Amino Acids (2008) 34: 337–346
DOI 10.1007/s00726-007-0560-x
Printed in The Netherlands

Amino Acids

The effect of glutamine supplementation and physical exercise on neutrophil function

Review Article

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Received January 10, 2007
Accepted May 1, 2007
Published online October 12, 2007;

Summary. Glutamine is the most abundant free amino acid in the body. Its primary source is skeletal muscle, from where it is released into the bloodstream and transported to a variety of tissues. Several studies have shown that glutamine is important for rat and human neutrophil function and that these cells utilize glutamine at high rates. Physical exercise has also been shown to induce considerable changes in neutrophil metabolism and function. As neutrophils represent 50–60% of the total circulating leukocyte pool and play a key role in inflammation, both physical exercise and glutamine might be expected to regulate the inflammatory process. In this review, the changes in neutrophil function induced by physical exercise and glutamine supplementation are compared.

Keywords: Neutrophil – Glutamine – Physical exercise – Phagocytosis – Apoptosis

Neutrophil function

During infection, neutrophil (polymorphonuclear leukocyte) production by bone marrow is increased due to the action of cytokines such as granulocyte/macrophage colony-stimulating factor (GM-CSF), leading to a significant elevation in circulating neutrophils (neutrophilia). Neutrophils are the first barrier against infection due to their ability to migrate rapidly into loci of infection where they phagocytize and kill pathogens. The sequence of events that occurs in neutrophil response to microbial invasion includes adherence, chemotaxis, phagocytosis, oxidative burst, degranulation, and microbial killing (Wolach et al., 1982). Neutrophils also play a key role in the early stages of the inflammatory response as chemotactic factors (including components of the complement cascade and chemokines) promote their migration from peripheral blood to extravascular tissue (Faurschou and Borregaard, 2003). Specific cytoskeletal rearrangements involving the polymerization of F-actin are required for this chemotactic response, allowing the cell to undergo sequential morphological changes from a rounded to an elongated shape that reflect the state of cell activation and an increased ability to migrate to the target sites (Damaj et al., 1996).

The migration of leukocytes out of blood vessels (a process known as extravasation) occurs in four steps. The first step involves the expression of P-selectin (CD62P) on the neutrophil surface. This can be induced by neutrophil exposure to complement, tumor necrosis factor-α (TNF-α) or lipopolysaccharide (LPS). Subsequently, these factors together with P-selectin induce the synthesis of a second selectin, E-selectin (CD62E) on the endothelial cell surface which recognizes the sulfated-sialyl-Lewis^X moiety of certain leukocyte glycoproteins. The interaction of both E-selectin and P-selectin with these glycoproteins allows neutrophils to adhere reversibly to the vessel wall, so that circulating leukocytes can be said to 'roll' along the endothelium (Gallin et al., 1999; Janeway et al., 2001). This adhesive interaction facilitates the next step in neutrophil migration, an association between specific leukocyte integrins (lymphocyte function-associated antigen-1 [LFA-1] and Mac-1 [i.e. CR3]) with molecules on the endothelial surface such as intracellular adhesion

molecule-1 (ICAM-1). LFA-1 and Mac-1 normally adhere only weakly to the endothelium, but IL-8 and other chemokines bound to proteoglycans on the endothelial cell surface trigger conformational changes in LFA-1 and Mac-1, greatly increasing the adhesive properties of the leukocyte, which then attaches firmly to the blood vessel lining (Gallin et al., 1999; Janeway et al., 2001).

The third step in leukocyte extravasation depends on both the adhesive interactions of neutrophils with endothelium via LFA-1 and Mac-1, and on an immunoglobulin-related molecule called PECAM (CD31) (platelet-endothelial cell adhesion molecule), which is expressed both on leukocytes and at the intercellular junctions of endothelial cells. These interactions enable the phagocyte to ''squeeze'' between the endothelial cells and then to penetrate the basement membrane with the aid of proteolytic enzymes released from the neutrophil in a process known as diapedesis. The final step in extravasation is the migration of neutrophils through the sub-endothelial connective tissue, and is induced by several chemokines, including IL-8 secreted by macrophages encountering pathogens at the site of infection. Once reaching an inflammatory site, neutrophils are able to eliminate pathogens by phagocytosis and by the release of specific antimicrobials into their immediate environment in the process of degranulation (Gallin et al., 1999; Janeway et al., 2001). Upon activation, individual neutrophils generate several toxic reactive oxygen species (ROS) and an array of proteolytic enzymes that act together to kill infectious agents (Weiss, 1989). The superoxide anion radical (or superoxide, $O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), the products of the univalent and bivalent reduction of oxygen (O_2), respectively, are produced during normal aerobic metabolism in mammalian cells and constitute physiological intracellular metabolites (Cadenas and Davies, 2000). Superoxide is generated through the mitochondrial electron transport chain, xanthine–xanthine oxidase, and cytochrome P450 (Cadenas and Davies, 2000). An additional source of H_2O_2, independent of mitochondrial respiration resides on the outer mitochondrial membrane where H_2O_2 is generated by the monoamine oxidase catalyzed oxidative deamination of biogenic amines in a direct two-electron reduction of O_2 to H_2O_2 (Hauptmann et al., 1996).

ROS generation involves the pathways described above in most cells including neutrophils. In neutrophils, however, the production of superoxide occurs mainly through NADPH-oxidase activation. NADPH-oxidase is a membrane-associated enzyme complex that catalyzes the one-electron reduction of oxygen to generate superoxide at the expense of NADPH (Babior, 1999). The NADPH-oxidase complex consists of two membrane-bound ($gp91^{phox}$, $p22^{phox}$) and three cytosolic ($p47^{phox}$, $p40^{phox}$, $p67^{phox}$) components plus Rap1a and Rac2. Upon activation of NADPH oxidase, $p47^{phox}$ is phosphorylated on specific sites and all components are translocated from the cytosol to form a complex with the cell membrane giving the enzyme complex the ability to convert O_2 to $O_2^{\bullet -}$ which is now localized in secretory granules (Babior, 1999).

The oxidizing agents generated by NADPH-oxidase activity include not only $O_2^{\bullet -}$ but H_2O_2, which is produced by dismutation of the $O_2^{\bullet -}$, and both $O_2^{\bullet -}$ and H_2O_2 in turn give rise to other ROS that are strong cytosolic oxidants, such as hypochlorous acid (formed by H_2O_2 and chloride ions under the action of myeloperoxidase released from neutrophil granules) and the hydroxyl radical (OH^-) (Babior, 1999, 2004).

