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Multidimensional analysis reveals increasing phenotypic changes in the total neutrophil compartment during 8 consecutive days of endurance exercise

Autor(es): Staveren, Selma van; Haaf, Twan ten; Klöpping, Margot; Tinnevelt, Gerjen; Ruiters, Karin de; Piacentini, Maria F.; Roelands, Bart; Meeusen, Romain; Koning, Jos de; Jansen, Jeroen; Vriesekoop, Nienke; Koenderman, Leo

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MULTIDIMENSIONAL ANALYSIS REVEALS INCREASING PHENOTYPIC CHANGES IN THE TOTAL NEUTROPHIL COMPARTMENT DURING 8 CONSECUTIVE DAYS OF ENDURANCE EXERCISE

Selma van Staveren¹; Twan ten Haaf²; Margot Klöpping¹; Gerjen Tinnevelt³; Karin de Ruiter⁴; Maria F. Piacentini⁵; Bart Roelands⁶; Romain Meeusen⁶; Jos de Koning²; Jeroen Jansen³; Nienke Vrisekoop¹; Leo Koenderman¹

Intensified endurance exercise is associated with increased risk of upper respiratory tract infections (URTI) likely caused by an impaired immune response (Niemann 2000). Peripheral blood neutrophil counts increase upon exercise and recover within 6-24 hours, this accounts both for anaerobic as well as aerobic exercise bouts (Robson et al. 1999). We have previously described the mobilization of 3 neutrophil subsets with different functional characteristics in response to acute inflammation such as evoked by systemic LPS challenge in healthy volunteers. These cells can be identified by differential expression of FcγRIII (CD16) and of L-selectin (CD62L) (Pillay et al. 2012). This study tested the hypothesis that intensified endurance exercise leads to impaired recovery of the innate immune response characterized by differential mobilization of neutrophil subsets normally absent in the peripheral blood.

The kinetics of the innate immune response were studied in 30 healthy amateur cyclists (11 female, 19 male) participating in an 8-day strenuous cycling tour (mean daily distance of 160 km and 2300 altimeters). The innate immune response was studied by analysis of peripheral blood neutrophils both in terms of absolute numbers as well as phenotype. Neutrophils were analyzed by flow cytometry with the use of the multidimensional analysis

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¹ UMC Utrecht, Department of Respiratory Medicine, Laboratory of Translational Immunology, The Netherlands.

² Vrije Universiteit Amsterdam, MOVE Research Institute Amsterdam, The Netherlands.

³ Radboud University Nijmegen, Analytical Chemistry, Institute for Molecules and Materials, The Netherlands.

⁴ LUMC Leiden, Department of Parasitology, The Netherlands.

⁵ University of Rome "Foro Italico", Department of Human Movement and Sport Sciences, Italy.

⁶ Human Physiology Research Group Vrije Universiteit Brussel, Brussels, Belgium.

Email: s.vanstaveren-2@umcutrecht.nl

method FLOOD (Jansen et al. 2016). The overnight recovery of the innate immune response was followed in the mornings of day 5 and day 8 and compared with the morning day 1 (baseline).

Repeated endurance exercise led to an increase in total neutrophil counts (1.26 fold-increase; 95%CI 1.01-1.51 $p=0.0431$) in the morning at day 8. Flow cytometry measurements revealed the appearance of 2 neutrophil subsets: CD16^{bright}CD62L^{dim} and CD16^{dim}CD62L^{bright}. Deep phenotyping of flow cytometry data revealed a complex change in neutrophil phenotype, characterized by decreased expression of both CD11b and CD62L and marked increased expression of LAIR-1, VLA-4 and CBRM1/5. The changes in expression were found on all neutrophils present in the blood. Strikingly, in strong contrast to our findings during LPS challenge, these neutrophils did not upregulate classical degranulation markers. In fact, our FLOOD analysis revealed that the exercise induced neutrophil phenotype did not overlap with the neutrophils after LPS challenge.

In conclusion, a buildup in changes in the neutrophil compartment was identified in the blood at day 5 and 8 of endurance exercise with a complex change in marker expression. Differential functioning of these changed granulocytes may play a role in the increased risk for URTI during intensified training.