The suppression of tumor necrosis factor–alpha production in response to pathogen stimulation by strenuous exercise and underlying mechanisms

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Abstract In general, physical activity reduces the risk of cancer and infectious diseases. However, strenuous exercise has been shown to transiently increase the risk of infection, and this is referred to as the “open window.” Indeed, intense exercise reduces the concentrations of several cytokines in plasma in response to pathogens. The mechanisms responsible for this observation may depend on exercise-induced increased stress hormone secretion, especially that of catecholamines. Exercise-induced catecholamines, acting through β-adrenergic receptors, have been found to be responsible for exercise-induced suppression of plasma tumor necrosis factor-alpha (TNF-α) after lipopolysaccharide (LPS) administration. In the signaling mechanisms of cells, there are no changes in the surface expression of Toll-like receptor (TLR) 4. Although there are also no changes in the LPS-induced TNF-α mRNA expression in tissues after exercise, the TNF-α content in the tissues of exercised animals is lower than that in the tissues of non-exercised animals. Therefore, a strenuous exercise-induced reduction in plasma TNF-α concentration, despite pathogen stimulation, depends on the translation of TNF-α in tissues.

Keywords: open window theory, lipopolysaccharide, Toll-like receptors (TLRs), catecholamines, exhaustive exercise, pro-inflammatory cytokine

Introduction Physical activity reduces the risk of cancer and infectious diseases1-3). However, strenuous exercise has been shown to transiently increase the risk of infection4). The “open window”5) theory of high intensity and exhaustive exercise-induced immunosuppression is supported by substantial research. Indeed, innate immune functions are known to be transiently reduced by strenuous exercise6-9). In animal experimental models, strenuous exercise induces increases in mortality, as well as worsening of infection with the influenza virus or herpes virus10). Recently, the mechanisms underlying exercise-induced immunosuppression have been investigated.

Strenuous exercise-induced immunosuppression The “open window” theory hypothesizes that short term suppression of the immune system occurs following acute bouts of endurance-exercise (Fig. 1)11). This window of opportunity may allow for increases in susceptibility to upper respiratory illnesses (URIs)12-15), although there is also negative evidence against this theory16). This theory suggests that viruses and bacteria may gain a foothold, thereby increasing the risk of subclinical and clinical infections. In fact, intense exercise reduces circulating T1 lymphocytes17), natural killer (NK) cell activity18) and the production of secretory IgA in the mucosa19). Therefore, many studies have reported decreases in immune function in response to exercise. However, the concentrations of several cytokines in plasma are increased by intense exercise20-23). In addition, only a few studies have reported changes in response to pathogen stimulation in vivo after the completion of exercise. Consequently, it has not yet been determined whether these observations reflect a correct immune function in vivo.

System of pathogen recognition in innate immunity Vertebrate immunity can be broadly categorized into
adaptive immunity and innate immunity. The innate immune system relies on pattern recognition receptors (PRRs) for the detection of pathogens. Pattern recognition receptors bind conserved molecular structures shared by large groups of pathogens, termed pathogen-associated molecular patterns. The Toll-like receptors (TLRs) are a recently discovered family of PRRs. Since the late 1990s, remarkable research on innate immunity has shown that many pathogens, including lipopolysaccharide (LPS), are recognized by TLRs. Toll is an essential receptor for host defenses against fungal infections in Drosophila24). In addition, the mammalian homolog of the Toll receptor has been shown to induce the expression of genes involved in inflammatory responses. In 1998, moreover, a point mutation in the TLR4 gene was identified in a murine strain that is unresponsive to LPS25). Based on the results of these studies, two professors won the Nobel Prize in Physiology or Medicine in 201126). At present, at least 11 mammalian TLRs have been discovered, and rapid progress has been made in our understanding of the innate immune system’s ability to sense invasion of microbial pathogens by TLRs27).

Fig. 1 The “open window” theory.5) The “open window” theory proposes that intense exercise increases the risk of infection and decreases the ability of the immune system to respond to pathogens. The “open window” theory is supported by the finding that exercise increases the production of pro-inflammatory cytokines, such as TNF-α, which can recruit immune cells to the site of infection. However, the open window may also be closed by the production of anti-inflammatory cytokines, such as IL-10, which can inhibit the activity of macrophages and other immune cells.