Another unstable and reactive free radical produced by immune and inflammatory cells is nitric oxide (NO). NO is synthesized from L-arginine and molecular oxygen by the enzymatic action of inducible nitric oxide synthase (iNOS) utilizing electrons donated by NADPH (Moncada et al., 1991). It acts as both a toxic agent directed against infectious organisms, and in the regulation of host immune function, thus acting as an anti-inflammatory or immunosuppressive agent via its inhibitory or apoptotic effects (see review Coleman, 2001). The NO produced by neutrophils acts synergistically with bacterial products and with cytokines to promote an increase of iNOS expression and consequently the production of additional NO (see review Armstrong, 2001). The nitric oxide generating system has also been suggested to function as an autacoid mechanism, regulating neutrophil activation during pathophysiological conditions in vivo (Nath and Powledge, 1997) in addition to acting directly in the killing of pathogens including bacteria and fungi (Malawista et al., 1992).

After killing the ingested microorganisms, neutrophils die by apoptosis and are phagocytosed by macrophages, thereby preventing the release of neutrophil contents to the extracellular environment that would otherwise induce inflammation (Scheel-Toellner et al., 2004). Neutrophil apoptosis can be activated by both intrinsic and extrinsic pathways (Kroemer, 2002). In the intrinsic process, the apoptotic agents lead to formation of a mitochondrial permeability transitional pore (MPTP). That is formed by a voltage dependent channel (VDAC), adenine nucleotide translocase (ANT), cyclophilin D and several proteins of the Bcl-2 family (Kroemer, 2002). Apoptotic cells undergo alterations in the MPTP that leads to the release into the cytoplasm of a number of intra-mitochondrial proteins

(including cytochrome c, apoptotic inducing factor [AIF], pro-caspases 3 and 9, the *second mitochondria-derived activator of caspases* [Smac, also known as DIABLO] and endonuclease G), that precedes the activation of caspases and endonucleases (Kroemer, 1998; Lemasters et al., 1998; Mayer and Oberbauer, 2003).

Glutamine metabolism in neutrophils

The importance of glutamine in cell function was first postulated by Krebs (1935). Later, Ardawi and Newsholme (1982) demonstrated that glutamine utilization is critical in providing energy and biosynthetic precursors for the proliferation of the immune cells (i.e. lymphocytes). Curi et al. (1997) studied glutamine function in rat neutrophils and were the first to demonstrate that the rate of glutamine utilization in these cells is higher than that of glucose. They found that glutamine was utilized by neutrophils at a rate of 12.8 $nmol \cdot min^{-1}$ per mg protein when added to the cells at a final concentration of 2 mM for 1 h. In contrast, glucose was utilized at only 7.7 $nmol \cdot min^{-1}$ per mg protein when cells were incubated in 5 mM glucose. The conversion of glucose to lactate in these cells was high (60% of the amount of glucose utilized), whereas [U–^{14}C] glucose decarboxylation was very low. Curi et al. (1997) also noted that the formation of ammonia represented ~27% of glutamine utilization in neutrophils and the conversion of glutamine to glutamate, aspartate, alanine, and lactate accounted for ~85% of the total amino acid utilized. Similar results have been reported for macrophages (Newsholme and Newsholme, 1989). We have also shown that the addition of glutamine to LPS-treated neutrophils significantly inhibits the production of cytokine-stimulated TNF-α (Pithon-Curi et al., 2002b). This study suggested that glutamine plays a significant role in the neutrophil-mediated response to infection and injury and supports an earlier study where the addition of glutamine to the culture medium of cultured neutrophils obtained from burn and post-operative patients, was shown to augment the bacterial killing activity of these cells (Furukawa et al., 1997, 2000). Castell et al. (2004) have shown in addition that phosphate-dependent glutaminase, a key enzyme in glutamine metabolism, is present on the surface of human neutrophils, suggesting that high rates of glutamine utilization in the human neutrophil might suppress the production of pro-inflammatory factors such as IL-8.

Glutamine has also been shown to be important in the production of ROS via. the NADPH-oxidase pathway. Several investigations have demonstrated that glutamine increases expression of the NADPH-oxidase components $p22^{phox}$, $p47^{phox}$ and $gp91^{phox}$ in rat neutrophils (Pithon-Curi et al., 2002a; Moinard et al., 2002). Moreover, Castell et al. (2004) have shown that glutamine (2 mM) increases the respiratory burst in human neutrophils stimulated with both phorbol myristate acetate (PMA) and formyl-methionyl-leucyl-phenylalanine (fMLP). Similar effects were observed by Mühling et al. (2002, 2005), who reported that exogenous L-alanyl-L-glutamine (1 mM) significantly increased neutrophil superoxide anion generation, H_2O_2 formation and the release of mieloperoxidase (MPO). More recently these authors have proposed that modulation of intracellular glutamine metabolization and/or de novo synthesis, and well as a blockade of important glutamine-dependent metabolic processes may lead to significant modifications of immune functions (Mühling et al., 2007).

As noted earlier, adhesion molecules play a key role in cell-to-cell and cell-to-extracellular matrix interactions. For example, ICAM-1 and VCAM-1 (vascular cell adhesion molecule-1) serve important functions in the adhesion of monocytes, lymphocytes, and neutrophils to activated endothelium (Carlos and Harlan, 1994; Jang et al., 1994). A study by Fukatsu et al. (2001) showed that, compared with conventional total parenteral nutrition, glutamine-supplemented parenteral nutrition in mice decreased the intestinal expression of ICAM-1, indicating the importance of glutamine on modulation of cellular adhesion, and therefore on neutrophil migration.

Effect of exercise on neutrophil function

Marked changes in blood leukocyte counts resulting from a single bout of high intensity exercise are well known and are due largely to the movement of neutrophils from the marginal pool to the circulating pool as a result of muscular action (Morozov et al., 2003; Mochida et al., 2007). Fielding et al. (1993) in addition showed that after intense acute exercise neutrophils infiltrate into the underlying tissue where they may release ROS and Wolach et al. (1998, 2000) demonstrated that fMLP-stimulated neutrophil chemotaxis was reduced 24 h after aerobic exercise in trained and untrained women. This group (Wolach et al., 2005) later reported that the decrease in chemotaxis after moderate exercise was transient, with a full recovery by 48 h. Several reports have also shown that exercise changes the expression of cell-adhesion molecules and consequently neutrophil movement into damaged tissue, including skeletal muscle (MacIntyre et al., 2001; Morozov et al., 2001; Mochida et al., 2007).

