Time to ‘couple’ redox biology with exercise immunology

Book Chapter for ‘Oxidative Eustress in Exercise Physiology’ – CRC Press

Alex J Wadley[1] & Steven J Coles[2]

[1]School of Sport, Exercise & Rehabilitation Sciences, University of Birmingham, Edgbaston, B15 2TT

[2]Institute of Science & the Environment, University of Worcester, Worcester, WR2 6AJ

Running Title: Redox exercise immunology

Word Count: 3032

Key Words: leukocyte, physical activity, immunity, thiols, reductive stress

Corresponding author
Dr Alex J Wadley
School of Sport, Exercise & Rehabilitations Sciences
University of Birmingham
Edgbaston
Birmingham, UK
B15 2TT
Email: a.j.wadley@bham.ac.uk
Phone: +44 (0)121 414 8011

Abstract

Regular moderate intensity exercise is known to have both anti-inflammatory and antioxidant roles in humans, whereas more intense exercise is commonly associated with both impaired immune function and heightened oxidative stress. The disciplines of immunology and redox biology are therefore regularly coinvestigated in the context of exercise; however, this has primarily been limited to monitoring changes in extracellular biomarkers (i.e. plasma, serum and saliva). Evidence indicates that redox reactions mediate multiple aspects of innate and adaptive immunity. Exercise modulates innate (e.g. wound healing) and adaptive (e.g. vaccination response) immunity; however, only recently has the link between altered immune cell redox state, immune cell function and exercise been established. It is noteworthy that accumulating evidence over the last 15 years indicates that redox reactions govern various physiological processes in skeletal muscle after exercise. A role for redox reactions (i.e. reversible cysteine thiol oxidation) in modulating the benefits of exercise on immunity therefore seems highly intuitive. In this chapter we review the current body of literature on the topic of redox exercise immunology and discuss how methodological issues largely explain the gaps in our knowledge. We end by suggesting how modern advances in biomedical sciences could benefit the field of redox exercise immunology and propose future areas of investigation, including immunometabolism and extracellular vesicle tissue crosstalk.

Book chapter subtitles

1. Introduction
2. Redox reactions and immunity
3. Global oxidation in immune cells after single bouts of exercise
4. Evaluating immune cell redox state after exercise
5. Single cell technologies
6. Future Perspectives
    I. Appreciation for Oxidative Eustress
    II. Immunometabolism
    III. Extracellular Environment
7. Conclusion

1. Introduction

Exercise immunology is a continually expanding discipline, with relevance to both sport performance (Walsh, 2018) and the management of chronic inflammatory disease (Gleeson *et al.*, 2011). Regular bouts of moderate-to-vigorous intensity exercise confer protection against common upper respiratory tract infections (e.g. rhinovirus and influenza) (Matthews *et al.*, 2002; Nieman *et al.*, 2011), as well as, offsetting the development of multiple immune-related chronic diseases (Peake *et al.*, 2017; Campbell and Turner, 2018; Simpson *et al.*, 2020). The benefits of regular exercise relate to changes in both body composition (i.e. reduced central fat and/or increased skeletal muscle mass) and a steady summation of changes to the immune system after each individual session (Gleeson *et al.*, 2011). The immune system is exquisitely sensitive to physical activity. Physiological changes in cardiac output, blood flow and shear stress, as well as biochemical changes in catecholamine (e.g. adrenaline) (Krüger *et al.*, 2008; Graff *et al.*, 2018) and cytokine concentrations (Bay *et al.*, 2020) are proposed mechanisms mediating the preferential mobilisation of highly functional immune cells into peripheral blood during exercise. This non-uniform response appears to be highly coordinated and is thought to prime the immune system to survey the body for tissue damage, infection and malignant transformation after each bout (Rooney *et al.*, 2018). The mechanisms underlying these events are continually being established. Notably, recent evidence has highlighted that shifts in the redox environment within immune cells influences immune cell trafficking after exercise (Petersen *et al.*, 2012; Michailidis *et al.*, 2013; Sakelliou *et al.*, 2016; A.J. Wadley *et al.*, 2018; Spanidis *et al.*, 2018).

The fields of exercise immunology and redox biology have been regularly intertwined over the last 30 years, primarily in the context of single bouts of exhaustive exercise or periods of high-volume training (collectively termed ‘arduous exercise’). A recurring and contentious topic in the field of exercise immunology is that arduous exercise might suppress immune function (Simpson *et al.*, 2020). Comparably, a notion in the field of redox biology posits that excessive reactive oxygen and nitrogen species (RONS) production after arduous exercise elicits *oxidative stress*, which has been associated with skeletal muscle fatigue, pain and delayed recovery (Powers, Talbert and Adhihetty, 2011). The aforementioned relationship is therefore highly intuitive. A body of evidence supports a role of redox reactions in governing multiple aspects of innate and adaptive immunity (Sies and Jones, 2020); however, the marked physiological changes that exercise causes to the immune system, coupled with the multiple challenges in quantitatively investigating cellular redox state after exercise (Cobley *et al.*, 2017; Nikolaidis, Margaritelis and Matsakas, 2020), makes redox exercise immunology and challenging field of investigation.

This chapter will summarise the body of literature on the topic of redox exercise immunology and suggest how modern technological advances in biomedical science offer scope to delineate the molecular pathways underpinning redox-driven changes in immunity after exercise. The concept of *oxidative eustress* will be emphasised following recent evidence from our laboratory highlighting that exhaustive exercise can elicit *reductive stress* in cytotoxic T cells. Given the unique metabolic demands of individual subsets of immune cells, including cytotoxic T cells, the field of *immunometabolism* will be briefly discussed as a topic for future investigation. By integrating these topics, major advances are permissible in the field of exercise physiology to benefit individuals across the spectrum of human health.