Functionally, the critical role of TLR4 in the recognition of the microbial component LPS has been characterized. TLR2 also recognizes peptidoglycan (PGN) and other varieties of microbial components. TLR3 is implicated in the recognition of double-stranded RNA (dsRNA) and viruses. The enforced expression of TLR5 induces a response to flagellin (FG), a monomeric constituent of bacterial flagella. TLR7 and human TLR8 are predicted to recognize the nucleic acid-like structure of a virus. This prediction was shown to be true based on the finding that TLR7 and human TLR8 recognize guanosine- and uridine-rich single-stranded RNA (ssRNA) from viruses such as the human immunodeficiency virus, vesicular stomatitis virus and influenza virus. ssRNA is abundant in the host, although host-derived ssRNA is usually not detected by TLR7 or TLR8. TLR9 is a receptor for CpG DNA. Bacterial DNA contains unmethylated CpG motifs, which confer immunostimulatory activity. In vertebrates, the frequency of CpG motifs is severely reduced and the cysteine residues of the CpG motifs are highly methylated, leading to abrogation of immunostimulatory activity. There are at least two types of CpG DNA, termed A/D-type CpG DNA and B/K-type CpG DNA27).

TLRs-mediated signaling pathways have been shown to exist based on the finding that TLR4 activates myeloid differentiation protein 88 (MyD88)-dependent and -independent pathways. MyD88 recruits IL-1 receptor-associated kinase (IRAK) and TNF receptor-associated factor (TRAF) upon ligand stimulation. TRAF activates inhibition of the nuclear factor-kappa B (IκB) kinase complex (IKK). IκBs are destroyed, allowing nuclear factor-kappa B (NF-κB) to translocate into nuclei. NF-κB controls inflammatory responses by inducing proinflammatory cytokines. In the MyD88-independent pathway, TIR-domain-containing adapter-inducing interferon-β (TRIF) and TRIF-related adaptor molecule (TRAM) mediate phosphorylation of IRF. Phosphorylated interferon regulatory factor (IRF) is dimerized and translocated into the nucleus to bind to DNA. The activation of IRF is required for the induction of type I IFN (Fig. 2)27).

**TNF-α response to strenuous exercise (in pathogen-free cases)**

Tumor necrosis factor-alpha (TNF-α) is a pro-inflammatory cytokine that is important for the initiation of the inflammatory response to infection28). Under normal conditions, plasma TNF-α is rarely detected and the TNF-α mRNA expression does not increase after intense exercise. In fact, exercise treatment does not change the TNF-α concentrations in plasma29,30). In some studies, no detectable or obvious changes in the levels of TNF-α were found in serum31). Malm32) suggested that the reported changes that occur in the plasma and serum TNF-α levels, in response to exercise, are equivocal. Moreover, Pedersen et al.33) concluded that in vitro data were not supported by in vivo experiments. Therefore, examining the impact of exercise on cytokine responses to bacterial and viral products is important in order to determine the role that exercise might play in altering the quantity or type of immune response to physiologically relevant pathogens.

**TNF-α in response to LPS after strenuous exercise**

Strenuous exercise has been shown to transiently increase the risk of infection6). Cytokines are important chemical regulators of the immune response that exert both local immune regulatory and systemic effects31). The plasma levels of multiple cytokines increase in response to infection. For example, infection promotes a strong inflammatory response characterized by increases in the levels of pro-inflammatory cytokines and chemokines in plasma24,34). TNF-α production by activated macrophages is a major mediator of many of the effects of LPS, a Gram-negative bacterial component, in vivo28). Remarkable reductions in the levels of TNF-α, in response to LPS, are observed following intense exercise. Indeed, in rats, the levels of plasma TNF-α are reduced from the baseline levels before exercise to approximately 10% of
those observed in sedentary controls. Bagby et al.\textsuperscript{35} reported that stressful exercise reduces the production of the pro-inflammatory cytokine TNF in response to LPS. Kita-mura et al.\textsuperscript{36} also found that exhaustive exercise reduces the LPS-induced plasma TNF-α levels to \textasciitilde10% of those seen in non-exercised controls. Several investigators have found that LPS-induced increases in the levels of TNF-α in rats and mice are inhibited by exhaustive exercise\textsuperscript{35-37}. Therefore, exhaustive exercise results in suppressed levels of TNF-α in response to LPS.