Therefore, release of the marginal pool of neutrophils appears to be coordinated with mechanisms that increase their extravasation. However, there has not been a general agreement as to which adhesion molecules play the major role in this. Smith et al. (1996) showed increased expression of CD11b in response to moderate exercise. However, Peake et al. (2004) found that after moderate exercise the expression of CD11b (integrin alpha M), CD16 (low-affinity immunoglobulin G [IgG] Fc receptor) and CD35 (erythrocyte complement receptor 1 or CR1) was unchanged, although 1 h afterwards a significant decrease was observed. The authors suggested that this reduction in expression of the receptors after exercise might be part of the inflammatory response in exercise-induced muscle damage.

A reduction in expression of L-selectin (CD62L) in neutrophils has been reported to occur immediately after intense exercise, followed by an increase during recovery (Kurokawa et al., 1995) whereas CD11a (integrin alpha L) remained unchanged. Chinda et al. (2003a) studying neutrophils from marathon runners reported that intense exercise decreased the expression of CD16, but did not affect CD11b expression. In another study Chinda et al. (2003b) showed decreased expression of CD11b and CD16 in neutrophils from judoists. On the other hand, Mochida et al. (2007) showed no change in expression of CD11b and CD16 after intensive pre-competition training in female judoists. Overall these studies would suggest that the effect of exercise on neutrophil function may vary with the athlete and the type of exercise. Wang and Liao (2004) reported decreases in P-selectin but increases in neutrophil-derived NO metabolites and von Willbrand factor (vWF) after moderate exercise. The authors speculated that by increasing NO release, moderate exercise may decrease the activity of adhesion molecules on platelets, thereby reducing platelet activation induced by shear stress and so suppressing neutrophil interaction with surface-adherent platelets.

Exhaustive intense physical exercise in athletes (80–100% VO_{2max}) can also induce degranulation of circulating neutrophils, causing an increase in plasma concentration of neutrophil activation markers including MPO, lactoferrin, and elastase (Gray et al., 1993; Robson et al., 1999; Pyne et al., 2000; Morozov et al., 2003, 2006; Inoue et al., 2004). Long duration (60–150 min) and moderate-intensity (50–60% VO_{2max}) exercise have also been shown to induce an increase of elastase activity in plasma (Pyne et al., 2000; Robson et al., 1999; Smith et al., 1996). Furthermore, plasma MPO, lactoferrin, IL6, IL8, G-CSF (granulocyte colony-stimulating factor), MCSF (monocyte/macrophage colony stimulating factor), and MCP-1 (monocyte chemoattractant protein 1) have been shown to increase after prolonged endurance exercise (marathon) (Suzuki et al., 2003), suggesting that endurance exercise could mediate the recruitment of neutrophils and monocytes that would increase tissue damage. On the other hand, Hack et al. (1992) showed that after intense exercise the functional capacity (i.e. phagocytic capacity and oxidative metabolism) of neutrophils is decreased. Furthermore Lagranha et al. (2005) showed that although acute intense exercise induces an increase in the production of ROS by neutrophils, this effect is not associated with alteration in expression of the components of NADPH-oxidase; $p22^{phox}$, $p47^{phox}$ and $gp91^{phox}$. Quindry et al. (2003) showed that the role of exercise in oxidative metabolism varies with exercise intensity, noting that significant oxidative stress in blood was present immediately after maximal intensity but not after submaximal exercise. Concomitant to the oxidative stress, neutrophilia and subsequent superoxide generation by neutrophils were also significantly increased. These findings suggest that an exercise-induced increase in neutrophil count may lead to an enhanced oxidative stress in blood. The authors also postulated that high-intensity exercise leads to an obligatory oxidative stress that may be necessary for adaptation and potentially for disease prevention.

Activation of protein kinase C (pkC) is one of the earliest events in the cascade leading to activation of neutrophil respiratory burst (Nauseef et al., 1991). Wang (2004) showed that a strenuous exercise reduced the translocation of $p47^{phox}$ to the neutrophil membrane by decreasing phosphorylation of the pkCζ isoform, but not of classical or novel pkC isoforms, thereby suppressing the extent of platelet-promoted neutrophil oxidant production. The author suggested that strenuous exercise attenuates the acute inflammatory response and innate immunity in vascular injury, leading to suppression of cellular defenses against invading bacteria that have transmigrated through vessels and entered the blood. Chinda et al. (2003a) studying neutrophils from marathon runners reported that intense exercise decreased oxidative burst activity, but caused no significant change in neutrophil phagocytic capacity. These authors later reported (Chinda et al., 2003b) that neutrophils from judoists showed an increased production of ROS and a decrease in phagocytic capacity compared with pre-training measurements. Futhermore, in vitro stimulation of neutrophil respiratory burst has been shown to be decreased immediately after an exhaustive high-intensity exercise (80–100% VO_{2max}) (Hack

et al., 1992; Robson et al., 1999). Other investigations suggesting a decrease in ROS after exercise were those of Lagranha et al. (2005) who showed that acute exercise in rats had no effect on neutrophil phagocytosis but induced a marked decrease in nitric oxide production due to a decrease in iNOS mRNA expression. As to any effect of exercise on phagocytosis, Blannin et al. (1996) and Hack et al. (1992) reported that immediately after both moderate (50–70% VO_{2max}) and maximal intensity exercise, neutrophil phagocytic activity is significantly increased and remained elevated for up to 24 h. Hack et al. (1994) in addition showed that neutrophil phagocytic activity at rest or 24 h after exercise was higher in athletes submitted to moderate training when compared with intense training.

