Amino Acids (2008) 35: 243–245
DOI 10.1007/s00726-007-0561-9
Printed in The Netherlands

Amino Acids

Neutrophil fatty acid composition: effect of a single session of exercise and glutamine supplementation

Short Communication

C. J. Lagranha [1], **T. C. Alba-Loureiro** [1], **E. F. Martins** [1], **T. C. Pithon-Curi** [2], and **R. Curi** [1]

[1] Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil
[2] Institute of Biomedical and Health Sciences, Cruzeiro do Sul University, São Paulo, Brazil

Received January 10, 2007
Accepted May 15, 2007
Published online August 28, 2007;

Summary. The fatty acid composition of immune cells appears to contribute to variations of cell function. The independent and combined effects of a single session of exercise (SSE) and glutamine supplementation (GS) on neutrophil fatty acid composition were investigated. Compared to control (no treatment given – i.e. neither SSE or GS), single session of exercise decreased myristic, palmitic and eicosapentaenoic (EPA) acids, and increased lauric, oleic, linoleic, arachidonic (AA) and docosahexaenoic (DHA) acids whereas glutamine supplementation combined with SSE (GS + SSE) increased oleic acid. Polyunsaturated/saturated fatty acid ratio and Unsaturation index were higher in neutrophils from the SSE and GS groups as compared with control. These findings support the proposition that SSE and GS may modulate neutrophil function through alterations in fatty acid composition.

Keywords: Fatty acid composition – Neutrophils – Glutamine supplementation – Single session of exercise

Introduction

Glutamine plays an important role in protein (as amino acid source), lipid (through NAD(P)H production required for lipid synthesis) and nucleotide synthesis (through purine and pyrimidine production), and influences NADPH oxidase activity (Newsholme et al., 2003; Curi et al., 2005) in neutrophils. Glutamine raises the in vitro bactericidal capacity and ROS production by neutrophils (Ogle et al., 1994). On the other hand, Pithon-Curi et al. (2003) showed that glutamine has a protective effect on neutrophil apoptosis. In spite of this, the mechanisms involved in the effect of glutamine on neutrophil function remain to be fully elucidated.

Physical exercise causes important changes in neutrophil function. Lagranha et al. (2004) showed that a single bout of intense exercise leads to apoptosis of neutrophils obtained from both sexually immature and mature rats. Concomitant with neutrophil death, a single session of exercise induces an increase in the production of ROS by neutrophils two hours after glutamine supplementation (GS) and 1 h after the beginning of single session exercise (Lagranha et al., 2005). These authors also demonstrated that GS caused a significant increase in the phagocytic capacity of neutrophil from rats submitted to a single session of exercise (SSE).

Fatty acids can be synthesized from glucose and glutamine in leukocytes (Curi et al., 1989; Homem de Bittencourt et al., 1993). Fatty acids are involved in several aspects of leukocyte function such as inflammation, cytokine release (Bahramian et al., 2004), adhesion molecule expression (Yaqoob, 1998), and induction of cell death (Lima et al., 2002). Kew et al. (2003) verified that fatty acid composition of immune cells from healthy subjects appear to contribute to variations of cell function that have an intimate involvement of the cell membrane. This information led us to examine the effect of AE and GS on fatty acid composition of rat neutrophils. Changes in fatty composition may be an important mechanism for the effect of AE and GS on neutrophil function.

Materials and methods

Male Wistar rats, weighing 200 ± 20 g (about 2 months of age) maintained on commercial chow (Nuvilab CR1, Nuvital Nutrientes Ltd.,

Curitiba, PR, Brazil), were used in this study and divided into four groups with: E – subjected to SSE; G – given GS but no SSE; GE – given GS and SSE; C – no treatment given (i.e. neither SSE nor GS). In the GS groups, glutamine was administered at 1 g/kg b.w. 1 h before starting the exercise session. Rats subjected to single session of exercise (SSE) were exercise on a treadmill (Inbramed, Model Standart, Brazil) at a speed of 0.9 km/h during 1 h. The protocol for SSE and GS was performed as described by Lagranha et al. (2004, 2005). Neutrophils were obtained by intraperitoneal injection of oyster glycogen solution (1%) 2 h after GS and 1 h after the beginning of the single session of exercise. Neutrophils accounted for 95% of the cells, with 5% of mononuclear cell contamination. The number of viable cells (>95% neutrophils) was determined in a Neubauer chamber under an optical microscope by Trypan blue exclusion. The experimental procedure of this study (number 187/2001) was approved by the Animal Care Committee of the Institute of Biomedical Sciences, Sao Paulo University.