2. Redox reactions and immunity

Redox reactions play a central role in modulating aspects of both innate and adaptive immunity (Nathan and Cunningham-Bussel, 2013; Pei and Wallace, 2018). RONS like hydrogen peroxide recruit phagocytes (e.g. neutrophils) to the site of infection, which then produce large amounts of superoxide (via NADPH oxidases), nitric oxide (via nitric oxide synthases) and hypochlorous acid (via myeloperoxidase) that can destroy foreign pathogens (Winterbourn, Kettle and Hampton, 2016). These RONS also support the release of extracellular traps, which further facilitate this process (Brinkmann *et al.*, 2004; Kenny *et al.*, 2017). Redox reactions have also been implicated in humoral immunity, with disulfide bond formation in the endoplasmic reticulum of plasma cells facilitating the correct folding of immunoglobulins prior to release (Anelli, Sannino and Sitia, 2015) and the process of wound healing, by remodeling the extracellular matrix to seal a wound (Cano Sanchez *et al.*, 2018). Interestingly, there is evidence that immune processes can be impaired by the blocking (Lévigne *et al.*, 2016) and overproduction of RONS. This highlights the critical balance between the production and scavenging of RONS in governing immune function. In this respect, there is evidence to support a role for glutathione and redox-sensitive proteins (e.g. redox enzymes and transcriptions factors) in governing the proliferation, survival and function of various immune cells (e.g. T cells, B cells and macrophages) (Sies and Jones, 2020).

There is data to support a beneficial role of single and regular bouts of exercise in modulating immunological processes, such as wound healing (Emery *et al.*, 2005) and antibody production (Eskola *et al.*, 1978), which are redox-regulated pathways as highlighted above. The next sections will evaluate the research investigating the effects of exercise on the redox status of immune cells, with a particular focus on the effects of single bouts of exercise.

3. Global oxidation in immune cells after single bouts of exercise

Single bouts of exercise have been shown to increase indices of *oxidative stress* in leukocytes isolated from whole blood after exercise cessation. For example, it has been reported that protein carbonylation and lipid peroxidation (i.e. broad global surrogates of oxidative damage) were higher in peripheral blood mononuclear cells (PBMCs) and neutrophils respectively after prolonged cycling (Sureda *et al.*, 2005; Tauler *et al.*, 2006). Furthermore, DNA strand breaks have been reported to be higher in PBMCs after brief exhaustive exercise (Peres *et al.*, 2020). Separate studies investigating various antioxidant enzymes (i.e. superoxide dismutase, glutathione peroxidase and peroxiredoxin) have indicated that their activity and protein expression also increase following different modes and intensities of exercise (Tauler *et al.*, 2006; Ferrer *et al.*, 2009; Turner *et al.*, 2011, 2013; A. J. Wadley *et al.*, 2015). These studies collectively indicate that proteins, lipids and DNA within bloodborne innate and adaptive immune cells are sensitive to oxidation after exercise, with a concurrent antioxidant enzyme response. However, when drawing comparisons between blood samples taken before, immediately and in the hours following exercise (Figure 1A and 1B), it is important to appreciate the vast heterogeneity of immune cells within blood, since the effects of oxidation may vary depending on the immune cell type.

Bouts of exercise evoke profound changes in the number and composition of immune cells within the bloodstream. During exercise, natural killer cells and lymphocytes with high functional capacity (i.e. high cytotoxicity and tissue migration potential) are preferentially

drawn into the circulation (Figure 1A and 1B) through dislodgment from the vascular endothelium and recruitment from marginal pools (Gustafson *et al.*, 2017). Depending on the type of exercise (i.e. intensity, duration) these immune cells can then leave the circulation ≈ 2-3 hours following cessation by migrating into various tissues (e.g. gut, lungs), thus lowering the number, and again, altering the composition of immune cells in the blood (Krüger *et al.*, 2008). Although the aforementioned studies indicate that the immune cell compartment of blood is more oxidised after exercise, they don't account for the well documented compositional changes in immune cells resulting from exercise, or identify how perturbations in the redox state of immune cells can functionally impact different aspects of cellular immunity (Figure 1).

4. Evaluating immune cell thiol redox state after exercise

It is now well established that 2-electron oxidants, such as hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) mediate a broad range of cellular processes (Ferreira and Reid, 2008; Reid, 2008; Lamb and Westerblad, 2011). These processes occur through reversible oxidation of redox-sensitive amino acids, such as tyrosine, methionine and cysteine. The latter has received a lot of attention over the last 10 years, with reversible cysteine oxidation implicated with a broad range of cellular processes, notably metabolism (Kolossov *et al.*, 2015) and signal transduction (Sobotta *et al.*, 2014). Cysteine contains a terminal sulphydryl (-SH) or 'thiol' group that is highly electronegative in nature. Cysteine oxidation is dependent on the pKa of the thiol, which itself is influenced by the amino acid sidechain microenvironment in which that 'particular' thiol resides. This means that for some proteins, solvent accessible cysteine thiols are 'redox active', conferring an ability to partake in cellular redox reactions, which may include the reduction of oxidising agents such as H_2O_2 and $ONOO^-$(Flohé *et al.*, 2011; Daiber *et al.*, 2013; Poole, 2015). Notably, reversible oxidation of cysteine thiols has been implicated with signal transduction in human skeletal muscle during exercise. Redox enzymes such as Peroxiredoxin (PRDX) are particularly important in this context, and are thought to act as transducers of oxidant signals ('redox relays') due to their high turnover rate of cellular H_2O_2 (Winterbourn, 2013, 2015; Wadley, Aldred and Coles, 2016). Recent data has also demonstrated that PRDX thiols can be oxidised in PBMCs after different types of exercise (Turner *et al.*, 2013; A.J. Wadley *et al.*, 2015).