There are reports that regular moderate physical activity is beneficial for good health by decreasing infection risk (Nieman and Henson, 1994; Nieman, 2000). This type of lower level exercise decreases the risk of infection by increasing the immune capacity, whereas conversely in athletes involved in high intensity exercise (pre-competition or overtraining), the risk of infection increases due to a reduction in immune function (Nieman and Henson, 1994). Indeed, Nieman (2000) and Pyne (1994) have suggested that moderate intensity exercise is a potent immune modulator. However, the effect of moderate intensity exercise on neutrophil function remains controversial. In this regard, Pyne et al. (1996) and Robson et al. (1999) reported that after moderate intensity exercise (50–60 VO_{2max}), of both short or long duration (40 or 150 min), neutrophil respiratory burst was decreased. By contrast, others have found an increase of neutrophil respiratory burst production after 60–90 min of moderate intensity exercise (50–70% VO_{2max}) (Smith et al., 1996). It has been suggested that these apparently opposite effects of moderate exercise may occur due to the duration of the effort (Robson et al., 1999). The specific form of moderate exercise taken also seems to affect neutrophil function. Bury et al. (1998) showed that moderate training does not affect the function of neutrophils from football players, as compared with the values obtained from individuals at rest. Pyne (1995) however observed activation of the neutrophils in swimmers submitted to moderate-intensity training.

Our group has observed that chronic moderate exercise does not induce a significant increase in the phagocytic capacity of neutrophils from immature (2-month old) rats but does increase the production of reactive oxygen species. Conversely, in neutrophils from mature (3-month old) rats, exercise induced an increase in neutrophil phagocytic capacity but had no effect on the production of reactive oxygen species (data not published). Therefore we can conclude that the effect of moderate-intensity exercise on immune function varies with the duration of the effort and the training state of the athletes.

Effect of glutamine supplementation on exercise-induced changes in neutrophil function

Human plasma glutamine concentrations are decreased by 20–25% after prolonged and exhaustive exercise (Castell et al., 1996; Castell and Newsholme, 1998; Cuisinier et al., 2001). Robson et al. (1999) showed that when exercise was prolonged, both plasma glutamine and neutrophil function were decreased after 1 and 2.5 h post-exercise. In conditions where plasma glutamine concentration is decreased, provision of this amino acid could be advantageous for cells of the immune system, including neutrophils (Castell and Newsholme, 2001). In support of this hypothesis, Ikeda et al. (2003) showed that glutamine supplementation abolishes the reduction of phagocytic capacity in neutrophils from patients with peritonitis after administration of total parenteral nutrition. As observed by Moinard et al. (2002), glutamine and arginine-enriched diets raise the respiratory burst of neutrophils. These authors also found that glutamine and arginine modulate the production of neutrophil ROS from stressed rats through a pathway involving polyamine and NOS. Furthermore Murphy and Newsholme (1997) showed that the rate of nitrite production (in the absence of extracellular arginine) was reduced by culturing macrophages or monocytes in the presence of the glutaminase inhibitor, 6-diazo 5-oxo norleucine, suggesting the importance of glutamine for NO production in leukocytes.

Lagranha et al. (2005) showed that glutamine supplementation both in rested rats and in rats submitted to acute exercise caused a significant increase in neutrophil phagocytic capacity. This study also demonstrated that acute exercise induced a marked decrease in NO production, but the rats that had received glutamine supplementation showed improvement of NO production when compared with neutrophils from non-supplemented exercised rats. To examine the possible mechanisms involved in neutrophil NO production, the expression of iNOS mRNA was then evaluated and found to decrease with exercise. Significantly, the exercise-induced reduction in iNOS transcript was abolished by glutamine supplementation.

As previously mentioned, we found that exercise raises the production of ROS by neutrophils (Lagranha et al., 2005). Glutamine administration caused a further increase

(by two-fold) in the production of reactive oxygen metabolites in neutrophils from exercised rats as compared with exercised rats not supplemented with glutamine. Glutamine also raised the production of ROS in neutrophils from rested rats. To evaluate the possible mechanisms involved the expression levels of the components of NADPH-oxidase; $p22^{phox}$, $p47^{phox}$ and $gp91^{phox}$ were determined. No changes in mRNA levels of these components were observed in neutrophils from exercised as compared to rested rats. However, in neutrophils from exercised rats supplemented with glutamine, there was a marked increase in expression of $p22^{phox}$, $p47^{phox}$ and $gp91^{phox}$ compared with non-supplemented animals (Lagranha et al., 2005).

Effect of intense exercise on neutrophil apoptosis

Apoptosis is a highly regulated form of cell death required for the normal development of multicellular organisms. The morphological features of apoptosis include condensation and marginalization of nuclear chromatin, DNA fragmentation, plasma membrane blebbing, and cell shrinkage (Kerr et al., 1972; Kroemer, 1998). Change in mitochondrial transmembrane potential (MTP), the driving force of cellular ATP formation, constitutes an obligate step in inducing apoptotic death. Mitochondrial depolarization precedes nuclear signs of apoptosis and the activation of endogenous endonucleases and caspases that results in irreversible DNA fragmentation (Kroemer, 1998).

Hsu et al. (2002) first showed changes in MTP in neutrophils taken from athletes submitted to different intensities of aerobic exercise. Lagranha et al. (2004) showed that a single bout of intense exercise leads to increase of apoptosis in neutrophils obtained from both sexually immature and mature rats and also that exercise leads to marked changes in expression of both pro- and antiapoptotic genes of neutrophils from mature rats. However, the effect of exercise on apoptosis-related gene expression was not observed in neutrophils from immature rats suggesting that the changes in pro- and antiapoptotic genes expression induced by exercise are dependent on the sexual maturity of the rats. This is supported by previous studies showing that testosterone can regulate expression of pro- and anti-apoptotic genes in leukocytes (Pearse et al., 1992).

Disruption of mitochondria and release of cytochrome c from mitochondria to cytosol is a triggering feature of apoptosis (Green and Reed, 1998). Cytochrome c leakage is closely associated with mitochondrial depolarization and a decrease in ATP synthesis and precedes the caspase activation that leads to degradation of target proteins and to chromatin condensation (Green and Reed, 1998; Kroemer, 1998; Fehrenbach et al., 2003). Our group has shown that exercise promotes a marked decrease of MTP in neutrophils from both immature and mature rats (Lagranha et al., 2004). Similar findings in MTP were obtained by Hsu et al. (2002) who postulated that intense exercise has cumulative effects on leukocyte mitochondrial function. However, in our study, an effect on neutrophil apoptosis was observed after only single bout of exercise. As to the mechanism(s) underlying these apoptotic changes, superoxide has been shown to regulate expression of pro- and anti-apoptotic genes and may induce disruption of MTP (Kroemer, 1998) and Lagranha et al. (2004) suggest that exercise-induced neutrophil apoptosis could be mediated by a substantial increase in the production of ROS.

