced. Furthermore, a functional activity measurement system of neutrophils including cell migration and reactive oxygen species (ROS) production, and preventive countermeasures against neutrophil-induced pathogenesis (e.g., polyphenols) are described.

**Keywords:** Exercise; Neutrophil; Oxidative stress; Muscle damage

### Introduction

In the blood circulation, there are five kinds of white blood cells (leukocytes), of which neutrophils (polymorphonuclear leukocytes) are the most abundant. Neutrophils not only play an important role in host defense against infectious microbes by migrating to the site of infection and producing reactive oxygen species (ROS) to kill invading microorganisms. They also mediate pathological processes and acute inflammatory tissue damage [1]. Migration of neutrophils to the tissue is the first step to induce local inflammation, and the overproduction of ROS by neutrophils leads to oxidative stress. Although neutrophils migrate into damaged tissues following injury, infiltration of neutrophils into damaged muscles within several hours after exercise has also been demonstrated [2]. In the exercise and sport science fields, we have reported that exhaustive exercise facilitates neutrophil activity, suggesting their involvement with muscle damage [3-12]. However, endogenous antioxidant capacity also increases following exercise, which can partially attenuate neutrophil activation and oxidative stress [13-16]. Because we observed complex phenomena centered on neutrophils following exercise, some novel technology was required to examine the neutrophil dynamics and functional modulation.

### Development of an Assessment Methodology

Since antioxidants are considered as one of the countermeasures against oxidative stress, it is necessary to develop an assessment methodology which replicates *in vivo* conditions more closely. Under these conditions, whether migrated neutrophils and ROS production are useful or not for the body can be delineated, and appropriate countermeasures against inflammation and oxidative stress can be proposed. Therefore, we developed a neutrophil activity measurement system that analyzes the migratory activity of neutrophils and their ROS production. In brief, this system involves layering mixed whole blood and luminol as a chemiluminogenic probe on transparent hydrogel to detect ROS by luminol-dependent chemiluminescence. This assay largely monitors myeloperoxidase (MPO)-dependent formation of highly toxic ROS, such as hypochlorous acid (HOCl).

Also, the cell count in the hydrogel can be quantified as a measure of the migratory activity of neutrophils. This new method can be applied not only for assessing the state of inflammation and oxidative stress *ex vivo*, but also as a screening system for predicting the effectiveness of antioxidant and anti-inflammatory substances *in vitro* [17-20].

### Application of the Experimental Approach

This methodology identified neutrophil activation *ex vivo* in the absence of changes in many cytokines and inflammatory markers after eccentric muscle-damaging contractions, highlighting the importance of neutrophil dynamics in the pathological process for exercise-induced muscle damage. Furthermore, we have demonstrated that enhanced neutrophil activity after intensive endurance exercise is associated with muscle and renal damage, which are observed in endurance athletes.

Many antioxidants such as plant extracts, especially polyphenols, have been screened *in vitro* for the prevention of lifestyle-related diseases, neoplastic diseases, as well as exercise-induced oxidative stress [17-19]. As a consequence, curcumin, a type of dietary polyphenol, displayed the most potent inhibitory action on neutrophil migration and ROS production among the tested antioxidants *in vitro* [19]. An additional plant extract, *Tabebuia avellanedae* (taheebo) extract also displayed inhibitory effects on neutrophil-related oxidative stress *ex vivo* [21].

### Related and Supplementary Findings

Apart from the above methodology, we have also investigated the role of neutrophils in the exercise-induced muscle damage. We demonstrated that neutrophils firstly migrate to the damaged muscle, attract macrophages, and induce inflammatory mediators and cytokines [12]. Although curcumin is known to protect against ischemia/reperfusion injury in rat skeletal muscle [22], we demonstrated exercise-induced oxidative stress was attenuated by prior curcumin ingestion *in vivo* in humans [23]. Taheebo extract includes polyphenols that were found to suppress not only oxidative stress, but also the production of inflammatory cytokines and prostaglandin E2 by blocking cyclooxygenase-2 (COX-2) in the same manner as anti-

inflammatory and pain-relieving drugs such as celecoxib [24,25]. Also, pretreatment with fucoidan, a known leukocyte-adhesion inhibitor, is reported to reduce muscle hyperalgesia induced by local administration of P2X3 agonist in a rat model [26]. This indicates that leukocyte adhesion and subsequent migration results in pain and inflammation, and that leukocyte adhesion and migration are the point of action to prevent inflammation and tissue damage.

Aside from functional foods, reducing exercise-induced dehydration by sports drink ingestion also inhibited neutrophil activation and cytokine release *in vivo* [27,28]. Further studies are needed to investigate which food and fluid ingredients and supplementation strategies are most effective to reduce inflammation, because severe systemic inflammation can cause multiple organ failure and heat stroke [29,30]. Therefore, these potential countermeasures not only help to reduce pathophysiological processes, but also lead to new research findings for the prevention of oxidative stress, inflammation, organ damage and dysfunction [31-35].

## Concluding Remarks

Strenuous exercise induces leukocytosis mainly due to neutrophilia in the systemic circulation, while neutrophil activity is associated with skeletal muscle damage and other internal organ dysfunctions. As for the underlying mechanisms, the research findings of exertional effects on systemic inflammation centered on neutrophils are described above, which are (together with cytokines) in line with the pathogenesis of multiple organ failure in systemic inflammation, heat stroke and sepsis. Herein, the benefits of measuring neutrophil functional activity, including cell migration and ROS production, and possible preventive countermeasures targeting pathogenesis have been described. Future studies are required to examine the validity of such prevention and treatment approaches, based on their mechanisms of action.

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