The fatty acids were extracted from neutrophils as previously described by Folch et al. (1957). After extraction and saponification, the fatty acids were derivatized and analyzed by high performance liquid chromatography (HPLC) using a Shimadzu model LC-10A. The samples were separated using a C8 column (25 cm × 4.6 cm ID, 5-μm particles) with a C8 pre-column (2.5 cm × 4.6 cm ID, 5-μm particles), at a flow rate of 1 mL/min of acetonitrile/water (77:23, v/v) and fluorescence detected (325 nm excitation and 395 nm emission). The fatty acids used as standards were: lauric, myristic, stearic, arachidonic, palmitic (ω-9), oleic (ω-9), linoleic (ω-6), eicosapentaenoic (ω-3) and docosahexaenoic (ω-3) acids (Sigma, St. Louis, MO, USA). The unsaturation index of the fatty acids present in neutrophils was calculated as following: the proportion of each fatty acid multiplied by the number of double bonds. The polyunsaturated/saturated (P/S) fatty acid ratio of the total fatty acids present in neutrophils was calculated as following: the total percentage of unsaturated fatty acids divided by the total percentage of saturated fatty acids.

Statistical analysis

All values are presented as mean + SEM. Differences between conditions were indicated by using one-way analysis of variance (ANOVA). Where a significant main effect was observed, multiple *t*-tests, employing Bonferroni corrections, were used to identify specific differences between conditions.

Results and discussion

A single session of exercise induced a decrease in myristic (1.7-fold), palmitic (2.4-fold) and in EPA (2.4-fold) acids, and caused a marked increase in lauric (2.3-fold), oleic (3.4-fold), linoleic (3-fold), AA (5.3-fold) and DHA (1.5-fold) acids (Fig. 1, E compared to C). Glutamine supplementation + SSE induced an increase of oleic (1.2-fold) in neutrophils, as compared to GS alone (Fig. 1, GE compared to G). Polyunsaturated fatty acids are able to modify both membrane phospholipid composition and fluidity of plasma membrane (Murphy, 1990). Glutamine supplementation alone induced a decrease in myristic (1.2-fold), palmitic (2-fold) and EPA (2.2-fold) acids as compared with no treatment (Fig. 1, G compared to C). On the other hand, GS caused an increase in lauric (4.5-fold), stearic (1.2-fold), oleic (2.4-fold), linoleic (2.2-fold) and arachidonic (4-fold) acids in rat neutrophil as compared with no treatment (Fig. 1).

The P/S ratio and unsaturation index were higher in neutrophils from E, G and GE groups as compared with the control group (Table 1). Exercise and glutamine supplementation combined resulted in increased P/S ratio and unsaturation index compared to no treatment. However, there was no marked additive effect of SSE and GS.

Changes in fatty acid composition affect the physical state of the membrane lipids and may modulate the function of membrane proteins (e.g. carrier mediated transport activity) (Brasitus et al., 1979; Benga and Homes, 1984). Moreover, immune cell function, such as phagocytosis, oxidative burst, proliferation and production of cytokines,