The role of cysteine thiols in regulating immune responses to exercise is a recent area of investigation, with emerging data indicating that exercise might cause *reductive stress* in immune cells after exercise (A.J. Wadley *et al.*, 2018; Spanidis *et al.*, 2018). A series of dietary intervention studies have indicated that supplementation with the thiol donor, N-acetylcysteine (NAC) can blunt immune cell mobilization patterns in response to both muscle-damaging (Michailidis *et al.*, 2013; Sakelliou *et al.*, 2016) and exhaustive aerobic exercise (Petersen *et al.*, 2012). Although the exact tissue uptake kinetics are unclear, this data suggests that alterations in the thiol redox state of immune cells or other tissues (i.e., increased reductive capacity) may regulate immunity after exercise. Spanidis et al, 2018 stratified participants into 'oxidative' and 'reductive' groups based on their individual changes from rest in erythrocyte reduced glutathione (GSH) concentration following a muscle-damaging protocol (i.e. increase in GSH was termed 'reductive') (Spanidis *et al.*, 2018). PBMCs isolated at the same timepoints from the 'oxidative' participants were more sensitive to *in vitro* oxidation and had lower catalase activity compared to 'reduced' participants. This suggests that the reductive capacity

of PBMCs could be important for governing their function, although the exact nature is unclear. Furthermore, both the above studies did not account for compositional shifts in immune cell populations after exercise.

Recently, our group validated a novel method to investigate changes in the thiol redox state of different T cell populations using digital flow cytometry (A.J. Wadley *et al.*, 2018). By incorporating the thiol specific probe, fluorescein-5 maleimide (F5M) into routine flow cytometry panels (excitation: λ488 nm; emission: λ525 nm) (A J Wadley *et al.*, 2018; Wadley, Morgan, *et al.*, 2019), we were able to label and identify intracellular proteins with solvent accessible reduced cysteines in distinct human immune cell populations before and after exercise. Within each cell population, a loss of F5M signal indicated thiol oxidation, whereas a gain in F5M signal indicated thiol reduction. By exploring changes in T cell populations in response to a cycling ramp test to exhaustion (N=20), we reported that T-cytotoxic ($CD8^+$) lymphocyte thiols became transiently *reductive* after exercise to a greater extent than T-helper ($CD4^+$) lymphocytes, both cell types indicating a state of *reductive stress*. We also identified a marked increase in the concentration of $CD8^+$ T cells with highly reduced thiols in post-exercise blood (termed *'$CD8^+$ $Reduced^+$'*). Flow cytometric exploration of the *$CD8^+$ $Reduced^+$* cell population showed less expression of the lymphoid homing receptor, C-C Chemokine Receptor-7 (CCR7) compared to *$CD8^+$ $Reduced^-$*, an immunophenotype suggestive of tissue migratory properties (Sallusto *et al.*, 1999). With our expanding knowledge of how specific immune populations with rapid effector functions are preferentially mobilized into the circulation during exercise, these data suggest that a more *reduced* cellular redox state could, in part, mediate immune cell mobilization and possibly extravasation. The appearance of *$CD8^+$ $Reduced^+$* in blood was very transient, falling back to resting concentrations within 15 minutes, which is difficult to interpret without further investigation. The response could signify a subtle, but transient deviation from *oxidative eustress* that elicits $CD8^+$ T cell functions or the rapid migration of *$CD8^+$ $Reduced^+$* from the circulation after exercise. It must be noted that $CD8^+$ T cells are also a heterogeneous population of lymphocytes, composed of naïve, memory and effector T cells. These cells all have differing basal redox states (Turner *et al.*, 2011) and metabolic phenotypes (discussed later) (Fox, Hammerman and Thompson, 2005; van der Windt and Pearce, 2012). Nonetheless, this method provides a platform for future studies to interrogate the redox environment within cells of the immune system after exercise to provide important mechanistic insight. This could involve staining for different immune cell populations (e.g., natural killer cells), other cysteine modifications (e.g., sulfenic acid) or other redox-sensitive amino acids (e.g., methionine and tyrosine).

5. Single cell approaches

Multiparameter flow cytometry is a well-established method for evaluating immunophenotypic and functional changes in PBMCs. Workflow protocols that incorporate standard immunophenotyping with intracellular cytokine analysis (e.g. Interleukin (IL)-2, IL-4, IL-10 and Interferon Gamma) have successfully identified transient changes in T cell function in response to different exercise intensities (LaVoy *et al.*, 2017). A similar approach has been used to evaluate natural killer and $CD8^+$ T cell functional activity in response to various exercise types. Here, lysosome associated membrane protein-1 (CD107a) on natural killer and $CD8^+$ T cells, as a marker cellular degranulation, displayed differential expression levels depending on exercise frequency and intensity (Broadbent and Coutts, 2017). Our approach uses multiparameter flow cytometry to evaluate the intracellular redox environment in

immunophenotypically distinct T cell populations (redox flow cytometry, see figure 1, panel C) (Wadley, Morgan, *et al.*, 2019). However, the experimental limit has been reached using conventional flow cytometry. As such cell immunophenotyping, functional and intracellular redox environment analysis have yet to be united in a single experimental platform.

The development of CyTOF™ mass cytometry may be a solution to current experimental limitations. This technology has permitted high resolution assessment of natural killer / T cell immunophenotype and functional analysis at the single cell level (Kay, Strauss-Albee and Blish, 2016; Brodie and Tosevski, 2018). Like traditional flow cytometry, CyTOF™ mass cytometry utilizes specific antibodies (labeled with metals in this instance) to detect the expression of various cellular antigens (Heck, Bishop and Ellis, 2019). Because the technology is based on time-of-flight mass spectrometry as a detection method, the limitations / complications associated with standard flow cytometry (e.g. spectral overlap) are ameliorated. Therefore, upwards of 50 different immunophenotypic and(or) functional molecules for a single cell may be detected simultaneously. So far protocols have been established to concurrently evaluate cytokines, transcription factors and immunophenotypic markers (Lin, Gupta and Maecker, 2015; Simoni *et al.*, 2018). In theory, the analysis of protein / peptide markers of the intracellular redox environment (e.g. peroxiredoxin, thioredoxin, glutaredoxin, glutathione) as well as redox-sensitive transcription factors implicated with *oxidative* and *reductive stress* (Bellezza *et al.*, 2018) could easily be incorporated into CyTOF™ mass cytometry workflows.