**Biological mechanisms underlying strenuous exercise-induced TNF-α suppression in response to LPS**

Stress hormones, including glucocorticoids and catecholamines, have been suggested to be potential mediators of immunosuppression\textsuperscript{38}. Indeed, these hormones suppress the production of pro-inflammatory cytokines (e.g. IL-1β, IL-6, TNF-α, IFN-γ) when administered in vitro and in vivo in animals and humans\textsuperscript{39-42}. The mechanisms responsible for this observation may depend on exercise-induced increased stress hormone secretion, such as that of glucocorticoids and catecholamines. It has been shown that adrenalectomy (ADX) partially attenuates exercise-induced suppression of the plasma levels of TNF-α in response to LPS, suggesting that adrenal products (e.g. glucocorticoids and/or catecholamines) play an important mechanistic role in the above observation (Fig. 3a)\textsuperscript{36}. In addition, it is well known that the levels of circulating corticosterone and catecholamines increase as a result of strenuous exercise\textsuperscript{43-45} or stress\textsuperscript{46,47}.

RU-486, a glucocorticoid receptor antagonist, does not block exercise-induced decreases in the LPS-induced plasma levels of TNF-α (Fig. 3b). Based on the findings that intense, prolonged exercise increases adrenal glucocorticoid production\textsuperscript{43}, and the fact that glucocorticoids are known to reduce macrophage production of pro-inflammatory cytokines such as TNF-α\textsuperscript{39}, glucocorticoids can be assumed to play a role in exhaustive exercise-induced reductions in TNF-α levels in response to LPS. Indeed, the plasma corticosterone concentrations are increased by exhaustive exercise. Furthermore, it has also been shown that dexamethasone (a glucocorticoid receptor agonist) treatment reduces the LPS-stimulated plasma levels of TNF-α in vivo\textsuperscript{41,48}. However, in exercised RU486-treated rats, partial attenuation of the effects of exercise is very small in comparison with ADX, indicating that other adrenal-dependent factors play a significant role.\textsuperscript{50,51}
role in attenuating exercise-induced reductions in LPS-stimulated levels of TNF-α. A previous study has also shown that the plasma corticosterone concentrations are increased by LPS treatment in rats\(^5\). Additionally, Bagby et al. reported that corticosterone concentrations are increased in rats immediately after exhaustive exercise. However, the corticosterone levels also increase to similar concentrations in rats that do not exercise after an LPS challenge\(^5\). Therefore, exhaustive exercise-induced corticosterone activity does not affect the TNF-α response to LPS. However, it is known that corticosterone is at least partly responsible for intense exercise-induced enhancement of phagocytosis and chemotaxis in macrophages\(^3\).

In contrast, pretreatment of rats with the beta-adrenergic receptor (β-AR) blocker propranolol almost completely reverses exercise-induced suppression of plasma TNF-α levels in response to LPS (Fig. 3b)\(^6\). Furthermore, atenolol almost completely attenuates exercise-induced suppression of plasma TNF-α levels in response to LPS. Treatment of rats with the β2AR blocker ICI 118,551 partially inhibits exercise-induced decreases in the LPS-induced TNF-α levels without affecting the response in sedentary control rats. Exercise-induced catecholamines, acting through β-ARs, are responsible for exercise-induced suppression of plasma TNF-α levels after LPS administration. It is well known that plasma catecholamine concentrations increase in response to strenuous exercise\(^1\,6\,3\,5\,1\). Changes in concentrations of noradrenaline, between exercised and non-exercised rats, are remarkable compared with those of corticosterone and adrenaline. Noradrenaline has a high affinity for β1AR, but little or no affinity for β2AR; whereas adrenaline has a high affinity for β2AR only\(^5\). Previous studies have reported that LPS-induced releases of TNF-α are inhibited by prior adrenal receptor blockade\(^2\,8\,5\). Catecholamines bind to α- and β-ARs on the external surface of cell membranes. These hormone-receptor complexes interact with G proteins and activate adenylyl cyclase, which converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). cAMP activates protein kinase A, which subsequently phosphorylates certain intracellular proteins, altering their activity\(^4\). Therefore, β1AR, which has affinity for both adrenaline and noradrenaline, may modulate the effects of catecholamines on exercise-induced changes in the TNF-α levels in response to LPS. Hence, catecholamines suppress TNF-α production in response to LPS\(^5\), then LPS-induced releases of TNF-α are inhibited by prior adrenal receptor blockade\(^5\). In fact, it has been reported that the exercise training-induced low expression of β-ARs might accelerate TNF-α production in response to LPS\(^6\).

