An immunometabolic signature of athletes susceptible to respiratory tract illness? Comment on: Evidence of immunometabolic dysregulation and airway dysbiosis in athletes susceptible to respiratory illness

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Respiratory tract illness (RTI), is a leading cause of missed training days and time lost while preparing for, and taking part in, sports competitions.[1] Among athletes, a multitude of factors probably influence the likelihood of developing a RTI, including sleep disruption, international travel, exposure to groups of people, psychological stress, inadequate nutrition and environmental extremes.[2] Historically, there has been concern that a very large volume of repeated vigorous exercise bouts (e.g., during weeks and months of training) could impair immune function, but this idea has been debated[3] and greater attention is turning towards inter-individual factors that might explain infection risk.[2] From a mechanistic standpoint, there is very limited evidence supporting a role for host factors influencing RTI susceptibility among elite athletes. Establishing markers which identify athletes who are prone to RTI is important for developing preventive strategies.

In this context, in a recent issue of *eBiomedicine* Cuthbertson *et al*[4] assessed a variety of immunometabolic factors among a cohort of Olympic athletes who had been clinically evaluated for susceptibility to RTI. Athletes exhibiting RTI susceptibility (defined as $\geq$ 4 RTI episodes within the previous 18 months; n22, described as highly susceptible) were compared with athletes who were unwell less frequently ($\leq$ 1 RTI episode within the previous 18 months; n=23, described as non-susceptible). Further comparisons were made with a healthy control group of 19 age- and sex-matched participants who undertook less than 4 hours of exercise per week. These control participants reported $\leq$ 2 RTI episodes within the previous 12 months. Participants underwent assessments for allergy, asthma and other airway diseases using questionnaires and respiratory testing. Peripheral blood mononuclear cells (PBMCs) were isolated for fresh immunological characterization and plasma was stored for metabolomic analyses. PBMCs were used for advanced multi-parameter B cell and T cell phenotyping using conventional flow cytometry and mass cytometry. In addition, PBMCs were challenged for 18 hours with a variety of stimulants and proinflammatory cytokines were measured in the supernatant. Oropharyngeal throat swabs were collected for bacterial identification, classification and quantitation with sequencing.

The main findings showed that athletes who were highly-susceptible to RTI exhibited lower memory regulatory T cells than non-susceptible athletes. In addition, when PBMCs were stimulated with Phorbol 12-myristate 13-acetate (PMA) and Ionomycin, highly-susceptible athletes exhibited a greater IL-6 and TNF-alpha response compared to non-susceptible athletes. Plasma metabolomic analyses indicated that RTI susceptibility was also associated with immune metabolic dysregulation, as shown by disturbances in plasma sphingolipid metabolism. A number of variables were different when comparing highly-susceptible athletes to healthy controls, but did not differentiate infection risk among athletes. Compared to healthy controls, highly-susceptible athletes had an expansion of $CD8^+$ central memory T cells, a higher ratio of these cells to memory regulatory T cells, greater production of IL-4 and IL-13 by PBMCs following PMA/Ionomycin challenge, and showed evidence of upper airway microbial dysbiosis, characterized by a reduction in bacterial biomass and diversity.

These results show that RTI-susceptible athletes exhibited a unique immunometabolic signature differentiating them from athletes who were less susceptible and also compared to healthy controls. What is the relevance of these findings to applied practice and elite sport? In the future, as emphasized by the authors, these immunometabolic factors could be used as biomarkers to identify athletes at risk of RTIs, and longer

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term, may even have importance as therapeutic targets. It could be argued that making advanced immunometabolic measurements in the general context of sport is expensive and challenging practically; however such procedures might be accessible for top-level athletes and elite sport organisations, such as those involved in this study.

Despite the promising findings of this study, even if replicated with a larger sample size, and in different settings, a very significant question remains: could an immunometabolic profile among athletes reported to be at risk of RTI be a case of inverse causality? In other words, could frequent RTI leave an immunometabolic "imprint" or "signature" representing a sign of historical repeated infections? Indeed, infection-associated immune profiles are well known with other viruses which – unlike the causative agents of RTI-mostly give rise to chronic or latent infections.[5,6] Thus, research aiming to establish a cause-and-consequence relationship between RTI risk and immunometabolic processes should be carefully interpreted. In the absence of randomized and controlled trials that manipulate experimental exposure to real infections in naturalistic settings – which of course, would be challenging to design and deliver in any context, especially elite sport- the answer to this question may not be revealed.

More broadly, the study by Cutherbertson *et al*[4] and RTI susceptibility in general is an example of conflict between the often-quoted Occam's razor (i.e., paraphrased as: the simplest explanation is most likely – there could be a single cause of multiple symptoms) and Hickam's dictum (i.e., paraphrased as: there could be multiple causes of multiple symptoms).[7-8] The commentary here emphasises an important point: although research may reveal what some people interpret to be a single cause that influences RTI risk among athletes (e.g., a hereditary/genetic alteration, as has been shown in other contexts[9-10]), and other research may question other single causes (e.g., extreme volumes of exercise and "over-training"[3]) it is most likely that multiple factors influence RTI risk in the general population and among athletes. Setting philosophical sayings, cause-and-effect, and multi-factorial influences aside, Cutherbertson *et al*[4] make an important step towards establishing an immunometabolic profile that could identify athletes who develop RTIs. Indeed, if these measurements were made routinely, they might help elite sporting organizations to adopt preventative strategies and identify individuals at most need of support.

Contributors

BG and JET equally contributed to this manuscript.

Declaration of interests

The authors have no conflict of interests.

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