Figure 1. Reductionist approach to conduct single cell analysis of immune cell redox state after exercise in humans

6. Future Perspectives

I. *Appreciation for Oxidative Eustress*

The evidence in this chapter highlights the need for further investigations in the field of redox exercise immunology. Dysregulation of the immune system is central to many chronic human diseases, with the role of *oxidative distress* now widely characterised (Nathan and Cunningham-Bussel, 2013; Sies and Jones, 2020). The role of *reductive stress* is less clear, but some recent evidence implicates *reductive stress* with enhanced T cell autoimmunity in rheumatoid arthritis (Weyand, Shen and Goronzy, 2018). Subtle or acute *reductive* shifts in immune cells are even less well characterised, with our findings following a single bout of exercise the first to our knowledge. The techniques outlined in section 5, as well as an appreciation for the scientific disciplines highlighted below could shed light into the functional significance of these results. It is important to highlight that acute shifts in both *oxidative* and *reductive* redox state may be important for changes in immunity after exercise.

II. Immunometabolism

Immunometabolism is a rapidly expanding field in immunological research, with rewiring of cellular metabolism now linked with modulating multiple immune processes (Dimeloe *et al.*, 2017). For example, T cells become highly reliant on glycolysis upon activation to support their effector functions (Fox, Hammerman and Thompson, 2005; van der Windt and Pearce, 2012).

Interestingly, emerging data indicate that shifts in cellular redox state also support this metabolic reprogramming (Muri and Kopf, 2020). Indeed, T cell activation, which involves antigen presentation to the T cell receptor (TCR) is enhanced and sustained by mitochondrial and NADPH oxidase derived ROS (Jackson *et al.*, 2004; Kamiński *et al.*, 2012). Following TCR signalling, CD28 ligation is the costimulatory signal needed to evoke the pronounced glycolytic shift. This is sustained through rapid glucose transport (GLUT1) and *reduction* of accumulating pyruvate to lactate, which maintains the cellular NAD^+: NADH ratio. Despite a lower ATP yield per molecule of glucose, aerobic glycolysis drives more rapid metabolism of glucose than mitochondrial oxidation. The metabolic shift also supports the provision of biosynthetic precursors (nucleotides, amino acids and fatty acids) via the pentose phosphate pathway (PPP) that sustain the formation of effector molecules for T cell functions (Vander Heiden, Cantley and Thompson, 2009; Macintyre and Rathmell, 2013). The PPP enhances nicotinamide adenine dinucleotide phosphate (NADPH) supply (van der Windt and Pearce, 2012), the crucial cofactor needed to provide reducing equivalent for multiple cellular antioxidant enzymes (e.g. peroxiredoxin, thioredoxin, glutaredoxin) and the abundant tripeptide, glutathione. This activation-induced shift in T cell metabolism therefore provides reductive capacity for these cells to modulate cellular RONS and thus maintain redox homeostasis, and functional capacity (Ma *et al.*, 2018). Interestingly, this evidence indirectly supports our findings of more reductive $CD8^+$ T cells in the circulation after exercise (*$CD8^+$ Reduced$^+$*) (A.J. Wadley *et al.*, 2018). Given that we know that exercise evokes a marked and preferential increase in immune cells that are highly primed for their effector functions, we can intuitively suggest that these cells would have a more glycolytic phenotype. Future work should intertwine the fields of immunology, metabolism and redox biochemistry to further understand the mobilization of specific immune cells after exercise.

III. Extracellular Environment

This chapter has exclusively focused on changes in redox state of immune cells on a cell-by-cell basis, but it is important to consider how the extracellular redox environment might be important in regulating immunity after exercise. Some cytosolic redox enzymes are released via non-classical pathways, associated with extracellular vesicles (EVs), such as exosomes and nanoparticles (Thierry Léveillard and Najate Aït-Ali, 2017). EV's are important mediators of cellular communication during exercise (Whitham *et al.*, 2018) and it is conceivable that 'redox cross talk' occurs between cells/ tissues after exercise. A series of redox enzymes (i,e, PRDX-1, PRDX-2, PRDX-5, PRDX-6, Thioredoxin (TRX)-1, Superoxide Dismutase (SOD)1 and SOD2) are known to be secreted in EVs via a non-classical route in response to stress. Interestingly, there is data to suggest that redox enzymes secreted via the classical secretory pathway (e.g. SOD3 and PRDX-4), but not the non-classical secretory pathway (e.g. TRX-1, PRDX-2 and TRX-Reductase) are released into plasma in response to exercise (Wadley, Keane, *et al.*, 2019). This suggests that non-classically secreted redox enzymes may be contained within EV's. In the context of immunity, PRDX-1 and PRDX-2 have been shown to be released in exosomes following exposure to inflammatory stimuli *in vitro* (Mullen *et al.*, 2015), with PRDX-1 also linked with cytokine production (Riddell *et al.*, 2010). The cargo of EV's needs to be explored after bouts of exercise to discern whether inter-cellular 'redox cross talk' occurs between cells of the immune system after exercise. It is important to note that other plasma proteins (Guseh *et al.*, 2020) and indeed metabolites (e.g. lactate) (Ratter *et al.*, 2018; Zwaag *et al.*, 2020) that are released during exercise may also play important roles in regulating redox-driven immunity after exercise.