Apoptosis and glutamine metabolism

Nunn et al. (1996) determined the endogenous concentration of various metabolites in human neutrophils undergoing apoptosis, including lactate and some amino acids. The endogenous concentration of lactate and glutamine was reduced by 45%, whereas that of arginine, glycine, alanine, aspartate, and glutamate was not modified. Thus, glutamine utilization appears to be increased in apoptotic neutrophils. On the other hand, glutamine has been shown to delay the occurrence of spontaneous apoptosis in neutrophils (Pithon-Curi et al., 2003) and the plasma concentration of glutamine has been correlated positively with MTP (Pithon-Curi et al., 2003). A reduction in MTP has been recognized as necessary for the commitment of cells to apoptosis (Phaneuf and Leeuwenburgh, 2001). Plasma glutamine is also inversely correlated with annexin V binding to externalized phosphatidylserine, and with chromatin condensation in both rat and human neutrophils (Pithon-Curi et al., 2003).

Chang et al. (2002) showed that glutamine decreases lymphocyte apoptosis due to an increased expression of anti-apoptotic genes. On the contrary, depletion of glutamine from the culture medium of Chinese hamster ovary cells has been shown to enhance apoptosis (Sanfeliu and Stephanopoulos, 1999). A possible mechanism for the antiapoptotic effect of glutamine could be the production of glutathione, which has been reported to stabilize neutrophil mitochondrial function and to delay apoptosis (O'Neill et al., 2000). The antioxidant effects of metabolites of glutamine such as glutathione thus may contribute to the effect of glutamine in reducing exercise-induced

Table 1. Summary of the experiments performed to show the effects of glutamine and exercise on neutrophil function

Glutamine concentration	Function	Effect on function	References
Glutamine effects			
0.5–1.0 mM	ATP and NADPH production	↑	Curi et al. (1999)
0.5–2.0 mM	Bacterial killing	↑	Furukawa et al. (2000)
0.5–2.0 mM	DNA fragmentation, chromatin condensation, phosphatidylserine externalization, mitochondrial despolarization	↓	Pithon-Curi et al. (2003)
1.0 mM	Superoxide production, hydrogen peroxide production, MPO activity	↑	Mühling et al. (2002, 2005)
1.0–2.0 mM	ROS production and NADPH components expression	↑	Pithon-Curi et al. (2002a)
2.0 mM	TNFα production	↓	Pithon-Curi et al. (2002b)
2.0 mM	IL8 production	↓	Castell et al. (2004)
2.0 mM	Respiratory burst	↑	Castell et al. (2004)
6.8 mM/kg per d	ROS production	↑	Moinard et al. (2002)
1g/kg supplementation	DNA fragmentation, chromatin condensation, phosphatidylserine externalization, mitochondrial despolarization	↓	Lagranha et al. (2004)
1g/kg supplementation	Phagocytosis and ROS production	↑	Lagranha et al. (2005)
2% GLN-enriched parenteral nutrition	ICAM-1 expression	↓	Fukatsu et al. (2001)
Exercise intensity	Function	Effect on function	References
Exercise effects			
Moderate	CD11b expression	↑	Smith et al. (1996)
Moderate	CD11b, CD16 and CD35 expression	↓	Peake et al. (2004)
Moderate	P-selectin expression	↓	Wang and Liao (2004)
Moderate	Chemotaxis	↓	Wolach et al. (1998, 2000, 2005)
Moderate	Respiratory burst	↓	Pyne et al. (1996) and Robson et al. (1999)
Moderate	Respiratory burst	↑	Smith et al. (1996)
Moderate	NO metabolites and vWF production	↑	Wang and Liao (2004)
Moderate and long duration	Elastase activity	↑	Smith et al. (1996), Robson et al. (1999) and Pyne et al. (2000)
Moderate and intense	Phagocytosis	↑	Hack et al. (1992) and Blannin et al. (1996)
Intense	CD11b and CD16 expression	↑	Gray et al. (1993)
Intense	CD16 expression	↓	Chinda et al. (2003a)
Intense	CD11b and CD16 expression	↓	Chinda et al. (2003b)
Intense	CD11b and CD16 expression	=	Mochida et al. (2007)
Intense	L-selectin expression	↓	Kurokawa et al. (1995)
Intense	Phagocytosis and respiratory burst	↓	Hack et al. (1992)
Intense	MPO, lactoferrin and elastase activity	↑	Gray et al. (1993), Camus et al. (1998), Robson et al. (1999), Pyne et al. (2000), Morozov et al. (2003, 2006) and Inoue et al. (2004)
Intense	Oxidative metabolism and superoxide production	↑	Quindry et al. (2003)
Intense	IL6, IL8, GCSF, MCSF, MCP-1	↑	Suzuki et al. (2003)
Intense	$p47^{phox}$ translocation and pkCζ expression	↓	Wang (2004)
Intense	ROS production, NO production, phagocytosis	↑, ↓, =	Lagranha et al. (2005)
Intense	ROS production	↓	Chinda et al. (2003a)
Intense	ROS production and phagocytosis	↑, ↓	Chinda et al. (2003b)
Intense	Respiratory burst	↓	Hack (1992) and Robson (1999)
Intense	DNA fragmentation and mitochondrial despolarization	↑	Hsu et al. (2002)
Intense	DNA fragmentation, chromatin condensation, phosphatidylserine externalization and mitochondrial despolarization	↑	Lagranha et al. (2004)

↑ Stimulation; ↓ inhibition; = no changes

TNFα Tumor necrosis factor α; *IL8* interleukin 8; *ROS* reactive oxygen species; *MPO* myeloperoxidase; *ICAM-1* intracellular adhesion molecule-1; *IL6* interleukin 6; *G-CSF* granulocyte colony-stimulating factor; *MCSF* monocyte/macrophage colony stimulating factor; *MCP-1* monocyte chemoattractant protein 1; *CD11b* integrin alpha M; *CD16* low-affinity immunoglobulin G [IgG] Fc receptor; *CD35* erythrocyte complement receptor 1 or CR1; *NO* nitric oxide; *vWF* von Willbrand factor; *pkCζ* protein kinase C zeta

neutrophil apoptosis. Glutamine administration may also be responsible for inducing protective effects on mitochondrial integrity, thereby also reducing exercise-induced apoptosis (Lagranha et al., 2004). However, glutamine administration also appears to be responsible for raising the production of reactive oxygen metabolites (Lagranha et al., 2004), so it is unlikely that the protective effect of glutamine on exercise-induced neutrophil apoptosis is mediated by a reduction in oxidative stress.