**Cell biological mechanisms underlying strenuous exercise-induced TNF-α suppression in response to LPS**

LPS binds to the membrane TLR4 on cells, then induces NF-κB activation, which leads to the expression of TNF-α mRNA\(^5\). After being translated from its mRNA, pro-TNF cleaves to its mature form by TNF-α converting enzyme (TACE), and TNF-α is then released into the extracellular space\(^4\). Although Colbert et al.\(^2\) reported that exercise-induced changes in the plasma cytokine concentrations are not dependent on tissue (liver, lung and muscle) mRNA expression in mice, they did not find any impact of pathogens, such as LPS, on exercise–induced cytokine production. Recently, Tanaka et al.\(^6\) reported that the cell-surface expression of TLR4 on macrophages is unchanged by exhaustive exercise (Fig. 4a). McFarlin et al.\(^6\) also found no changes in the monocyte (CD14\(^+\)) cell surface expression of TLR4 following acute exercise. Therefore, exercise-induced TNF-α suppression is not caused by changes in the expression of the LPS receptor TLR4 on the cell surface. Despite tremendous advances in our understanding of the role of TLRs in host defenses and the specific signaling events that are initiated following TLR activation\(^5\), the effects of exercise on TLR expression are poorly understood. Recently, in human subjects, TLR4 expression in monocytes was slightly, but significantly, decreased by strenuous exercise\(^6\). However, it is not possible to collect a sufficient number of tissue macrophages when using human models\(^6\). Additionally, monocytes are a less mature form of macrophage\(^5\). Therefore, exercise-induced suppression of TNF-α concentrations in plasma, in response to LPS, may not de-

![Fig. 3](image-url) The effects of adrenalectomy and GCR (RU-486), β-AR (propranolol), β1AR (atenolol) and β2AR (ICI 118,551) antagonists on the plasma TNF-α response to LPS after exhaustive exercise\(^6\).

(a) The plasma TNF-α concentrations are shown as absolute values. SHAM: exhaustively exercised rats after sham operation, ADX: exhaustively exercised rats after adrenalectomy. **p<0.01 vs SHAM. (b) The values are expressed as the percentage of inhibition from the TNF-α concentrations in the non-exercised (N-Ex) vehicle controls in each group. The values are expressed as the mean ± S.E.M. *p<0.05 and **p<0.01, as compared with the N-Ex rats in each drug-treated group.

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pend on LPS receptor expression on tissue macrophages.

Although LPS primarily binds to TLR4/CD14 cells, such as macrophages, and expresses TNF-α mRNA via NF-κB activation, TNF-α mRNA expression, in response to LPS in the liver, lungs and spleen, is not decreased by exhaustive exercise (Fig. 4b). Clearly, suppression of the plasma and tissue levels of TNF-α in response to LPS does not result from the TNF-α mRNA expression in any of these tissues. Therefore, the LPS-induced TNF-α concentrations in plasma are inhibited by exhaustive exercise, although the TNF-α mRNA expression in tissues is nevertheless maintained. Interestingly, the LPS-induced TNF-α content in tissues is greatly inhibited by exhaustive exercise (Fig. 4c). NF-κB is a transcription factor that plays a key role in the regulation of the immune response. It is activated by LPS and other inflammatory stimuli, leading to the expression of pro-inflammatory genes, including TNF-α.