7. Conclusion

The redox environment of immune cells is sensitive to changes in both *oxidative* and *reductive stress*; however, accurate evaluation of these changes and their functional significance *in vivo* is a challenge. In the field of exercise physiology, this is further complicated by changes in the composition of leukocytes measured before and after exercise. The use of flow cytometric methods and single cell technologies such as CyTOF™ mass cytometry offer huge potential to unravel this complex picture, placing redox exercise immunology at the dawn of a new era.

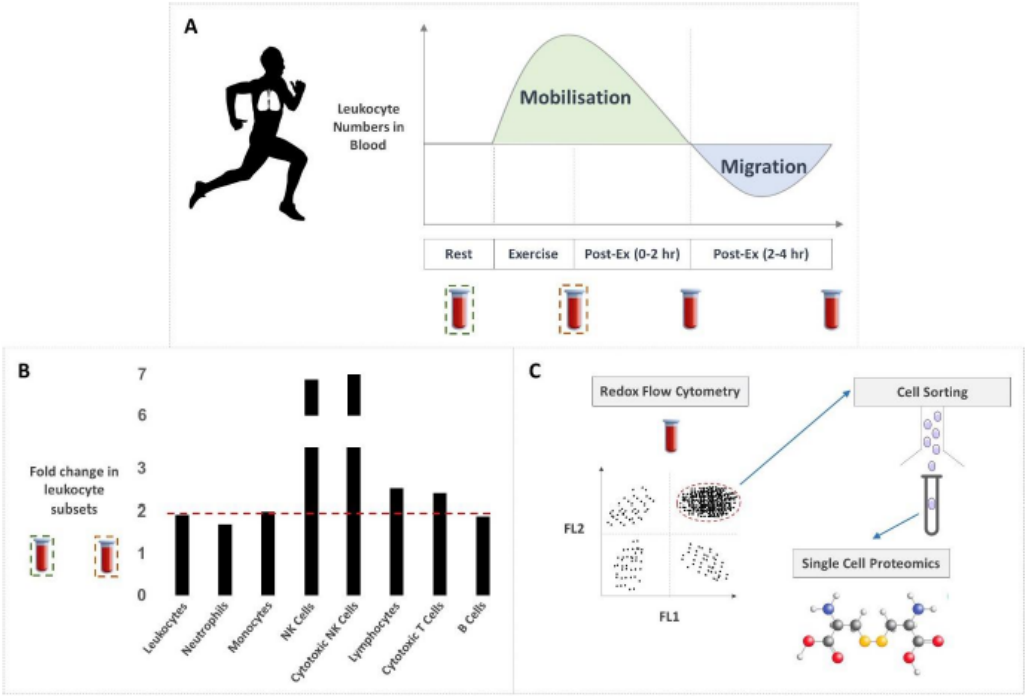

Figure 1: Reductionist approach to conduct single cell analysis of immune cell redox state after exercise in humans. (A) Exercise evokes powerful physiological changes on the number of immune cells in peripheral blood. Notably, leukocyte number increases during and after moderate-to-vigorous exercise (mobilisation) and then falls below resting levels in the hours after exercise cessation (migration). (B) Graphical figure indicating how exercise-induced leukocytosis is not a uniform response. Fold change in blood cell concentrations immediately after a high intensity bout of cycling (20 minutes at 80% VO_2max) relative to resting values has been extracted from 3 published articles (variance not included due to graphical representation) (A J Wadley *et al.*, 2015; Turner, Spielmann, *et al.*, 2016; Turner, Wadley, *et al.*, 2016). NK: Natural Killer (C) By employing suitable cell sorting methods, single cell proteomics can be used to determine exercise-driven redox modifications and their relevance to immune function.

References

Anelli, T., Sannino, S. and Sitia, R. (2015) 'Proteostasis and "redoxtasis" in the secretory pathway: Tales of tails from ERp44 and immunoglobulins.', *Free radical biology & medicine*, 83, pp. 323–30. doi: 10.1016/j.freeradbiomed.2015.02.020.

Bay, M. L. *et al.* (2020) 'Human immune cell mobilization during exercise: effect of IL-6 receptor blockade.', *Experimental physiology*, 105(12), pp. 2086–2098. doi: 10.1113/EP088864.

Bellezza, I. *et al.* (2018) 'Nrf2-Keap1 signaling in oxidative and reductive stress.', *Biochimica et biophysica acta. Molecular cell research*, 1865(5), pp. 721–733. doi: 10.1016/j.bbamcr.2018.02.010.

Brinkmann, V. *et al.* (2004) 'Neutrophil extracellular traps kill bacteria.', *Science (New York, N.Y.)*, 303(5663), pp. 1532–5. doi: 10.1126/science.1092385.

Broadbent, S. and Coutts, R. (2017) 'Intermittent and graded exercise effects on NK cell degranulation markers LAMP-1/LAMP-2 and CD8+CD38+ in chronic fatigue syndrome/myalgic encephalomyelitis.', *Physiological reports*, 5(5). doi: 10.14814/phy2.13091.

Brodie, T. M. and Tosevski, V. (2018) 'Broad Immune Monitoring and Profiling of T Cell Subsets with Mass Cytometry.', *Methods in molecular biology (Clifton, N.J.)*, 1745, pp. 67–82. doi: 10.1007/978-1-4939-7680-5_4.

Campbell, J. P. and Turner, J. E. (2018) 'Debunking the myth of exercise-induced immune suppression: Redefining the impact of exercise on immunological health across the lifespan', *Frontiers in Immunology*, 9(APR), pp. 1–21. doi: 10.3389/fimmu.2018.00648.

Cano Sanchez, M. *et al.* (2018) 'Targeting Oxidative Stress and Mitochondrial Dysfunction in the Treatment of Impaired Wound Healing: A Systematic Review.', *Antioxidants (Basel, Switzerland)*, 7(8). doi: 10.3390/antiox7080098.

Cobley, J. N. *et al.* (2017) 'Exercise redox biochemistry: Conceptual, methodological and technical recommendations', *Redox Biology*. Elsevier B.V., 12(March), pp. 540–548. doi: 10.1016/j.redox.2017.03.022.