Glutamine metabolism can result in formation of other amino acids, such as aspartate. It also leads to an increase in ATP and NADPH production (Curi et al., 1999), which are known to regulate mitochondrial function (Curi et al., 1999). Changes in the ATP/ADP ratio have been recognized as a major determinant of cell death, with a moderate decrease of ATP content leading to apoptosis, whereas a marked decrease of this nucleotide causes necrosis (Green and Reed, 1998; Kroemer, 1998). Finally, there is evidence that glutamine is involved in the control of the activities of apoptosis signal-regulating kinase I and stress-activated protein kinase (Ko et al., 2001).

Concluding remarks

The effects of high-intensity physical exercise and glutamine supplementation on neutrophil function are summarized in Table 1. Exercise induces significant changes in neutrophil function which may be either beneficial or harmful depending on intensity and duration. Glutamine also regulates neutrophil activity and may counteract the effects of exercise on specific neutrophil functions such as apoptosis and nitric oxide production. These observations suggest that careful consideration should be given to neutrophil functions in athletes particularly those using glutamine supplementation as part of their regimen.

Acknowledgements

The authors are grateful to J. R. Mendonça, E.P.P. Santos, and G. de Souza, for technical assistance. Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Pesquisa (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Projetos de Apoio a Núcleos de Excelência (Pronex) for support our research.

References

Ardawi MS, Newsholme EA (1982) Maximum activities of some enzymes of glycolysis, the tricarboxylic acid cycle and ketone-body and glutamine utilization pathways in lymphocytes of the rat. Biochem J 208: 743–748

Armstrong R (2001) The physiological role and pharmacological potential of nitric oxide in neutrophil activation. Int Immunopharmacol 1: 1501–1512

Babior BM (1999) NADPH oxidase: an update. Blood, 93: 1464–1476

Babior BM (2004) NADPH oxidase. Curr Opin Immunol 16: 42–47

Blannin AK, Chatwin LJ, Cave R, Gleeson M (1996) Effects of submaximal cycling and long-term endurance training on neutrophil phagocytic activity in middle aged men. Br J Sports Med 30: 125–129

Bury T, Marechal R, Mahieu P, Pirnay F (1998) Immunological status of competitive football players during the training season. Int J Sports Med 19: 364–368

Cadenas E, Davies KJA (2000) Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med 29: 222–230

Carlos TM, Harlan JM (1994) Leukocyte-endothelial adhesion molecules. Blood 84: 2068–2101

Castell L, Vance C, Abbott R, Marquez J, Eggleton P (2004) Granule localization of glutaminase in human neutrophils and the consequence of glutamine utilization for neutrophil activity. J Biol Chem 279: 13305–13310

Castell LM, Newsholme EA (1998) Glutamine and the effects of exhaustive exercise upon the immune response. Can J Physiol Pharmacol 76: 524–532

Castell LM, Newsholme EA (2001) The relation between glutamine and the immunodepression observed in exercise. Amino Acids 20: 49–61

Castell LM, Poortmans JR, Newsholme EA (1996) Does glutamine have a role in reducing infections in athletes? Eur J Appl Physiol Occup Physiol 73: 488–490

Chang WK, Yang KD, Chuang H, Jan JT, Shaio MF (2002) Glutamine protects activated human T cells from apoptosis by up-regulating glutathione and Bcl-2 levels. Clin Immunol 104: 151–160

Chinda D, Nakaji S, Umeda T, Shimoyama T, Kurakake S, Okamura N, Kumae T, Sugawara K (2003a) A competitive marathon race decreases neutrophil functions in athletes. Luminescence 18: 324–329

Chinda D, Umeda T, Shimoyama T, Kojima A, Tanabe M, Nakaji S, Sugawara K (2003b) The acute response of neutrophil function to a bout of judo training. Luminescence 18: 278–282

Coleman JW (2001) Nitric oxide in immunity and inflammation. Int Immunopharmacol 1: 1397–1406

Cuisinier C, Ward RJ, Francaux M, Sturbois X, de Witte P (2001) Changes in plasma and urinary taurine and amino acids in runners immediately and 24 h after a marathon. Amino Acids 20: 13–23

Curi R, Newsholme P, Pithon-Curi TC, Pires-de-Melo M, Garcia C, Homem-de-Bittencourt PI Jr, Guimaraes AR (1999) Metabolic fate of glutamine in lymphocytes, macrophages and neutrophils. Braz J Med Biol Res 32: 15–21

Curi TC, De Melo MP, De Azevedo RB, Zorn TM, Curi R (1997) Glutamine utilization by rat neutrophils: presence of phosphate-dependent glutaminase. Am J Physiol 273: C1124–C1129

Damaj BB, McColl SR, Neote K, Hebert CA, Naccache PH (1996) Diverging signal transduction pathways activated by interleukin 8 (IL-8) and related chemokines in human neutrophils. IL-8 and Gro-alpha differentially stimulate calcium influx through IL-8 receptors A and B. J Biol Chem 271: 20540–20544

Faurschou M, Borregaard N (2003) Neutrophil granules and secretory vesicles in inflammation. Microbes Infect 5: 1317–1327

Fehrenbach E, Veith R, Schmid M, Dickhuth HH, Northoff H, Niess AM (2003) Inverse response of leukocyte heat shock proteins and DNA damage to exercise and heat. Free Radic Res 37: 975–982

Fielding RA, Manfredi TJ, Ding W, Fiatarone MA, Evans WJ, Cannon JG (1993) Acute phase response in exercise. III. Neutrophil and IL-1 beta accumulation in skeletal muscle. Am J Physiol 265: R166–R172

Fukatsu K, Lundberg AH, Kudsk KA, Hanna MK, Johnson CD, Wu Y, Wilcox HG, Zarzaur BL (2001) Modulation of organ ICAM-1 expression during IV-TPN with glutamine and bombesin. Shock 15: 24–28

Furukawa S, Saito H, Inaba T, Lin MT, Inoue T, Naka S, Fukatsu K, Hashiguchi Y, Han I, Matsuda T, Ikeda S, Muto T (1997) Glutamine-enriched enteral diet enhances bacterial clearance in protected bacterial

peritonitis, regardless of glutamine form. JPEN J Parenter Enteral Nutr 21: 208–214

Furukawa S, Saito H, Inoue T, Matsuda T, Fukatsu K, Han I, Ikeda S, Hidemura A (2000) Supplemental glutamine augments phagocytosis and reactive oxygen intermediate production by neutrophils and monocytes from postoperative patients in vitro. Nutrition 16: 323–329

