Effects of long distance walking analyzed by multidimentional flow cytometry analysis of neutrophils

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The innate immune system plays a major role in health, as it is the means to prevent infections and keep homeostasis. The neutrophil is an very important effector cell in the innate immune response. It is known that intense physical exercise induces changes in the neutrophil count and activity, but how exactly this affects the immunoresponse of different types of subjects is not fully understood.

In the current study, we analyzed a set of 45 people (mean age 63.8 ± 6.9, 35% females) who participated in the "4-Day Marches", an annual four-day walking event in Nijmegen, The Netherlands. Blood samples were collected from the subjects at baseline and shortly after each day of walking (30, 40 or 50 km). They were measured on-site, using a fully automated load-and-go Multicolor Flow Cytometer (MFC) in a mobile lab. Measurements were done with and without adding a bacterial stimulus that mimics a response to an infection.

The resulting MFC data were analyzed using two chemometric methods recently developed at Radboud University for such purposes, named ECLIPSE and DAMACY, respectively. The former combines Principal Component Analysis (PCA) with Kernel Density Estimates to distinguish and characterize the treatment-related cells from the control ones. The latter applies Partial Least Squares-Discriminant Analysis (PLS-DA) on the PCA scores to build a model that best distinguishes the response subjects from the controls.

These methods enabled to delineate the main response pattern for the data set under study, showing that four days of chronic exercise seem associated with initial activation of the neutrophil compartment followed by partial normalization at day 3-4. Subject-specific differences could also be observed, and the main modes of response were highlighted by clustering analysis. We also observed an interesting similarity between the response from several days of walking and the samples activated with the bacterial stimulus.