Daiber, A. *et al.* (2013) 'Protein tyrosine nitration and thiol oxidation by peroxynitrite-strategies to prevent these oxidative modifications.', *International journal of molecular sciences*, 14(4), pp. 7542–70. doi: 10.3390/ijms14047542.

Dimeloe, S. *et al.* (2017) 'T-cell metabolism governing activation, proliferation and differentiation; a modular view.', *Immunology*, 150(1), pp. 35–44. doi: 10.1111/imm.12655.

Emery, C. F. *et al.* (2005) 'Exercise accelerates wound healing among healthy older adults: a preliminary investigation.', *The journals of gerontology. Series A, Biological sciences and medical sciences*, 60(11), pp. 1432–6. doi: 10.1093/gerona/60.11.1432.

Eskola, J. *et al.* (1978) 'Effect of sport stress on lymphocyte transformation and antibody formation.', *Clinical and experimental immunology*, 32(2), pp. 339–45.

Ferreira, L. F. and Reid, M. B. (2008) 'Muscle-derived ROS and thiol regulation in muscle fatigue.', *Journal of applied physiology (Bethesda, Md. : 1985)*, 104(3), pp. 853–60. doi: 10.1152/japplphysiol.00953.2007.

Ferrer, M. D. *et al.* (2009) 'Antioxidant regulatory mechanisms in neutrophils and lymphocytes after intense exercise.', *Journal of sports sciences*, 27(1), pp. 49–58. doi: 10.1080/02640410802409683.

Flohé, L. *et al.* (2011) 'A comparison of thiol peroxidase mechanisms.', *Antioxidants & redox signaling*, 15(3), pp. 763–80. doi: 10.1089/ars.2010.3397.

Fox, C. J., Hammerman, P. S. and Thompson, C. B. (2005) 'Fuel feeds function: energy metabolism and the T-cell response.', *Nature reviews. Immunology*, 5(11), pp. 844–52. doi: 10.1038/nri1710.

Gleeson, M. *et al.* (2011) 'The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease', *Nature Reviews Immunology*, 11, pp. 607–615.

Graff, R. M. *et al.* (2018) 'β 2 -Adrenergic receptor signaling mediates the preferential mobilization of differentiated subsets of CD8+ T-cells, NK-cells and non-classical monocytes in response to acute exercise in humans', *Brain, Behavior, and Immunity*, 74(July), pp. 143–153. doi: 10.1016/j.bbi.2018.08.017.

Guseh, J. S. *et al.* (2020) 'An expanded repertoire of intensity-dependent exercise-responsive plasma proteins tied to loci of human disease risk.', *Scientific reports*, 10(1), p. 10831. doi: 10.1038/s41598-020-67669-0.

Gustafson, M. P. *et al.* (2017) 'A systems biology approach to investigating the influence of exercise and fitness on the composition of leukocytes in peripheral blood', *J Immunother Cancer*, 5(30), pp. 1–14.

Heck, S., Bishop, C. J. and Ellis, R. J. (2019) 'Immunophenotyping of Human Peripheral Blood Mononuclear Cells by Mass Cytometry.', *Methods in molecular biology (Clifton, N.J.)*, 1979, pp. 285–303. doi: 10.1007/978-1-4939-9240-9_18.

Vander Heiden, M. G., Cantley, L. C. and Thompson, C. B. (2009) 'Understanding the Warburg effect: the metabolic requirements of cell proliferation.', *Science (New York, N.Y.)*, 324(5930), pp. 1029–33. doi: 10.1126/science.1160809.

Jackson, S. H. *et al.* (2004) 'T cells express a phagocyte-type NADPH oxidase that is activated after T cell receptor stimulation.', *Nature immunology*, 5(8), pp. 818–27. doi: 10.1038/ni1096.

Kamiński, M. M. *et al.* (2012) 'T cell activation is driven by an ADP-dependent glucokinase linking enhanced glycolysis with mitochondrial reactive oxygen species generation.', *Cell reports*, 2(5), pp. 1300–15. doi: 10.1016/j.celrep.2012.10.009.

Kay, A. W., Strauss-Albee, D. M. and Blish, C. A. (2016) 'Application of Mass Cytometry (CyTOF) for Functional and Phenotypic Analysis of Natural Killer Cells.', *Methods in molecular biology (Clifton, N.J.)*, 1441, pp. 13–26. doi: 10.1007/978-1-4939-3684-7_2.

Kenny, E. F. *et al.* (2017) 'Diverse stimuli engage different neutrophil extracellular trap pathways.', *eLife*, 6. doi: 10.7554/eLife.24437.

Kolossov, V. L. *et al.* (2015) 'Thiol-based antioxidants elicit mitochondrial oxidation via respiratory complex III.', *American journal of physiology. Cell physiology*. doi: 10.1152/ajpcell.00006.2015.

Krüger, K. *et al.* (2008) 'Exercise-induced redistribution of T lymphocytes is regulated by adrenergic mechanisms.', *Brain, behavior, and immunity*, 22(3), pp. 324–38. doi: 10.1016/j.bbi.2007.08.008.

Lamb, G. D. and Westerblad, H. (2011) 'Acute effects of reactive oxygen and nitrogen species on the contractile function of skeletal muscle.', *The Journal of physiology*, 589(September 2010), pp. 2119–2127. doi: 10.1113/jphysiol.2010.199059.

LaVoy, E. C. *et al.* (2017) 'T-cell redeployment and intracellular cytokine expression

following exercise: effects of exercise intensity and cytomegalovirus infection.', *Physiological reports*, 5(1). doi: 10.14814/phy2.13070.

Lévigne, D. *et al.* (2016) 'NADPH oxidase 4 deficiency leads to impaired wound repair and reduced dityrosine-crosslinking, but does not affect myofibroblast formation.', *Free radical biology & medicine*, 96, pp. 374–84. doi: 10.1016/j.freeradbiomed.2016.04.194.