Gallin JI, Goldstein IM, Snyderman R (1999) Inflammation-basic principles and clinical correlates, 3rd edn. Raven Press, New York

Gray AB, Telford RD, Collins M, Baker MS, Weidemann MJ (1993) Granulocyte activation induced by intense interval running. J Leukoc Biol 53: 591–597

Green DR, Reed JC (1998) Mitochondria and apoptosis. Science 281: 1309–1312

Hack V, Strobel G, Rau JP, Weicker H (1992) The effect of maximal exercise on the activity of neutrophil granulocytes in highly trained athletes in a moderate training period. Eur J Appl Physiol Occup Physiol 65: 520–524

Hack V, Strobel G, Weiss M, Weicker H (1994) PMN cell counts and phagocytic activity of highly trained athletes depend on training period. J Appl Physiol 77: 1731–1735

Hauptmann N, Grimsby J, Shih JC, Cadenas E (1996) The metabolism of tyramine by monoamine oxidase A/B causes oxidative damage to mitochondrial DNA. Arch Biochem Biophys 335: 295–304

Hsu TG, Hsu KM, Kong CW, Lu FJ, Cheng H, Tsai K (2002) Leukocyte mitochondria alterations after aerobic exercise in trained human subjects. Med Sci Sports Exerc 34: 438–442

Ikeda S, Kudsk KA, Le T, Zarzaur BL, Johnson CD (2003) Glutamine improves impaired cellular exudation and polymorphonuclear neutrophil phagocytosis induced by total parenteral nutrition after glycogen-induced murine peritonitis. Shock 19: 50–54

Inoue H, Sakai M, Kaida Y, Kaibara K (2004) Blood lactoferrin release induced by running exercise in normal volunteers: antibacterial activity. Clin Chim Acta 341: 165–172

Janeway CA Jr, Travers P, Walport M, Shlomchik MJ (2001) Immunobiology: the immune system in health and disease, 5th edn. Garland Publishing, New York

Jang Y, Lincoff AM, Plow EF, Topol EJ (1994) Cell adhesion molecules in coronary artery disease. J Am Coll Cardiol 24: 1591–1601

Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26: 239–257

Ko YG, Kim EY, Kim T, Park H, Park HS, Choi EJ, Kim S (2001) Glutamine-dependent antiapoptotic interaction of human glutaminyl-tRNA synthetase with apoptosis signal-regulating kinase 1. J Biol Chem 276: 6030–6036

Krebs HA (1935) Metabolism of amino-acids: the synthesis of glutamine from glutamic acid and ammonia, and the enzymic hydrolysis of glutamine in animal tissues. Biochem J 29: 1951–1969

Kroemer G (1998) The mitochondrion as an integrator/coordinator of cell death pathways. Cell Death Differ 5: 547

Kroemer G (2002) Introduction: mitochondrial control of apoptosis. Biochimie 84: 103–104

Kurokawa Y, Shinkai S, Torii J, Hino S, Shek PN (1995) Exercise-induced changes in the expression of surface adhesion molecules on circulating granulocytes and lymphocytes subpopulations. Eur J Appl Physiol Occup Physiol 71: 245–252

Lagranha CJ, Senna SM, de Lima TM, Silva EP, Doi SQ, Curi R, Pithon-Curi TC (2004) Beneficial effect of glutamine on exercise-induced apoptosis of rat neutrophils. Med Sci Sports Exerc 36: 210–217

Lagranha CJ, de Lima TM, Senna SM, Doi SQ, Curi R, Pithon-Curi TC (2005) The effect of glutamine supplementation on the function of neutrophils from exercised rats. Cell Biochem Funct 23: 101–107

Lemasters JJ, Nieminen AL, Qian T, Trost LC, Elmore SP, Nishimura Y, Crowe RA, Cascio WE, Bradham CA, Brenner DA, Herman B (1998) The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy. Biochim Biophys Acta 1366: 177–196

MacIntyre DL, Sorichter S, Mair J, Berg A, McKenzie DC (2001) Markers of inflammation and myofibrillar proteins following eccentric exercise in humans. Eur J Appl Physiol 84: 180–186

Malawista SE, Montgomery RR, van Blaricom G (1992) Evidence for reactive nitrogen intermediates in killing of staphylococci by human neutrophil cytoplasts. A new microbicidal pathway for polymorphonuclear leukocytes. J Clin Invest 90: 631–636

Mayer B, Oberbauer R (2003) Mitochondrial regulation of apoptosis. News Physiol Sci 18: 89–94

Mochida N, Umeda T, Yamamoto Y, Tanabe M, Kojima A, Sugawara K, Nakaji S (2007) The main neutrophil and neutrophil-related functions may compensate for each other following exercise-a finding from training in university judoists. Luminescence 22: 20–28

Moinard C, Caldefie-Chezet F, Walrand S, Vasson MP, Cynober L (2002) Evidence that glutamine modulates respiratory burst in stressed rat polymorphonuclear cells through its metabolism into arginine. Br J Nutr 88: 689–695

Moncada S, Palmer RM, Higgs EA (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 43: 109–142

Morozov VI, Pryatkin SA, Kalinski MI, Rogozkin VA (2003) Effect of exercise to exhaustion on myeloperoxidase and lysozyme release from blood neutrophils. Eur J Appl Physiol 89: 257–262

Morozov VI, Tsyplenkov PV, Golberg ND, Kalinski MI (2006) The effects of high-intensity exercise on skeletal muscle neutrophil myeloperoxidase in untrained and trained rats. Eur J Appl Physiol 97: 716–722

Mühling J, Nickolaus KA, Halabi M, Fuchs M, Krull M, Engel J, Wolff M, Matejec R, Langefeld TW, Welters ID, Menges T, Dehne MG, Sablotzki A, Hempelmann G (2005) Alterations in neutrophil (PMN) free intracellular alpha-keto acid profiles and immune functions induced by L-alanyl-L-glutamine, arginine or taurine. Amino Acids 29: 289–300

Mühling J, Burchert D, Langefeld TW, Matejec R, Harbach H, Engel J, Wolff M, Welters ID, Fuchs M, Menges T, Krull M, Hempelmann G (2007) Pathways involved in alanyl-glutamine-induced changes in neutrophil amino- and alpha-keto acid homeostasis or immunocompetence. Amino Acids 33: 511–524