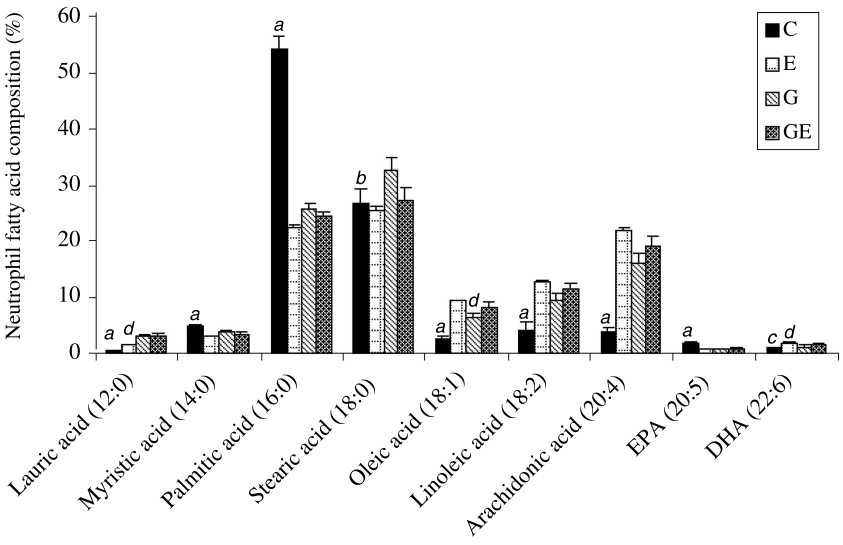

Fig. 1. Fatty acid composition of neutrophils (1×10^6 cells). Groups: *C* no treatment given (i.e. neither SSE nor GS); *E* subjected to SSE; *G* given GS but no SSE and *GE* given GS and SSE. The values are presented as mean ± SEM of two determinations from at least three animals in each group. *a* $p < 0.05$ as compared to E and G groups; *b* $p < 0.05$ as compared to G group; *c* $p < 0.01$ as compared to E group and *d* $p < 0.05$ as compared to GE group. *EPA* eicosapentaenoic acid; *DHA* docosahexaenoic acid

Table 1. Polyunsaturated/saturated fatty acid ratio and unsaturaton index in neutrophils from the studied groups

	C	E	G	GE
P/S ratio	0.16	0.89	0.52	0.71
Unsaturation index	44.6	138.0	101.8	121.7

P/S ratio Polyunsaturated/saturated fatty acid ratio. Groups: *C* no treatment given (i.e. neither SSE nor GS); *E* subjected to SSE; *G* given GS but no SSE; *GE* given GS and SSE. The values are presented as mean ± SEM of two determinations from at least three animals in each group

appear to be more strongly influenced by cell membrane fatty acid composition (Kew et al., 2003). Alterations in fatty acid composition of rat neutrophils induced by SSE and GS may be an important mechanism for the impairment of neutrophil function observed under these conditions.

Acknowledgements

Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Pesquisa (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) support this research.

References

Bahramian N, Ostergren-Lunden G, Bondjers G, Olsson U (2004) Fatty acids induce increased granulocyte macrophage-colony stimulating factor secretion through protein kinase C-activation in THP-1 macrophages. Lipids 39: 243–249

Benga G, Homes RP (1984) Interaction between components in biological membranes and their implications for membrane function. Prog Biophys Mol Biol 43: 195–257

Brasitus TA, Schaechter D, Mamouneas TG (1979) Functional interactions of lipids and proteins in rat intestinal microvillus membranes. Biochemistry 18: 4136–4144

Curi R, Williams JF, Newsholme EA (1989) Formation of ketone bodies by resting lymphocytes. Int J Biochem 21: 1133–1136

Curi R, Lagranha CJ, Doi SQ, Sellitti DF, Procopio J, Pithon-Curi TC, Corless M, Newsholme P (2005) Molecular mechanisms of glutamine action. J Cell Physiol 204: 392–401

Folch J, Lee M, Stanley GHA (1957) A simple method for isolation and purification of total lipid from animal tissue. J Biol Chem 226: 497–503

Homem de Bittencourt PI Jr, Peres CM, Yano MM, Hirata M, Curi R (1993) Pyruvate is a lipid precursor for rat lymphocytes in culture. Evidence for a lipid exporting capacity. Biochem Mol Biol Int 30: 631–641

Kew S, Banerjee T, Minihane AM, Finnegan YE, Willians CM, Calder P (2003) Relation between the fatty acid composition of peripheral blood mononuclear cells and measures of immune cell function in healthy, free-living subjects aged 25–72 y. Am J Clin Nutr 77: 1278–1286

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

Lima TM, Kanunfre CC, Pompéia C, Verlengia R, Curi R (2002) Ranking the toxicity of fatty acids on Jurkat and Raji cells by flow cytometric analysis. Toxicol In Vitro 16: 741–747

Murphy MG (1990) Dietary fatty acids and membrane protein function. J Nutr Biochem 1: 68–79

Newsholme P, Lima MM, Procopio J, Pithon-Curi TC, Doi SQ, Bazotte RB, Curi R (2003) Glutamine and glutamate as vital metabolites. Braz J Med Biol Res 36: 153–163

Ogle CK, Ogle JD, Mao JX, Simon J, Noel JG, Li BG, Alexander JW (1994) Effect of glutamine on phagocytosis and bacterial killing by normal and pediatric burn patient neutrophils. J Parent Ent Nutr 18: 128–133

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

Yaqoob P (1998) Lipids and the immune response. Curr Opin Clin Nutr Metab Care 1: 153–161

Authors' address: Claudia J. Lagranha, Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, Av. Prof. Lineu Prestes 1524, 05508-900 Butantan, SP, Brazil,
Fax: +55-11-3091-7285, E-mail: lagranha@hotmail.com