In the cytoplasm, pro-TNF is processed by a cellular enzyme called TNF-α converting enzyme (TACE). The mature TNF is then released into the extracellular space. Therefore, the exercise-induced suppression of TNF-α in tissues, together with the lack of changes in TNF-α mRNA expression, indicates enhanced degradation of proteins. As already mentioned above, catecholamines activate cAMP via adrenergic receptors. In addition, it has been reported that heat shock protein 72 (HSP72), which is induced by acute exercise, prevents the post-translational release of TNF. Furthermore, prostaglandin E2 (PGE2), which markedly increases immediately after intense exercise, also inhibits LPS-induced TNF-α production. PGE2 increases intracellular cAMP, which inhibits LPS-induced TNF-α production in macrophages.

There are no differences in the soluble TNFR concentrations in plasma between Ex and Non-Ex groups (Fig. 4d). Therefore, soluble TNF receptor (sTNFR), which inactivates and enhances the renal clearance of systemic TNF, is not affected by exercise. The LPS-induced soluble TNFR concentrations in plasma are not increased by exhaustive exercise. Soluble TNFR strongly inactivates systemic TNF and enhances its renal clearance. In particular, TNFR1/p55, which was measured as TNFR in the liver, lungs and spleen in the Ex and N-Ex mice. Percentage of soluble TNFR in plasma. These results suggest that

Fig. 4 The effects of exhaustive exercise on the LPS signaling pathway. (a): TLR4 expression on the surface of splenic macrophages (CD14+) in the exercised (Ex) and non-exercised (N-Ex) mice. The cell surface expression of TLR4 was measured using flow cytometric analysis. (b): The effects of LPS injection on TNF-α mRNA expression in the liver, lungs and spleen in the Ex and N-Ex mice. The values of TNF-α mRNA expression in each tissue, as a percentage of the values in the Ex mice compared with that of the values in the N-Ex mice, are shown. (c): Tissue TNF-α content in response to LPS in the liver, lungs and spleen in the Ex and N-Ex mice. Percentage of TNF-α content in the Ex mice compared with that in the N-Ex mice. (d): Soluble TNFR (sTNFR) concentrations in plasma in response to LPS in the Ex and N-Ex mice. Percentage of plasma sTNFR concentrations in the Ex mice compared with that in the N-Ex mice. Values are expressed as the mean ± S.E.M. The white columns show the values in the N-Ex mice and the black columns show the values in the Ex mice. *p<0.05, **p<0.01.

Fig. 5 A schematic depiction of the suppressive effects of exhaustive exercise on TNF-α production in the LPS signaling pathway. LPS: lipopolysaccharide, TLR4: toll-like receptor 4, NF-κB: nuclear factor kappa B, TNF-α: tumor necrosis factor-α, TNFR: TNF receptor, sTNFR: soluble TNFR and mTNFR: membrane TNFR.
Destruction of the structure of muscle fibers after intense exercise may be a useful factor for immune cells that cause inflammation. If an "open window" state does not occur, the inflammatory condition may induce pain, fever, and edema in many places, including the muscle. Whether catecholamine secretion, which is controlled by the central system, controls this phenomenon, is more significant for mammalian exercise. Moreover, since this control is caused by the inhibition of a post-transcriptional mechanism, its recovery is more rapid compared with the suppression of gene expression. This reaction might contribute to repair of damaged muscle. However, it does not mean that the compensatory actions of the innate immune function do not exist after strenuous exercise. In fact, after strenuous exercise, recruitment of neutrophils and NK cells, and enhancement of macrophage phagocytosis, are known to occur. Surprisingly, these actions are regulated by the mechanisms that have been cleverly orchestrated. Namely, in macrophages, while production of inflammatory cytokines is inhibited, phagocytosis is enhanced by strenuous exercise. IL-6, which is derived from skeletal muscle, increases rapidly during intense exercise and controls excessive systemic inflammation by exerting anti-inflammatory effects. Coming from a perspective of the central control of the innate immune host defense during exercise, we can be surprised by the wonders of these biological functions.
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