Murphy C, Newsholme P (1997) Glutamine as a possible precursor of L-arginine and thus nitric oxide synthesis in murine macrophages. Biochem Soc Trans 25: 404S

Nath J, Powledge A (1997) Modulation of human neutrophil inflammatory responses by nitric oxide: studies in unprimed and LPS-primed cells. J Leukoc Biol 62: 805–816

Nauseef WM, Volpp BD, McCormick S, Leidal KG, Clark RA (1991) Assembly of the neutrophil respiratory burst oxidase. Protein kinase C promotes cytoskeletal and membrane association of cytosolic oxidase components. J Biol Chem 266: 5911–5917

Newsholme P, Newsholme EA (1989) Rates of utilization of glucose, glutamine and oleate and formation of end-products by mouse peritoneal macrophages in culture. Biochem J 261: 211–218

Nieman DC (2000) Special feature for the Olympics: effects of exercise on the immune system: exercise effects on systemic immunity. Immunol Cell Biol 78: 496–501

Nieman DC, Henson DA (1994) Role of endurance exercise in immune senescence. Med Sci Sports Exerc 26: 172–181

Nunn AV, Barnard ML, Bhakoo K, Murray J, Chilvers EJ, Bell JD (1996) Characterisation of secondary metabolites associated with neutrophil apoptosis. FEBS Lett 392: 295–298

O'Neill AJ, O'Neill S, Hegarty NJ, Coffey RN, Gibbons N, Brady H, Fitzpatrick JM, Watson RW (2000) Glutathione depletion-induced neutrophil apoptosis is caspase 3 dependent. Shock 14: 605–609

Peake J, Wilson G, Hordern M, Suzuki K, Yamaya K, Nosaka K, Mackinnon L, Coombes JS (2004) Changes in neutrophil surface

receptor expression, degranulation, and respiratory burst activity after moderate- and high-intensity exercise. J Appl Physiol 97: 612–618

Pearse MJ, O'Bryan M, Fisicaro N, Rogers L, Murphy B, d'Apice AJ (1992) Differential expression of clustering in inducible models of apoptosis. Int Immunol 4: 1225–1231

Phaneuf S, Leeuwenburgh C (2001) Apoptosis and exercise. Med Sci Sports Exerc 33: 393–396

Pithon-Curi TC, Levada AC, Lopes LR, Doi SQ, Curi R (2002a) Glutamine plays a role in superoxide production and the expression of p47phox, p22phox and gp91phox in rat neutrophils. Clin Sci (Lond) 103: 403–408

Pithon-Curi TC, Trezena AG, Tavares-Lima W, Curi R (2002b) Evidence that glutamine is involved in neutrophil function. Cell Biochem Funct 20: 81–86

Pithon-Curi TC, Schumacher RI, Freitas JJ, Lagranha C, Newsholme P, Palanch AC, Doi SQ, Curi R (2003) Glutamine delays spontaneous apoptosis in neutrophils. Am J Physiol Cell Physiol 284: C1355–C1361

Pyne D (1995) Effects of an intensive 12-wk training program by elite swimmers on neutrophil oxidative activity. Med Sci Sports Exerc 27: 536–542

Pyne DB (1994) Regulation of neutrophil function during exercise. Sports Med 17: 245–258

Pyne DB, Baker MS, Smith JA, Telford RD, Weidemann MJ (1996) Exercise and the neutrophil oxidative burst: biological and experimental variability. Eur J Appl Physiol Occup Physiol 74: 564–571

Pyne DB, Smith JA, Baker MS, Telford RD, Weidemann MJ (2000) Neutrophil oxidative activity is differentially affected by exercise intensity and type. J Sci Med Sport 3: 44–54

Quindry JC, Stone WL, King J, Broeder CE (2003) The effects of acute exercise on neutrophils and plasma oxidative stress. Med Sci Sports Exerc 35: 1139–1145

Robson PJ, Blannin AK, Walsh NP, Castell LM, Gleeson M (1999) Effects of exercise intensity, duration and recovery on in vitro neutrophil function in male athletes. Int J Sports Med 20: 128–135

Sanfeliu A, Stephanopoulos G (1999) Effect of glutamine limitation on the death of attached Chinese hamster ovary cells. Biotechnol Bioeng 64: 46–53

Scheel-Toellner D, Wang K, Assi LK, Webb PR, Craddock RM, Salmon M, Lord JM (2004) Clustering of death receptors in lipid rafts initiates neutrophil spontaneous apoptosis. Biochem Soc Trans 32: 679–681

Smith JA, Gray AB, Pyne DB, Baker MS, Telford RD, Weidemann MJ (1996) Moderate exercise triggers both priming and activation of neutrophil subpopulations. Am J Physiol 270: R838–R845

Suzuki K, Nakaji S, Yamada M, Liu Q, Kurakake S, Okamura N, Kumae T, Umeda T, Sugawara K (2003) Impact of a competitive marathon race on systemic cytokine and neutrophil responses. Med Sci Sports Exerc 35: 348–355

Wang JS (2004) Strenuous, acute exercise suppresses polymorphonuclear leukocyte respiratory burst under adherence to surface-adherent platelets in men. Thromb Haemost 92: 1076–1085

Wang JS, Liao CH (2004) Moderate-intensity exercise suppresses platelet activation and polymorphonuclear leukocyte interaction with surface-adherent platelets under shear flow in men. Thromb Haemost 91: 587–594

Weiss SJ (1989) Tissue destruction by neutrophils. N Engl J Med 320: 365–376

Wolach B, Baehner RL, Boxer LA (1982) Review: clinical and laboratory approach to the management of neutrophil dysfunction. Isr J Med Sci 897–916

Wolach B, Sonnenschein D, Gavrieli R, Chomsky O, Pomeranz A, Yuli I (1998) Neonatal neutrophil inflammatory responses: parallel studies of light scattering, cell polarization, chemotaxis, superoxide release, and bactericidal activity. Am J Hematol 58: 8–15

Wolach B, Falk B, Gavrieli R, Kodesh E, Eliakim A (2000) Neutrophil function response to aerobic and anaerobic exercise in female judoka and untrained subjects. Br J Sports Med 34: 23–28

Wolach B, Gavrieli R, Ben-Dror SG, Zigel L, Eliakim A, Falk B (2005) Transient decrease of neutrophil chemotaxis following aerobic exercise. Med Sci Sports Exerc 37: 949–954

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