Lin, D., Gupta, S. and Maecker, H. T. (2015) 'Intracellular Cytokine Staining on PBMCs Using CyTOF™ Mass Cytometry.', *Bio-protocol*, 5(1). doi: 10.21769/BioProtoc.1370.

Ma, R. *et al.* (2018) 'A Pck1-directed glycogen metabolic program regulates formation and maintenance of memory CD8+ T cells.', *Nature cell biology*, 20(1), pp. 21–27. doi: 10.1038/s41556-017-0002-2.

Macintyre, A. N. and Rathmell, J. C. (2013) 'Activated lymphocytes as a metabolic model for carcinogenesis.', *Cancer & metabolism*, 1(1), p. 5. doi: 10.1186/2049-3002-1-5.

Matthews, C. E. *et al.* (2002) 'Moderate to vigorous physical activity and risk of upper-respiratory tract infection.', *Medicine and science in sports and exercise*, 34(8), pp. 1242–8. doi: 10.1097/00005768-200208000-00003.

Michailidis, Y. *et al.* (2013) 'Thiol-based antioxidant supplementation alters human skeletal muscle signaling and attenuates its inflammatory response ... signaling and attenuates its inflammatory response and recovery after', *Am J Clin Nutr*, 98, pp. 233–45. doi: 10.3945/ajcn.112.049163.

Mullen, L. *et al.* (2015) 'Cysteine Oxidation Targets Peroxiredoxins 1 and 2 for Exosomal Release through a Novel Mechanism of Redox-Dependent Secretion', (10), pp. 98–108. doi: 10.2119/molmed.2015.00033.

Muri, J. and Kopf, M. (2020) 'Redox regulation of immunometabolism.', *Nature reviews. Immunology*. doi: 10.1038/s41577-020-00478-8.

Nathan, C. and Cunningham-Bussel, A. (2013) 'Beyond oxidative stress: an immunologist's guide to reactive oxygen species.', *Nature reviews. Immunology*, 13(5), pp. 349–61. doi: 10.1038/nri3423.

Nieman, D. C. *et al.* (2011) 'Upper respiratory tract infection is reduced in physically fit and active adults.', *British journal of sports medicine*, 45(12), pp. 987–92. doi: 10.1136/bjsm.2010.077875.

Nikolaidis, M. G., Margaritelis, N. V and Matsakas, A. (2020) 'Quantitative Redox Biology of Exercise.', *International journal of sports medicine*, 41(10), pp. 633–645. doi: 10.1055/a-1157-9043.

Peake, J. M. *et al.* (2017) 'Recovery of the immune system after exercise.', *Journal of applied physiology (Bethesda, Md. : 1985)*, 122(5), pp. 1077–1087. doi: 10.1152/japplphysiol.00622.2016.

Pei, L. and Wallace, D. C. (2018) 'Mitochondrial Etiology of Neuropsychiatric Disorders.', *Biological psychiatry*, 83(9), pp. 722–730. doi: 10.1016/j.biopsych.2017.11.018.

Peres, A. *et al.* (2020) 'DNA damage in mononuclear cells following maximal exercise in sedentary and physically active lean and obese men.', *European journal of sport science*, pp. 1–10. doi: 10.1080/17461391.2020.1801850.

Petersen, A. C. *et al.* (2012) 'Infusion with the antioxidant N -acetylcysteine attenuates early adaptive responses to exercise in human skeletal muscle', *Acta Ohysiol*, 204, pp. 382–392. doi: 10.1111/j.1748-1716.2011.02344.x.

Poole, L. B. (2015) 'The basics of thiols and cysteines in redox biology and chemistry.', *Free*

radical biology & medicine, 80, pp. 148–57. doi: 10.1016/j.freeradbiomed.2014.11.013.

Powers, S. K., Talbert, E. E. and Adhihetty, P. J. (2011) 'Reactive oxygen and nitrogen species as intracellular signals in skeletal muscle.', *The Journal of physiology*, 589(Pt 9), pp. 2129–2138. doi: 10.1113/jphysiol.2010.201327.

Ratter, J. M. *et al.* (2018) 'In vitro and in vivo Effects of Lactate on Metabolism and Cytokine Production of Human Primary PBMCs and Monocytes.', *Frontiers in immunology*, 9, p. 2564. doi: 10.3389/fimmu.2018.02564.

Reid, M. . (2008) 'Free radicals and muscle fatigue: Of ROS, canaries, and the IOC', *Free Radic Biol Med.*, 44(2), pp. 169–179.

Riddell, J. R. *et al.* (2010) 'Peroxiredoxin 1 stimulates secretion of proinflammatory cytokines by binding to TLR4.', *Journal of immunology (Baltimore, Md. : 1950)*, 184(2), pp. 1022–30. doi: 10.4049/jimmunol.0901945.

Rooney, B. V *et al.* (2018) 'Lymphocytes and monocytes egress peripheral blood within minutes after cessation of steady state exercise: A detailed temporal analysis of leukocyte extravasation.', *Physiology & behavior*, 194, pp. 260–267. doi: 10.1016/j.physbeh.2018.06.008.

Sakelliou, A. *et al.* (2016) 'Evidence of a Redox-Dependent Regulation of Immune Responses to Exercise-Induced Inflammation', *Oxidative Medicine and*, pp. 1–19.

Sallusto, F. *et al.* (1999) 'Two subsets of memory T lymphocytes with distinct homing potentials and effector functions', *Nature*, 401, pp. 708–712.

Sies, H. and Jones, D. P. (2020) 'Reactive oxygen species (ROS) as pleiotropic physiological signalling agents.', *Nature reviews. Molecular cell biology*, 21(7), pp. 363–383. doi: 10.1038/s41580-020-0230-3.

Simoni, Y. *et al.* (2018) 'Mass cytometry: a powerful tool for dissecting the immune landscape.', *Current opinion in immunology*, 51, pp. 187–196. doi: 10.1016/j.coi.2018.03.023.

Simpson, R. J. *et al.* (2020) 'Can exercise affect immune function to increase susceptibility to infection?', *Exercise immunology review*, 26, pp. 8–22.

Sobotta, M. C. *et al.* (2014) 'Peroxiredoxin-2 and STAT3 form a redox relay for H2O2 signaling', *Nature Chemical Biology*, 11(1), pp. 64–70. doi: 10.1038/nchembio.1695.

Spanidis, Y. *et al.* (2018) 'Exercise-Induced Reductive Stress Is a Protective Mechanism against Oxidative Stress in Peripheral Blood Mononuclear Cells.', *Oxidative medicine and cellular longevity*, 2018, p. 3053704. doi: 10.1155/2018/3053704.

Sureda, A. *et al.* (2005) 'Relation between oxidative stress markers and antioxidant endogenous defences during exhaustive exercise.', *Free radical research*, 39(12), pp. 1317–24. doi: 10.1080/10715760500177500.

Tauler, P. *et al.* (2006) 'Increased lymphocyte antioxidant defences in response to exhaustive exercise do not prevent oxidative damage.', *The Journal of nutritional biochemistry*, 17(10), pp. 665–71. doi: 10.1016/j.jnutbio.2005.10.013.

Thierry Léveillard and Najate Aït-Ali (2017) 'Cell Signaling with Extracellular Thioredoxin and Thioredoxin- Like Proteins: Insight into Their Mechanisms of Action', *Oxidative Medicine and Cellular Longevity*, 2017(Article ID 8475125), p. 11 pages. doi: 10.1155/2017/8475125.

Turner, J. E. *et al.* (2011) 'Assessment of oxidative stress in lymphocytes with exercise.', *Journal of applied physiology (Bethesda, Md. : 1985)*, 111(1), pp. 206–11.

Turner, J. E. *et al.* (2013) 'The antioxidant enzyme peroxiredoxin-2 is depleted in lymphocytes seven days after ultra-endurance exercise.', *Free radical research*, 47(10), pp. 821–8.

Wadley, A. J. *et al.* (2015) 'Monitoring changes in thioredoxin and over-oxidised peroxiredoxin in response to exercise in humans', *Free Radical Research*, 49(3), pp. 290–298. doi: 10.3109/10715762.2014.1000890.

Wadley, A.J. *et al.* (2015) 'The impact of intensified training with a high or moderate carbohydrate feeding strategy on resting and exercise-induced oxidative stress', *European journal of applied physiology*.

Wadley, A J *et al.* (2018) 'Detecting intracellular thiol redox state in leukaemia and heterogeneous immune cell populations: An optimised protocol for digital flow cytometers.', *MethodsX*, 5, pp. 1473–1483. doi: 10.1016/j.mex.2018.10.013.

Wadley, A.J. *et al.* (2018) 'Preliminary evidence of reductive stress in human cytotoxic T-cells following exercise', *Journal of applied physiology*.

Wadley, A. J., Keane, G., *et al.* (2019) 'Characterisation of extracellular redox enzyme concentrations in response to exercise in humans', *Journal of Applied Physiology*, In Press.

Wadley, A. J., Morgan, R. G., *et al.* (2019) *Using flow cytometry to detect and measure intracellular thiol redox status in viable T cells from heterogeneous populations*, *Methods in Molecular Biology*. doi: 10.1007/978-1-4939-9463-2_5.

Wadley, A. J., Aldred, S. and Coles, S. J. (2016) 'An unexplored role for Peroxiredoxin in exercise-induced redox signalling?', *Redox Biology*, 8. doi: 10.1016/j.redox.2015.10.003.

Walsh, N. P. (2018) 'Recommendations to maintain immune health in athletes.', *European journal of sport science*, 18(6), pp. 820–831. doi: 10.1080/17461391.2018.1449895.

Weyand, C. M., Shen, Y. and Goronzy, J. J. (2018) 'Redox-sensitive signaling in inflammatory T cells and in autoimmune disease.', *Free radical biology & medicine*, 125, pp. 36–43. doi: 10.1016/j.freeradbiomed.2018.03.004.

Whitham, M. *et al.* (2018) 'Extracellular Vesicles Provide a Means for Tissue Crosstalk during Exercise', *Cell Metabolism*. Elsevier, 27(1), pp. 237-251.e4. doi: 10.1016/j.cmet.2017.12.001.

van der Windt, G. J. W. and Pearce, E. L. (2012) 'Metabolic switching and fuel choice during T-cell differentiation and memory development.', *Immunological reviews*, 249(1), pp. 27–42. doi: 10.1111/j.1600-065X.2012.01150.x.

Winterbourn, C. C. (2013) *The biological chemistry of hydrogen peroxide*. 1st edn, *Methods in Enzymology*. 1st edn. Elsevier Inc. doi: 10.1016/B978-0-12-405881-1.00001-X.

Winterbourn, C. C. (2015) 'Are free radicals involved in thiol-based redox signaling?', *Free Radical Biology and Medicine*. Elsevier, 80, pp. 164–170. doi: 10.1016/j.freeradbiomed.2014.08.017.

Winterbourn, C. C., Kettle, A. J. and Hampton, M. B. (2016) 'Reactive Oxygen Species and Neutrophil Function.', *Annual review of biochemistry*, 85, pp. 765–92. doi: 10.1146/annurev-biochem-060815-014442.

Zwaag, J. *et al.* (2020) 'Involvement of Lactate and Pyruvate in the Anti-Inflammatory Effects Exerted by Voluntary Activation of the Sympathetic Nervous System.', *Metabolites*, 10(4). doi: 10.3390/metabo10040148.